TWIN RESEARCH FOR EVERYONE

From Biology to Health, Epigenetics, and Psychology

Edited by Adam D. Tarnoki, David L. Tarnoki, Jennifer R. Harris, and Nancy L. Segal

Twin Research for Everyone

From Biology to Health, Epigenetics, and Psychology

Twin Research for Everyone

From Biology to Health, Epigenetics, and Psychology

Edited by

Adam D. Tarnoki
Medical Imaging Centre, Semmelweis University, Hungarian Twin Registry, Budapest, Hungary

David L. Tarnoki
Medical Imaging Centre, Semmelweis University, Hungarian Twin Registry, Budapest, Hungary

Jennifer R. Harris
Centre for Fertility and Health, The Norwegian Institute of Public Health, Oslo, Norway

Nancy L. Segal
Department of Psychology, California State University, Fullerton, CA, United States

ACADEMIC PRESS
An imprint of Elsevier
elsevier.com/books-and-journals

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1650, San Diego, CA 92101, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

ISBN: 978-0-12-821514-2

Publisher: Stacy Masucci
Acquisitions Editor: Peter B. Linsley
Editorial Project Manager: Samantha Allard
Production Project Manager: Omer Mukthar
Cover Designer: Vicky Pearson Esser

Typeset by Aptara, New Delhi, India

Contents

Contributors

Catarina Almqvist
Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; Pediatric Allergy and Pulmonology Unit at Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden

Juko Ando
Keio Univerity, Tokyo, Japan

Meike Bartels
Department of Biological Psychology, Faculty of Behavioural and Movement Sciences, Vrije Universiteit, Amsterdam, The Netherlands; Amsterdam Public Health Research Institute, Amsterdam University Medical Centre, Amsterdam, The Netherlands

Munkh-Erdene Bayartai
The University of Sydney, Charles Perkins Centre, Sydney School of Health Sciences, Faculty of Medicine and Health

Christopher R. Beam
University of Southern California, Department of Psychology

Adriene M. Beltz
Department of Psychology, University of Michigan, MI, United States

Sheri A. Berenbaum
Department of Psychology, The Pennsylvania State University, University Park, PA, United States; Department of Pediatrics, The Pennsylvania State University, Hershey, PA, United States

Dorret I. Boomsma
Netherlands Twin Register, Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; Amsterdam Public Health Research Institute, Amsterdam, The Netherlands; Amsterdam Reproduction and Development Research Institute, Amsterdam, The Netherlands

Isabell Brikell
Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden

Minh Bui
Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia

Lucas Calais-Ferreira
Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia; Centre for Adolescent Health, Murdoch Children's Research Institute, Melbourne, VIC, Australia

Kaare Christensen
The Danish Twin Registry, Department of Public Health, University of Southern Denmark, Odense, Denmark

Robin P. Corley
Institute for Behavioral Genetics, University of Colorado Boulder, CO, United States

Victoria K. Cortessis
Department of Population and Public Health Science, Keck School of Medicine, University of Southern California, Los Angeles, CA, United States; Department of Obstetrics & Gynecology, Keck School of Medicine, University of Southern California, Los Angeles, CA, United States

Wendy Cozen
Division of Hematology-Oncology, Department of Medicine, School of Medicine, University of California at Irvine, Orange, CA, United States; Department of Pathology, School of Medicine, University of California at Irvine, Orange, CA, United States; Chao Family Comprehensive Cancer Center, University of California at Irvine, Orange, CA, United States

Jeffrey M. Craig
Environmental & Genetic Epidemiology Research, Molecular Immunity, Royal Children's Hospital, Parkville, VIC, Australia; IMPACT Institute, School of Medicine, Deakin University, Geelong, VIC, Australia

Lisabeth Fisher DiLalla
Southern Illinois University School of Medicine

David L. DiLalla
Southern Illinois University Carbondale

Conor V. Dolan
Netherlands Twin Register, Department of Biological Psychology, Vrije Universiteit Amsterdam, and Amsterdam Public Health (APH), Amsterdam, The Netherlands

James G. Dowty
Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia

Jeremy A. Elman
Department of Psychiatry, University of California San Diego; Center for Behavior Genetics of Aging, University of California San Diego

Vivienne F.C. Esser
Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia

Paulo H. Ferreira
The University of Sydney, Charles Perkins Centre, Sydney School of Health Sciences, Faculty of Medicine and Health

Deborah Finkel
Department of Psychology, Indiana University Southeast, United States; Institute for Gerontology, School of Health and Welfare, Jönköping University, Sweden

Irene Giardina
Department of Obstetrics and Gynecology, Centre of Perinatal and Reproductive, Medicine, University of Perugia, Italy

Fiona A. Hagenbeek
Netherlands Twin Register, Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; Amsterdam Public Health Research Institute, Amsterdam, The Netherlands

Irén Haltrich
2nd Department of Pediatrics, Semmelweis University, Budapest, Hungary

Aino Heikkinen
Institute for Molecular Medicine Finland FIMM, HiLIFE, University of Helsinki, Helsinki, Finland

John L. Hopper
Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia

Jouke-Jan Hottenga
Netherlands Twin Register, Department of Biological Psychology, Vrije Universiteit Amsterdam, and Amsterdam Public Health (APH), Amsterdam, The Netherlands

Yoon-Mi Hur
College of General Education, Kookmin University, Seoul, South Korea

Matthew R. Jamnik
Southern Illinois University Carbondale

Kaushalya Jayaweera
Institute for Research & Development in Health & Social Care

Jaakko Kaprio
Institute for Molecular Medicine FIMM, University of Helsinki, Helsinki, Finland

Alice J. Kim
University of Southern California, Department of Psychology

Esther Lam
Division of Hematology-Oncology, Department of Medicine, School of Medicine, University of California at Irvine, Orange, CA, United States; Chao Family Comprehensive Cancer Center, University of California at Irvine, Orange, CA, United States

Henrik Larsson
Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden; School of Medical Sciences, Örebro University, Sweden

Soo Ji Lee
Genome and Health Big Data Lab, Graduate School of Public Health, Seoul National University, Seoul, Korea; Institute of Health and Environment, Seoul National University, Seoul, Korea

Anna Lengyel
2nd Department of Pediatrics, Semmelweis University, Budapest, Hungary

Shuai Li
Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia

Paul Lichtenstein
Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

Levente Littvay
Central European University

John C. Loehlin
University of Texas at Austin, Department of Psychology, Austin, TX, USA

Yuk Jing Loke
Environmental & Genetic Epidemiology Research, Molecular Immunity, Royal Children's Hospital, Parkville, VIC, Australia

Sara Lundgren
Institute for Molecular Medicine Finland FIMM, HiLIFE, University of Helsinki, Helsinki, Finland

Thomas M. Mack
Department of Population and Public Health Science, Keck School of Medicine, University of Southern California, Los Angeles, CA, United States

Sue Malta
Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia

Riley L. Marshall
Southern Illinois University Carbondale

Nicholas G. Martin
Genetic Epidemiology Group, QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

Matt McGue
The Danish Twin Registry, Department of Public Health, University of Southern Denmark, Odense, Denmark; Department of Psychology, University of Minnesota, Minneapolis, MN, United States

Sarah E. Medland
Mental Health & Neuroscience Department, QIMR Berghofer Medical Research Institute, QLD, Australia

Brittany L. Mitchell
Mental Health & Neuroscience Department, QIMR Berghofer Medical Research Institute, QLD, Australia

Elena Cristina Mitrea
Lucian Blaga University of Sibiu

Namitha Mohandas
Environmental & Genetic Epidemiology Research, Molecular Immunity, Royal Children's Hospital, Parkville, VIC, Australia; Department of Paediatrics, University of Melbourne, Parkville, VIC, Australia; IMPACT Institute, School of Medicine, Deakin University, Geelong, VIC, Australia

José J. Morosoli
Mental Health & Neuroscience Department, QIMR Berghofer Medical Research Institute, QLD, Australia

Julia Métneki
Retired, Twin Researcher, Foundation of Hungarian Twin Registry; Hungarian Twin Registry, Budapest, Hungary

Tuong L. Nguyen
Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia

Francisca J. Niculae
Department of Psychology, California State University, Fullerton, CA, United States

Veronika Odintsova
Netherlands Twin Register, Department of Biological Psychology, Vrije Universiteit Amsterdam, and Amsterdam Public Health (APH), Amsterdam, The Netherlands; National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I.Kulakov of Ministry of Healthcare of the Russian Federation, Moscow, Russia

Miina Ollikainen
Institute for Molecular Medicine Finland FIMM, HiLIFE, University of Helsinki, Helsinki, Finland

Juan R. Ordoñana
Department of Human Anatomy and Psychobiology, University of Murcia, Murcia Institute for BioHealth Research (IMIB-Arrixaca-UMU), Murcia, Spain

Emily Pali
Southern Illinois University Carbondale

Matthew S. Panizzon
Department of Psychiatry, University of California San Diego; Center for Behavior Genetics of Aging, University of California San Diego

Hang A. Park
Genome and Health Big Data Lab, Graduate School of Public Health, Seoul National University, Seoul, Korea; Department of Emergency Medicine, Hallym University Dongtan Sacred Heart Hospital, Hwaseong-si, Korea

Nancy L. Pedersen
Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden; Department of Psychology, University of Southern California, United States

Holly T. Pham
Department of Psychology, The Pennsylvania State University, University Park, PA, United States

Tinca J.C. Polderman
Amsterdam UMC, Vrije Universiteit Amsterdam, Child and Adolescent, Psychiatry & Psychosocial Care, Amsterdam Public Health; Vrije Universiteit Amsterdam, Clinical and Developmental Psychology, Amsterdam, The Netherlands

René Pool
Netherlands Twin Register, Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; Amsterdam Public Health Research Institute, Amsterdam, The Netherlands

András Pári
Pázmány Péter Catholic University (PPKE), Maria Kopp Institute for Demography and Families (KINCS), Budapest, Hungary

Monica Rankin
International Council of Multiple Birth Organisations (ICOMBO)

Gian Carlo Di Renzo
Department of Obstetrics and Gynecology, Centre of Perinatal and Reproductive, Medicine, University of Perugia, Italy

Chandra A. Reynolds
Department of Psychology, University of California – Riverside, United States

Maryam Salehi
Division of Hematology-Oncology, Department of Medicine, School of Medicine, University of California at Irvine, Orange, CA, United States; Chao Family Comprehensive Cancer Center, University of California at Irvine, Orange, CA, United States

Nancy L. Segal
California State University, Fullerton and Director of the Twin Studies Center, CA, USA; Department of Psychology, California State University, Fullerton, CA, United States

Karri Silventoinen
Population Research Unit, Faculty of Social Sciences, University of Helsinki, Helsinki, Finland

P. Eline Slagboom
Leiden University Medical Centre, Leiden, The Netherlands

Gary Steinman
Department of Obstetrics & Gynecology, Hadassah Hospital—Hebrew University, Jerusalem, Israel

Garth Stephenson
IMPACT Institute, School of Medicine, Deakin University, Geelong, VIC, Australia

Athula Sumathipala
Institute for Research & Development in Health & Social Care; School of Medicine, Faculty of Medicine & Health Sciences, Keele University

Joohon Sung
Genome and Health Big Data Lab, Graduate School of Public Health, Seoul National University, Seoul, Korea; Institute of Health and Environment, Seoul National University, Seoul, Korea

Carole Tafforin
Ethospace, Research and Study Group in Human and Space Ethology, Toulouse, France

Adam D. Tarnoki
Medical Imaging Centre, Semmelweis University, Hungarian Twin Registry, Budapest, Hungary

David L. Tarnoki
Medical Imaging Centre, Semmelweis University, Hungarian Twin Registry, Budapest, Hungary

Valentina Tosto
Department of Obstetrics and Gynecology, Centre of Perinatal and Reproductive, Medicine, University of Perugia, Italy; Department of Obstetrics and Gynecology, Giannina Gaslini Children's Hospital, Genova, Italy

Valentina Tsibizova
Almazov State Perinatal Research Centre, St Petersburg, Russia

Peter Varjassy
Retired, Chief Physician, Medical Historian

Eero Vuoksimaa
Institute for Molecular Medicine Finland, University of Helsinki, Finland

Gonneke Willemsen
Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; Amsterdam Public Health Research Institute, Amsterdam, The Netherlands

Yen Ting Wong
IMPACT Institute, School of Medicine, Deakin University, Geelong, VIC, Australia

Eco de Geus
Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; Amsterdam Public Health Research Institute, Amsterdam, The Netherlands

Lianne P. de Vries
Department of Biological Psychology, Faculty of Behavioural and Movement Sciences, Vrije Universiteit, Amsterdam, The Netherlands; Amsterdam Public Health Research Institute, Amsterdam University Medical Centre, Amsterdam, The Netherlands

Jenny van Dongen
Netherlands Twin Register, Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; Amsterdam Public Health Research Institute, Amsterdam, The Netherlands; Amsterdam Reproduction and Development Research Institute, Amsterdam, The Netherlands

Margot P. van de Weijer
Department of Biological Psychology, Faculty of Behavioural and Movement Sciences, Vrije Universiteit, Amsterdam, The Netherlands; Amsterdam Public Health Research Institute, Amsterdam University Medical Centre, Amsterdam, The Netherlands

Editor Biographies

Dr Adam D. Tarnoki is an assistant professor, radiologist, and twin researcher at Semmelweis University Medical Imaging Center and Department Head, Department of Radiology at National Institute of Oncology, Budapest, Hungary. He is a founding member of the Hungarian Twin Registry. He has presented more than 200 international lectures and has authored over 150 publications. He is a board member of the Hungarian Society of Radiologists and teaches radiology in English, German, and Hungarian at Semmelweis University. Dr. Tarnoki serves as vice-president of the Hungarian Medical Association of America Hungary Chapter. He has also served as a member of the local organizing committee and Faculty of the 3rd World Congress on Twin Pregnancy and the 15th Congress of the International Society for Twin Studies (ISTS) in Budapest, Hungary, 2014, and the local host of the 19th International Congress on Twin Studies hold online in November 2021. He serves as deputy editor-in-chief of the *Hungarian Radiology Journal*, and he is the president of the International Society of Twin Studies (ISTS) since 2022.

Dr David L. Tarnoki is an assistant professor, radiologist, and twin researcher at Semmelweis University Medical Imaging Center and Head of Oncologic Imaging Diagnostic Center, National Institute of Oncology, Budapest, Hungary. He is a founding member of the Hungarian Twin Registry and has presented more than 200 international lectures and authored over 150 publications. He is the board member of the Hungarian Society of Radiologists and teaches radiology in English, German, and Hungarian at Semmelweis University. Dr. Tarnoki serves as secretary of the Hungarian Medical Association of America Hungary Chapter and is a member of the Academy of Young Researchers, Hungarian Academy of Sciences. He has also served as a member of the local organizing committee and Faculty of the 3rd World Congress on Twin Pregnancy and the 15th Congress of the International Society for Twin Studies (ISTS) in Budapest, Hungary, 2014, and the local host of the 19th International Congress on Twin Studies hold online in November 2021. He is the secretary of the International Society of Twin Studies (ISTS) since 2022.

Dr Jennifer R. Harris is currently a research director at the Centre for Fertility and Health at the Norwegian Institute of Public Health in Oslo, Norway. She has interdisciplinary training in life-span development and genetics. She has been conducting twin research throughout her academic career. She is the past president of the International Society for Twin Studies, a recipient of the James Shields Award for outstanding research in behavioral genetics, and is the scientific director of the Norwegian Twin Registry. Dr. Harris has a broad commitment to the wider scientific community and serves on several expert panels, boards, steering groups, scientific advisory committees, and editorial boards.

Dr Nancy L. Segal is currently a professor of psychology at California State University, Fullerton, and Director of the Twin Studies Center. She has authored over 250 scientific articles and book chapters, as well as seven books on twins, most recently, Deliberately Divided: Inside the Controversial Study of Twins and Triplets Adopted Apart. Her 2012 book, Born Together-Reared Apart: The Landmark Minnesota Twin Study, won the 2013 William James Award from the American Psychological Association. Dr. Segal has received several international awards including the James Shields Award for Lifetime Contributions to Twin Research (International Society for Twin Studies) and the International Making a Difference Award (Multiple Births Canada). She is also the 2016 recipient of the prestigious Wang Family Excellence Award from California State University. Dr. Segal has contributed to national and international media, including the New York Times and the Washington Post. She has been a frequent guest on national and international television and radio programs, such as the Today Show, Good Morning America, The Martha Stewart Show, the Oprah Winfrey Show, The Forum (BBC), and the Hidden Brain National Public Radio.

Preface

The creation of *Twin Research for Everyone* originated from a Hungarian twin research book, edited by Adam and David Tarnoki. It quickly became clear that a book on twin research, targeted to students and scholars brought together cutting-edge research from international contributors to the widest possible audience, as needed. The Tarnokis noted the necessity for such a book and through discussions with Nancy Segal during a 2015 twin research conference in Osaka, Japan, plans for this book began to take shape. Segal enthusiastically supported this idea and agreed to serve as a co-editor. Jennifer Harris, the president of the *International Society for Twin Studies*, was also asked to be an editor. Once the four editors selected the topical content, invitations for chapters were sent to leading researchers in the field. All invited authors accepted the invitation to contribute to this unique book. Two of Elsevier's editors—Samantha Allard and Peter B. Linsley—gladly took on the project and worked with us over the 3-year period required for its completion. We hope your reading experience will be informative and inspiring!

Introduction to twin research for everyone: From biology to health, epigenetics, and psychology

Adam D. Tarnoki[a], David L. Tarnoki[a], Jennifer R. Harris[b], Nancy L. Segal[c]

[a]*Medical Imaging Centre, Semmelweis University, Hungarian Twin Registry, Budapest, Hungary*

[b]*Centre for Fertility and Health, The Norwegian Institute of Public Health, Oslo, Norway*

[c]*Department of Psychology, California State University, Fullerton, CA, United States*

Twin research has transformed our understanding of the influences that affect human health, development and aging. As illustrated by this book, this is a particularly exciting time to be studying twins. Novel analytical methods coupled with large and diverse types of data collected in twin studies worldwide provide new and unique opportunities for elucidating the complex factors that help answer some of the most important questions confronting science. For example, why do we age differently or what factors explain human variation in health outcomes? Twinning rates have soared dramatically in recent years, at least in western nations. Why that has happened is a fascinating story that you will learn about in this book. More and more countries are establishing twin registries[1] and researchers all over the world are using twin models to disentangle genetic and environmental influences on behavioral, medical, and physical traits. However, interest in twins has also grown among political scientists, religious scholars and economic researchers who study twins to learn more about the bases of political attitudes, participation in religious activities and how years of education affect earnings. The editors of *Twin Research for Everyone: From Biology to Health, Epigenetics, and Psychology* believe that virtually any study can be made more informative when data from identical (monozygotic or MZ) and fraternal (dizygotic of DZ) twin participants are included. We hope that when you finish reading this book you will understand the value of twin studies and why they continue to play such an important role in research across a wide span of scientific inquiry.

It is important to recognize that findings from twin studies are not just for twins—most research findings, except for those related to the unique conceptions and birth events of twins, apply to everyone. The classic twin design—comparing the degree of resemblance between identical and fraternal twin pairs–is best thought of as a model for identifying and understanding the influences that explain why we differ from one another across a broad spectrum of human traits. However, growing up as a twin is another key aspect of twin studies that continues to be the focus of a great deal of research. Not everyone is raised alongside a same-age brother or sister, a situation that warrants special attention from parents, educators and counselors. You will learn what having a twin is like in the chapter, "Growing Up as Twins: The Perspectives of Twin Researchers." The contributors to this chapter, some of whom are editors of this book, became interested in twin studies because of their personal experiences

and knowledge of what twinship means, and their desire to know more about who they are and how they got that way.

The sections and chapters of *Twin Research for Everyone* unfold in a logical sequence. After this Introduction, we begin with Section I on background. This section includes Chapter 1, on the history of twin studies, that describes how the field began and how it has progressed. Chapter 2 looks at twinning rates throughout the world. Identical twinning occurs at about the same rate regardless of country or ethnicity, but the fraternal (non-identical) twinning rate varies a great deal. This discussion is followed by Chapter 3 on twin registries worldwide that explains why twin registries are such valuable resources that help researchers conduct their work.

Section II focuses on the phenomenology of twinning. Chapter 4 explores biological aspects of twinning, chapter 5 concerns the management of twin pregnancies, and chapter 6 provides special insights related to conjoined twins. Section III features chapters that address different aspects of twins in families. Chapter 7 presents personal twin and family perspectives, which may be most interesting for those readers who are twins or twin parents themselves. Chapter 8 discusses issues relevant to the parenting of twins, and Chapter 9 focuses on the very interesting topics surrounding reared-apart twins and switched-at-birth twins. Chapter 10 covers research topics related to opposite-sex twins, which is a group that is often neglected in the twin-based literature.

Section IV presents twin methodologies. These chapters are intended for all students, but especially future twin researchers and statisticians. Chapter 11 looks closely at the establishment and management of twin registers. Chapter 12 introduces basic and advanced concepts and analytical approaches used in twin research. This background is important for understanding results from twin studies which are described in several later chapters in the book. Chapter 13 provides an overview of findings from twin studies of complex traits and diseases that are based largely on the approaches described in chapter 12. This includes discussions of sex differences in heritability and gene-environment interplay. Chapter 14 enriches the methodological content and describes the use of twin studies to make inferences about causation. This section ends with Chapter 15 which discusses the use of clinical trials in twin research.

Section V focuses on twin studies of behavior and summarizes findings spanning a wide range of behavioral traits. Chapter 16 is concerned with what twin studies tell us about the social sciences, especially political attitudes, Chapter 17 discusses the development of childhood psychiatric disorders, and Chapter 18 focuses on well-being. However, there is much more to come. Chapter 19 offers an overview of personality research, Chapter 20 examines psychopathology, Chapter 21 discusses cognitive aging, and Chapter 22 addresses tobacco use and smoking behavior. You will see that a twin research perspective adds immeasurably to what we know, and what we can know, about these behavioral traits.

Section VI of *Twin Research for Everyone* includes chapters on health-related topics. Chapter 23 reviews anthropometric studies of twins, Chapter 24 examines cardiovascular characteristics, and Chapter 25 offer insights from pediatric twin studies. Health-related topics are continued in Chapter 26 which addresses twin-singleton

differences, Chapter 27 considers studies of puberty, and Chapter 28 presents twin studies of musculoskeletal traits. This section concludes with additional areas of interest. Chapter 29 highlights contributions of twin studies to cancer epidemiology, Chapter 30 focuses on epigenetic studies of neurodevelopment (epigenetics is a topic revisited in the next section) and Chapter 31 reviews how twin studies have contributed to our knowledge about dementia.

Section VII is the penultimate part of this book, taking a close look at twin research that uses diverse types of genetic and other omic data. Chapter 32 describes the new role of twin studies in multinomics, Chapter 33 provides additional information on epigenetics, and Chapter 34 discusses how a co-twin control study of space travel was used to explore changes in telomeres as reflective of aging. The last chapters in this section include Chapter 35 which considers environmental factors affecting neurodevelopmental disorders, Chapter 36 which introduces the microbiome and twin studies, and Chapter 37 which describes chromosomal disorders.

Section VIII is the final portion of *Twin Research for Everyone*. It provides a summary and concluding statement jointly authored by the four editors, commenting on what we have learned from twin studies, and what new avenues they may hold in the future. The appendix provides various resources, such as twin-based books, websites and organizations which should be helpful to many of you. But before you begin reading, it is important to know and acknowledge some of the brightest and darkest events that have occurred over the years. The bright times help us understand how and why twin research has progressed as it has, while the dark times are reminders of the care and attention that our twin research participants and their families deserve. References are provided below for anyone wishing additional information on these topics.

There have been mostly bright moments in the history of twin studies that we can all celebrate—among them are Sir Francis Galton's discovery of the twin method in 1875; the first report of reared-apart twins in 1922; formation of the *International Society for Twin Studies (ISTS)* in 1974; launching of the International Twin Workshops on Statistical Genetic Methods for Human Complex Traits, which started in 1987 and continues today to train researchers worldwide interested in learning twin methodology, and the 340-day stay of identical twin Scott Kelly at the International Space Station, in 2016, while his brother Mark remained on earth for comparative study. Unfortunately, there are also some dark sides to twin research that warrant mention so they will never be repeated. They include the brutal medical experiments conducted on twin children during the Holocaust by Dr. Josef Mengele, at the Auschwitz-Birkenau concentration camp[2]; a tragic longitudinal comparison of an accidentally castrated identical male twin, raised as a girl, and his uncastrated co-twin[3]; a controversial study of separated infant twins in New York City who were secretly studied for twelve years without their families' knowing they were twins[4,5]; and a heated debate over whether IQ data gathered on separated British twins reflected intentional fraud or clerical error.[6] You can read about these events in the references cited below. It is the responsibility of all researchers to make certain that such activities, conducted in the name of research, are never repeated.

To help understand the myriad aspects and complexity of issues related to twins, either as a parent, a researcher, a twin or an interested individual, we recommend this comprehensive book to you. We hope that as you read *Twin Research for Everyone*, you will sense that certain chapters were written by researchers who are twins themselves, who are great enthusiasts of twin topics. We hope this compilation of work sheds light on why twin research is so vital, illustrates the enormous contributions twin research has made across many fields of study, and highlights why twin registries are, and will continue to be, invaluable research resources for years to come.

Chapter reviewers

Professor Juko Ando (Japan)
Professor Nándor Ács (Hungary)
Dr. Edward Bell, University of Western Ontario (Canada)
Professor Dorret I Boomsma (The Netherlands)
Professor Jeffrey Craig (Australia)
Professor Kaare Christensen (Denmark)
Dr. Noémi Császár-Nagy (Hungary)
Dr. Lucas Calais Ferreira (Australia)
Dr. Bianka Forgó (Sweden, Hungary)
Dr. Chika Honda (Japan)
Professor Jaakko Kaprio (Finland)
Dr. Robert Krueger (USA)
Dr. Robert Lyle (Norway)
Professor Hermine Maes (USA)
Professor Nick Martin (Australia)
Professor Matt McGue (USA)
Dr. Emanuela Medda (Italy)
Dr. Júlia Métneki (Hungary)
Dr. Joseph Nedelec (USA)
Professor Nancy Pedersen (Sweden)
Dr. Marton Piroska (Hungary)
Professor Ragnhild Bang Nes (Norway)
Klara Vitkova Rulikova (Czech Republic)
Professor Richard Saffery (Australia)
Professor Julie Aitken Schermer (Canada)
Professor Harold Snieder (Netherlands)
Professor Antonia Stazi (Italy)
Dr. Garth Stephenson (Australia)
Dr. Elena Szabová, CSc. (Slovakia)
Professor Sára Tóth (Hungary)
Dr. Tony Vernon (USA)

References

1. Hur Y-M, Bogl LH, Ordoñana JR, et al. Twin family registries worldwide: an important resource for scientific research (special issue). *Twin Res Hum Genet*. 2019;22(6):427–437.
2. Segal NL. Holocaust twins: their special bond. *Psychol Today*. 1985;19(8):52–58.
3. Diamond M, Sigmundson HK. Sex reassignment at birth: long-term reviews and clinical implications. *Arch Ped Adol Med*. 1997;151(3):298–304.
4. Segal NL. More thoughts on the Child Development Center Twin Study. *Twin Res Hum Gen*. 2005;8(3):276–281.
5. Segal NL. *Deliberately Divided: Inside the Controversial Study of Twins and Triplets Adopted Apart.* Lanham, MD, Rowman & Littlefield, 2021.
6. NJ Mackintosh, ed. *Cyril Burt: Fraud or Framed?* Oxford: Oxford University Press; 1995.

SECTION

Background 1

CHAPTER

History of Twin Studies 1

John C. Loehlin
University of Texas at Austin, Department of Psychology, Austin, TX, USA

1.1 Twins

A small proportion of human pregnancies result in two offspring—something like 1% of pregnancies worldwide, varying across subpopulations in the range of ten percent to six-tenths of one percent. Multiple births greater than two do occur but are much rarer. A pair of offspring from a single human pregnancy are called *twins*.

Twins are likely to be recognized as a distinct category in any population large enough for the occurrence of a number of multiple births. For example, in Greek mythology, Castor and Pollux are twins with bonds so close that when Castor dies, Pollux gives up half of his immortality to be with his brother. In Nigerian mythology, the Ibeji are twins of joy and happiness and are viewed as one soul shared between two bodies. Other instances of twins are found in the legends and lore of ancient Egypt, Syria, Norse mythology, Central America, the Jews, and Hinduism. As this suggests, twins are often accorded special status.

1.2 Twin studies

Studies of twins (beyond studies of the twinning process itself) have been stimulated by the recognition that twin pairs fall mostly into two categories: identical (one egg, Monozygotic, MZ) twins and fraternal (two-egg, Dizygotic, DZ) twins. MZ twins originate in the fertilization of one egg by one sperm and a subsequent splitting of the developmental process into two individuals with the same genetic characteristics. DZ twins represent the fertilization of one egg by one sperm and another egg by another sperm in close enough succession to result in a single pregnancy, consisting of a pair of individuals who resemble ordinary brothers and sisters in having one gene from each parent in each pair. Thus, if a trait is heavily influenced by genes, identical twins, because of their identical genetic origin, will be tend to be more alike on the trait than fraternal twins, who only show the genetic resemblance of ordinary siblings.

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00025-8

Ordinary sibling pairs, because they share parents, have a genetic resemblance of about one-half—varying somewhat with *assortative mating*, the degree of genetic similarity of parents on the trait in question. For a trait for which assortative mating is negligible, the difference in resemblance between MZ and DZ twins estimates one-half of the genetic influence on that trait in that population. Doubling that difference yields the *heritability*, an estimate of the proportion of the variance of the trait due to the genes. For example, if we consider a trait in which spouse correlations are fairly small, such as extraversion (0.06–0.12 in one summary of US and British studies),[1] MZ correlations of 0.48, 0.52, 0.48, and 0.53 in US samples occurred in conjunction with DZ correlations of 0.06, 0.04, 0.10, and 0.25. Literal application of the "double the difference" rule would yield heritability estimates of 0.84, 0.96, 0.76, and 0.56 for extraversion. The correlations are based on fairly modest twin samples (in the range 34–152), so the correlations—and hence the heritability estimates—should not be taken as precise, but together they suggest a substantial genetic component for the trait of Extraversion.

1.3 History of twin studies

The history of twin studies may be looked at along various dimensions. First, twin studies got more subtle over time, taking into account various factors that might bias their estimates, such as assortative mating of parents for the trait in question, or the fact that twin pairs are always both the same age, whereas ordinary sib pairs are always of different ages, or the fact that MZ pairs are always of the same sex whereas about half of DZ pairs are opposite-sexed. Second, the studies often have not been simple twin studies, but have included groups other than twins, such as adopted children, other biological children of the twins' parents, other relatives, the children of MZ and DZ twins, etc. These can permit tests of some of the assumptions underlying simple twin studies. Third, twin resemblances and some of the other comparisons can be followed longitudinally to see if heritabilities increase or decrease over time or with development. And finally, twin samples have got larger—much larger—as they have been based on national birth records or registration for military service, rather than twins located, for example, in a few local high schools. The development of sophisticated statistical techniques, especially statistical equation modeling (SEM), makes hypothesis-testing with multivariate data structures possible. This is accompanied by the development of user-friendly computer package programs such as Mplus (Muthén and Muthén, 2017)[2] and OpenMx (Neale et al., 2017).[3]

The history of scientifically based twin studies may as well be started with Francis Galton (1822–1911),[4] a half-cousin of Charles Darwin. Galton was concerned with the relative roles of nature and nurture in shaping human traits and recognized the value of resemblances among different classes of relatives for this purpose—inventing the correlation coefficient in the process. He never did a twin study in the modern sense but did notice a key feature for such studies: the existence of a substantial subgroup among twin pairs in which the twins of a pair were extraordinarily similar in appearance and behavior (i.e., MZ twins).

1.4 Early twin studies of cognition and personality

Early twin studies proper included those of Merriman (1924),[5] Lauterbach (1925),[6] Tallman (1928),[7] and Holzinger (1929)[8] on intelligence as measured by IQ tests. All of them found higher correlations for MZ than DZ pairs, and thus evidence for the heritability of cognitive ability.

Many twin studies have been carried out for personality and temperament measures. A fairly typical early example was that of Carter (1935),[9] using the six scales of the Bernreuter temperament test: neuroticism, self-sufficiency, introversion, dominance, self-confidence, and sociability. The study included 55 MZ and 44 same-sex DZ pairs. The twin correlations ranged from 0.44 to 0.71 for the MZs and −0.14 to 0.41 for the same-sex DZs, higher for the MZs than the DZs on every scale. A simple "twice the difference" procedure yields heritabilities in the range 0.20–1.16 across the traits, a range presumably inflated by sampling and measurement error but suggesting the appreciable heritability of traits of this kind.

1.5 Combining other relatives with twins

Measuring twins' parents as well as twins allows assessment of the degree of assortative mating for the trait in the population in question, and hence the reasonableness of the "twice the difference" approach to heritability estimation. The difference between adoptive siblings' resemblance (assumed to be due to shared family environment, not genes) and that of MZ pairs (shared family environment, plus genes) provides a direct estimate of genetic effects on a trait.

Thus adding other relatives to a twin study, or combining twin, adoption, and family studies, may verify the assumptions or allow adjustment for the biases in twin studies taken alone.

1.6 Heritability over age

One can compare heritability estimates from twins at different ages. Sometimes this involves a comparison of heritability estimates obtained from different twin samples that are of different ages. Sometimes this involves a single sample of twins tested repeatedly across age. An example of this is the Louisville Twin Study (Wilson and Matheny, 1986),[10] in which the similarity of twins was assessed from birth to age 15, going from near zero heritabilities at birth to 0.32 at age 7 to 0.68 at age 15.

An example of the comparison of twin samples of various ages is found in a consortium of 11,000 pairs of twins from four countries (Haworth et al., 2010),[11] which obtained heritabilities for intellectual ability in childhood, adolescence, and young adulthood of 0.41, 0.55, and 0.66, respectively.

One interpretation of the heritability increases in either type of study is that individuals have an increasing control of their exposure to environments as they grow

older, and increasingly the environments chosen are consistent with the individual's genetic predispositions.

1.7 Increasing sample sizes

This may be illustrated by the difference in the number of twin pairs in the 1935 study by Carter, 99 pairs, and the 2010 study by Haworth et al., 11,000 pairs, an increase by a factor of over 100.

1.8 Twin studies nowadays

Due to recent advances in molecular genetics, a brand new perspective has emerged in twin studies, an epigenetic study using the discordant MZ method[12]. Epigenetics is the study of postnatal chemical changes such as methylation and histone modification in DNA sequences that control gene expression. Because MZ siblings share the same DNA information, significant phenotypic differences between MZ siblings can be due to differences in epigenetic modifications. Although hundreds of discordant twin studies have been conducted so far, few robust conclusions have been established because of the rarity of such MZ pairs. The DISCOTWIN consortium (Willemsen et al., 2015[13]; Avery and Duncun, 2019[14]), which covers the twin registries of Europe and Australia, is promising.

1.9 Summary

We consider twin studies to be studies that compare the different resemblances of MZ and DZ twins on a human trait, in order to obtain an estimate of its heritability—the genetic contribution to individual differences on the trait. Many such studies have been done on many psychological traits since the 1920s.

A number of trends can be observed in twin studies. First, they have been combined with the resemblances of other kinds of relatives in adoption and family studies to test and adjust for various potential biases in twin studies taken alone. Second, they have been carried out at different ages, to test for changes in heritability during development. Third, with the increase in national twin registries and international collaboration they have become much, much larger. And finally, they have tended to result in the conclusion that the genes make a substantial contribution to individual differences in most human psychological traits.

References

1. Loehlin JC. *Genes and Environment in Personality Development*. Newbury Park, CA: Sage; 1992:24–5.

2. Neale MC, Hunter MD, Pritkin J, et al. OpenMx 2.0: Extended structural equation and statistical modeling. *Psychometrika*. 2016;81:535–549.
3. Muthén LK and Muthén BO. *Mplus Statistical Analysis with Latent Variables: User's Guide* (Version 8) (2017), https://www.statmodel.com/download/usersguide/MplusUserGuideVer_8.pdf
4. Galton F. The history of twins as a criterion of the relative powers of nature and nurture. *J Royal Anthrop Inst Great Britain and Ireland*. 1876;6:391–406.
5. Merriman C. The intellectual resemblance of twins. *Psychol Monogr*. 1924;33:1–58.
6. Lauterbach CE. Studies in twin resemblance. *Genetics*. 1925;10:525–568.
7. Tallman GG. A comparative study of identical and non-identical twins with respect to intelligence resemblances. *J Consult Psychol.* 1957; 21:355–357.
8. Holzinger KJ. The relative effect of nature and nurture influences on twin differences. *J educ Psychol*. 1929;20:241–248.
9. Carter HD. Twin similarities in emotional traits. *Char Pers*. 1935;4:61–78.
10. Wilson RS, Matheny Jr AP. Behavior-genetics research in infant temperament: the Louisville twin study. In: Plomin R, Dunn J, eds. *The Study of Temperament*. 1986;81–96.
11. Haworth CMA, Wright MJ, Luciano M, et al. The heritability of general cognitive ability increases linearly from childhood to young adulthood. *Mol Psychiatry*. 2010;15:1112–1120.
12. Tan Q, Christiansen L, Hjelmborg JvB, Christensen K. Twin methodology in epigenetic studies. *J Exp Biol*. 2015;218:134–139.
13. Willemsen G, Ward KJ, Bell CG, et al. The concordance and heritability of Type 2 Diabetes in 34,166 twin pairs from international twin registers: The Discordant Twin (DISCOTWIN) Consortium. *Twin Res Human Genetics*. 2015;18:762–771.
14. Avery AR, Duncan GE. Heritability of Type 2 diabetes in the Washington State Twin Registry. *Twin Res Hum Genet*. 2019;22:95–98.

CHAPTER

Prevalence of twinning worldwide

2

András Pári
Pázmány Péter Catholic University (PPKE), Maria Kopp Institute for Demography and Families (KINCS), Budapest, Hungary

2.1 How is that possible? One delivery and two childbirths at least

The birth of twins has always been surrounded by curiosity. If we look through the history of mankind or at different points of the world, the birth of twins in all societies was surrounded by a sense of peculiarity. In many cultures and religions, the birth of twin was coupled with admiration as it was considered the gift of God, but in many places, it was met with fear and rejection of the community. It is important to highlight that higher (older) age of mothers giving birth to twins—which is a very important factor—occurs in several places, but perhaps the very first written memory is the history of Rebecca, the mother of Jacob and Esau from the Bible (Gen. 25, 21–26). In the world of mythology, twins, with a special appearance that we often find depicted in art, are not hard to find (e.g., Remus and Romulus were the twin sons of Mars and Rhea Silvia (Livy); famous Mayan demigods were Hunahpu and Xbalanque, referred to as the Hero twins (Rideout [2015]). Nowadays in movies, twins are equally as ubiquitous (e.g., Fred and George Weasley—Harry Potter Movies; Luke Skywalker and Leia Organa—Star Wars Movies).

Professional twin research was first started in the 19th century by Sir Francis Galton,[1] an English anthropologist. He was the first to state that if identical twins[a] developed from the outset under the same environmental effects, they would be completely the same. However, he emphasized that this cannot be achieved in real life, as even identical twins have bigger or smaller differences, and since their genetic substance (genome) is the same, the difference can only be traced back to the environment. His recognition has great importance in the field of inheritance research today.

In Europe, especially in the Scandinavian countries, twin-birth ratios were high in the late 19th and early 20th centuries (Eriksson–Abbott–Kostense–Fellman [1995]).[2] Galton's name, mentioned above, is linked to the start of medical-biological twin research,

[a] Biological typing of twins: twin births make up the vast majority of multiple births (97%–98%). The literature distinguishes between two types of twins: monozygotic (monozygotic, MZ) and dicotyledonous (dizygotic, DZ) twins.

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00026-X

but Dionys Hellin should also be highlighted, who, as a research physician, observed the following statistical regularity and summarized the named formula (Hellin's law) in 1895. (If for every n number of single births there is a double twin birth, and then for every n^2 get pregnant one triple, and all n^3 a quadruple plural delivery is given.) It is important to note that the rule is for spontaneous conceptions; Hellin noted that in contemporary Europe at the end of the 19th century, the number was generally $n = 89$. Of course, this varied from country to country: a lower value could be used in the Scandinavian countries and a higher value in the southern and eastern countries (Fig. 2.1 (Hellin, 1895)).[b]

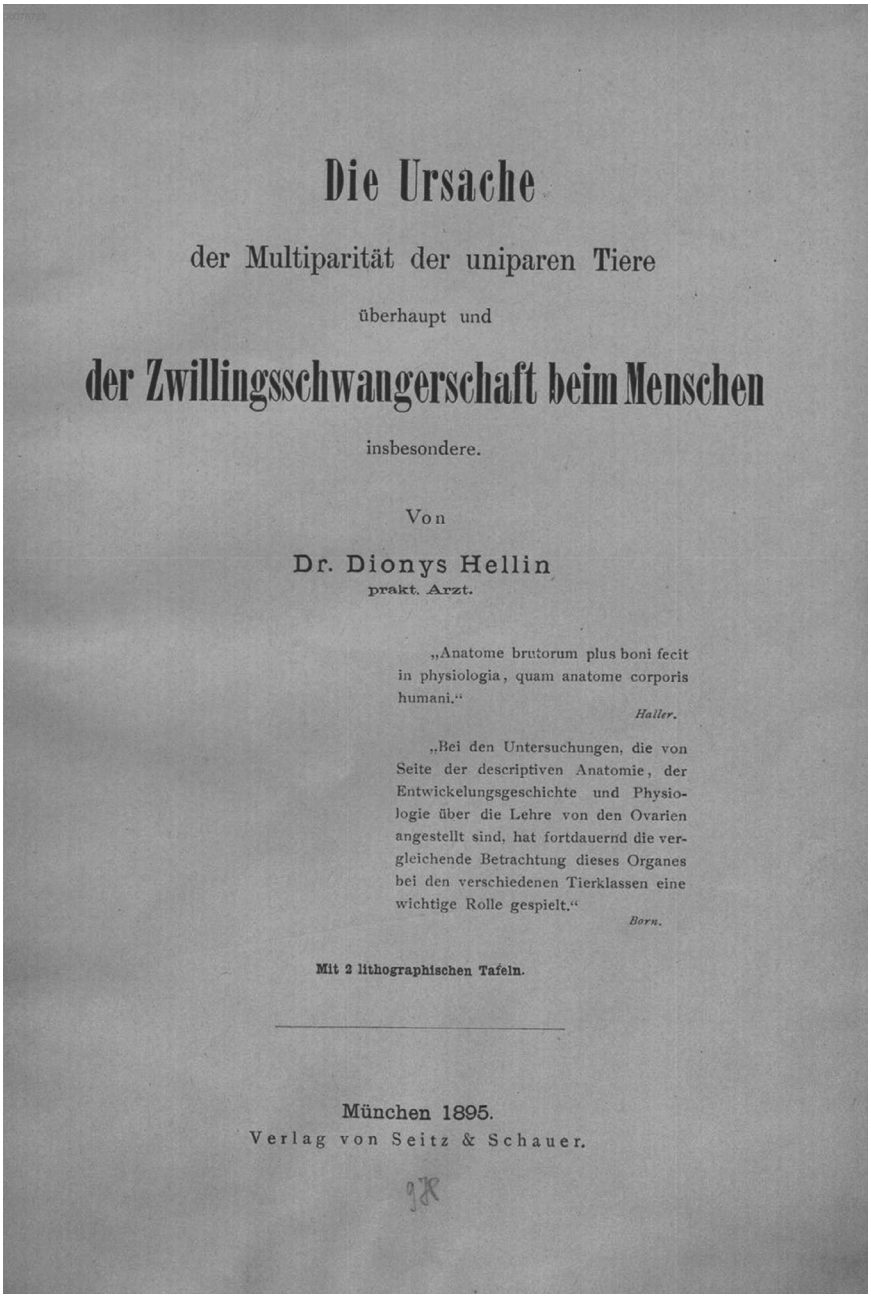
On the chronological order of twin births: it was already observed after the First World War[3]; Pison–Monden–Smits[4] that the twin birth rate (TBR) significantly increased during the years of the war. It is due to the fact that while the younger (typically aged 18–30) men were at war for years, their typically young wives bore fewer children than in previous years; meanwhile, the birth rate in the "older" (31+) age groups stayed standard. Therefore there was a natural increase of childbirths among older-aged women. This female cohort—for biological reasons—was more likely to have twins, so naturally in wars the TBR jumped. This did not happen any differently in World War II. But in those ages data collection stalled in several countries, therefore we don't have specific data for some countries around 1942–1945. Interestingly, however, the TBRs in the last quarter century are as high as during the wars in some European countries, such as in Hungary.[5] From the late 1940s onwards, there was a generally declining trend, and this lasted until the 1970s. This coincides with the baby boom period after World War II, when the younger women in their early years had a higher proportion of children than the older age cohort (Wood, 1997[6]).

In the 1960s, the twinning rate was very low due to the fact that mothers had their children at a younger age, and the fertility rate dropped significantly, which has a noticeable effect on the twin births rate also. At that time, a serious decline in the fertility rate occurred, and families with more than three children were few.[c]

In the 1980s, with the exception of some industrialized and economically developed countries, the rate of twin births stagnated (Pison-D'Addato, 2006).[7] By the mid-1990s, the highest rates of the century had been measured in several countries. In developing and underdeveloped countries, the TBRs are generally lower than average, with the exception of Africa, where TBRs are around 50 twin births per thousand live births. The average age of mothers at childbearing in the case of twin

[b] Hellin, D. *Die Ursache der Multiparität der uniparen Tiere überhaupt und der Zwillingsschwangerschaft beim Menschen insbesondere*. Seitz und Schauer, p. 25. (1895) "Während man sagen kann, dass beim Menschen durchschnittlich eine Zwillingsgeburt auf etwa 89 einfache Geburten vorkommt tritt eine Drillingsgeburt auf $(89)^2$ einfache Geburten auf, eine Vierlingsgeburt auf $(89)^3$; überhaupt, soweit dies in Grenzen der Möglichkeit liegt, erscheint eine x fache Geburt auf $(89)^{x-1}$ einfache Geburten."

[c] "Women whose first children were twins reached upon the first pregnancy—or the second, for those who wanted three children—the desired number of children. These women were less likely to have subsequent pregnancies than those who had the same number of pregnancies, but no twins. Thus, women predisposed to having twins were less and less represented in birth orders following the first, since they were more likely to control their pregnancies: as a result, the twinning rate declined"[8] p. 4).



Die Ursache

der Multiparität der uniparen Tiere

überhaupt und

der Zwillingsschwangerschaft beim Menschen

insbesondere.

Von

Dr. Dionys Hellin
prakt. Arzt.

„Anatome brutorum plus boni fecit in physiologia, quam anatome corporis humani."
Haller.

„Bei den Untersuchungen, die von Seite der descriptiven Anatomie, der Entwickelungsgeschichte und Physiologie über die Lehre von den Ovarien angestellt sind, hat fortdauernd die vergleichende Betrachtung dieses Organes bei den verschiedenen Tierklassen eine wichtige Rolle gespielt."
Born.

Mit 2 lithographischen Tafeln.

München 1895.
Verlag von Seitz & Schauer.

FIG. 2.1 The first page of Hellin's study on the frequency of twin births (1895).

births is important for two reasons: on the one hand, the older age of mothers plays a significant role at the first childbearing, on the other hand, older maternal age is also reflected in the statistics even if the mother has already given birth to several children. In developed countries, there is a tendency for mothers to postpone the birth of their first child, while in less developed and some developing countries, the average age of mothers increases due to having more children. In the latter, the date of the first childbearing occurs at a younger age. In some Asian countries such as Japan—a developed country—extremely few twins are born. This is likely attributable to the fact that there is a difference in between races biologically: the proportion of twins is high among the African population and low among the Asian population.[8]

The twinning rate depends on many different factors, among which the age of the mother, the order of birth, and fertility rate are the most important factors. The average age of the mother at childbirth (AAoM) has varied in the past 70 years. In the 1950s, the mean age was close to 28, then during the 70s, it decreased by 1.5–2 years. A rapid increase followed in the 80s and at the end of 1990, the AAoM was over 29. Indeed, older women tend to have twins more likely than younger women. The highest rate is among 35 to 39-year-olds, while for those aged over 40 years, the natural twinning rates decreased markedly (Fellman–Eriksson).[9]

In Europe, live births averaged 19 twin births per thousand in the 1980s and then 24 per thousand in the 1990s. After the turn of the millennium, the proportion of twin and multiple births was around 27–28‰ based on the 2004 data (EU Perinatal Health Report[10]). In that study, the focus of the growth of TBR is on artificial insemination and increasing number of mothers having children at older age. After a decade, we witnessed a significant change in twin births in Europe.[d]

In recent decades, in addition to the declining birth rate, the number of twin births has steadily increased slightly, so the TBR has naturally jumped as a result of these two opposing effects. In case of twins and multiple births—twin births where more than two children are born—the most influential factor is indeed the age of the mother. The study of historical trends, from Galton and Hellin to the Scandinavian researchers who dealt with the issue, agrees about the importance of mother's age at births as a demographic factor. (Eriksson–Fellman,[11] Rachootin–Olsen [1980], Eriksson–Abbott–Kostense–Fellman,[2] Wood [1997],[6] Pison,[7] Pison–Couvert,[12] Fellman–Eriksson,[13] Martin–Hamilton–Osterman,[14] Pison–Monden–Smits [2015],[15] Pári [2015][16]).

[d] "Perinatal complications associated with multiple births impose considerable costs on health services, families, and societies. Accordingly, the high rates due to either delayed childbearing or subfertility management raise questions about the need for policies to encourage earlier childbearing and to prevent multiple pregnancies in assisted conception. The decrease in twinning rates in some countries may be the result of policies to reduce the risks of multiple births for women undergoing subfertility procedures; more knowledge about how these policies are contributing to the changes in the multiple birth rate would be useful for health professionals and policy makers." (Perinatal Health Report, 2015 p. 38).

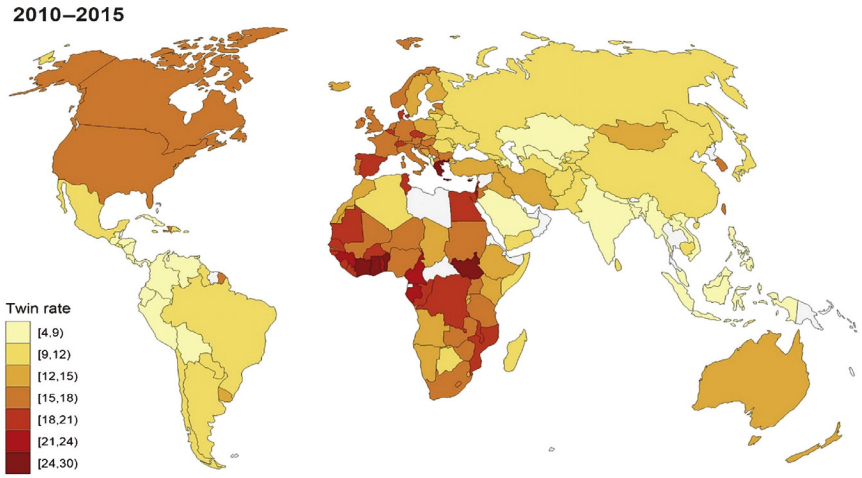


FIG. 2.2 Twin deliveries per 1000 total deliveries in 1980–1985 (Monden–Pison–Smits).[25]
Source: Human Reproduction, pp. 1–1, 2021 doi:10.1093/humrep/deab029.

2.2 Questions of the methodology of twinning rate

Twins are typically considered as medical cases and appeared as single statistical data, but we should not forget that—at least—two children are born at almost the same time.[e,17,18] It is important to differentiate twin deliveries from twin births, statistically twin births show almost doubled number of rates. It means that from 100 twin deliveries, an average of 194–197 children are born alive (taking into account child mortality—which may reduce the number, and the number of multiples (triplets, quadruplets, etc.) also, which could increase this number) (Fig. 2.2).

It is also important to note that both twin delivery and twin birth data are recorded in several countries, however, current statistical laws and data collections in some countries only allow access to twin birth data through census data. Unfortunately, this is why registries and databases are incomplete. So twin registers are very important, from which we learn extremely important and gap-filling data about the living conditions, diseases, sociological, demographic, biological, and genetic characteristics of twin families and twins. Furthermore, it is important to note that in countries where the record of twin births is not accurate, the number of twin births can be approachable by an approximate estimate. All this can be estimated from the fertility rate of the given country, the AAoM, the infant and perinatal mortality rates. Based on the results of previous large-scale research studies or statistical data surveys, and examining the twin birth trends observed in the given region, a relatively accurate

[e] Twins born typically 15–30 min apart (Lindroos et al., 2018[17]; Axelsdóttir–Ajne, 2019.[18]).

estimate can be given of a countries or regions with a lack of data. Changes in the social, economic and political, legislation situation, as well as environmental factors are difficult to estimate accurately for twin births, but it may have effects on twin births (Hur-Kwon, 2005[19]; Gan–Wu–Tu-Zheng, 2007[20]; Pári, 2014[21]).

All EU member states cover births and deaths in a civil registration system. Data usually include citizens and permanent residents in these obligatory registries. Non-residents are universally excluded. The registration system is the source of the number of live births, stillbirths, infant deaths, and maternal deaths also. Some may also provide data about background characteristics, such as maternal age, parity, plurality (singleton, twin, or triplet or higher-order pregnancies) or birth weight, nationality of the mother, recent and previous residence of the mother. They include some data on fathers also, but the principle of "*mater certus pater incertus est" (mother is certain, father is uncertain [at the childbirth])*[f] encounters difficulties.

Reflecting the Roman principle we know less about fathers than mothers, so more detailed demographic and biological research on twin fathers is yet to come, but it would be very important to map twin families. On one hand, the principle no longer applies since the end of 1970s, mostly attributable to in vitro fertilization. The views of motherhood changed[g] the previous principle derived from Roman law. It has been recognized "that advances in medicine have already displaced the principle yet the law has not caught up with the changing nature of motherhood while acknowledging the need to keep the definition of fatherhood and parenthood up to date."[22] p. 383). Yet we found very little research on this topic, specifically focusing on twin births.

2.3 Effect of assisted reproductive treatment

Family policies and regulations can also affect the number of twin births. Family benefits play a role when there are incentives or disincentives to having a child. If family support and subsidies are linked to the mothers' childbearing age, or if mothers who are about to have a child are encouraged to have a child at a younger age, it will also reduce the chances of having twins, because the mother's age at childbearing plays a decisive role in twin conceptions. At the heart of all this is the social or family policy preferences of states, which primarily serve interests related to the economy, welfare, and the sustainability of society.

The socio-demographic and policy factors are various and could impact the rates of multiple pregnancy. There are different cultures, legislation, and methods of supporting assisted reproduction treatments in the European countries

[f] "*Mother is always certain and the father is uncertain*" (latin). The conclusive presumption (*praesumptio iuris et de iure*), also known as an irrebuttable presumption, is a type of presumption used in several legal systems.

[g] Remark on this legal question is surrogacy requires a shift in the presumption of motherhood from a presumption based on maternity (giving birth) to one based on the ultimate care and nurture role (motherhood).

(Heino–Gissler–Hindori-Mohangoo et al.[23] The average number of embryos transferred and mothers' age at childbearing widely varies and this has implications for multiple birth rates.

It is widely believed–but not true–that more children are now born as twins from assisted reproductive treatment (ART) than a "single" child. As a result of artificial procedures (assisted reproductive procedures such as insemination, medical treatment) in the last decade and a half in Europe and the United States, 20%–30% more twins were born as a whole (Martin et al., 2012).[14] But it is important to note that most twins are still born naturally, not with ART. Official statistics do not record whether live-born children whether conceived naturally or artificially, but estimates have been made of this (Zeitlin–Mohangoo, 2008[24]).

It is not clear that the increase in the number of twin births was the result of an assisted reproduction procedure, as maternal average age is also constantly rising and this has already been shown to have an effect on twin births.[21] Due to the lack of official data and research, it is difficult to ascertain but it seems that artificial reproductive techniques (ART) were not yet significant among all deliveries in 2010.[h]

One consequence of ART is an increase in the number of twin births, unless only one embryo was implanted. Mothers with multiple pregnancies are ten times more likely to have a premature birth and the offspring is four times more likely to die in infancy. Twin mothers have a higher risk of morbidity and mortality, while twins have a higher risk of congenital anomalies and weight loss.

Mode of delivery shows correlation with twin pregnancy (Perinatal Health Report, 2004, 2010, 2015) and the vaginal twin deliveries decreased in the past centuries. In Europe, specifically Cyprus, the cesarean mode of delivery (57% in 2015) is more common than vaginal delivery, as seen in Romania (47% in 2015). Of note, the two countries have totally different twinning rates. Cyprus has a very high twin and triplets birth ratio, unlike Romania. In the latter country, the mean age of mothers at birth is lower than the European average; as a result of this, the number of twin births is also lower in proportion to the population. Globally, however, the number of cesarean sections in Romania is very high, primarily to reduce the risk of twin births and to avoid infant mortality. Cesarean sections also dominate in most twin births all around the world.

2.4 One out of twenty-eight births

Since the 1980s, the proportion of twin births has risen by one-third, meaning that there are twelve twin births per thousand births today compared to the previous nine. TBRs were low in Asia and South America, moderate in Europe and North America, and high in many African countries. In recent decades, the proportion of twin births has been increasing in wealthy countries and higher income (developed) regions of

[h] "Up to 5 to 6% of births in some countries may occur after use of some form of ART, although the use of the less invasive procedures is under-reported in most data systems or not reported at all. Births after in vitro fertilisation (IVF) account for 2%–4% of all births." (Perinatal Health Report, 2010, p. 17.)

the world, due to the more frequent use of assisted reproduction procedures and the older age of mothers at childbirth. But even so, Asia and Africa currently give birth to 80% of the world's twins (Monden–Pison–Smits, 2021).[25]

An average 140 million children were born worldwide in 2015–2020 period (UN Population database[i]), of which slightly more are estimated 3.9 million children were born as members of a pair of twins, bringing the average TBR to 27.93‰.[j] There is a large difference between the continents concerning TBRs and the proportion of women giving birth in an older—when compared to the average—age. There is a tendency for more than one fifth (23%) of mothers in countries with higher TBRs to be 30 years of age or older, and the high fertility rate also has a significant effect on twinning rate.

The incidence of twin births has continued to vary widely in the recent years worldwide. The highest proportions continue to be seen in African countries: Nigeria (73.6 per thousand), Burundi (61.2 per thousand), and Somalia (59.2 per thousand). The lowest values are found in Asian countries. The three lowest ratios are held by Democratic People's Republic of Korea (8.2 per thousand), Singapore (9.4 per thousand), and Mongolia (9.5 per thousand).[k]

There is a strong correlation between TBRs and fertility rates ($R^2 = 0.7$). Therefore countries with higher fertility rates also have higher TBRs (Fig. 2.3). However, this is not necessarily true for all countries, as high fertility rates in several Asian countries (e.g., Afghanistan, Pakistan, Mongolia, Turkmenistan) are not coupled with high TBRs.

Most twins (approximately 475,000) were born in India in 2018. More than 340,000 were born in Nigeria and 273,000 in China, as one member of twin pairs each year. Interestingly, the TBR was below average: 16.7 per thousand in India, 50.4 per thousand in Nigeria, and 17.1 per thousand in China in the same year. In the countries with the 10 highest TBRs (not ratios), the average TBR was well above average (27.93‰): 35.6 per thousand. It should be noted that these 10 countries account for more than half of the world's twin population, or nearly 2 million people (50.5%). Actually, in the 10 countries with the highest TBRs, only 10% of the world's twin population is born (391,000).

---

[i] Source: https://population.un.org/wpp/Download/Standard/Fertility/.

[j] In most countries, twin birth rates have been estimated because, on the one hand, or no twin birth data are available and, on the other hand, relatively old data (e.g., census data) are available. Twin birth rates were therefore standardized for 2018 to be derived from fertility rates (World Bank 2018 data) and maternal average age at childbirth (UN 2008 country-specific data) and infant mortality rates (2015–2020 UN average). During the standardization, the estimated ratios were adjusted with the twin birth or twin birth trends observable on the basis of the data of the previous years and decades.

[k] UNICEF methodology paper found that assessment of the effect of twin births and "other multiple births are generally low across countries though there is marked variation in these rates. The highest rate of twinning is observed in Benin (55.3 twins per 1000 live births), followed by Ghana and Cameroon (43.3 and 42.8 twins per 1000 live births). The lowest rates are in Bolivia, the Philippines and Honduras, with rates below 15 twins per 1000 live births. In 10 countries, no triplet births were recorded and in the remaining countries, rates were below 2 per 1000 live births, except in Jordan (2.2 per 1000 live births). These rates are so low that any effect on low birth weight estimates are expected to be minimal."[26] p.6).

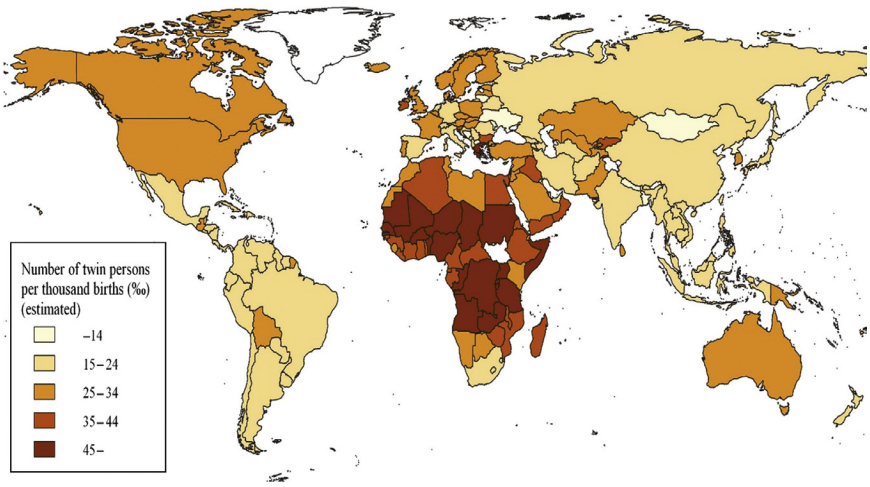


FIG. 2.3 The estimated rate of twin births between 2011 and 2019 by countries (per thousand births). Source: Worldbank, United Nations Population Database and National Statistical Offices data (From 2011 to 2019) own estimation.

A *Hungarian example* (Pári, 2011, 2014)[21] shows that the age of the mother can be related to several sociological factors:

- The number of previous pregnancies played a major role in having twins after the fifth or subsequent birth before the 1990s. After that, the twin rate from first pregnancies began to rise and in the 2000s became the most frequent. The effect of the number of previous pregnancies did not disappear, but having twins from the first or second delivery became dominant.
- The extension of the mother's childbearing age plays a major role in this. Also, as a result of the expansion of higher education that began in the early 1990s in Hungary, more women study at college or university. The chance of having twins is not strongly correlated with educational attainment, but rather with the mother's age and genetically inherited factors. Yet the time spent in higher education usually results in a rising age for childbearing, which has a determining role in the number of twin deliveries.
- There was no significant difference in twinning rates between the types of settlements (e.g., smaller towns and villages, capital) before the mentioned period. The twinning rate varied between 18‰ and 25‰. After the mid-1990s, however, the twinning rate was above the national average in the capital city and in towns with county rank, while in smaller towns and villages, although the twinning rate also increased, it was below the average.

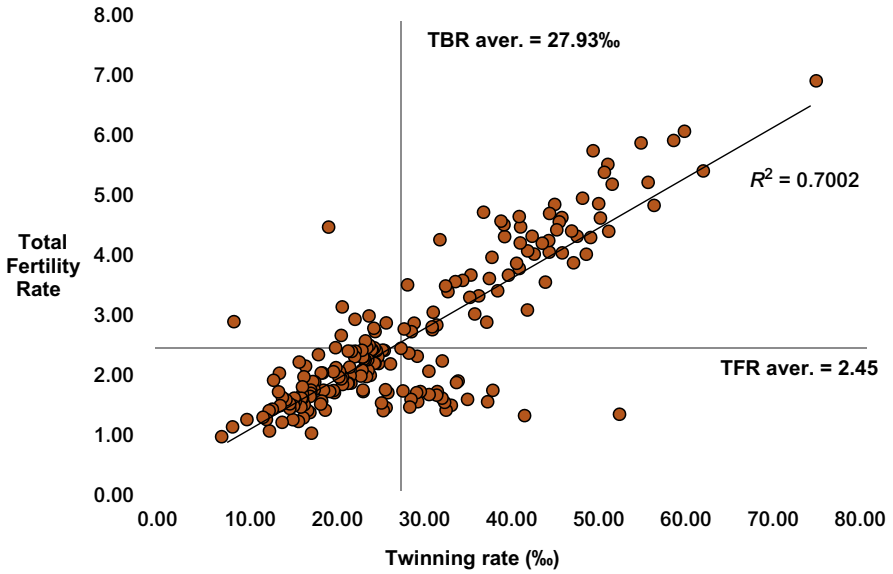


FIG. 2.4 Correlation between total fertility rate (*TFR*) and twin births rate (*TBR*). Source: World Bank, Fertility indicators, 2018 and own estimated calculation.

Fig. 2.4 shows that there is some correlation (R^2 = 0.14) between TBRs and the AAoM worldwide, but the correlation is not as strong as the number of births.[1]

This is therefore less correlation than the relationship between fertility rate or number of births and TBR. As we have seen, in several countries with high fertility meaning the fourth or fifth child (e.g. African countries), the AAoM reaches the fertility rate of those with lower births (e.g., most European countries). The fertility and the number of previous births have a statistically significant effect (Fig. 2.5).

According to the United Nations database, the (46) countries with 30 years and higher in AaoM show an average 2.86 total fertility rate, which is 0.41 higher (16.7% higher) than the world average (TFR = 2.45). The "youngest" mothers—those (25) countries where the AAoM is 27 years and younger—are seen at 1.83 TFR (25.4% lower). Compared to the countries of mothers of 30 years and older, the younger mother group gives birth, statistically, to exactly one less child.

It is important to highlight that the TBR is even higher in the countries where the ratio of "older (30+) aged" mothers are higher (TBR = 32.37‰) than in the countries where the proportion of mothers 27 years and younger (TBR = 20.17‰).

[1] Note that this number shows the national averages worldwide.

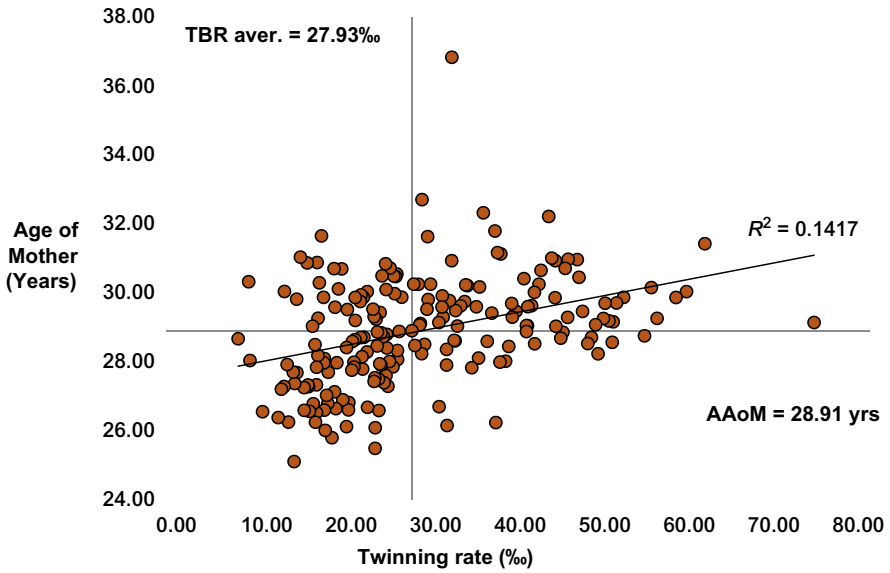


FIG. 2.5 Correlation between average age of mother at childbirths (AAoM) and twin births rate (TBR). Source: UN Population database and own estimated calculation.

Acknowledgments

I would like to thank the help to research analysts—Franciska Nagyné Keserü, Anikó Varga, Andrea Dadányiné Tóth, Szilvia Szombathelyi—of the State Secretary of Family and Youth Affairs in the Ministry of Human Capacities (Hungary) who collected twin related data from National Censuses and from Statistical and Demographical Yearbooks worldwide.

Supported by the KDP-2021 Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.

References

1. Galton F. The history of twins, as a criterion of the relative powers of nature and nurture, *Fraser's Mag*, vol. 12 (pg. 566-576) Reprinted with revisions in Galton F. The history of twins, as a criterion of the relative powers of nature and nurture. *J Anthropol Inst*. 1875;5:391–406 1875 (Reprinted in *Int J Epidemiol* 2012;41:905–11).
2. Eriksson AW, Abbott C, Kostense PJ, Fellman JO. Secular changes of twinning rates in Nordic populations. *Acta Genet Med Gemellol (Roma)*. 1995;44:141–162.
3. Saile T. A többes születések statisztikája. *Statisztikai Szemle*. 1928;1:33–43.
4. Pison G, Monden C, Smits J. Twinning rates in developed countries: trends and explanations. *Popul. Dev. Rev*. 2015;41:629–649. Wiley-Blackwell. https://onlinelibrary.wiley.com/doi/full/10.1111/j.1728-4457.2015.00088.x <hal-02078718>.
5. Pári A. A megemelkedett ikerszületési arányszámok körülményei és területi vonatkozásai Magyarországon. *Területi Statisztika*. 2011;14:620–637 (51.) évf. 6. szold.

6. Wood Rebecca Trends in multiple births, 1938–1995. Population Trends. 1997;87:29–35. PMID: 9134573. In press.
7. Pison G, D'addato AV. Frequency of twin births in developed countries. *Twin Res Human Gen*. 2006;9:250–259.
8. Pison G. Nearly half of the world's twins are born in Africa. *Population et Sociétés*. 2000:1–4. https://www.ined.fr/fichier/s_rubrique/18789/publi_pdf2_pop_and_soc_english_360.en.pdf (accessed July 10, 2020).
9. Fellman J, Eriksson AW. Standardization of the twinning rate. *Hum Biol*. 1990;62:803–816.
10. Euro-Peristat Project, with SCPE, Eurocat, Euroneostat. (2004, 2010, 2015). European Perinatal Health Report. Retrieved from www.europeristat.com https://www.europeristat.com/images/doc/EPHR/european-perinatal-health-report.pdf https://www.europeristat.com/images/doc/EPHR2010_w_disclaimer.pdf https://www.europeristat.com/images/EPHR2015_web_hyperlinked_Euro-Peristat.pdf (accessed 14 April 2022).
11. Eriksson AW, Fellman J. Twinning and legitimacy. *Hereditas*. 1967;57:395–402.
12. Pison G, Couvert N. The frequency of twinbirths in France. The triple influence of biology, medicine and family behaviour. *Population-E*. 2004;59(6):765–794.
13. Fellman J, Eriksson AW. Maternalage and temporal effects on stillbirth rates. *Twin Res Human Gen*. 2008;11:558–566.
14. Martin JA, Hamilton BE, Osterman MJK (2012): Three decades of twin births in the United States, 1980–2009. NCHS Data Brief. No. 80. http://www.cdc.gov/nchs/data/databriefs/db80.htm.
15. Pison Gilles, Monden Christaan, Smits Jeroen Twinning rates in developed countries: trends and explanations. Population and Development Review. 2015;41(4):629–649. doi:https://doi.org/10.1111/j.1728-4457.2015.00088.x. https://doi.org/10.1111/j.1728-4457.2015.00088.x, 2015. [Accessed 14 April 2022]. In press.
16. Pári András A Hellin-szabály napjainkban, avagy megjósolható-e az ikerszületések aránya? KAPOCS. 2015;14(3):31–35. 66. https://efolyoirat.oszk.hu/02900/02943/00067/pdf/EPA02943_kapocs_2015_3_031-035.pdf, 2015. [Accessed 14 April 2022]. In press.
17. Lindroos L, Elfvin A, Ladfors L, et al. The effect of twin-to-twin delivery time intervals on neonatal outcome for second twins. *BMC Pregnancy Childbirth*. 2018;18:36. https://doi.org/10.1186/s12884-018-1668-6 (accessed 14 April 2022).
18. Axelsdóttir I, Ajne G. Short-term outcome of the second twin during vaginal delivery is dependent on delivery time interval but not chorionicity. *J Obstet Gynaecol*. 2019;39:308–312. https://doi.org/10.1080/01443615.2018.1514490.
19. Hur Y, Kwon J. Changes in twinning rates in South Korea: 1981–2002. *Twin Res Human Gen*. 2005;8(1):76–79. https://doi.org/10.1375/twin.8.1.76.
20. Gan J, Wu Z, Tu Z, Zheng J. The comparison of twinning rates between urban and rural areas in China. *Twin Res Human Gen*. 2007;10:633–637. https://doi.org/10.1375/twin.10.4.633.
21. Pári A. Main characteristics of hungarian twin and multiple births in official statistics. *Twin Res Human Gen*. 2014;17:359–368. https://www.cambridge.org/core/journals/twin-research-and-human-genetics/article/main-characteristics-of-hungarian-twin-and-multiple-births-in-official-statistics/A4C95DAB10C0BBB472BDE2033F42173D (accessed 14 April 2022).
22. D'alton-Harrison R. Mater semper incertus est: Who's your mummy? *Med Law Rev*. 2014;22:357–383. 2014. https://doi.org/10.1093/medlaw/fwt047.

23. Heino A, Gissler M, Hindori-Mohangoo AD, et al. Variations in multiple birth rates and impact on perinatal outcomes in Europe. *PLoS One*. 2016;11:e0149252. https://doi.org/10.1371/journal.pone.0149252.
24. Zeitlin J, Mohangoo A (eds.) (2008): European perinatal health report. EURO-PERISTAT Project. Work document http://www.europeristat.com/images/doc/EPHR/european-perinatalhealth-report.pdf (accessed May 14, 2020).
25. Monden C, Pison G, Smits J. Twin Peaks: more twinning in humans than ever before. *Hum Reprod*. 2021;36(6):1666–1673. https://doi.org/10.1093/humrep/deab029.
26. UNICEF (2016): Assessment of the effect of twin births, references periods and birth subsets on low birth weight estimates, MICS Methodological Papers, No. 3.

Further readings

Fellman J, Eriksson AW. On the History of Hellin's Law. *Twin Res Human Gen*. 2009;12:183–190.

Hellin D (1895): Die Ursache der Multiparität der uniparen Tiere überhaupt und der Zwillingsschwangerschaft beim Menschen insbesondere. Seitz und Schauer, München

Livius Titus Book 1, Chapter 4. The History of Rome. Harvard University Press; 1919. In press.

Rideout Benjamin, Twins in Mesoamerica as a Symbol of Contrasting Duality. Spectrum. 2015;4(1):5. doi:https://doi.org/10.1016/B978-0-12-820047-6.00004-7. In press.

United Nations, Population and ferility database, https://www.un.org/en/development/desa/population/publications/dataset/fertility/data.asp.

United Nations, Demographic Yearbook (2018) https://unstats.un.org/unsd/demographic-social/products/dyb/dyb_2018/ (accessed July 28, 2020).

World Bank - World development indicatiors database https://data.worldbank.org/.

CHAPTER

Twin family registries worldwide

3

Yoon-Mi Hur[a,*], Veronika V. Odintsova[b,c,*], Juan R. Ordoñana[d,*], Karri Silventoinen[e,*], Gonneke Willemsen[b,*]

[a]*College of General Education, Kookmin University, Seoul, South Korea*

[b]*Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*

[c]*Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology, Moscow, Russia*

[d]*Department of Human Anatomy and Psychobiology, University of Murcia, Murcia Institute for BioHealth Research (IMIB-Arrixaca-UMU), Murcia, Spain*

[e]*Population Research Unit, Faculty of Social Sciences, University of Helsinki, Helsinki, Finland*

**All authors equally contributed to this chapter.*

3.1 Introduction

During the last 65 years, twin registries have contributed much to our knowledge about genetic and environmental influences on all aspects of human behavior, physical characteristics, and health. The establishment of twin registries, then and now, is driven by the realization that a large sample size is critical to study the genetic basis of complex, heterogeneous, and polygenic traits and diseases. While the first twin registries were established in Europe, we now have twin registries on six continents, exceeding more than 60 twin registries across 26 countries (see Fig. 3.1 and Table 3.1).

Many twin registries are not limited to twins but also actively recruit family members of twins, changing into twin family registries, thereby enhancing the analytic power and the potential to study the processes of intergenerational associations and differences. The sample sizes of these twin family registries differ greatly, from a few hundred to over 250,000 participants, covering all ages. Since 2002, the journal of the *International Society for Twin Studies* (*Twin Research and Human Genetics)* has periodically published special issues on twin family registries in the world.[1–4] Fig. 3.2 shows the increases in the total number of participants (twins and their family members) in twin registries from 2002 to 2019 special issues. Between 2002 and 2019, the number of participating twins and their families increased over four times. Although a few registries have discontinued their activities during this period, the overall growth is due to an increase in the number of participants within existing twin registries as well as the incorporation of new twin registries over the years.

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00009-X

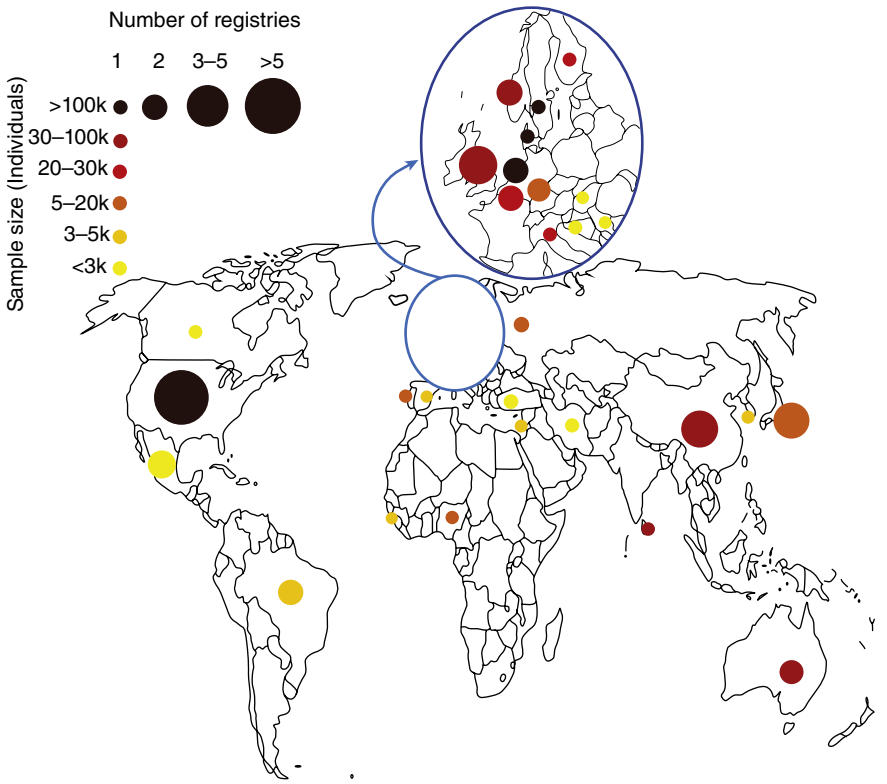


FIG. 3.1 Worldwide distribution of twin registries.

Table 3.1 provides an overview of twin registries across the world. In this chapter, we will briefly discuss registries per continent, touching upon the history, the variety of recruitment, and data collected. In this way, we hope to share with you the many opportunities for scientific study that twin registries continue to offer.

3.2 Twin family registries across the continents

3.2.1 Europe

The history of twin registries starts in Europe. The first official twin registry in the world was the Danish Twin Registry in 1954.[5,6] Originally established with the specific aim of studying cancer in a restricted cohort, its scope was broadened in time to many other aspects of health and lifestyle. This registry also grew to include a large percentage of the Danish twin population, recruited through a linkage with various registration systems such as church and conscription records, and national

Table 3.1 An overview of twin registries worldwide.

Country	Name of the registry (or running head)	Target region	Major recruitment methods	Total sample size	Subjects	Age in years	ZYG	Major phenotypes	DNA (biosample collection)	Reference/s and website
Australia	Twins Research Australia	National	Twin Pregnancy Booklet, internet, media, facebook, MBA	45,000 pairs	MZ, DZ, OSDZ	All ages	Q+ DNA	Health, psychological traits	Yes	42 www.twins.org.au
	Peri/Postnatal Epigenetic Twin Study (PETS)	Melbourne	Mothers recruited in pregnancy	250 pairs	MZ, DZ, OSDZ & their parents	10–12	DNA	Cardiometabolic, neuro-developmental	Yes	45 https://www.mcri.edu.au/peripostnatal-epigenetic-twins-study-pets
	Brisbane Longitudinal Twin Study	Brisbane & Queensland	Schools & media posting	7000	MZ, DZ, OSDZ & their siblings & parents	12–25	DNA	Melanoma, personality, cognitive abilities, psychiatric symptoms, neuroimaging, biochemistry	Yes	43 www.qtwin.org.au
	The Academic Development Study of Australian Twins	National	Twins registered with Twin Research Australia	4336	MZ, DZ, OSDZ, triplets, siblings	8–14	Q+ DNA	Reading, writing, spelling, grammar and numeracy, health	No	44 https://www.une.edu.au/about-une/faculty-of-medicine-and-health/school-of-psychology/research/the-australian-twin-study-of-the-naplan

(Continued)

Table 3.1 An overview of twin registries worldwide. *Continued*

Country	Name of the registry (or running head)	Target region	Major recruitment methods	Total sample size	Subjects	Age in years	ZYG	Major phenotypes	DNA (biosample collection)	Reference/s and website
Belgium	The East Flanders Prospective Twin Survey	East Flanders	Birth records	20,070	MZ, DZ, OSDZ & triplets	0–46	DNA, chor	Pre- perinatal influences on behavior and diseases	Yes	17 www.twins.be
	TwinssCan	East Flanders	East Flanders Prospective Twin Survey	1202	MZ, DZ, OSDZ & their families	15–35	Q+ DNA	Psychopathology	Yes	20
Brazil	University of Sao Paulo Twin Panel	National	University of Sao Paulo; media	4826	MZ, DZ, OSDZ, triplets+	All ages	Q+DNA	Psychological traits, anthropometric variables	Yes	71 https://www.paineluspdegemeos.com.br/
Canada	Quebec Newborn Twin Study	Quebec	Birth records	1324	MZ, DZ, OSDZ	0–19	DNA	Cognitive, behavioral, and social-emotional components of developmental health	Yes	36
China	Chinese National Twin Registry	National	Center for Disease Control, media	61,566	MZ, DZ, OSDZ, triplets+	All ages	Q+ DNA	Diseases, public health variables	Yes	50
	Beijing Twin Study	Beijing	Public schools	1387 pairs	MZ, DZ, OSDZ, triplets+	10–18	Q+ DNA	Psychopathology, psychological traits	Yes	51
	Guangzhou Twin Eye Study	Guangzhou	Guangzhou City Bureau of Statistics	1300 pairs	MZ, DZ, OSDZ, triplets+	7–30	DNA	Ocular data, anthropometry, cardiovascular risk factors	Yes	52

Country	Name of the registry (or running head)	Target region	Major recruitment methods	Total sample size	Subjects	Age in years	ZYG	Major phenotypes	DNA (biosample collection)	Reference/s and website
Denmark	Danish Twin Registry	National	Church records, Danish civil registration system, Conscription register, MBA	175,518	MZ, DZ, OSDZ, triplets+ & their families	10–100+	Q+DNA	Diseases, lifestyle/health-related behaviors, aging, cognitive and physical abilities, depression symptomatology, socio economic status	Yes	6 https://www.sdu.dk/en/Om_SDU/Institutter_centre/Ist_sundhedstjenesteforsk/Centre/DTR.aspx
England	Twins Early Development Study	National	Birth records	16,000 pairs	MZ, DZ, OSDZ	2–21	Q+DNA	Cognitive, emotional, and behavioral development	Yes	15 https://www.teds.ac.uk
	Children of the Twins Early Development Study	National	TEDS	554	children of TEDS	0–11	Q+DNA	Child psychopathology, temperament, cognitive development	No	16 https://www.teds.ac.uk/coteds
	TwinsUK	National	Media campaign	14,686	MZ, DZ, OSDZ	18–82	Q+DNA	Complex diseases and aging	Yes	12 http://twinsuk.ac.uk/
Finlanda	FinnTwin16	National	Central Population Register of Finland	30,527	MZ, DZ, OSDZ, triplets+ & their siblings, parents	16–35	Q+DNA	Substance use/dependence, lifestyle, mental & somatic health, psychosocial and socioeconomic traits	Yes	10 www.twinstudy.helsinki.fi

(Continued)

Table 3.1 An overview of twin registries worldwide. *Continued*

Country	Name of the registry (or running head)	Target region	Major recruitment methods	Total sample size	Subjects	Age in years	ZYG	Major phenotypes	DNA (biosample collection)	Reference/s and website
Germany	German Twin Family Panel	National	Community registration office	4097 pairs & families	MZ,SSDZ & their siblings, parents,partners	5–25	Q+DNA	Social inequalities	Yes	19 https://www.twin-life.de/en
	Study of Personality Architecture and Dynamics	National	Media, twin clubs, city registration offices	1962	MZ,DZ, OSDZ, triplets+ & their spouses, children, parents	14–94	Q	Personality and related traits	No	80 www.speady.de/studies/?lang=en
Guinea-Bissau	Guinea-Bissau Twin Registry	Center & six suburban areas of Bissau	Hospital, population-based	3600	MZ, DZ, OSDZ & singleton controls	0 to young adults	Q+DNA	Metabolic disease, childhood twin mortality	Yes	66
Hungary	Hungarian Twin Registry	National (population based from 2021)	Media, previous databases, twin registries. From 2019: national database	1044 pairs	MZ, DZ, OSDZ & their families	All ages	Q	Health related variables & diseases (e.g., radiogenomics, musculoskeletal, cardiovascular and respiratory diseases, etc.), psychology, sociology etc.	Yes	ikrek.semmelweis.hu
Israel	Longitudinal Israeli Study of Twins	National	The Ministry of Interior	1657 families	MZ, DZ, OSDZ & their parents	3–15	Q+DNA	Prosocial behavior, empathy, temperament, values, parenting	Yes	60 https://soclabweb.wixsite.com/home/home

Country	Name of the registry (or running head)	Target region	Major recruitment methods	Total sample size	Subjects	Age in years	ZYG	Major phenotypes	DNA (biosample collection)	Reference/s and website
Italy	Italian Twin Registry	National	Municipality registry offices, maternity hospitals	29,000	MZ, DZ, OSDZ & their families	0–95	Q+DNA	Mental health, psychological traits, health related variables	Yes	22 https://scic.iss.it/gemelli/
Iran	Isfahan Twins Registry	National	Welfare agencies, public health homes, public & private nursing homes	1000	MZ, DZ, OSDZ, triplets+	All ages	Q+DNA, Nail, hair	Health and lifestyle related variables, behaviors and disease	Yes	61
Japan	Keio Twin Research Center	National	Government resident register	10,691 pairs	MZ, DZ, OSDZ	3–52	Q+DNA	Psychological traits, education related variables, mental health	Yes	54
	Osaka University Center for Twin Research	National	Media, posters	3000	MZ, DZ, OSDZ	All ages	DNA	Physical growth, health, dental phenotype	Yes	55
	West Japan Twins and Higher Order Multiple Births Registry	National	MBA, public health centers	12,041	MZ, DZ, OSDZ, triplets+	0–40	Q	Maternal and child health of families with multiples; physical growth	No	56

(Continued)

Table 3.1 An overview of twin registries worldwide. *Continued*

Country	Name of the registry (or running head)	Target region	Major recruitment methods	Total sample size	Subjects	Age in years	ZYG	Major phenotypes	DNA (biosample collection)	Reference/s and website
Korea, Republic	South Korean Twin Registry	National	Schools, maternity hospitals, MBA	4058	MZ, DZ, OSDZ	1–30	Q	Psychological traits, mental health	No	57
Mexico	Mexico Twin Registry (MexTR)	State of Jalisco	Public records of university students, MBA, maternity hospital	under plan	MZ,DZ, OSDZ	All ages	NA		under plan	72
	Mexican Twin Registry (TwinsMX)	National	Social media, public campaigns	145	MZ, DZ, OSDZ	18–60	Q	Somatic and mental health psychometrics, lifestyle	Yes	73 https://twins-mxofficial.unam.mx/
The Netherlands	Twin Longitudinal Investigation of Fetal Discordance	National	Hospital	plan to have 100 pairs+	Mono-chorionic twin pairs	Prenatal to 8	Chor	Fetal growth, cardiovascular diseases, neurodevelopmental impairment	Yes	18 www.twin-lifestudy.info
	The Netherlands Twin Register	National	City councils; commercial birth felicitation service; Dutch society of parents of multiples	255,785	MZ, DZ, OSDZ & their families	All ages	Q+DNA	Psychological variables, mental health, physical growth	Yes	8 http://www.tweelingenregister.org/.
Nigeria	Nigerian Twin and Sibling Registry	Lagos State, Abuja, FTC	Schools	5323	MZ, DZ, OSDZ, triplets, & singletons	10–21	DNA	Psychological traits, mental health	Yes	67

Country	Name of the registry (or running head)	Target region	Major recruitment methods	Total sample size	Subjects	Age in years	ZYG	Major phenotypes	DNA (biosample collection)	Reference/s and website
Norway	Oslo University Adolescent and Young Adult Twin Project	National	Birth records	4668 twin pairs & families	MZ, DZ, OSDZ & their families	12–22	Q+DNA	Psychological variables (personality), mental health	No	21
	Norwegian Twin Registry	National	Birth records, record linkages of national registries	32,664	MZ, DZ, OSDZ	28 and older	Q+DNA	Somatic & mental health	Yes	9
Portugal	Portuguese Healthy Family Study	National	Public schools	12,385	singleton children & their parents, sibling pairs	All ages	NA	Physical activity, body composition and physique, fitness & metabolic syndrome	No	24
Russia	Russian Twin School Registry	National	Public schools	5000	MZ, DZ, triplets	7-18	Q	Psychological characteristics, anthropometric measures, mental & somatic health	Yes	27 http://www.protwins.ru
Serbia	Serbian Twin Registry	National	Public campaigns, media, twin festival	1658	MZ, DZ, OSDZ, & their family members	All ages	Q+DNA	Psychological characteristics, anthropometric measures, mental & somatic health	Yes	81 http://www.blizanci.rs
Spain	Murcia Twin Registry	Regional (Murcia)	University, birth records	3545	MZ, DZ, OSDZ, triplets+	20+	Q+DNA	Health related variables	Yes	23 https://www.um.es/registro-gemelos/

(Continued)

Table 3.1 An overview of twin registries worldwide. *Continued*

Country	Name of the registry (or running head)	Target region	Major recruitment methods	Total sample size	Subjects	Age in years	ZYG	Major phenotypes	DNA (biosample collection)	Reference/s and website
Sri Lanka	Sri Lankan Twin Registry	National	Birth record, hospitals, media	34,280	MZ, DZ, OSDZ, singletons	All ages	Q	Mental disorders & metabolic syndrome	Yes	58 https://www.ird.lk/sltr/
Sweden	Swedish Twin Registry	National	Birth records	216,258	MZ, DZ, OSDZ	All ages	Q+DNA	Mental & somatic diseases, behavior	Yes	7 http://ki.se/en/research/the-swedish-twin-registry
USA	Arizona Twin Project	Arizona	Birth records	700	MZ, DZ, OSDZ	1–11	Q+DNA	Developmental psychopathology & somatic health	Yes	82
	Avera Twin Register	National	Media campaign	838	MZ, DZ, triplets+, siblings, & their parents	All ages	Q+DNA	Lifestyle, aging, diseases	Yes	83 www.avera.org/twin-register
	Boston University Twin Project	Massachusetts	Birth records	310 pairs	MZ, same-sex DZ	birth to age 5	DNA	Temperament and related behaviors	Yes	84
	CATSLife	Colorado	Adoption agencies	776	Mostly adoptees & their birth and adoptive parents	0–40	NA	Behavioral development, cognitive aging, health	Yes	85
	Colorado Twin Registry	Colorado	Schools, birth records	4500	MZ, DZ, OSDZ & their families	0–40	Q+DNA	Psychological traits (cognitive abilities, substance use and abuse, health etc.)	Yes	86 https://www.colorado.edu/ibg/research/human-research-studies/colorado-twin-registry

Country	Name of the registry (or running head)	Target region	Major recruitment methods	Total sample size	Subjects	Age in years	ZYG	Major phenotypes	DNA (biosample collection)	Reference/s and website
	Early Growth and Development Study	National	Adoption agencies	2456	Mostly adoptees & their birth and adoptive parents and siblings	0–20	NA	Temperament, behavior problems, mental health, obesity, achievement	Yes	87 https://www.egdstudy.org/
	Florida State Twin Registry	Florida	schools	5593	MZ, DZ, OSDZ, triplets+	11–22	Q	Reading development, school achievement, behaviors,	No	88
	Fullerton Virtual Twin Project	National	Media, multiple birth organizations, personal referrals	169	virtual twins	4.01–54.84	NA	Psychological traits	No	89
	Louisville Twin Study	Kentucky	LTS database	1770	MZ, DZ, triplets+, siblings, children of	All ages	Q+DNA	Psychological, physical growth	Yes	90
	Michigan State University Twin Registry	Michigan	Birth records, university	30,000	MZ, DZ	3–55	Q+DNA	Internalizing and externalizing psychopathology	Yes	91 https://msutwinstudies.com/
	Minnesota Center for Twin and Family Research	Minnesota	Birth records	23,199	MZ,DZ, adoptees	7 to old adults	Q+DNA	Substance use and related psychopathology	Yes	31 https://mctfr.psych.umn.edu/
	Mid-Atlantic Twin Registry of Virginia Commonwealth University	Virginia, North & South Carolina	Birth records, schools	54,042	MZ, DZ, triplets+ & their families	All ages	Q+DNA	Developmental psychopathology	Yes	34

(Continued)

Table 3.1 An overview of twin registries worldwide. *Continued*

Country	Name of the registry (or running head)	Target region	Major recruitment methods	Total sample size	Subjects	Age in years	ZYG	Major phenotypes	DNA (biosample collection)	Reference/s and website
	NAS-NRC Twin Registry & Duke Twin Study of Memory in Aging	National	Birth records linked with army records	31,848	male MZ & DZ	15–82	Q+DNA	Anthropometric, health and mortality, education and earnings	Yes	32
	National Project on Achievement in Twins	National	Schools	2514	MZ,DZ, OSDZ	4.25–14.25	Q	Reading development, school achievement, behaviors,	No	37 http://www.idcd-lab.com/natpat-twin-project.html
	Pennsylvania Longitudinal Study of Parents and Children	Pennsylva-nia state	Schools, birth records	2260	MZ, DZ, OSDZ, triplets+ & their parents	0–88	Q	Mental & somatic health, prosocial traits	No	92
	Project Talent Twin and Sibling Study	National	Schools	5003	MZ, DZ, OSDZ, triplets+ & their siblings	14–78	Q & photo	Cognition, personality, education, occupation, activities, health, aging	No	38 projecttalent.org
	Southern Illinois Twins/Triplets and Siblings Study	Illinois	Media, birth records	1175	MZ,DZ, OSDZ, triplets+, siblings	1–20	Q+DNA	Childhood aggression, parent-child interaction, emotional development	Yes	93 https://www.siumed.edu/playlab/twin-play-lab.
	Vietnam Era Twin Study of Aging	National	Army records	1230	male MZ, DZ	50–70	DNA	Cognitive and brain aging, Alzheimer's disease	Yes	35

Country	Name of the registry (or running head)	Target region	Major recruitment methods	Total sample size	Subjects	Age in years	ZYG	Major phenotypes	DNA (biosample collection)	Reference/s and website
	Washington State Twin Registry	Washington State	Department of licensing	9668 pairs	MZ,DZ, OSDZ	All ages	Q+DNA	A variety of somatic and mental health outcomes	Yes	94 ttps://wstwinregistry.org/
	Wisconsin Twin Project	Wisconsin	Birth records	5000 pairs	MZ, DZ, OSDZ	Prenatal to 24	Q+DNA	Temperament, affective neuroscience, developmental psychopathology, puberty	Yes	95 https://goldsmithtwins.waisman.wisc.edu/
Consortia	Collaborative Project of Development of Anthropometrical Measures in Twins (CODATwins)	24 countries	Twin registries in the participating countries	489,981	MZ, DZ, OSDZ	0 to about 90	Q+DNA	Height, BMI, education, smoking		79
	Interplay of Genes and Environment across Multiple Studies (IGEMS)	Australia, Denmark, Finland, Sweden, USA	Twin registries in the participating countries	76,233	MZ, DZ, OSDZ	14–103	Q+DNA	Dementia, mortality, physical, SES, & psychological functioning		76 https://dornsife.usc.edu/labs/igems/
	Nordic Twin Study on Cancer (NorTwinCan)	Denmark, Finland, Norway, & Sweden	Twin registries in the participating countries	315,413	MZ, DZ, OSDZ		Q+DNA	Cancer		75

Note. Numbers in "total sample size" refer to individual twins unless "pairs" is stated. ZYG, zygosity assessment methods; MZ, monozygotic twins, DZ, dizygotic twins; OSDZ, opposite-sex dizygotic twins; SSDZ, same-sex dizygotic twins; MBA, multiple birth association; Chor, chorionicity; Q, questionnaire method; NA, not applicable; Q+DNA= questionnaire supplemented by DNA testing; [a]Finland has two other twin family registries: see Rose et al.[96] for the FinnTwin12 cohort and Kaprio et al.[97] for the Older Finnish Twin Cohort. Table was adapted from Table 2 in Hur et al.[4], with permission.

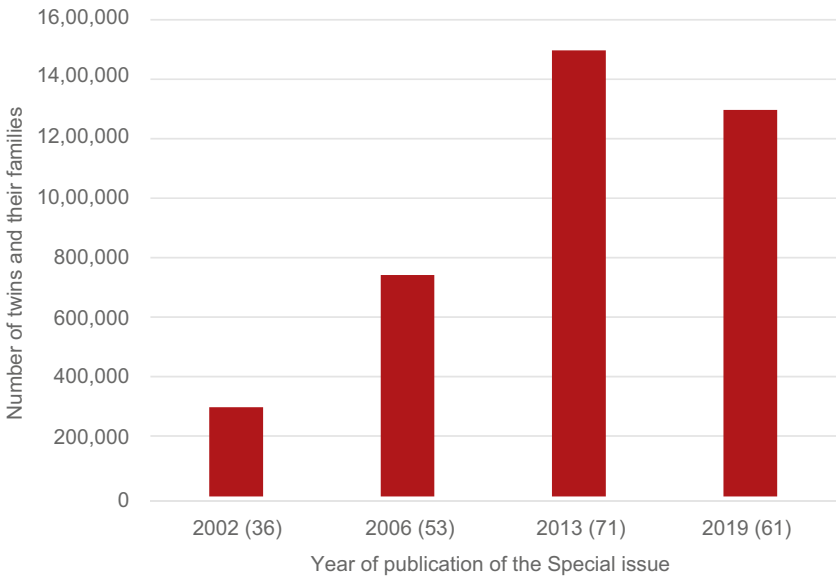


FIG. 3.2 Increases in the number of participants (twins and their families) reported in special issues of *Twin Research and Human Genetics* from 2002 to 2019. The number of twin registries in each special issue is in parenthesis.

health registries in Denmark. National twin registries in other European countries quickly followed, first primarily in the Nordic countries but later also in other parts of Europe. As evident from Table 3.1, Europe at the time of this chapter still contains the most twin registries across the continents, as well as the two largest registries in the world: the Swedish Twin Registry (STR)[7] and the Netherlands Twin Register (NTR),[8] both including more than 200,000 participants from all ages. While the STR has more information on the elderly, the NTR is unique in the large number of newborn twins it has followed across the lifespan from the late 1980s onwards. This allows for the study of the influence of genetic and environmental factors on many aspects of the development from early childhood into adulthood.

Though smaller in size than their neighbors, the two other Nordic twin registries, the Norwegian[9] and the Finnish Twin Registries,[10] are still very large and extensive in their data collection. As in most twin registries, general longitudinal surveys are sent out periodically while detailed phenotypic information may be obtained in specific projects, such as the volumes of fat and lean body mass via body scan, cholesterol, and glucose levels via blood samples, as well as the outcomes of cognitive tests and brain activity via electroencephalography (EEG) recordings. In addition, many cohorts collect genotypic and epigenetic data on their participants. Depending on the focus and costs, such information may be available for all those in the registry or for only a selected group. For instance, the Finnish Twin Registry obtained body scans and fat biopsies for a subset of monozygotic (MZ) twins discordant for obesity, allowing for more insight into the biological mechanisms associated with obesity.[11]

Many twin registries continuously expand the information they collect based on scientific developments. A good example of this is TwinsUK,[12] which recently collected stool samples from many of its participants, leading to the first publications on the heritability and molecular genetics of the microbiome.[13,14]

Twin registries may also develop in other ways. The Twins Early Development Study (TEDS)[15] was established in 1994 in the UK to study the emotional and cognitive development in young twins, but as the participants grew older and started their own families, the children of the TEDS participants led to a third separate registry.[16] Other twin registries have also started to follow the offspring of twins, but none made this such an explicit aim as the Children of the TEDS.[16]

There are three types of MZ twins based on the time of zygotic division: the dichorionic-diamniotic pairs (splitting before the 4th day after fertilization), the monochorionic-diamniotic pairs (splitting between the fourth and the seventh day after fertilization), and the monochorionic-monoamniotic pairs (splitting after the eighth day after fertilization).[17] As MC twins share a single placenta, they are frequently exposed to a vastly discordant prenatal environment due to complications. The TwinLIFE[18] identifies (at least) 100 monochorionic (MC) twin pairs born in Dutch hospitals, stores their biological samples at birth, and follows the twins from the prenatal period into childhood longitudinally. By studying intrapair differences in DNA methylation and future health development, the TwinLIFE can provide powerful insight into the effects of pre- and peri-natal environments on later health.

With also a large population registry in Belgium,[17] unique in its access to perinatal and placental information, and a recently established German registry with a focus on socioeconomic circumstances,[19] and additional smaller twin cohorts in Belgium and Norway focusing on psychopathology,[20,21] it is evident that Northern and Western Europe are well represented in twin registries. Fortunately, over the past years, twin registries have also been established in Southern Europe, and twin registries in Italy,[22] Spain,[23] and Portugal[24] with a strong focus on health and health-related behaviors, are rapidly increasing in numbers and data collection. More recently, the Hungarian Twin Registry was established which has a strong focus on health and disease but also collects psychosocial information on its participants.[25] Of interest is the Russian twin registry, which in part represents Europe but also reflects Asian cultures. The Russian Federation actually has a very long and rich history of twin studies with many prominent names and findings,[26] but a nationwide representative school-aged twin registry only developed over the last decade.[3,26] So far, the Russian School Twin Registry (RSTR) has recruited 5.000 twins from 7 to 17 years of age, of different ethnicities and culturally distinct populations, but it expects to include more than 100.000 school-aged twins to study the role of the interplay between genetic and environmental factors in the development of academic achievement.[27,28] Another twin registry located both in Europe and Asia is the Turkish Twin Study[29] which specifically focuses on the role of cultural differences in the etiology of health problems.

Hopefully, the expansion across the eastern and southern parts of Europe will continue, but it is certain that the European twin registries will continue to contribute much to our knowledge about genetic and environmental influences on behavior and health.

3.2.2 North America

Even though the history of modern twin studies in North America is younger than in Europe, during the last decades a vigorous tradition of twin research has developed in North America as demonstrated by a large number of twin cohorts. There are at least 25 independent twin cohorts in the United States and two twin cohorts in Canada (see Table 3.1). An early milestone for the twin studies in the United States was the establishment of the Minnesota Study of Twins Reared Apart (MISTRA) in 1979 which studied adult twins separated early in infancy and reared in different families.[30] This project has provided important contributions to our understanding of the role of genetic factors in the variation of psychological traits. The first studies published in the early 1980s were based on only a small number of reared-apart MZ pairs but still suggested that genetic factors play an important role in the variation of complex behavioral traits. However, the study on the heritability of IQ published in 1990 including data from over 100 reared-apart twin pairs or triplets clearly demonstrated the large influence of genetic differences on the individual variation in cognitive abilities.[30] In addition to MISTRA, there were several studies of twins raised together at the University of Minnesota, which were later integrated into the Minnesota Center for Twin and Family Research (MCTFR).[31] Although the main focus of the MCTFR is substance use and related psychopathology, it has investigated psychological adjustment, personality, cognitive ability, brain function, and other physiological traits longitudinally. However, the history of US twin studies goes further back in time, and actually the oldest systematically collected measures of twins are available from a study started in 1965.[32] This study made use of the military records collected in the past for US veterans. This resulted for example in height and BMI measurements in twins conducted in the early 1940s and thus allows estimating the heritability for traits obtained two decades earlier.[33]

Currently, the US twin registries include a wide variety of cohorts, each having own specific strengths. The largest cohort is the Mid-Atlantic Twin Registry[34] having a sample size of more than 50,000 twins, triplets, and their family members. Most of the North American cohorts are relatively small having less than 5000 and in many cohorts less than 1000 twin individuals or relatives. However, the smaller sample sizes compared to many European twin cohorts is in many cohorts compensated by intensive clinical measures often not available for bigger cohorts. For example, the Vietnam Era Twin Study of Aging includes detailed measures of brain based on neuroimaging as well as cognitive tests.[35]

North American cohorts often focus in their data collection on a specific state or another well-defined geographic area, such as Greater Montreal area of Quebec Newborn Twin Study.[36] However, there are a few exceptions in the US cohorts. Two US cohorts represent male war veterans: the National Academies of Science - National Research Council (NAS-NRC) twin registry[32] and the Vietnam Era Twin Study of Aging.[35] National school records have been used in sampling twins in the National Project on Achievement in Twins[37] as well as in the Project Talent Twin and Sibling Study.[38] Further, some cohorts were developed focusing on groups otherwise underrepresented in scientific research, such as the Carolina African American Twin

Study of Aging[39] and the Texas Twin Project,[40] which includes overrepresentation of ethnic minorities.

The number of traits collected in North American twin studies is vast, covering all aspects of human development, behavior, and health, although most studied topics have been psychological traits including cognitive development and school achievement. Psychopathology, especially, substance use/abuse (including tobacco and alcohol) and personality measures also received much attention. While traits related to physiology (with the exception of psychophysiological measures) and physical diseases have also been included in North American cohorts, these traits are less intensively studied than in European cohorts. This reflects the tradition in North America that twin research has been mainly linked to psychological research and many twin cohorts have been collected at departments of psychology. However, like European counterparts, many Northern American registries now also include genotypic and epigenetic information.

3.2.3 Australia

The origin of twin registries in Australia dates back to the 1970s when the first state registries were founded, which then received support from the National Health and Medical Research Council. The twinning rate in Australia is 16.2 per 1000 births, which is comparable to those in European countries.[41] The largest Australian national registry up to date, the Australian Twin Registry, now renamed as Twins Research Australia (TRA),[42] is located in Melbourne, Victoria, and enrolled more than 40,000 twin pairs and higher-order multiples of all ages over its 40-year history, representing approximately 20% of twin pairs in Australia. For the past decades, TRA has studied health and many diseases including cancers. While playing an active research role itself, TRA provides news and information to twin members and their families through its website, and promotes twin community forum to influence practice and policy on twins in Australia. TRA also supports twin researchers and prospective students from multiple disciplines by providing open access to its volunteer twin membership and training for statistical analyses for twin studies.

Another large Australian registry—Brisbane Longitudinal Twin Study (BLTS)—is located in Queensland at the South East coast and recruited around 5000 twins and siblings at 12 years old between 1992 and 2018.[43] Over the past years the BLTS contributed to the understanding of the role of genetic factors in melanoma risk (mole study), personality traits, psychiatric symptoms, and IQ; and conducted studies in the fields of neuroimaging, haematology, biochemistry, and ophthalmology. Several specialized registries in Australia covered specific topics. For example, the Academic Development Study of Australian Twins (ADSAT) longitudinally studied educational achievement in twin pairs, higher-order multiplies and siblings with literacy and numeracy testing in different grades.[44] The Peri/Postnatal Epigenetic Twins Study (PETS) recruited mothers of 250 twin pairs during pregnancy and have studied on genetic and intrauterine components of variation in the human neonatal epigenome, fetal programming, and early factors of later diseases.[45] Other registries include the Teeth

and Faces of Twins Registry[46] focusing on the study of genetic, epigenetic and environmental influences on dentofacial structures and oral health and the Australian Twin and Ophthalmic Traits Registry,[47] focusing on eye physiology and visual disorders.

Australian twin registries have played a prominent role in twin research and their researchers have made important contributions, especially in the development of methodology for causal inference, polygenic risk scores, integrative omics analysis, and combination of twin data with other kinds of data from different sources (such as geospatial and environmental data).

3.2.4 Asia and Middle East

Asians, especially East Asians, have the lowest natural twin birth rates in the world, with a rate of 4 to 6 pairs per thousand births.[48] Recently, however, due to the increased use of assisted reproductive technologies, twin birth rates have increased sharply across many East Asian countries, facilitating development of twin studies. Twin birth rates in Middle East remain poorly understood. However, a rate of 14.4 pairs per thousand birth has been reported recently.[49]

Large-scale, population-based twin registries are currently maintained in China, Japan, and South Korea in East Asia and Sri Lanka in South Asia. The Chinese National Twin Registry,[50] currently the largest twin register in Asia, recruits twins mainly through the Centers for Disease Control throughout the country, and focuses on disease and public health issues. In addition, two other regional registries are available to study psychopathology and ophthalmological traits in Chinese children and adolescents.[51,52] For example, the Guangzhou Twin Eye Study research team recently showed that within discordant MZ twin pairs, the more myopic twin was associated with performing more near-work activities than their co-twins, confirming environmental risk factors in myopia.[53]

Japan has currently three active registries: Keio Twin Research Center (KTRC),[54] Osaka University Center for Twin Research (Osaka Twin Registry),[55] and West Japan Twins and Higher Order Multiple Births Registry (West Japan Registry).[56] These registries use different ascertainment schemes and study somewhat different phenotypes. While the KTRC focuses on psychological and education-related traits, the Osaka Twin Registry and the West Japan registry concentrates on health issues and physical growth. The West Japan registry is unique in that they have a very large number of higher-order multiples and can serve as a valuable resource to study specific concerns regarding higher-order multiples.

The South Korean Twin Registry (SKTR)[57] is a nationwide volunteer registry founded in 2001. It includes twins from preschool to young adulthood age, with the aim of studying genetic and environmental influences and their interplays in psychological and mental health traits in South Koreans. The SKR has demonstrated that heritability for personality traits from children to young adults and symptoms of various types of psychopathology are comparable to those found in many western twin studies.[57]

A nationwide population-based twin registry was established in Sri Lanka in 1997 to study genetic and environmental etiologies of mental disorders and metabolic

syndrome in low- and middle-income countries.[58] The Sri Lankan Twin Registry currently includes over 30,000 twins and singletons of all ages. The registry team recently found that while the levels of risk factors for the metabolic syndrome in Sri Lankan twins were similar to those found in western samples, the prevalence of depressive/anxiety symptoms was lower in Sri Lankans than in Westerners.[59]

In the Middle East there are currently two twin registries. The Longitudinal Israeli Study of Twins (LIST)[60] and the Isfahan Twin Registry (ITR) in Iran.[61] The LIST identified twins born in Israel in 2004 and 2005, and has studied prosocial behavior and related traits longitudinally since 2007. Although mail surveys have been conducted regularly, experimental and observational data of social behavior have been collected longitudinally, which are major strengths of the LIST. Additionally, the Isfahan Twin Registry (ITR) was launched in Iran in 2017 to study (epi)genetic causes of diseases, especially cancer, diabetes, and cardiovascular diseases in Iranians. The registry now includes more than 1000 participants and collects biological samples, medical records, and questionnaire data from twins and multiples to establish a biobank.

3.2.5 Africa

Africa has the highest twin birth rates in the world,[48] with rates varying from 20 pairs to 49 pairs per thousand births.[62,63] The reasons for these high rates include a higher level of follicle-stimulating hormone in African women as compared to women of other ethnic origins,[64] diet, and high parity.[65] Despite these high twin birth rates, few twin studies have been conducted in Africa due to lack of research infrastructure and facilities, political instability, and high rates of illiteracy. Only two twin registries are currently established on the African continent: the Guinea-Bissau Twin Registry (GBTR)[66] and the Nigerian Twin and Sibling Registry (NTSR).[67]

The GBTR was founded in Bissau in 2009. In collaboration with the Bandim Health Project that maintains the health and demographic surveillance system (HDSS) in Bissau, the registry has collected medical data and biological materials from newborns to 30 years of age ($N > 3600$) since its inception. The GBTR aims to investigate risk factors for newborn twin mortality and the etiology of metabolic disorders specific to African populations. A recent GBTR study reported higher body fat percentage and glucose levels in both the fasting and postprandial state for twins compared to singletons, which the researchers suggest may be due to suboptimal nutrition during twin pregnancy.[68]

The NTSR was initiated in 2010 to study genetic and environmental influences and their interplays for psychological and mental health traits in Nigerian children and adolescents. Because the birth registration system is not well developed in Nigeria, twins in the NTSR are identified through public schools. As of 2019, over 5000 twins and their families have been registered with the NTSR.[67] Even though living conditions in sub-Saharan Africa are vastly different from those in many developed countries, the findings from the NTSR samples to date suggest that genetic and environmental influences on cognitive abilities, prosocial behavior, religious attendance, and family environments are largely similar to those reported in twin samples from developed countries.[67]

3.2.6 Latin America and the Caribbean

The history of twin registries in Latin America and the Caribbean (LAC) is still to be written, and the absence of data has left out Central and South American populations as well as Mexico and the Caribbean from the global research scheme and international collaborative projects. Fortunately, very recently, valuable initiatives have started to consolidate and give a much needed impulse to this area of research.

LAC has a peculiar amalgam of people with Indigenous and European origin, together with African ancestry in some of its countries, what makes this vast region a genetic melting pot with an enormous research potential. It is also a region of interest from an environmental standpoint, as it shares broad cultural commonalities, while keeping an extremely rich diversity of local milieus. As such, the emerging contribution of twin research in LAC countries would be an important addition to the international research resources.

However, the record of twin registries in LAC has been, so far, irregular. Early initiatives included Chilean[69] as well as Cuban registries,[70] the latter comprising a large sample of >55,000 twins, but, as yet these registries have not published any research results in peer-reviewed international scientific journals. More recently, a parallel initiative appeared in Brazil: the University of Sao-Paulo Twin Panel,[71] which was closely followed by two incipient registries in Mexico: the Mexican Twin Registry[72] and TwinsMX.[73] This has opened new perspectives for twin studies in LAC countries, and as they increase in size, they should be able to make meaningful contributions to twin studies in the near future.

3.3 International consortia

The fact that many twin registries collected similar phenotypic and genotypic information allows for meaningful collaborations between the registries, offering the opportunity to increase statistical power by increasing sample size and to study interactions between genes and exposures to diverse environmental conditions. One of the early collaborative efforts pooled Nordic population-based twin registries (Denmark, Finland, Norway, and Sweden) linked with the country-specific national cancer and cause-of-death registries to analyze the heritability of cancer incidence.[74] This consortium (NorTwinCan) has recently been re-established (NorTwinCan) to include more than 300,000 twins and 58 years of follow-up, on average, and studies the genetic and environmental etiology of cross-cancer associations.[75]

Interplay of Genes and Environment across Multiple Studies (IGEMS) is a consortium of 18 twin studies from 5 different countries (Sweden, Denmark, Finland, United States, and Australia) established to investigate the nature of gene-environment (GE) interplay in physical and psychological functioning, dementia, and mortality.[76] Fifteen of these studies are longitudinal, with follow-up as long as 59 years after baseline. The IGEMS now includes over 76,000 participants aged 14–103 years at intake.

A major effort in the field of comparative twin studies was Genome EU twin consortium combining twin data from seven European countries and Australia.[77] This project led to a series of papers comparing heritability estimates for a number of traits across the countries, showing that participating countries are very similar in heritability for height, BMI, and sport participation. This project was further continued within the European Network for Genetic and Genomic Epidemiology (ENGAGE Consortium) focusing more on molecular genetics.[78] The largest collaborative consortium to date is the COllaborative project of Development of Anthropometrical measures in Twins (CODATwins) which as its target to pool together all twin cohorts in the world having information on body composition, parental and own education, and smoking.[79] Currently, the CODATwins database includes around 1 million height and weight measures on almost 500,000 twins from 54 twin projects representing 24 countries. In addition, many of the twin registries also contribute to consortia, which are not twin-specific, by adding their data or the outcomes of genomewide association analyses to those of non-twin populations, thereby increasing the power for gene finding.

3.4 Concluding remarks

Twin registries have come a long way from the establishment of the first register in 1954. As illustrated in Fig. 3.1 and Table 3.1, twin registries are now present across the entire world, though with a focus on Western societies. While it will be important to ensure other parts of the world will be better represented, the wealth of data available in combination with the large sample size presents a source of important scientific findings.

The phenotypes studied in the registries cover almost all types of behavioral traits as well as mental health and various complex diseases. Notable is that several twin family registries are able to link their cohorts with national demographic, social, and public health registries, which may not only help in increasing their sample sizes and the representativeness of the population under study but may also further enrich the data available for individuals in the registry. Consistent with current trends in genetic research, a growing number of twin registries have been incorporating molecular genetic measures to the spectrum of available information. Many of them now include data from genome-wide or exome microarrays, whole-genome sequencing, or methylation analyses. Together with the impressive amount of phenotypical information accumulated, this has enhanced the already high potential for collaboration, both nationally and internationally, of twin registries.

The biggest threat for twin registries is a lack of funding. Setting up and maintaining a twin family register requires a long-term financial commitment which few institutions are prepared to make and many registries are therefore dependent on research grants to keep the twin registry going. The fact that so many twin registries are still ongoing and new registries still emerge is evidence of the great scientific value represented by twin registries.

References

1. Busjahn A. Twin registers across the globe: what's out there in 2002? *Twin Research and Human Genetics*. 2002;5:v–vi.
2. Busjahn A, Hur Y-M. Twin registries: an ongoing success story. *Twin Research and Human Genetics*. 2006;9:705.
3. Hur Y-M, Craig JM. Twin registries worldwide: an important resource for scientific research. *Twin Research and Human Genetics*. 2013;16:1–12.
4. Hur YM, Bogl LH, Ordoñana JR, Taylor J, Hart SA, Tuvblad C, Ystrom E, Dalgård C, Skytthe A, Willemsen G. Twin family registries worldwide: an important resource for scientific research. *Twin Research and Human Genetics*. 2019;22(6):427–437.
5. Skytthe A, Kyvik K, Holm NV, Vaupel JW, Christensen K. The Danish Twin Registry: 127 birth cohorts of twins. *Twin Research and Human Genetics*. 2002;5:352–357.
6. Pedersen DA, Larsen LA, Nygaard M, Mengel-From J, McGue M, Dalgård C, Hvidberg L, Hjelmborg J, Skytthe A, Holm NV, Kyvik KO, Christensen K. The Danish Twin Registry: an updated overview. *Twin Research and Human Genetics*. 2019;22:499–507.
7. Zagai U, Lichtenstein P, Pedersen NL, Magnusson PKE. The Swedish twin registry: content and management as a research infrastructure. *Twin Research and Human Genetics*. 2019;22:672–680.
8. Ligthart L, van Beijsterveldt CEM, Kevenaar ST, de Zeeuw E, van Bergen E, Bruins S, Pool R, Helmer Q, van Dongen J, Hottenga JJ, Van't Ent D, Dolan CV, Davies GE, Ehli EA, Bartels M, Willemsen G, de Geus EJC, Boomsma DI. The Netherlands twin register: longitudinal research based on twin and twin-family designs. *Twin Research and Human Genetics*. 2019;22:623–636.
9. Nilsen T, Brandt I, Harris JR. The Norwegian twin registry. *Twin Research and Human Genetics*. 2019;22:647–650.
10. Kaidesoja M, Aaltonen S, Bogl LH, Heikkilä K, Kaartinen S, Kujala UM, Kärkkäinen U, Masip G, Mustelin L, Palviainen T, Pietiläinen KH, Rottensteiner M, Sipilä PN, Rose RJ, Keski-Rahkonen A, Kaprio J. FinnTwin16: A longitudinal study from age 16 of a population-based Finnish twin cohort. *Twin Research and Human Genetics*. 2019;22:530–539.
11. Muniandy M, Heinonen S, Yki-Järvinen H, Hakkarainen A, Lundbom J, Lundbom N, Kaprio J, Rissanen A, Ollikainen M, Pietiläinen KH. Gene expression profile of subcutaneous adipose tissue in BMI-discordant monozygotic twin pairs unravels molecular and clinical changes associated with sub-types of obesity. *International Journal of Obesity (Lond)*. 2017;41:1176–1184.
12. Verdi S, Abbasian G, Bowyer RCE, Lachance G, Yarand D, Christofidou P, Mangino M, Menni C, Bell JT, Falchi M, Small KS, Williams FMK, Hammond CJ, Hart DJ, Spector TD, Steves CJ. TwinsUK: The UK adult twin registry update. *Twin Research and Human Genetics*. 2019;22:523–529.
13. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, Beaumont M, Van Treuren W, Knight R, Bell JT, Spector TD, Clark AG, Ley RE. Human genetics shape the gut microbiome. *Cell*. 2014;159(4):789–799.
14. Goodrich JK, Davenport ER, Beaumont M, Jackson MA, Knight R, Ober C, Spector TD, Bell JT, Clark AG, Ley RE. Genetic determinants of the gut microbiome in UK twins. *Cell Host & Microbe*. 2016;19(5):731–743.
15. Rimfeld K, Malanchini M, Spargo T, Spickernell G, Selzam S, McMillan A, Dale PS, Eley TC, Plomin R. Twins early development study: a genetically sensitive investigation

into behavioral and cognitive development from infancy to emerging adulthood. *Twin Research and Human Genetics*. 2019;22(6):508–513.
16. Ahmadzadeh YI, Eley TC, Plomin R, Dale PS, Lester KJ, Oliver BR, McMillan A, McAdams TA. Children of the twins early development study (CoTEDS): a children-of-twins study. *Twin Research and Human Genetics*. 2019;22(6):514–522.
17. Derom C, Thiery E, Rutten BPF, Peeters H, Gielen M, Bijnens E, Vlietinck R, Weyers S. The east flanders prospective twin survey (EFPTS): 55 years later. *Twin Research and Human Genetics*. 2019;22(6):454–459.
18. Groene SG, Todtenhaupt P, van Zwet EW, van Pel M, Berkhout RJM, Haak MC, Roest AAW, Lopriore E, van Klink JMM, Heijmans BT. TwinLIFE: the twin longitudinal investigation of FEtal discordance. *Twin Research and Human Genetics*. 2019;22(6):617–622.
19. Mönkediek B, Lang V, Weigel L, Baum MA, Eifler EF, Hahn E, Hufer A, Klatzka CH, Kottwitz A, Krell K, Nikstat A, Diewald M, Riemann R, Spinath FM. The German twin family panel (TwinLife). *Twin Research and Human Genetics*. 2019;22(6):540–547.
20. Pries LK, Snijders C, Menne-Lothmann C, Decoster J, van Winkel R, Collip D, Delespaul P, De Hert M, Derom C, Thiery E, Jacobs N, Wichers M, Guloksuz S, van Os J, Rutten BPF. TwinssCan - Gene-environment interaction in psychotic and depressive intermediate phenotypes: risk and protective factors in a general population twin sample. *Twin Research and Human Genetics*. 2019;22(6):460–466.
21. Torgersen S, Waaktaar T. The Oslo University adolescent and young adult twin project: recruitment and attrition. *Twin Research and Human Genetics*. 2019;22(6):641–646.
22. Medda E, Toccaceli V, Fagnani C, Nisticò L, Brescianini S, Salemi M, Ferri M, D'Ippolito C, Alviti S, Arnofi A, Stazi MA. The Italian twin registry: an update at 18 years from its inception. *Twin Research and Human Genetics*. 2019;22(6):572–578.
23. Ordoñana JR, Carrillo E, Colodro-Conde L, García-Palomo FJ, González-Javier F, Madrid-Valero JJ, Martínez Selva JM, Monteagudo O, Morosoli JJ, Pérez-Riquelme F, Sánchez-Romera JF. An update of twin research in Spain: The Murcia Twin Registry. *Twin Research and Human Genetics*. 2019;22(6):667–671.
24. Pereira S, Santos C, Katzmarzyk PT, Maia J. Familial Resemblance in body shape and composition, metabolic syndrome, physical activity and physical fitness: a summary of research in Portuguese families and siblings. *Twin Research and Human Genetics*. 2019;22(6):651–659.
25. Tarnoki AD, Tarnoki DL, Forgo B, Szabo H, Metneki DJ, Littvay L. The Hungarian Twin Registry update: Turning from a voluntary to a population-based registry. *Twin Research and Human Genetics*. 2019;22(6):561–566.
26. Segal NL, Senina IN. Russian twin studies: colleagues, controversies, case studies and current events. *Twin Research and Human Genetics*. 2019;22(6):53–64.
27. Kovas Y, Galajinsky EV, Boivin M, Harold GT, Jones A, Lemelin JP, Luo Y, Petrill SA, Plomin R, Tikhomirova T, Zhou X, Malykh S. The Russian school twin registry (RSTR): project PROGRESS. *Twin Research and Human Genetics*. 2013;16(1):126–133.
28. Kovas Y, Tikhomirova T, Selita F, Tosto MG, Malykh S. How genetics can help education. In: Yulia K., Malykh S., Gaysina D., eds. *Behavior Genetics for Education*. London: Palgrave Macmillan; 2016: 1–23.
29. Oncel SY, Aliev F. Turkish twin study: an initial step. *Twin Research and Human Genetics*. 2013;16(1):333–335.
30. Bouchard Jr TJ, Lykken DT, McGue M, Segal NL, Tellegen A. Sources of human psychological differences: the Minnesota Study of Twins Reared Apart. *Science*. 1990;250(4978):223–228.

31. Wilson S, Haroian K, Iacono WG, Krueger RF, Lee JJ, Luciana M, Malone SM, McGue M, Roisman GI, Vrieze S. Minnesota Center for Twin and Family Research. *Twin Research and Human Genetics*. 2019;22(6):746–752.
32. Gatz M, Plassman BL, Tanner CM, Goldman SM, Swan GE, Chanti-Ketterl M, Walters EE, Butler DA. The NAS-NRC twin registry and Duke twins study of memory in aging: An update. *Twin Research and Human Genetics*. 2019;22(6):757–760.
33. Silventoinen K, Jelenkovic A, Sund R, Yokoyama Y, Hur YM, Cozen W, et al. Differences in genetic and environmental variation in adult body mass index by sex, age, time period, and region: an individual-based pooled analysis of 40 twin cohorts. *American Journal of Clinical Nutrition*. 2017;106:457–466.
34. Lilley ECH, Morris AT, Silberg JL. The Mid-Atlantic Twin Registry of Virginia Commonwealth University. *Twin Research and Human Genetics*. 2019;22(6):753–756.
35. Kremen WS, Franz CE, Lyons MJ. Current status of the Vietnam Era Twin Study of Aging (VETSA). *Twin Research and Human Genetics*. 2019;22(6):783–787.
36. Boivin M, Brendgen M, Dionne G, Ouellet-Morin I, Dubois L, Pérusse D, Robaey P, Tremblay RE, Vitaro F. The Quebec Newborn Twin Study at 21. *Twin Research and Human Genetics*. 2019;22(6):475–481.
37. Hart SA, Martinez K, Kennedy PC, Ganley CM, Taylor J. The National Project on achievement in twins. *Twin Research and Human Genetics*. 2019;22(6):761–764.
38. Prescott CA, Walters EE, Arpawong TE, Zavala C, Gruenewald TL, Gatz M. The project talent twin and sibling study: zygosity and new data collection. *Twin Research and Human Genetics*. 2019;22(6):769–778.
39. Whitfield KE. A registry of adult African American twins: the Carolina African American twin study of aging. *Twin Research and Human Genetics*. 2013;16(1):476–480.
40. Harden KP, Tucker-Drob EM, Tackett JL. The Texas twin project. *Twin Research and Human Genetics*. 2013;16(1):385–390.
41. Pison G, D'Addato AV. Frequency of twin births in developed countries. *Twin Research and Human Genetics*. 2006;9(2):457–466.
42. Murphy K, Lam J, Cutler T, Tyler J, Calais-Ferreira L, Little C, Ferreira P, Craig JM, Scurrah KJ, Hopper JL. Twins research Australia: a new paradigm for driving twin research. *Twin Research and Human Genetics*. 2019;22(6):438–445.
43. Mitchell BL, Campos AI, Rentería ME, Parker R, Sullivan L, McAloney K, Couvy-Duchesne B, Medland SE, Gillespie NA, Scott J, Zietsch BP, Lind PA, Martin NG, Hickie IB. Twenty-Five and up (25Up) study: a new wave of the Brisbane Longitudinal Twin Study. *Twin Research and Human Genetics*. 2019;22(3):154–163.
44. Larsen SA, Little CW, Grasby K, Byrne B, Olson RK, Coventry WL. The academic development study of Australian twins (ADSAT): research aims and design. *Twin Research and Human Genetics*. 2020;23(3):165–173.
45. Leong P, Loke YJ, Saffery R, Silk T, Burnett A, Josev E, Scurrah K, Theda C, Czajko A, Pua EP, Seal ML, Craig JM. Epigenetic influences on neurodevelopment at 11 years of age: protocol for the longitudinal Peri/Postnatal Epigenetic Twins Study at 11 years of age (PETS@11). *Twin Research and Human Genetics*. 2019;22(6):446–453.
46. Hughes TE, Townsend GC, Pinkerton SK, Bockmann MR, Seow WK, Brook AH, Richards LC, Mihailidis S, Ranjitkar S, Lekkas D. The teeth and faces of twins: providing insights into dentofacial development and oral health for practising oral health professionals. *Australian Dental Journal*. 2014;59(Suppl 1):101–116.
47. Schache M, Baird PN. The Australian twin registry as a resource for genetic studies into ophthalmic traits. *Twin Research and Human Genetics*. 2013;16(1):52–57.

48. Bulmer MG. *The Biology of Twinning in Man*. Oxford: Clarendon Press; 1970.
49. Smits J, Monden C. Twinning across the developing world. *PLoS One*. 2011;6(9):e25239.
50. Huang T, Gao W, Lv J, Yu C, Wu T, Wang S, Liao C, Meng L, Wang D, Wang Z, Pang Z, Yu M, Wang H, Wu X, Dong Z, Wu F, Jiang G, Wang X, Liu Y, Deng J, Lu L, Cao W, Li L. The Chinese National Twin Registry: A unique data source for systems epidemiology of complex disease. *Twin Research and Human Genetics*. 2019;22(6):482–485
51. Bi D, Li X, Chen J, Jiang N, Zhang J. The Beijing twin study (BeTwiSt): an update. *Twin Research and Human Genetics*. 2019;22(6):486–491.
52. Ding X, Wang W, Scheetz J, He M. The Guangzhou twin eye study: 2019 update. *Twin Research and Human Genetics*. 2019;22(6):492–498.
53. Ding X, Hu Y, Guo X, Guo X, Morgan I, He M. Possible causes of discordance in refraction in monozygotic twins: Nearwork, time outdoors and stochastic variation. *Investigative Ophthalmology and Visual Science*. 2018;59(13):5349–5354.
54. Ando J, Fujisawa KK, Hiraishi K, Shikishima C, Kawamoto T, Nozaki M, Yamagata S, Takahashi Y, Suzuki K, Someya Y, Ozaki K, Deno M, Tanaka M, Sasaki S, Toda T, Kobayashi K, Sakagami M, Okada M, Kijima N, Takizawa R, Murayama K. Psychosocial twin cohort studies in Japan: the Keio Twin Research Center (KoTReC). *Twin Research and Human Genetics*. 2019;22(6):591–596.
55. Honda C, Watanabe M, Tomizawa ROsaka Twin Research Group, Sakai N. Update on Osaka University twin registry: an overview of multidisciplinary research resources and biobank at Osaka University Center for Twin Research. *Twin Research and Human Genetics*. 2019;22(6):597–601.
56. Yokoyama Y. The New West Japan twins and higher order multiple births registry. *Twin Research and Human Genetics*. 2019;22(6):602–605.
57. Hur Y-M, Kang MC, Jeong H-U, Kang IC, Kim JW. The South Korean Twin Registry. *Twin Research and Human Genetics*. 2019;22(6):606–608.
58. Jayaweera K, Warnakula L, Dissanayake L, Adikari A, Siribaddana S, Zavos HMS, Rijsdijk F, Zunszain PA, Pariante CM, Glozier N, Hotopf M, Sumathipala A. The Sri Lankan twin registry biobank: South Asia's first twin biobank. *Global Health Epidemiology and Genomics*. 2020;9(5):e5.
59. Jayaweera K, Aschan L, Pannala G, Adikari A, Glozier N, Ismail K, Pariante CM, Rijsdijk F, Siribaddana S, Zavos HMS, Zunszain PA, Sumathipala A, Hotopf M. The Colombo twin and singleton follow-up study: a population based twin study of psychiatric disorders and metabolic syndrome in Sri Lanka. *BMC Public Health*. 2018;18(1):145–159.
60. Vertsberger D, Abramson L, Knafo-Noam A. The Longitudinal Israeli study of twins (LIST) reaches adolescence: genetic and environmental pathways to social, personality and moral development. *Twin Research and Human Genetics*. 2019;22(6):567–571.
61. Gharipour M, Haghjooy Javanmard S, Mansourian M, Sabri M, Khosravi E, Dianatkhah M, Sadeghian L, Jabarzare Z, Sarrafzadegan N. Isfahan Twins Registry (ITR): an invaluable platform for epidemiological and epigenetic studies: design and methodology of ITR. *Twin Research and Human Genetics*. 2019;22(6):579–582.
62. MacGillivray I. Epidemiology of twin pregnancy. *Seminars in Perinatology*. 1986;10: 4–8.
63. Pison G. Twins in Sub-Saharan Africa: frequency, social status and mortality. In: van de Walle E, Pison G, Sala-Diakanda M, eds. *Mortality and Society in Africa*. Oxford: Clarendon Press; 1992:253–278.
64. Nylander PPS. Serum levels of gonadotropins in relation to multiple pregmany in Nigeria. *Br. J. Obstet. Gynaecol*. 1973;80(7):651–653.

65. Akinboro A, Azeez MA, Bakare AA. Frequency of twinning in southwest Nigeria. *Indian Journal of Human Genetics*. 2008;14:41–47.
66. Bjerregaard-Andersen M, Gomes GM, Hennild DE, Jensen DM, Christensen K, Sodemann M, Beck-Nielsen H, Aaby P. The Guinea-Bissau twin registry update: a platform for studying twin mortality and metabolic disease. *Twin Research and Human Genetics*. 2019;22(6):554–560.
67. Hur YM, Jeong HU, Kang MC, Ajose F, Kim JW, Beck JJ, Hottenga JJ, Mbarek H, Finnicum CT, Ehli EA, Martin NG, de Geus EJ, Boomsma DI, Davies GE, Bates T. The Nigerian twin and sibling registry: an update. *Twin Research and Human Genetics*. 2019;22(6):637–640.
68. Hennild DE, Bjerregaard-Andersen M, Joaquim LC, Christensen K, Sodemann M, Beck-Nielsen H, Jensen DM. Prevalence of impaired glucose tolerance and other types of dysglycaemia among young twins and singletons in Guinea-Bissau. *BMC Endocrine Disorders*. 2016;16:46.
69. Ivanovic DM, Llop ER, Alvear JA, Pérez HT, Díaz NS, Leyton BD, Almagià AF, Larraín CG, Alvarez JE, Herrera YF, Hazbun EL. Chilean school-age children twin registry: planning, sampling and implications. *Twin Research and Human Genetics*. 2006;9:743–746.
70. Marcheco-Teruel B, Cobas-Ruiz M, Cabrera-Cruz N, Lantigua-Cruz A, García-Castillo E, Lardoeyt-Ferrer R, Robaina-Jiménez Z, Fuentes-Smith E, Morales-Calatayud F, Lemus-Valdés MT, Portuondo-Sao M, Comas-Pérez L, Pérez-Crispí JM, Díaz-De Villal T, Icart-Perera E, Jordán-Hernández A, Lage-Castellanos A, Rabell-Piera S, Llibre-Rodríguez Jde J, Valdés-Sosa P, Valdés-Sosa M. The Cuban twin registry: initial findings and perspectives. *Twin Research and Human Genetics*. 2013;16:98–103.
71. Otta E, Fernandes EDS, Bueno JA, dos Santos KL, Segal NL, Lucci TK, Ferreira IF, Cesar GC, David VF, Tatit DP, Short PCA, Fernandes LO, Crispim AC, Moretto MLT, Andrade NC, Corte S, Tobo PR, Barrichello CR, de Sousa RCG, Júnior MDS, Bussab VS, Ribeiro FJL. The University of São Paulo Twin Panel: current status and prospects for Brazilian twin studies in behavioral research. *Twin Research and Human Genetics*. 2019;22(6):467–474.
72. Cervantes-Cardona GA, Cervantes-Nápoles TE, Cervantes-Guevara G, Manzo-Palomera OR, González-Ojeda A, Silva-González G, Fuentes-Orozco C, Sánchez-Michel Á, Nápoles-Echauri AC. First steps of the Mexico Twin Registry (RGMex-MexTR) at the University of Guadalajara. *Twin Research and Human Genetics*. 2019;22(6):609–610.
73. Leon-Apodaca AV, Chiu-Han E, Ortega-Mora I, Román-López TV, Caballero-Sánchez U, Aldana-Assad O, Campos AI, Cuellar-Partida G, Ruiz-Contreras AE, Alcauter S, Rentería ME, TwinsMX Medina-Rivera A. Uncovering the basis of health and disease in the Mexican population. *Twin Research and Human Genetics*. 2019;22(6):611–616.
74. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K. Environmental and heritable factors in the causation of cancer–analyses of cohorts of twins from Sweden, Denmark, and Finland. *N. Engl. J. Med*. 2000;343:78–85.
75. Harris JR, Hjelmborg J, Adami HO, Czene K, Mucci L, Kaprio J. Nordic twin study of cancer (NorTwinCan) collaboration. *Twin Research and Human Genetics*. 2019;22(6):817–823.
76. Pedersen NL, Gatz M, Finch BK, Finkel D, Butler DA, Dahl Aslan A, Franz CE, Kaprio J, Lapham S, McGue M, Mosing MA, Neiderhiser J, Nygaard M, Panizzon M, Prescott CA, Reynolds CA, Sachdev P, Whitfield KE. IGEMS: The consortium on interplay of genes and environment across multiple studies—an update. *Twin Research and Human Genetics*. 2019;22(6):809–816.

77. Peltonen L. GenomEUtwin: a strategy to identify genetic influences on health and disease. *Twin Research*. 2003;5:354–360.
78. European Network for Genetic and Genomic Epidemiology (ENGAGE). http://www.euengage.org/.
79. Silventoinen K, Jelenkovic A, Yokoyama Y, Sund R, Sugawara M, Tanaka M, et al. The CODATwins Project: the current status and recent findings of collaborative project of development of anthropometrical measures in twins. *Twin Research and Human Genetics*. 2019;22(6):800–808.
80. Kandler C, Penner A, Richter J, Zapko-Willmes A. The study of personality architecture and dynamics (SPeADy): a longitudinal and extended twin family study. *Twin Research and Human Genetics*. 2019;22(6):548–553.
81. Smederevac S, Mitrović D, Sadiković S, Milovanović I, Branovački B, Dinić BM, Nikolašević Z, Kodžopeljić J, Ignjatović VB, Semnic M, Stankov TV, Vučinić N, Oljača M, Pajić D, Stojadinović A, Krstić T, Milutinović A. Serbian twin registry. *Twin Research and Human Genetics*. 2019;22(6):660–666.
82. Lemery-Chalfant K, Oro V, Rea-Sandin G, Miadich S, Lecarie E, Clifford S, Doane LD, Davis MC. Arizona twin project: specificity in risk and resilience for developmental psychopathology and health. *Twin Research and Human Genetics*. 2019;22(6):681–685.
83. Kittelsrud JM, Ehli EA, Petersen V, Jung T, Beck JJ, Kallsen N, Huizenga P, Holm B, Davies GE. Avera twin register growing through online consenting and survey collection. *Twin Research and Human Genetics*. 2019;22(6):686–690.
84. Saudino KJ, Ganiban JM. The Boston University Twin Project (BUTP) II. *Twin Research and Human Genetics*. 2019;22(6):691–694.
85. Wadsworth SJ, Corley RP, Munoz E, Trubenstein BP, Knaap E, DeFries JC, Plomin R, Reynolds CA, Team CATSLife. CATSLife: a study of lifespan behavioral development and cognitive functioning. *Twin Research and Human Genetics*. 2019;22(6):695–706.
86. Corley RP, Reynolds CA, Wadsworth SJ, Rhea SA, Hewitt JK. The Colorado twin registry: 2019 update. *Twin Research and Human Genetics*. 2019;22(6):707–715.
87. Leve LD, Neiderhiser JM, Ganiban JM, Natsuaki MN, Shaw DS, Reiss D. The early growth and development study: a dual-family adoption study from birth through adolescence. *Twin Research and Human Genetics*. 2019;22(6):716–727.
88. Taylor J, Martinez K, Hart SA. The Florida state twin registry. *Twin Research and Human Genetics*. 2019;22(6):728–730.
89. Segal NL, Niculae FJ. Fullerton virtual twin project: overview and 2019 update. *Twin Research and Human Genetics*. 2019;22(6):731–734.
90. Davis DW, Turkheimer E, Finkel D, Beam C, Ryan L. The Louisville twin study: past, present and future. *Twin Research and Human Genetics*. 2019;22(6):735–740.
91. Burt SA, Klump KL. The Michigan State University twin registry (MSUTR): 15 years of twin and family research. *Twin Research and Human Genetics*. 2019;22(6):741–745.
92. Ramos AM, Chen T, Hatemi PK, Cleveland HH, Neiderhiser JM. The Pennsylvania longitudinal study of parents and children (PALSPAC) twin registry. *Twin Research and Human Genetics*. 2019;22(6):765–768.
93. DiLalla LF, Jamnik MR. The Southern Illinois twins/triplets and siblings study (SITSS): a longitudinal study of early child development. *Twin Research and Human Genetics*. 2019;22(6):779–782.
94. Duncan GE, Avery AR, Strachan E, Turkheimer E, Tsang S. The Washington state twin registry: 2019 update. *Twin Research and Human Genetics*. 2019;22(6):788–793.

95. Schmidt NL, Lemery-Chalfant K, Goldsmith HH. Wisconsin twin project overview: temperament and affective neuroscience. *Twin Research and Human Genetics*. 2019;22(6):794–799.
96. Rose RJ, Salvatore JE, Aaltonen S, Barr PB, Bogl LH, Byers HA, Heikkilä K, Korhonen T, Latvala A, Palviainen T, Ranjit A, Whipp AM, Pulkkinen L, Dick DM, Kaprio J. FinnTwin12 Cohort: an updated review. *Twin Research and Human Genetics*. 2019;22(6):302–311.
97. Kaprio J, Bollepalli S, Buchwald J, Iso-Markku P, Korhonen T, Kovanen V, Kujala U, Laakkonen EK, Latvala A, Leskinen T, Lindgren N, Ollikainen M, Piirtola M, Rantanen T, Rinne J, Rose RJ, Sillanpää E, Silventoinen K, Sipilä S, Viljanen A, Vuoksimaa E, Waller K. The Older Finnish Twin Cohort - 45 years of follow-up. *Twin Research and Human Genetics*. 2019;22(6):240–254.

SECTION

Phenomenon of Twinning

2

CHAPTER

Biology of natural twinning

4

Gary Steinman
Department of Obstetrics & Gynecology, Hadassah Hospital—Hebrew University, Jerusalem, Israel

4.1 Introduction

Both human resistance and healthcare services have evolved to the point where twin gestations are more likely to be born alive and in good condition than in earlier times. Because of the space-saving size of the female reproductive organs, pregnancy-related problems can arise before, during, or after parturition. During gestation, several types of complications can occur with twins that are rarely, if ever, seen in one-baby pregnancies.

Crowding reduces the available uterine interior surface area per fetus, thereby diminishing nutrient flow between the placenta and each fetus in a twin gestation, compared to typical singletons. Because of the physical and environmental stresses, maturation of each twin baby's lungs is accelerated over that of solo fetuses. As a result, "term" pregnancy for a twin set is typically 37 instead of 40 weeks, as with singletons. For triplets, it is 34 weeks and for quadruplets it is 31 weeks, on average. Although the multiple babies are usually smaller because of the shortened gestational period, survival based on pulmonary function is typically superior to with singletons for the same length of time.[1]

In contrast, the occurrence of all multifetal pregnancies decreases as the number of babies increases per gestation. Part of this is due to the fact that multiple fertilizations become less and less frequent as the set number rises. Secondly, especially in the case of MZ multiples, the uteroplacental contact area servicing each baby following implantation decreases, thereby diminishing the level of support and nourishment for each fetus.[1]

The disappearance of one of the two babies in as many as 5 out of 6 twin sets before 20 weeks of gestation attests to the problems encountered early by multiples (see "vanishing twin" discussion later). It has been conjectured that if all such twin pregnancies survived until viability, there would be more than six multiple births for every now.[2]

At birth, the hazard of delivery of twins vaginally instead of cesarean section adds to the risk of not being born alive *and* uninjured. In many twin gestations, the gravida is twice as likely to experience preeclampsia, a serious blood pressure problem of pregnancy, in contrast to one with just a single baby (see discussion at the end of this chapter).

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00021-0

In summary, pregnancies consisting of more than one fetus pose many issues which are not encountered or are more infrequent than with one baby alone from the start.

4.2 Defining factors

This discussion will be limited to multifetal gestations that arise from unassisted rather than laboratory-based or drug-promoted conceptions. Currently, the birth rate of naturally conceived twins is roughly half as those produced in vitro in medically advanced countries (approximately 1.5% of all gestations). There are generally two types of twin sets (exceptions will be discussed later):

1. *Monozygotic* (MZ)—a conceptus resulting from the union of one ovum and one sperm, which divides into two beings within the first two weeks of gestation.
2. *Dizygotic* (DZ)—two embryos produced by the union of two separate spermatozoa joining with two discrete ova (gametes).

This coming together of two human gametes (ovum and sperm), each with 23 different chromosomes, reestablishes the characteristic 23 chromosomal pairs/cell (zygote) following fertilization. Another possibility is a multifetal pregnancy which bears a singleton plus a twin/triplet set concurrently in the same uterus. Such a case combines monozygosity with dizygosity in the same overall pregnancy. By the time of delivery, such a set could result in MZ or DZ twins, depending on which single baby may have not survived along the way.[3]

Later in this discussion, we will consider biological exceptions to these distinctions, especially in relatively homogeneous socio-cultural populations within defined geographic locales. In the majority of such neonatal MZ and/or DZ pairs, specific physical characteristics of the babies copying one another in some or all physical or behavioral aspects can be identified. For example, one member of an MZ pair can be an organ donor, if needed, for the other member. This is not typically true in DZ sets because of antigenic dissimilarities of the members between each other (It is interesting to note that the gravida and her developing fetus may have some antigenic differences between them, making the baby a "foreign body" in her, but yet not purging its presence in most cases because of the separated circulations in the placenta).

In almost all twin pregnancies, there are two membrane sacs surrounding the conceptuses (Varieties of multiple twin pregnancies are noted in Fig. 4.1). One membrane, the amnion, encompasses each baby; the other, the chorion, surrounds all the fetuses in many monozygotic gestations and both fetuses in dizygotic conceptions. Some monozygotic pairs share a single placenta. If a monozygotic twin pregnancy has no amniotic membrane separating the two fetuses, there is a serious risk of their cords getting tangled with each other. Thus, a DCMZ one-egg twin set would be dichorionic, monozygotic and an MCMZ set would be monochorionic, monozygotic.[3]

The mother's prior history of breastfeeding can increase the probability of conceiving twins in her next pregnancy. Many women feel that as long as they continue lactating, they are shielded from further pregnancies. While lactation does delay the

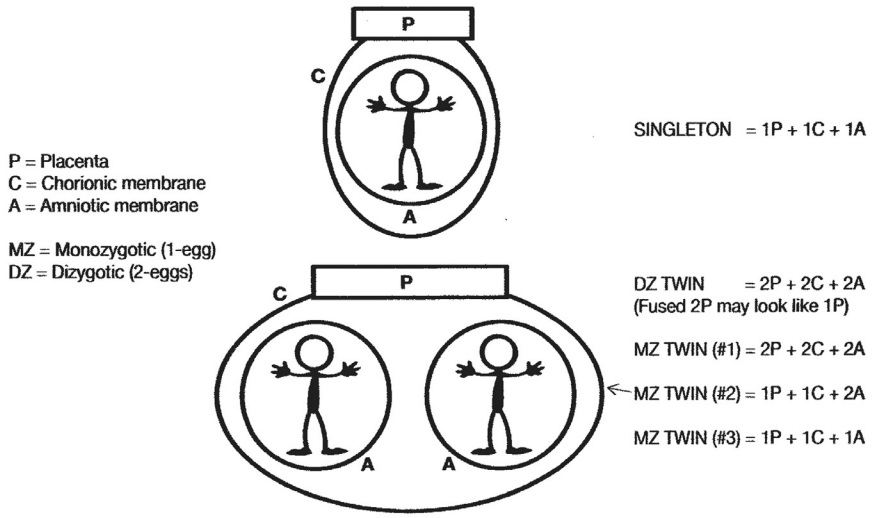


FIG. 4.1 Schematic drawing of the most common types of twin pregnancies based on placental numbers, membrane distributions, and separations between fetuses.

A representation of a singleton gestation is shown for comparison.

postpartum resumption of ovulation, in contrast to no breastfeeding, it does not stop reproductive capabilities forever unless the gravida becomes menopausal before attempting the next conception. Women who have ever lactated have persistently higher blood IGF (insulin-like growth factor) levels than those who have never done so. Mothers who had given birth to more than one child and had breastfed were more likely, based on the number of prior months lactating, to conceive twins now than those women who had not done so.[3]

4.3 Conception issues

In our modern world, there is a trend toward later marriage, and many women choose to delay their childbearing for career, educational, monetary, or personal reasons. For example, in 1994, the percentage of women having their first baby over the age of 30 was five times higher than in 1969.[3] This inclination appears to be continuing today. The age at which a woman eventually decides to conceive, her "blood" relatives' reproductive history, the number of children she elects to have, and the amount of breast-feeding she did previously can have a direct bearing on her chance of twinning in a subsequent pregnancy.

For a dizygotic twin gestation to result, two viable eggs and a good supply of active sperm must be available concurrently. Fertilization typically occurs in as little

as 5–15 min after vaginal deposition of semen. Unlike sperm, which are constantly being produced from precursor cells, a woman is born with all the eggs she will ever have naturally. The ovarian reserve of available follicles decreases rapidly with age. A female fetus at four months of gestation is estimated to have 3,500,000 eggs in her ovaries, while a woman aged 39–45 years has around 11,000. Whereas about 15 follicles are typically recruited monthly in the ovaries of young women, usually only one is mobilized per menstrual cycle after age 40.[2,4]

Inheritance also plays a role in determining the probability of the twinning process taking place, especially with dizygotic twins. For example, this twinning tendency has been studied by examining bovine (cow) populations. Twinning is uncommon among cows, relying on a special gene functioning on chromosome #5. However, the more times a particular cow is pregnant, the more likely it is to conceive twins.[5]

It is well known that the tendency to produce twins is greater in certain human families. For many years, the twinning trait was believed to be inherited only in cases of DZ pairs. However, a recent investigation identified several families containing as many as five spontaneous sets of MZ twins.[4] If based only on random occurrence, one would expect 268 separate gestations to yield just two sets of MZ twins within a single extended family. Women who have conceived twins naturally (without fertility treatment) have a greater chance of having a second set in a later pregnancy than a multiparous (mother of many children) female who has had only singletons. Of additional interest is the observation that 30% of fathers who have produced twin offspring have genetically connected male relatives who have also produced twins.[6] It could be concluded that twinning is a reproductive trait that is passed on via the procreative qualities of both genders. These findings suggest that the tendency to produce twins has an inherited genetic component that runs along with both the male and the female lines.

Although an MZ set originates with both children bearing the same genetic composition, it is not uncommon for such a pair to have small but perceptible physical differences by the time of birth. Thus, the final expression of physical traits in humans is the result of *nature* (heredity, as determined by genes) in combination with *nurture* (gestational environment). For example, fingerprints are primarily the result of genetic determinants. The basic initial expression of the prints in identical fetuses in the entire set is essentially the same. However, intrauterine events can modify the translation of the nuclear codes somewhat. The fingertips of the babies possess genetically determined pads at 3 months or so which evolve into individualized fingerprints by birth. Each baby of a given set of monozygotic quadruplets after birth has slightly different print patterns, apparently due to environmental factors such as surrounding intrauterine forces and pressures.[2,7] As the saying goes: no two people have exactly the same set of fingerprints.

In biomedical studies, it is desirable to test MZ twin sets who appear to be identical in all aspects. If so, one member of the pair can be given a new drug or treatment being evaluated for a special application, and the other member is given a seemingly similar placebo. In this way, all biological variables are apparently the same in both individuals of the set except for the presence or absence of the new modality. For

example, in 2015–16, one member of a monozygotic twin set was placed on the International Space Station and the other remained on Earth. At the end of the 342-day trip, the one who orbited was studied and was found to have undergone a number of physiological changes that the other had not experienced.[8]

Today, the gold standard for zygosity determination in the laboratory is examining the similarity of particular DNA sequences in chromosomes from within the cell nuclei. Technicians make many copies of the twins' DNA from small samples, and the specific sequences of the polynucleotides are tagged. The samples are then compared by looking at how they react by separation in an electrical field. If the two individuals are indeed genetically alike, their tagged DNA fragments will yield the same electrophoretic pattern.

The definition of a *twin* pregnancy can sometimes be ackward. It is commonly accepted that such a double pregnancy is one in which the union of sperm and egg(s) occurred at about the same time. However, there are cases where one of the two babies is delivered prematurely for medical reasons and the second is able to remain in the uterus for additional days or weeks. What should be taken as their birthdays? If different, are they technically "twins"? In actuality, the only time the question of zygosity is pertinent medically is if organ transplant from one person to the other in this pair becomes necessary at a later date. An additional example of this is the genetic study of a set of DZ twins who have one mother but each carries the genes of different fathers. This apparently resulted from fertilization of each taking place within a number of hours by two separate males.[2]

4.4 Maternal dietary factors affecting the frequency of multifetal gestations

1. The rate of twinning has been found to be related in part to a woman's blood level of *insulin-like growth factor-1* (IGF1) which, in many cases, is correlated with maternal height. This enhancing factor is released by the liver in proportion to the level of her pituitary growth hormone (GH). IGF is one of the treatments used to medically promote the ovarian release of ova. A statistical study showed that there is a direct relationship between the frequency of twinning and the mean *height* of women in selected populations. Countries with taller women tend to have higher twinning rates. Related to this is the significant IGF content of milk. Countries that have higher percentages of multifetal pregnancies are also those known to have elevated milk consumption compared to other countries. Cattle with the highest twinning rate also have the highest mean serum IGF level. In some cases, this appears due to the prophylactic use of GH, given to cattle to increase amounts of meat and milk produced.[9–11]
2. During *famines*, the rate of twinning in particular typically decreases. IGF is a polypeptide containing both essential and non-essential amino acids (Essential amino acids are not produced *de novo* in humans but must be acquired in the

Sample Country	Milk Protein Consumed (in grams/day)	Twin Rate per 1,000 Births
Finland	30.4	12.2
Denmark	16.8	9.6
Belgium	11.6	7.3
Spain	8.2	5.9

FIG. 4.2 Effect of mean milk consumption on dizygotic twinning.

diet, in contradistinction to non-essential amino acids which can be produced from available basic metabolites). Hence, a protein-poor diet, among other phenomena, should decrease the twinning rate in particular. In fact, this is what was observed in Holland during World War II, when severe starvation was experienced due to sharply reduced food supplies. The rate of twinning fell about 30% below its pre-War value. The rate of twinning after the end of the War rose significantly with improved nutrition.[9]

3. *Vegans* consume no food products that originate from animals, including milk. The rate of twinning among vegan mothers was compared with the general population whose diets were inclusive of all forms of omnivore and vegetarian components. In the general population, the natural twinning rate was 1.9%, whereas with vegans it was 0.4%.[9]
4. The *gender ratio* of several sets of MZ twins has been found to be 0.60, favoring females, rather than the anticipated value of 0.50. These findings are consistent with the model of twinning as a consequence of a depressed calcium in the prenidation environment and its effect on embryonic intercellular bonding. Because the X chromosome is larger than the Y, the cells of males can apparently reproduce faster than females prior to endometrial implantation. As a result, female embryos would remain longer in a low calcium environment than males, promoting the division of their cells prior to implantation. This would enhance the frequency of female MZ twinning versus male.[5]

4.5 Maternal physical factors and the rate of twinning

1. A relationship was also found between the chance of twinning and *race*. When comparing African–Americans, Caucasians, and Asians, the first group had the highest mean IGF level and the Asians the lowest. The general rate of twinning, when compared to race, showed the same trend. For unknown reasons, triplet gestations in Yoruba Africans are predominantly three-egg sets, whereas Caucasian triplets in the United States are most commonly dizygotic. In Japan, although infrequent, spontaneous triplets are most often one-egg pregnancies.[2]

2. A number of studies have reported a direct correlation between *height* and the level of insulin-like growth factor in the mother's blood. This hormone can be used to treat unusually short youngsters. IGF acts directly on the bones and muscles to stimulate growth, among many effects. In one such study of births in 32 countries, the maternal height was compared to the rate of birth of twins and triplets. The overall trend was that taller women tended to exhibit higher twinning rates. The mean maternal height in Denmark was 167.0 cm, whereas in Libya it was 153.5 cm. In Denmark, at that time the twinning rate was 19.9 per 1000 live births, whereas in Libya it was 11.5 per 1000.[2] In another investigation in the United States, the mean height of mothers of twins/triplets was 164.8 cm, whereas with singleton deliveries only the mean height was 161.8 cm ($P < 0.005$).[10]
3. In addition to the risks encountered in a multifetal pregnancy already discussed here, two more items that need examination are placental abruption and fetal growth retardation. Although these problems can also be encountered in a singleton gestation, they are more common in multifetal pregnancies. Fetal growth retardation can result from placental insufficiency or an inadequate junctioning between the placenta and the uterine wall. Disconnection of the placenta with the uterine wall is especially dangerous for the gravida because of the rapid rate of blood loss it causes. This phenomenon can occur with high blood pressure or trauma to the uterus.[4]

4.6 Biological factors tending to increase twinning

The probability of twinning (spontaneous/natural conception, in contradistinction to medically induced) is affected by family history, race, parity, prior pregnancies, maternal age at conception, and diet.

1. In the United States in 2001, 2 in 50 people were *dizygotic* twins and 2 in 150 were *monozygotic* twins. In Japan, most twins are monozygotic. Worldwide today, it is estimated there are 70 sets of monozygotic quadruplets, whereas it was concluded that there were 60 such sets in 1998.[2]
2. If a woman's sister or mother delivered dizygotic twins, her *chance* of conceiving twins is doubled. If she has already delivered dizygotic twins, her chance of having another set spontaneously is four times higher. If a woman is a dizygotic twin herself, she has a 1-in-17 chance of giving birth to a set of twins.[2]
3. The tendency for double ovulation is *inherited* through the mother's line. As we learned in a previous discussion, the reproductive faculties of the father's male relatives also appear to play a role with him in producing twin offspring.
4. Female fertility, in general, decreases progressively from the mid-twenties until menopause. Follicle stimulating hormone (FSH) induces the ovary to supply eggs that may finally be ovulated for fertilization. Although the overall level of IGF decreases from puberty until death, FSH abruptly rises beginning at age

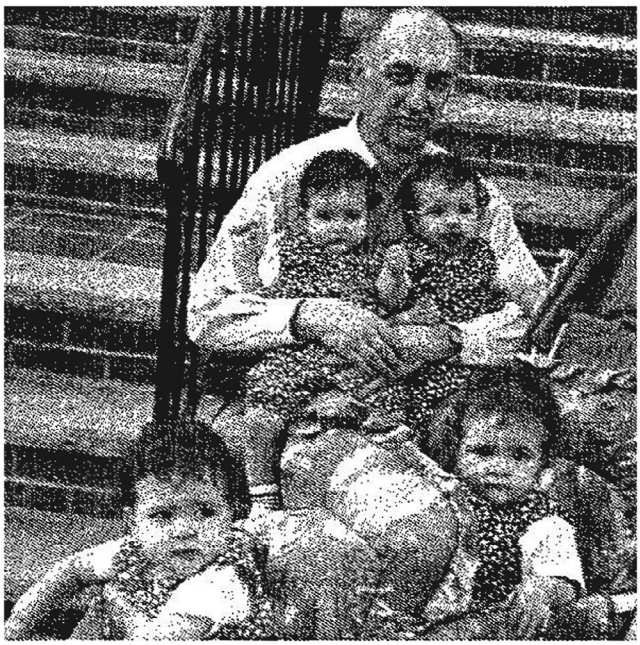


FIG. 4.3 Spontaneous monozygotic quadruplets.

Dr. Steinman in the Spring of 1998 with the monozygotic Borges quadruplets, whom he delivered in March, 1997 (printed with permission).

35 in anticipation of menopause. This may explain why there is an increase in twinning between 35 and 39, with peaking around 37 years. Because of the premenopausal FSH surge noted earlier, *older* women (especially between 35 and 39 years of age) have twins more often than younger women. Furthermore, the more children a woman has, the more likely she is to eventually have a set of twins.[2]

5. Contrary to popular belief, twinning does NOT skip generations.

Although these rates are prone to change in the future for a number of reasons, they give an appraisal that certain women currently are more likely to have twins. What may happen to any particular woman is difficult to predict. For example, the chance of delivering a boy is slightly higher than giving birth to a girl, but we all know of large families with exclusively one gender or the other.

In summary, the rate of spontaneous twinning appears usually to be affected by one or more of the following maternal factors:

1. Family history.
2. Race.
3. Genetic predisposition.
4. Maternal age.

5. Parity.
6. Prior or present lactation.
7. Nutritional adequacy.
8. Maternal dairy product consumption.
9. Maternal height.
10. Previous twin birth.

Factors 1–3 are fixed at birth, whereas the last seven factors are adjustable by variable environmental conditions, prior pregnancies, and personal lifestyle decisions. Height potential is inherited, but its achievement is affected by environmental factors such as diet and health maintenance.[2]

Many studies have been carried out looking for possible genetic factors governing the likelihood of production of twin gestations. Attention has been given to the possible effect of single nucleotide polymorphism (SNP). Most SNPs are silent since 98% occur in the intron or intergeneic regions of the DNA. To be classified as a polymorphism, the allele of interest, with a single nucleotide replaced by another, must have an occurrence frequency of 1% or more. Two such SNPs of putative interest in generating DZ twins are rs11031006-G and rs17293443-C. The former increases a woman's chance of delivering twins by 18% and the latter by 9%. Also, a region of chromosome 15q22.33 may participate in this reproductive modification. However, it is a possible for a gravida to bear DZ twins but not have either of these chromosomal variants.[12,13]

In Caucasian populations, natural twins (i.e., not medically promoted) typically amount to about 1%–3% of all births. An isolated village named Linha Sao Pedro in Brazil, populated predominantly by German immigrants, was found to have births that yielded 10% twins, half of which were monozygotic. Following World War II, Jospeh Mengele, a doctor who had experimented on twins in concentration camps during the War, escaped to South America. It was speculated that he had treated the local Brazilian population to enhance the likelihood for twinning, such as with GH. However, careful genetic studies revealed that twinning in that isolated village predated WW II by many years. This is a good example of the *Founder Effect*, a type of loss of genetic diversity which happens when a small population is separated from a larger gene pool.[14–16]

Whereas the rate of natural monozygotic twinning is relatively constant, the rate of DZ gestations can be variable between populations, totaling 1%–3% overall. Of interest is the rate of twinning in particular families, which may run in certain families for reasons yet to be determined.[17]

4.7 Some unique complications in twin pregnancies

1. *Vanishing* twin—Especially in dizygotic pregnancies, each twin may not develop as successfully as its partner, as noted earlier. In such a case, an ultrasound study may visualize two fetuses at 6–8 weeks of gestation, followed by the gravid woman observing a nonspecific episode of slight cramping

and spotting. A repeat ultrasound at 20 weeks or so will observe only one intrauterine fetus. In most cases, no specific cause for the loss of the other is identified. The incidence of viable multifetal gestations (MZ or DZ) becomes much smaller as the total size of the set enlarges. This is because one or more fetuses reach viability before the onset of parturition, whereas the second member of the twin set did not survive through the 20th week of gestation. In general, multifetal gestations are more likely to result in pregnancy loss (miscarriage) than singles. This may be the result of compromised uterine/placental circulation in a crowded multiple gestational environment. This scenario is estimated to occur in five out of six twin sets. Thus, for every one set of twins that survives until delivery, five experience the loss of one member of the pair. Because of this, the rate of natural twinning may actually be closer to 9%.[2]

2. *Conjoined* twins—In the case of monozygotic pregnancies, a rare complication found in only 0.25% of MZ pairs is the physical union of the two fetuses with various combinations of midline attachment to each other (e.g., abdomen-to-abdomen, as observed with the original Siamese Twins). The potential for survival is poor and 40% are stillborn because of the sharing of vital organs. All conjoined sets originate from one egg, develop with one set of placental membranes (MCMZ), and are predominantly female (75% of the time). Since such twins usually have normal chromosomes, the cause of this phenomenon is most likely a developmental issue such as delayed implantation, rather than a genetic error. This may be related to deficient calcium levels which reduce the adherence of embryonic cells, resulting in incomplete separation of blastomeres.[18]

   The specific cause of conjoining is not currently known, though scientists have at least two theories that attempt to explain this structural mistake. The two fetuses can be joined almost anywhere in the midline. In the first theory, the separation (fission) of an early embryo into two entities—usually in the latter half of the second week of gestation—is incomplete. Seventy-seven percent of conjoined sets are mirror images of each other, compared with 22% of unconnected monozygotic twin pairs, supporting the fission model of development. In the other theory, separated embryonic "discs" secondarily join (fuse) for unknown reasons. Both the fission and fusion theories are supported by real-life examples.[18]
3. A *chimera* is an ordinary person except that various part of his/her body actually came from a twin partner or from the mother. Such departures from the usual may occur in MZ or DZ twin gestations. Because of the irregular distribution of surface cells, a characteristic mosaicism may occur in skin coloration. A chimera might also be composed of both male and female cells in each member of a boy–girl dizygotic twin gestation.[2]
4. A *molar* pregnancy is one in which the gestational cells, especially from a malformed placenta, contain abnormal chromosomes inherited from the father. It is also known as a hydatidiform mole. Such a gestation may occur

with a normal single fetus as well as twins. Some cases bear malignant cells (choriocarcinoma) that were one of the first cancers to be arrested with chemotherapy. The mother's uterus is often large for gestational age. Grape-like tissue is sometimes passed vaginally and can alert the physician to this problem. Pregnancy-induced hypertension (preeclampsia), which typically appears in the third trimester, may exist in a molar pregnancy before 20 gestational weeks have passed. Human chorionic gonadotropin, the hormone commonly identified to diagnose any pregnancy, is unusually high in the case of a mole. It is essential to empty the uterus as soon as possible in these cases because of the risk of maternal hemorrhage, uterine rupture, or metastatic disease.[2]

5. *Twin-to-twin transfusion syndrome* (TTTS)—"When the time came for her to give birth, there were twin boys in her womb. The first to come out was red"—Book of Genesis.[19] As a result of sharing a single placenta, the blood supplies of identical monochorionic twin fetuses could become interconnected. Blood can be transferred disproportionately from one twin (the "donor") to the other (the "recipient") due to an imbalanced shared placental flow. This caused the donor twin (Jacob) to have a decreased blood volume (appears white). The blood volume of the recipient twin (Esau) was increased (appeared red), straining this fetus's heart which is working hard to pump the increased amount of blood, leading to heart failure. If untreated, the survival rate for TTTS twins is approximately 10%–15%. If the pregnancy appears to be at risk currently, the joined placental circulations can be disconnected by laser cauterization *in utero*, thereby increasing the chance of mutual survival markedly.[2]
6. *Heterotopic twin pregnancy*—Events within the reproductive tract are typically controlled by hormones. However, in some cases, physical obstructions or defects within the tract can alter the usual paths followed by the fertilized egg. Normally, the ovum is released from the gonad, is fertilized in the space between the ovary and the fimbriated end of the Fallopian tube, travels down the tube, and finally enters the uterine interior where implantation takes place. In humans, this overall process takes about 5 days. However, in some cases, impediments, especially from prior infections within the tube, can alter or completely impede the free passage of the fertilized egg. With partial injury, the first zygote may reach the uterine interior and the second is hindered in its passage through the Fallopian tube. Like any ectopic pregnancy, the second zygote can implant, grow, and cause rupture and hemorrhage from the tube. Ultrasound instrumentation is now sensitive enough to diagnose preoperatively the locus of each member of this unusual twin gestation. In the current state of medical progress, the tubal gestation cannot be saved and must be removed for the gravida's safety. In many cases, the intrauterine pregnancy can be retained and a term singleton delivery would be possible. Heterotopic twinning occurs in about one out of 5000 natural pregnancies. An extreme case is when both fetuses implant outside the uterus (e.g., a double tubal pregnancy). Though

rarely, ectopic gestations can also develop at other sites outside the uterus, such as the abdominal cavity or the ovary itself.[2,6]

7. *Boy-girl monozygotic twins*—True monozygotic twins are always of the same gender; dizygotic twins may be the same or opposite. One genetic error that sometimes appears in MZ twin embryos involves the sex (23rd) chromosome. If the Y chromosome in a human male conceptus (XY) fails to remain with the developing embryo, an XO baby results. It bears a total of 45 chromosomes instead of the usual 46. This is known as Turner Syndrome and leads to a miscarriage of that fetus 98% of the time. If it survives, the resulting newborn has the external appearance of a female but lacks functioning reproductive organs. If the Turner baby happens to be the womb mate to a normal XY fetus, the result will be one normal male and one defective female, whereas the babies had started out the pregnancy as a monozygotic, same sex (XY) pair.[6]

4.8 Maternal risks with a twin pregnancy

In general, twin pregnancies bear greater risks for the gravida than a singleton gestation.[4,9] The following are maternal complications that affect the gravida of multifetal gestations more often than with singletons. For example, preeclampsia is a condition most commonly found in pregnancy, whose frequency is higher with multifetal pregnancies than singletons:

1. Preeclampsia.
2. Gestational diabetes.
3. Iron-deficiency anemia.
4. Compromised breathing.
5. Blood vessel hematomas.
6. Worsening of preexisting medical problems.
7. Uterine rupture, especially after a previous c/section.
8. Excessive postpartum bleeding.
9. Pulmonary edema/dyspnea.
10. Cardiac decompensation.

Twins/triplets are more often delivered by cesarean section than singletons. This is major abdominal surgery with its attendant possible complications. In addition, having had such a surgical delivery at least once before often decreases the total number of further pregnancies a woman can tolerate without major risks to her health and well-being. Accordingly, it is often a last-minute decision if the babies can be delivered surgically or vaginally, depending on their final delivery positions, fetal heart rate status, and the personal preference and health status of the gravida. Much like the delivery of a singleton, it is foolhardy to expect that all potential unanticipated emergencies can be dealt with effectively in a setting other than a well-equipped and professionally staffed hospital.

4.9 Conclusions and prospectus

With the several issues described here, it is clear that there are many physiological differences between singleton and twin pregnancies, in origin, maintenance, and completion. Whereas they all start out with the union of sperm and egg(s), the subsequent events in twin gestations are often unlike those in singleton pregnancies, turning out to be more uniquely complicated. Medical or surgical manipulations to overcome reproduction problems can add to the list of potential difficulties affecting the final outcome. For example, the rate of twinning is higher in in vitro fertilization than natural reproductive efforts. In addition, recent advances with detection visualization and antenatal surgical correction of such congenital abnormalities can increase the survival and quality of life of anomalous babies (e.g., sacrococcygeal teratoma, congenital cystic lung malformations, and myelomeningocele). If both fetuses in a twin set are affected, the risk of antepartum intervention is doubled.[20,21]

Explorations into the means for overcoming prefertilization hurdles are not always successful, in spite of the application of the most efficacious modes known. Further research serves to increase the likelihood of reproductive success and decrease the problems that may arise from such natural gestations.[22–24] For example, one investigation examined why the twinning rates are higher in environmentally challenging places than elsewhere.[25] In such locations, women tend to breastfeed their offspring longer, which usually correlates with elevated serum IGF. This could also be the result of defensive responses to indure the harsher living conditions. The average life span there is shorter than in more economically advanced countries. In general, individuals with higher IGF levels tend to live shorter lives. Hence, the correlation with IGF and twinning is apparently corroborated.

Finally, several inherited, behavioral, and environmental characteristics which are apparently influential in women with a higher than usual capacity to produce twins were enumerated in this chapter. One additional trait, IQ, was reported to average 131 in women birthing twins/triplets, whereas the general American population displays a mean value of 98.[24] IGF1 is known to have several biological functions in humans. Consequentially, it could be postulated that elevated intelligence quotients and delivering twins are parallel products of the same growth factor.

Acknowledgments

The author wishes to thank Roberta Zuckerman for her helpful discussions about the presentation of this chapter, as well as Aviva Adler, librarian, for her cooperative assistance in retrieving relevant literature references.

References

1. Steinman G, Valderrama E. Mechanisms of twinning. III. Placentation, calcium reduction and modified compaction. *J Reprod Med*. 2001;46:995–1002.
2. Steinman, G. Womb Mates, Baffin Books Publishing, New York, NY, 2007, pp.23, 28–31,37,44, 53, 55, 67–80, 94, 105, 107.
3. Steinman G. Mechanisms of twinning. IX. Influence of prior lactation. *Annals Nutr Dis Therapy*. 2017;4:1047–1050.
4. Cunningham, FG, Leveno, KJ, Bloom, SL, et al., eds. Williams Obstetrics, McGraw-Hill, 25th ed., 2018. Pp. 124-145, 863-899.
5. Steinman G. Mechanisms of twinning. IV. Sex preference and lactation. *J Reprod Med*. 2001;46:1003–1007.
6. Steinman G. Mechanism of twinning. X. The male factor. *J Reprod Med*. 2008;563:681–684.
7. Steinman G. Mechanism of twinning. I. Effect of environmental diversity on genetic expression in monozygotic multifetal pregnancies. *J Reprod Med*. 2001;46(5):467–472.
8. Daley J. Test on astronaut and twin brother highlight spaceflight's human impact. *Sci Am*. 2019.
9. Steinman G. Mechanisms of twinning. VII. Effect of diet and heredity on the human twinning rate. *J Reprod Med*. 2006;51(5):405–410.
10. Steinman G. Mechanisms of twinning. VIII. Maternal height, insulinlike growth factor and twinning rate. *J Reprod Med*. 2006;51(9):694–698.
11. Steinman G. Can the chance of having be modified by diet? *Lancet North Am Ed*. 2006;367:1461–1462.
12. Mbarek H, Steinberg S, Nyholt DR, et al. Identification of common genetic variants influencing spontaneous dizygotic twinning and female fertility. *Amer J Human Genetics*. 2016;98:898–908.
13. Boomsma DI. Genetics of human DZ twinning. *Twin Res Human Genetics*. 2020;23:74–76.
14. Cyranoski D. Developmental biology: two by two. *Nature*. 2009;458:826–829.
15. Salomon, M. Kral, K. Explorer: Nazi Mystery: Twins from Brazil. National Geographic Television. 8/26/09.
16. Tabliani-Ribeiro A, Oliveira M, Sassi AK, et al. Twin town in south Brazil: A Nazi's experiment or a genetic founder effect? *PLoS One*. 2011;6 020328.
17. Hoekstra C, Zhao ZZ, Lambalk DB, et al. Dizygotic twinning. *Human Reprod Update*. 2008;14:37–47.
18. Steinman G. Mechanism of twinning. V. Conjoined twins, stem cells and the calcium model. *J Reprod Med*. 2002;47:313–321.
19. The old testament. Book of genesis. 25:26.
20. Kabagambe SK, Jensen GW, Chen YJ, et al. Fetal surgery for myelomeningocele: a systematic review and meta-analysis of outcomes in fetoscopic versus open repair. *Fetal Diagn Ther*. 2018;43:161–174.
21. Maselli KM, Badillo A. Advances in fetal surgery. *Annals Transl Med*. 2016;4(20):394–413.
22. Steinman G. Mechanisms of twinning. II. Laterality and intercellular bonding in monozygotic twinning. *J Reprod Med*. 2001;46(5):473–479.
23. Steinman G. Mechanisms of twinning. VI. Genetics and the etiology of monzygotic twinning in *in vitro* fertilization. *J Reprod Med*. 2003;48(8):583–590.
24. Steinman G. Mechanisms of twinning. XI. Do mothers of twins have higher IQs? The IGF connection. *J Reprod Med*. 2020;64(2):147–150.
25. Steinman G. Why the twinning rate is higher in Africa than elsewhere. *J Reprod Med*. 2009;54(10):609–616.

CHAPTER

Management and outcome of twin pregnancies

5

Gian Carlo Di Renzo[a], Valentina Tosto[a,c], Irene Giardina[a], Valentina Tsibizova[b]

[a]*Department of Obstetrics and Gynecology, Centre of Perinatal and Reproductive, Medicine, University of Perugia, Italy*

[b]*Almazov State Perinatal Research Centre, St Petersburg, Russia*

[c]*Department of Obstetrics and Gynecology, Giannina Gaslini Children's Hospital, Genova, Italy*

5.1 Introduction

The management of multiple pregnancies during gestation, labor and delivery, which has long been a key skill for the obstetrician, is never more important than in the current era.

Multiple pregnancies are increasing globally, in keeping with lyfestle changes, an increase in maternal age, and in the use of assisted reproductive techniques (ART). However, multiple pregnancies contribute disproportionately to prematurity and perinatal mortality and morbidity.

Whereas in the past multiple pregnancy often went undiagnosed until delivery, today improved antenatal management, including ultrasound and monitoring, allow a comprehensive and individualized plan for the mode and timing of each pregnancy control and delivery. Twin pregnancies are at increased risk for preterm birth, intrauterine growth restriction (IUGR), and other conditions such as hypertensive disorders, gestational diabetes. Monochorionic twins have additional risks for death and morbidity, primarily because of the twin-to-twin transfusion syndrome (TTTS) and congenital abnormalities.

In order to develop excellence in the antenatal and perinatal management of multiple pregnancies, it is important to consider several key points: chorionicity, fetus growth, adequate monitoring of pregnancy, fetus presentation, planned timing of delivery, planned place of birth offering the most appropriate intrapartum maternal and fetal monitoring.

5.2 Antenatal care

5.2.1 Diagnosis and chorionicity

The ultrasound evaluation of the first trimester in a twin pregnancy has the purpose of determining the correct gestational period, chorionicity, and amnionicity. For

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00028-3

TABLE 5.1 Recommended weight gain in twin pregnancies based on the pregravidic BMI.[2]

Pregravidic BMI	Weight gain
Normal weight 18.5–24.9 kg/m^2	17–25 Kg
Overweight 25–29.9 kg/m^2	14–23 Kg
Obesity ≥30 Kg/m^2	11–19 Kg

BMI, body mass index.

dating, the major fetal crown-rump length is taken into account, using the same biometric curves as the single pregnancy; in the case of a discrepancy equal to or greater than 7 days compared to the time of amenorrhea, even in a twin, the pregnancy must be renewed. In pregnancy obtained by ART, the gestational period is always defined from the date of the oocyte collection. To define chorionicity and amnionicity, an ultrasound evaluation within 14 + 0 weeks of gestation allows to obtain a sensitivity and specificity, respectively, of 90% and 99%.[1] In the case of dichorial pregnancy it will be possible to detect two placentas and two amniotic bags, each delimited by its own chorion and its own amniotic membrane. If the placentas are not separate but contiguous, it is necessary to look for the "lambda sign" as the chorions at the meeting point of the two placentas rise up forming a triangular space and a thick septum between the two gestational chambers, with a typical lambda appearance. In the event that the amniotic membranes end up on the placental surface forming a right angle, this sign, called the "T," must induce the operator to diagnose monochorionic pregnancy.[1] If it is not possible to diagnose chorionicity, pregnancy should be managed as monochorionic. The lack of visualization of amniotic membranes between the twins must raise the suspicion of monoamniotic monochorionic pregnancy. All these features are essential to organize a specific management of the pregnancy.

5.2.2 Nutritional advice

The adoption and/or adjustment of a healthy nutritional program for multiple pregnancy seems effective in reducing adverse outcomes. In a twin pregnancy, where the needs are greater, nutritional advice on the quantity and quality of the macro and micronutrients is essential and should be included in the care program. The ideal situation should be a preconceptional counseling on lifestyle habits, weight and diet patterns, physical activity in order to promote adequate behaviors and prepare women for a pregnancy as best as possible. As with single pregnancy, the recommended weight gain is based on the pregravidic body mass index—BMI (Table 5.1).[2]

5.2.3 Chromosomal screening: new challenges?

Multifetal pregnancies have an increased risk of morphological abnormalities and aneuploidies. The counseling relating to screening tests and diagnostic tests for aneuploidies is considerably complex due to some peculiar factors of twinning such as chorionicity, the operator's technical ability, the feasibility of taking the sample, spontaneous or ART pregnancy, and the possible implications in the event of a chromosomal abnormality affecting only one of the twins.[3] The combined screening of the first trimester (nuchal translucency + PAPP-A + beta-hCG) has in pregnancy twin a lower performance than a single pregnancy, but it is still effective with a detection rate of approximately 86% and 5% of false positives.[4] One of the main reasons for the reduced performance of the combined test in twin pregnancies is the difficult interpretation of the biochemical component, as each twin contributes in variable part to the concentration of the analytes without the possibility of determining their coindividual concentration. Further evidence are needed, but performance of these tests seems to be similar to singletons. Currently, we are witnessing the spread of "cell free" DNA analysis (cfDNA) performed on maternal blood to estimate the risk of aneuploidies, although this is a promising field, further studies are needed.[5,6] Le Conte et al showed that in twin pregnancy without fetal ultrasound abnormality, cfDNA screening for trisomies 21, 18, and 13 had a high success rate and good performance. Therefore, in routine practice, cfDNA analysis could be considered as a first- or second-line screening test.[7] Moreover, experts are exploring the potential role of noninvasive prenatal paternity testing by maternal DNA sequencing (NIPPT).[8] Invasive procedures for diagnosis of aneuploydies is available. The risk of pregnancy loss following invasive testing—chorionic villus sampling or amniocentesis—appears to be greater in twin than in singleton pregnancies (2% following chorionic villus sampling and 1.5%–2% following amniocentesis.[8,9]

5.2.4 Monitoring: timing and frequency

Experts frequently debate on how often to monitor twin pregnancies: how many visits? How many ultrasounds? In light of what has been reported by several authoritative health organizations, the following seems to be regrettable with adequate flexibility to modify as needed.

Clinical checks in uncomplicated dichorionic-diamniotic pregnancies will be scheduled monthly with an assessment of the pregnant woman's weight and blood pressure. Ultrasound assessments should follow the scheme proposed in Table 5.2 with an increase in checks in case complications arise. Having an increased risk of morbidity and fetal mortality, monochorionic-diamniotic pregnancies should be referred to referral centers. In the absence of complications also for monochorionic-diamniotics, the clinical evaluation will be monthly and the ultrasound evaluation will be more tightened (about bi-weekly) in order to early detect any transfusion between twins.[1] Monochorionic-monoamniotic pregnancy is a rare occurrence, about 1% of monozygotic pregnancies, and is associated with a high risk of fetal and perinatal mortality. The optimal methods and frequency intervals of the monitoring that will be carried out in reference centers with specific experience for these pregnancies and personalized.[9]

TABLE 5.2 Frequency of ultrasound evaluation of uncomplicated dichorionic-diamniotic pregnancy.

11–14 weeks	• Diagnosis • Labeling • Chorionicity-amnionicity • Chromosomal screening
20–22 weeks	• Fetal morfology and biometry • Amniotic fluid • Cervicometry
24–26 w, 28–30 w, 32–34 w, 36–37 w	• Fetal biometry • Amniotic fluid • Doppler flussimetry

5.2.5 Laboratory investigations

The laboratory tests are almost superimposable to those of a single pregnancy. The performance of the blood count at 20–24 weeks is indicated to identify patients who need folic acid and iron supplementation, due to the higher incidence of anemia in multiple pregnancies. The examination should be repeated at 28 and 34 weeks.[9] Although twinning represents a risk factor for gestational diabetes, currently the indications for undergoing oral load with 75 g of glucose are the same as for single pregnancy.[10]

5.3 Antenatal complications

5.3.1 Chorionicity

Chorionicity plays an important role in determining the type and severity of twin pregnancy complications, based on different placenta anatomy and physiology, and thus pathological paths. These discordances are related to unequal placental mass function and distribution (dichorionic-diamniotic pregnancies) and to vascular anastomoses (monochorionic twins). Dichorionic-diamniotic twin pregnancies are mainly complicated by fetal IUGR and maternal diseases such as hypertensive disorders and gestational diabetes (increased diabetogenic placental hormones). On the other hand, monochorionic twins are subjected to specific complications which originate in either imbalance or abnormality of the single placenta serving two twins. Almost all monochorionic twins have inter-twin vascular anastomoses: arterio-arterial (A-A) anastomoses and veno-venous anastomoses (V-V) are superficial connections, traveling across the placenta surface, without interruption between the two cord inserction; arterio-venous (A-V) anastomoses have a deep course (Fig. 5.1). There are marked variations in the number, size and distribution of these anastomoses, thus explaining differences in developing specific complicantions. This unequal placental sharing can cause complications including TTTS, twin

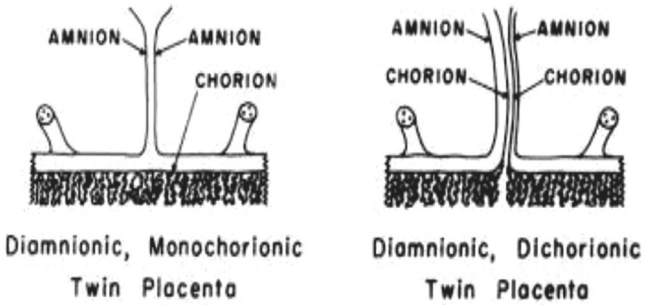


FIG. 5.1 Difference in dichorionic and monochorionic placentation.

TABLE 5.3 Main antenatal twin pregnancy complications.

Dichorionic-diamniotc	Monochorionic-diamniotic	Monochoriotic-monoamniotic
• Intrauterine growth restriction (discordant growth) • Congenital abnormalities • Hypertensive disorders • Gestational diabetes (increased diabetogenic placental hormones)	• Congenital abnormality • Intrauterine growth restriction (discordant growth) • Twin-to-twin transfusion syndrome (TTTS) • TAPS sequence • TRAP sequence • Cerebral palsy	• As monochorionic-diamniotic twins + umbilical cord entanglement, compression

TTTS, twin-to-twin transfusion syndrome; TAPS, anemia-polycythemia sequence; TRAP, inverted arterial perfusion sequence.

anemia-polycythemia sequence (TAPS), selective IUGR or twin reversed arterial perfusion sequence (TRAP). Monochorionicity also makes the management of these specific complications as well as that of a severe malformation in one twin hazardous since the spontaneous death of one twin exposes the co-twin to a risk of exsanguination into the dead twin and its placenta. The keystone of their management comes down to either surgical destruction of the inter-twin anastomoses on the chorionic plate when aiming at dual survival or selective and permanent occlusion of the cord of a severely affected twin aiming at protecting the normal co-twin. This can be best achieved by fetoscopic selective laser coagulation and bipolar forceps cord coagulation, respectively (Table 5.3).

5.3.2 Fetal growth restriction (FGR)

The ultrasound estimate of the fetal weight (EFW) in the twin pregnancy is less accurate than the single pregnancy. One of the reasons should be sought in the use of the same fetal growth curves among the de types of pregnancies. However, it is known that, especially in the third trimester, twins tend to reduce their growth

rate and this reduction is even more evident in monochorionic pregnancies. For this reason, specific growth curves have recently been proposed for multiple pregnancies customized to the obstetric and parental characteristics.[11,12]

FGR is defined as the finding of a fetus with an estimated weight <10th percentile, with an EFW discordance between the twins >25%. When the discrepancy of EFW exceeds 20%, pregnancy should already be considered as having an increased risk of negative outcome**.** After detection of a fetus with growth restriction, it is important to look for the cause with an accurate morphological examination, the search for viral infections or, if it is considered appropriate, an amniocentesis to identify any chromosomal aberrations that justify the delay in growth. The discrepancy of EFW should be calculated and documented at each ultrasound starting from 20 weeks and if the discrepancy is ≥25%, the patient should be referred to a third level center.[3] The surveillance of twin pregnancy pregnancies with FGR does not differ from that of single pregnancy, providing for monitoring of the fetal biophysical profile and hemodynamics in the umbilical artery, the middle cerebral artery and in the venous duct every 2 weeks, intensifying controls based on the extent of the delay. The selective fetal growth restriction could represent a problem for management, because of the potential need to anticipate birth for the restricted fetus, while the other fetus is healthy. In these cases, strict surveillance is essential and healthcares have to balance risks and benefits of their choices.

Childbirth, if fetal conditions permit, should not be anticipated before the 32–34 weeks of gestation.

5.4 Specific monochorionic pregnancy complications

5.4.1 Fetal-fetal transfusion syndrome (TTTS)

All monochorionic pregnancies have vascular anastomoses that connect the two fetal circulations and that can be responsible for TTTS, a hemodynamic imbalance that led to adverse pregnancy outcomes for both fetuses. TTTS affects 10-15% of monochorionic pregnancies and is associated with high perinatal mortality and morbidity.

The anastomoses in a TTTS placenta are different from the uncomplicated monochorionic placentas. There are three types of anastomoses: artery-to-artery (AA), vein-to-vein (VV), and artery-to-vein (AV) anastomoses. AA and VV anastomoses form direct communications on the surface of the chorionic plate and are bidirectional. AV anastomoses are located deep in the placental tissue and are obligate unidirectional. The AV anastomosis itself occurs at a capillary level deep in the shared placental lobule. AV anastomoses always direct flow from one twin to the other, while AA and VV anastomoses allow flow in both directions, depending on intertwin pressure gradients. An AA anastomosis can function as an AV anastomosis from twin 1 to 2 as well as from twin 2 to 1 and is in fact a flexible AV anastomosis. The bidirectional AA anastomosis can thus compensate for the imbalanced flow through the unidirectional AV anastomoses (Fig. 5.2).

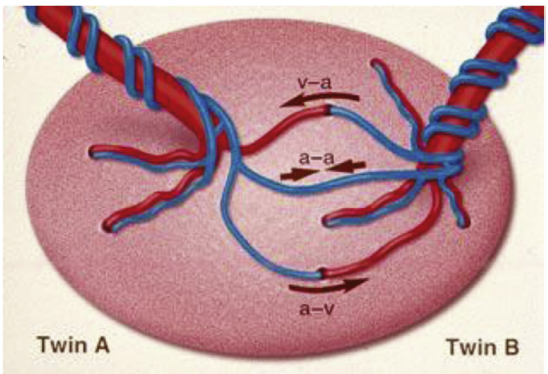


FIG. 5.2 Monochorionic twins anastomoses.

If left untreated, it leads to fetal death in 90% of cases with morbidity rates, in case of survival, reaching 50%. Diagnosis and staging follow Quintero's criteria (Table 5.4) whose rapid analysis allows us to see how the EFW discordance between twins is not a diagnostic parameter. Despite its widespread diffusion, this staging does not allow an accurate prediction of the neonatal outcome or of the chronological evolution of the pathology since the five stages are not always consecutively present in the TTTS. In monoamniotic monochorionic pregnancy, the ultrasound features of TTTS are the polyhydramnios in the common sac and the different bladder sizes in the two fetuses.[3] A timely diagnosis allows for the possible fetoscopic treatment by laser ablation of the anastomosis with the survival of both twins in 60%–70% of cases and at least one of the twins in 80%–90% of cases, ablation is indicated in all stages but generally stage I is managed conservatively with close ultrasound surveillance. If laser ablation is not possible a therapeutic option is a multiple amnioreduction starting from 26 weeks.[3]

TABLE 5.4 Quintero's classification system.[3,8]

Stage	Classification
I	Polyhydramnios-oligohydramnios sequence
	- receiving twin with DVP> 8 cm before 20 weeks
	- receiving twin with DVP> 10 cm after 20 weeks
	- donor twin with DVP <2 cm
II	The donor twin's bladder is not visible on ultrasound
III	Hemodynamic abnormalities in one or both twins
	- diastolic flow in art. umbilical absent or inverted
	- wave a in the inverted venous duct
	- pulsatile flow in the umbilical vein
IV	Hydrops of one or both twins
V	Death of one or both twins

DVP, deepest vertical pocket.

5.4.2 Anemia-polycythemia sequence (TAPS)

TAPS is due to the presence of small arteriovenous anastomoses (<1 mm) that allow a slow transfusion between the twins causing a discrepancy between the hemoglobin levels at birth, the donor twin has a picture of chronic anemia while the recipient is polycythemic. The incidence of spontaneous TAPS in monochorionic-diamniotic pregnancies is 5%, iatrogenic forms appear in 13% of laser ablation for TTTS.[3] The diagnosis involves monitoring the systolic peak speed in the middle cerebral artery (MCA-PVS), a value >1.5 MoM indicates anemia while a value <1 MoM indicates polycythemia. Other ultrasound signs are a hyperechoic and thick placenta in the donor twin, anechoic and thin in the recipient which also has a liver with a "starry sky" appearance due to the hyperechogenicity of the vessels of the portal system.[3] The evolution of the clinical picture, in severe forms, sees the donor's cardiac impairment, resulting in dropsy and fetal death. There is not much evidence regarding the outcome and case management of TAPS, therefore treatment must be individualized on a case-by-case basis, opting for vigilant waiting, early delivery, laser ablation or an anemic twin transfusion in utero. Fig. 5.3 shows differences in TTTS and TAPS pathophysiology.

5.4.3 Inverted arterial perfusion sequence (TRAP sequence)

The TRAP sequence is a rare complication where an acardial twin mass is perfused by the "pump" twin which is apparently normal, perfusion occurs retrograde through arteriovenous anastomoses at the point of common cord insertion. The proposed pathogenesis is the association of paired AA and VV anastomoses through the placenta combined with delayed cardiac function of one of the embryos early in pregnancy. The picture evolves into progressive high-output heart failure in the twin "pump" and its death in 30% of cases treated conservatively. Presently the most commonly used technique for TRAP sequence is intrauterine radiofrequency ablation (RFA) of the cord of the recipient or cord ligation by fetoscopic procedure with survival rates of the "pump" twin of >80%. The management of these pregnancies should be entrusted to third-level centers.[3]

5.4.4 Monoamniotic twins and cord entanglement

Monoamniotic twins are rare, they occur in about 1 in 8000 pregnancies and, as such, constitute 5% of monochorionic pregnancies and are at extremely high risk of pregnancy complications and fetal loss. Female fetuses predominate, only 25%–35% being male pairs. These pregnancies can be diagnosed reliably by ultrasound in most cases. Umbilical cord entanglement is present in almost all monoamniotic twins when it is systematically evaluated by ultrasound and color Doppler. Sulindac (a COX2 inhibitor) has been suggested as a means of medical amnioreduction (decreasing urine output from fetuses) to diminish fetal mobility and thereby cord entanglement, but acute intertwin transfusion is most likely an important cofactor and intrauterine demise can occur despite sulindac administration. Also, due to potential

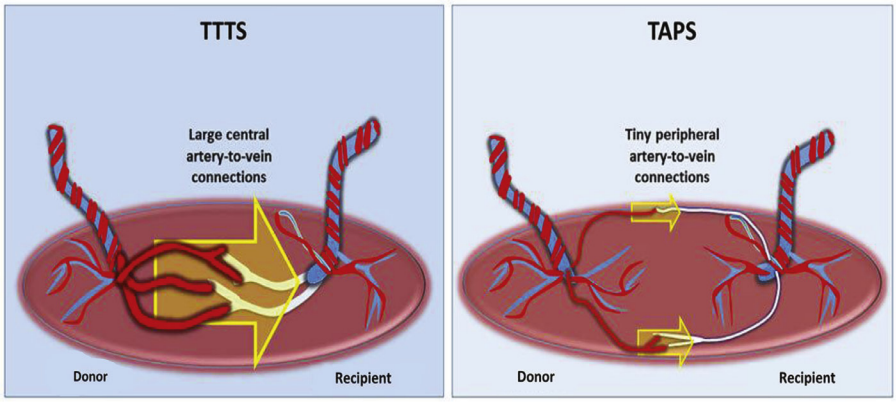


FIG. 5.3 Scheme of pathophysiology of TTTS and TAPS in monochorionic twins.

side-effects and no definite proof of benefit, the use of sulindac has not gained widespread popularity. Elective preterm birth (<33 weeks) and careful surveillance of monoamniotic twins may improve survival rates.

5.4.5 Cerebral palsy risk

Adverse neurodevelopmental outcomes are associated with twin pregnancies, considering the high risk of prematurity. In addition, several studies reported a higher risk of cerebral palsy (CP) in complicated monochorionic twin pregnancies and in vanishing twin syndrome (VTS). The aetiology of spastic CP, in the majority of cases, is not known but the general consensus is that cerebral impairment occurs prepartum. In monochorionic twin pregnancies, death of one twin late in gestation is recognized as being an important risk factor for the surviving co-twin to have CP. Ischemic processes and/or thrombotic causes were proposed as responsible for this adverse outcome. Nowadays, progresses in manage complicated twin pregnancies offer improved chances to reduce this complication (i.e., fetal neuroprophylaxis, laser therapy or vascular anastomoses ligature).

The VTS is a not unusual complication of twin pregnancy. It occurs mainly in the first trimester, less frequently in the second and third trimester. The early VTS is usually associated with favorable pregnancy outcome. On the opposite, the second or third trimester VTS is related to higher risks for pregnancy due to thromboplastin embolization events and great risk for surviving twin. At this regard, studied described a significant correlation between VTS and CP since the late 1990s.[13] Later, Pinborg et al observed that one in 10 IVF singletons originates from a twin gestation. Spontaneous reductions that occur at >8 weeks gestation are one of the causes for the higher risk of adverse obstetric outcome in IVF singletons; OR for CP resulted 1.9 (95% CI 0.7–5.2). Furthermore, we observed a correlation between the onset of spontaneous reduction, i.e. the later in pregnancy the higher the risk of neurological sequelae ($r = -0.09$; $P = 0.02$).[14]

5.4.6 Antenatal preventive interventions

Preterm birth, spontaneous or iatrogenic, is one of the main complications of twin pregnancy. Second trimester (18–24 weeks) cervical length screening by transvaginal ultrasound scan should be offered. In asymptomatic women, a cervical length threshold of 20 mm or less should be used.[9,15] In symptomatic women, cervical length screening has a poor predictive value for preterm birth in twins. However, even if women at increased risk of preterm birth with twins are accurately identified, there is no effective preventive strategy (this includes progesterone, bed rest, Arabin pessary, and oral tocolytics).[9,16,17] Recently, Merced *et al.* presented the results of a randomized controlled trial designed to ascertain whether cervical pessary could be useful in preventing PTB in twin pregnancies: significant differences were observed in PT rate before 34 weeks between the pessary group (16.4%) versus the control group (32.3%) after a threatened preterm labor episode.[18] Anyway, further investigations are needed in this field. Routine administration of corticosteroids is not recommended, which should be reserved for symptomatic cases and with the real risk of imminent childbirth· Steroids should be given if delivery is expected before 34 weeks, or if cesarean section (CS) is planned before 37 weeks' gestation. Repeat courses of steroids in case of threatened preterm birth should be based on individual circumstances and should no longer be routine practice.[9]

In the late 1990s, studies demonstrated the use of magnesium sulfate for the primary prevention of CP in preterm infants. However, since CP is a result of multiple interacting risk factors rather than of a single cause, it is unlikely that antenatal magnesium sulfate administration alone can prevent all cases of this illness in preterm infants. WHO recommended for both singleton and multiple pregnancies the use of magnesium sulfate for fetal protection from neurological complications in case of imminent (within the next 24 h) preterm birth <32 weeks of gestation. Several regimens were proposed and one of the most used is 4 g iv over 20 minutes and then 1 g/h for 8 to 24 h.[19] For the prevention of hypertensive disorders in pregnancy, it is possible to advise the pregnant woman to take 150 mg of aspirin starting from 11 weeks. The risk factors to be identified in this case are nulliparity, maternal age over 40 years, familiarity with preeclampsia, an interval with the previous pregnancy of 10 years and a BMI > 35.[9,20] The Fetal Medicine Foundation (FMF) algorithm identifies high-risk population for pre-eclampsia (PE) in the first trimester and suggests aspirin prophylaxis. It combines maternal history, mean arterial pressure, sierological marker (placental growth factor -PLGF) and ultrasound marker (uterine artery PI) to define a level of risk.[21] Home bed rest is not evaluated in randomized clinical trials, and hospital bed rest results not helpful.

5.5 Peripartum care

Preventive pianification of timing, place and mode of delivery are crucials aspects in twin pregnancies in order to achieve the best outcomes for mothers and babies.

5.5.1 Timing of birth

Women who come into the multiple pregnancy clinic commonly ask when their babies will be delivered.

Once informed them of the very significant risk of preterm delivery in all multiple pregnancies, clinicians can then move on to discussing the optimal timing of delivery. This timing is determined by the balance of competing risks to the fetus: the increasing risk of intrauterine demise or neurological injury as the pregnancy progresses is weighed against the risk of prematurity associated with mortality and morbidity for the neonate. For twin pregnancies, the balance of risks varies with chorionicity. For dichorionic-diamniotic twin pregnancies, the risks are balanced at 37 weeks with an increase in perinatal mortality for pregnancies continuing beyond 38 weeks. In monochorionic pregnancies, the risk of intrauterine mortality seems to exceed the risk of neonatal mortality after 36 weeks.[22] National guidelines recommend delivery between 37 and 38 completed weeks of gestation and monochorionic-diamniotic twin pregnancies from 36 to 37 weeks.[23] Monochorionic-monoamniotic pregnancies are subject to additional severe risks compared to diamniotic pregnancies, with around 18% of fetuses suffering intrauterine demise. In these pregnancies, CS should be planned between 32–34 weeks of gestation.[24]

The delayed delivery (asynchronous delivery) is possible in specific situations, especially in multiples. Our experience (data not published) between 2000 and 2008 registered 18 cases of delayed-interval delivery in multiple pregnancies: 14 women with a twin pregnancy and 4 women with triplets. All women delivered their first twin between 16 and 22 weeks and then were treated according to internal protocol. Outcomes were: four first newborns survived despite the severe prematurity; average of the delivery of the remaining fetus(es) was postponed of about 26 days (35% underwent CS); the survival rate of the 22 babies born after interval was 65%; neonatal follow up at 1 year showed uneventful development in 18 babies; 5 babies suffered from handicaps due to prematurity/immaturity despite the postponement (CP, retinopathy, bronchopulmonar dysplasia, motor retardation).

5.5.2 Mode of delivery and induction of labor

The mode of delivery in twin pregnancy is largely discussed, due to several attitudes and clinical choices often taken by obstetricians. Topics of discussion were/are prophylactic CS, lack of confidence to assist vaginal delivery and to perform internal manouvers, fear of medical-legal problems.[25] Anyway, for uncomplicated dichorionic-diamniotic twins, if the leading twin is cephalic it is reasonable to aim for vaginal delivery. If twin one is non-cephalic, CS is probably the safer option (Fig. 5.4). In many countries, monochorionic-diamniotic twins will commonly be delivered by CS; however, when uncomplicated, the option of vaginal birth could be considered also in monochorionic-diamniotic pregnancies. There is a possible risk of acute twin-to-twin transfusion syndrome occurring during labor, although the real risk of this complication appears to be small.[9,26] Planned CS for monochorionic-monoamniotic pregnancies is required for too high risks of intrapartum complications. Table 5.5

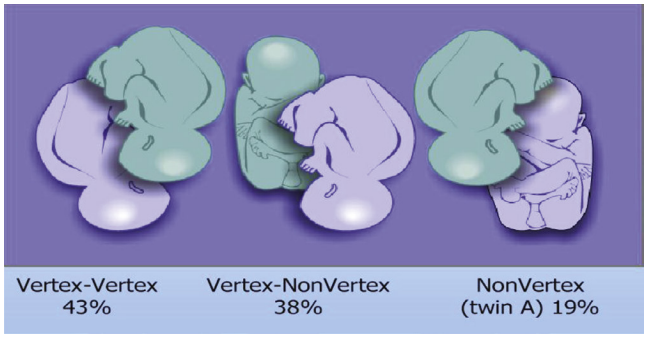


FIG. 5.4 Twin presentations at term.

TABLE 5.5 Mode of delivery for twin pregnancy according to fetuses presentation and chorionicity.

Presentation	Mode of delivery
VERTEX A/VERTEX B	VAGINAL DELIVERY
VERTEX A/NON VERTEX B controversial…	1. VAGINAL DELIVERY OF AMANAGEMENT OF B: • BREECH EXTRACTION • EXTERNAL CEPHALIC VERSION (successful–vaginal; unsuccessful–cesarean section) 2. CESAREAN SECTION
NON VERTEX A	CESAREAN SECTION
Chorionicity	Mode of delivery
DICHORIONIC-DIAMNIOTIC	VAGINAL DELIVERY is a good option in absence of other maternal/fetuses' contraindications
MONOCHORIONIC-DIAMNIOTIC controversial…	1. VAGINAL DELIVERY is possibile: consider higher risk of acute TTTS in labor; consider eventual active management to reduce interdelivery interval; required high experienced and trained team 2. CESAREAN SECTION is an option; many settings prefer prophylactic CS
MONOCHORIONIC-MONOAMNIOTIC	PLANNED CESAREAN SECTION is the safest option

A: first twin; B: second twin; CS, cesarean section.

resumes the mode of delivery suggested for twin pregnancies based on chorionicity and fetal presentation. Continuous fetal heart monitoring (cardiotocography) is recommended during labor. Intrapartum ultrasound should be a useful tool to guide the obstetrics-midwife. Vaginal delivery of the second twin usually occurs within 30 minutes of the first twin. Blood products should be always available and provision of intravenous fluid admistration is useful as supporting therapy. Active management of the delivery of the second twin is recommended to avoid a prolonged interval.[9,26]

About the induction of labor, studies reported different opinions. Several papers described a relatively good and safe profile of this practice to improve chances for vaginal delivery. For example, Zafman et al. observed that labor induction in twin gestations improved maternal outcomes and had similar neonatal outcomes compared with planned CS.[27] Inversely, Grossman et al observed that labor induction in twins was associated with increased maternal morbidity compared to planned CS. The increase in adverse maternal outcomes was due to those who underwent an induction of labor and ultimately required CS.[28]

5.6 Peri-conceptional period: a "key window" of intervention?

Nowadays, we know a lot about twin pregnancies and medical progresses led to great improvements in management and outcomes, both for the mother and the babies.

A significant number of multiple pregnancies derived from assisted reproductive technologies and ART policies vary from country to country, leading to a great eterogeneity in clinical cases, managements and outcomes.

The peri-conceptional period is emerging as a critical moment for the possible "prevention" of multiple pregnancies and therefore of the maternal-fetus-neonatal risks. For example, counseling and the correct selection of the population affected by infertility is essential for the reduction of multifetal pregnancy rates. A scrupulous selection of women based on their specific infertility problem (anovulatory, unexplained infertility, polycistosis ovary syndorme—PCOS), age, bodyweight should allow clinicians to make reasoned choices regarding the pharmacological stimulation protocols and the ART procedure. In the obese patient and, with less evidence, in the overweight patient, the reduction in body weight correlates directly with the pregnancy and live birth rates.[29] Therefore, the couple must be adequately informed of the path to follow, of the highest risk of obtaining a multifetal pregnancy by ART and which in this case would require "ad hoc" management. In women with good reproductive potential, an in vitro fertilization cycle with transfer of two embryos, compared to the transfer of a single embryo, increases the probability that twin pregnancy occurs, but at the same time increases the risk of preterm birth, increasing thus the probability of unfavorable neonatal outcome. The number of embryos to be transferred should be defined on the basis of successful predictive models. In women with a good prognosis, the only strategy to minimize the risk of multifetal pregnancy is the use of the transfer of a single

TABLE 5.6 Practice advice for a "good" twin pregnancy management.

Practice advice
Antenatal care
• dating and definiton of chorionicity (dichorionic versus monochorionic)
• accurate counselling about increased risks for:
- miscarriage
- aneuploidy (include information on I trimester screening—combined test and/or non-invasive prenatal test–and invasive diagnosis techniques)
- fetal structural anomalies
- meternal anemia
- preterm birth
- fetal growth disorders or other specific twin pregnancy abnormalities
- thromboembolism risk
- intrapartum complications (cesarean section, post-partum hemorrhage, maternal transfusion)
• refer to perinatal centre of adequate level in management of twin pregnancy
• monitoring: timing, frequency of evaluations established on several issues:
- chorionicity (dichorionic-diamniotic versus monochorionic-diamniotic versus monochorionic-monoamniotic)
- uncomplicated versus complicated
• prophylactic interventions:
- preterm birth prevention–scarce evidence for progesterone, cervical cerclage, pessary, tocolytic drugs
- if short cervix (<25mm) detected by US scan, micronized vaginal progesterone decreases risk of preterm birth and ameliorates neonatal mortality and morbidity
- corticosteroids administration - no as a routine tool; administered if delivery is expected <34 weeks, <37 weeks for planned CS
- magnesium sulphate for cerebral palsy prevention (<32 weeks)
• Specialized and individualized management based on case-by-case needs
Intrapartum care
Induction of labor/pregnancy interruption if pregnancy reaches 38 weeks in dichorionic diamniotic; 36 weeks in monochorionic diamniotic; 33 weeks in monochorionic monoamniotic
• adequate setting and experienced healthcare team for assisting twin delivery (skilled obstetrics and midwives, neonatologist, neonatal intensive care unit, anesthesist)
• preinduction of labor with baloon/dilapan/amniorexis is preferable, when indicated
• timing: related to pregnancy course (fetal and/or maternal complications) and chorionicity
• mode of delivery:
- vaginal delivery a good option (first twin cephalic–both dichorionic and monoamniotic-diamniotic). Continuos fetal surveillance. Active management of second delivery if needed. Immediate CS accessibility
- CS if both twins or first twin not cephalic
- CS if monochoriotic-monoamniotic
- active management of third stage (high risk for post-partum hemorrhage)
• group B Streptococcus (GBS) prophylaxis if preterm
• oxytocin may be used
• blood products available
• intrapartum ultrasound
• post-partum hemorrhage prophylaxis

embryo in fresh cycles, although this procedure is associated with lower rates of live births compared to the transfer of two embryos.[29] Embryo transfer policies vary from country to country. Even in this area, however, there are significant legislative differences, which regulate this practice. ART with two embryos transfer is a standard line-guide in many infertility centers. Embryo tranfer trends changed worldwide in the last few years and multiple pregnancies (three or > three fetuses) decreased. Multifetal fetal is considered a iatrogenic complication in ART and in some countries embryo reduction is available. It is the procedure more used to eliminate supernumerary embryos by potassium chloride or salt solution injection in fetal heart, under ultrasonography guide. However, where spontaneous multifetal or ART pregnancy has occurred, the prevention of possible complications is based on the clinical-laboratory-instrumental management following validated national and international guidelines.

5.7 Conclusion

Nowadays, twin pregnancies are a more frequent reality in the obstetric daily practice and require a great attention in the management, being associated with higher risks for maternal-fetal-neonatal complications compared to singleton pregnancy. Monochorionic twin pregnancies need to highest attention, should be referred to specialized clinics/tertiary level centers, and receive a multidisciplinary management during pregnancy and at delivery.

However, specialized and individualized antenatal and intrapartum care are at the basis of a good management in order to achieve the best outcomes (Table 5.6).

References

1. ISUOG Practice Guidelines: role of ultrasound in twin pregnancy. *Ultrasound Obstet. Gynecol*. 2016;47:247–263.
2. Luke B. Nutrition for multiples. *Clin Obstet Gynecol*. 2015;58:585–610.
3. Gestione della Gravidanza Multipla. Raccomandazioni SIGO, AOGOI, AGUI, Fondazione Confalonieri Ragonese. 2016. www.sigo.it.
4. Prats P, Rodrıguez I, Comas C, et al. Systematic review of screening for trisomy 21 in twin pregnancies in first trimester combining nuchal translucency and biochemical markers: a meta-analysis. *Prenat. Diagn*. 2014;34:1077–1083.
5. Motevasselian M, Saleh Gargari S, Younesi S, et al. Non-invasive prenatal test to screen common trisomies in twin pregnancies. *Mol Cytogenet*. 2020;13:5.
6. Yin Y, Zhu H, Qian Y, et al. Noninvasive prenatal screening for twin pregnancy: an analysis of 2057 cases. *Zhejiang Da Xue Xue Bao Yi Xue Ban*. 2019;48:403–408.
7. Le Conte G, Letourneau A, Jani J, et al. Cell-free fetal DNA analysis in maternal plasma as screening test for trisomies 21, 18 and 13 in twin pregnancy. *Ultrasound Obstet Gynecol*. 2018;52:318–324.
8. Xie Y, Qu N, Lin S, et al. Noninvasive prenatal paternity testing by maternal plasma DNA sequencing in twin pregnancies. *Electrophoresisi*. 2020. http://doi.org/10.1002/elps.202000036 [Epub ahead of print].

9. Good clinical practice advice: Management of twin pregnancy. FIGO Working Group on good clinical practice in maternal fetal medicine. *Int J Obstet Gynecol*. 2019. doi:10.11002/ijgo.12742.
10. Gallot D, Vélémir L, Delabaere A, et al. Which invasive diagnostic procedure should we use for twin pregnancies: chorionic villous sampling or amniocentesis? *J Gynecol Obstet Biol Reprod*. 2009;38:S39–S44.
11. NICE multiple pregnancy: evidence update. 2013.
12. Ghi T, Prefumo F, Fichera A, et al. Development of customized fetal growth charts in twins. *Am J Obstet Gynecol*. 2017;216:514e1–514e17.
13. Pharoah PO, Cooke RW. A hypothesis for the aetiology of spastic cerebral palsy—the vanishing twin. *Dev Med Child Neurol*. 1997;39:292–296.
14. Pingborg A, Lidegaard O, la Cour Freiesleben N, et al. Consequences of vanishing twins in IVF/ICSI pregnancies. *Hum Reprod*. 2005;20:2821–2829.
15. Conde-Agudelo A, Romero R, Hassan SS, et al. Transvaginal sonographic cervical length for the prediction of spontaneous preterm birth in twin pregnancies: A systematic review and meta-analysis. *Am J Obstet Gynecol*. 2010;203:e1–e12.
16. Romero R, Nicolaides K, Conde-Agudelo A, et al. Vaginal progesterone in women with an asymptomatic sonographic short cervix in the midtrimester decreases preterm delivery and neonatal morbidity: a systematic review and metaanalysis of individual patient data. *Am J Obstet Gynecol*. 2012;206:e1–e19.
17. Romero R, Conde-Agudelo A, El-Refaie W, et al. Vaginal progesterone decreases preterm birth and neonatal morbidity and mortality in women with a twin gestation and a short cervix: An updated meta-analysis of individual patient data. *Ultrasound Obstet Gynecol*. 2017;49:303–314.
18. Merced C, Goya M, Pratcorona L, et al. Cervical pessary for preventing preterm birth in twin pregnancies with maternal short cervix after an episode of threatened preterm labor: randomised controlled trial. *Am J Obstet Gynecol*. 2019;221. 55.e7-55.e8, and 55.e9.
19. WHO recommendation on the use of magnesium sulfate for fetal protection from neurological complications. 2015. Extranet.WHO.int
20. National Collaborating Center for Women's and Children's Health (UK). Multiple Pregnancy: The Management of Twin and Triplet Pregnancies in the Antenatal Period. London: RCOG Press; 2011.
21. The Fetal Medicine Foundation (FMF). The assessment of risk for preeclampsia. Fetalmedicine.org.
22. Cheong-See F, Schuit E, Arroyo-Manzano D, et al. Prospective risk of stillbirth and neonatal complications in twin pregnancies: systematic review and meta- analysis. *BMJ*. 2016;354:i4353.
23. NICE: Multiple pregnancy: antenatal care for twin and triplet pregnancies. National Institute for Health and Care Excellence—Guidance and Guidelines. 2011.
24. Van Mieghem T, De Heus R, Lewi L, et al. Prenatal Management of Monoamniotic Twin Pregnancies. *Obstet Gynecol*. 2014;124:498–506.
25. Barrett JF, Hannah ME, Hutton EK, et al. Twin Birth Study Collaborative Group. A randomized trial of planned cesarean or vaginal delivery for twin pregnancy. *N Engl J Med*. 2013;369:1295–1305.
26. Townsend R, Khalil A. Multiple pregnancy—labor and delivery. *Good Practice and Malpractice in Labor and Delivery, chapter 21*. Edra Edition; 2019.

27. Zafman KB, Rebarber A, Melka S, et al. Induction of Labor versus cesarean delivery in twin pregnancies. *Am J Perinatol*. 2019. http://doi.org/10.1055/s-0039-1693695 [Epub ahead of print].
28. Grossman TB, Tesfamariam R, Chasen ST, et al. Maternal morbidity of induction of labor compared to planned cesarean delivery in twin gestations. *J Matern Fetal Neonatal Med*. 2019;6:1–6. http://doi.org/10.1080/14767058.2019.1688291 [Epub ahead of print].
29. Di Renzo GC, Di Fabrizio L, Bavetta F, et al. Gestione della gravidanza multipla. *Medicina periconcezionale, chapter 28*. Minerva Medica Pbl; 2020.

CHAPTER

Conjoined twins

6

Julia Métneki[a], **Peter Varjassy**[b]
[a]*Retired, Twin Researcher, Foundation of Hungarian Twin Registry*
[b]*Retired, Chief Physician, Medical Historian*

6.1 Introduction

The mystery and secret of conjoined twins (CT) has interested people since ancient times. In addition to their unusual and stunning appearance, they were viewed with dread, as their birth was a sign of impending catastrophe or "punishment of God" for human sins.

In recent decades we hear more news about CT cases; they became the focus of intensive media interest. The growing attention can be attributed to the fact that with the development of medicine, more and more attempts are being made to successfully separate them. Furthermore, due to the Internet, all news becomes immediately available worldwide.

6.2 History of conjoined twins

Several archaeological findings testify to the existence CT in human beings dating back thousands of years. Among their artistic representations, numerous idols (mainly stone and ceramic figurines, rock paintings, cave drawings) have been found all over the world (Middle East, Polynesian Islands, Australia, Central America, Europe) in stone age settlements, caves, tombs; these were mainly double-headed creatures.[1,2]

The earliest example of CT is a marble statuette of "the double goddess" dating from 6500 BC in Çatal Höyük, Turkey.[3] In a small Mexican village (Tlatilco) that existed about 3000 years ago the excavations has also revealed many small female figurines of a wide range of facial and cranial duplications. The faces and heads from Tlatilco are particularly interesting because they are developmentally and proportionately correct, surprisingly.[4,5]

The *oldest written record* of the birth of a two-headed boy dates from 375 AD; they died three days apart at the age of two.[6] One of the *earliest monuments* in Europe comes from Biddenden in England. Born in 1100, joined at the hips and shoulders, Elisa and Mary Chulkhurst were the most famous, long-lived twin couple before the famous Siamese twins, who are recorded in the chronicles. They died at the age of 34, 6 h apart. The surviving sister refused separation surgery to save her life,

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00012-X

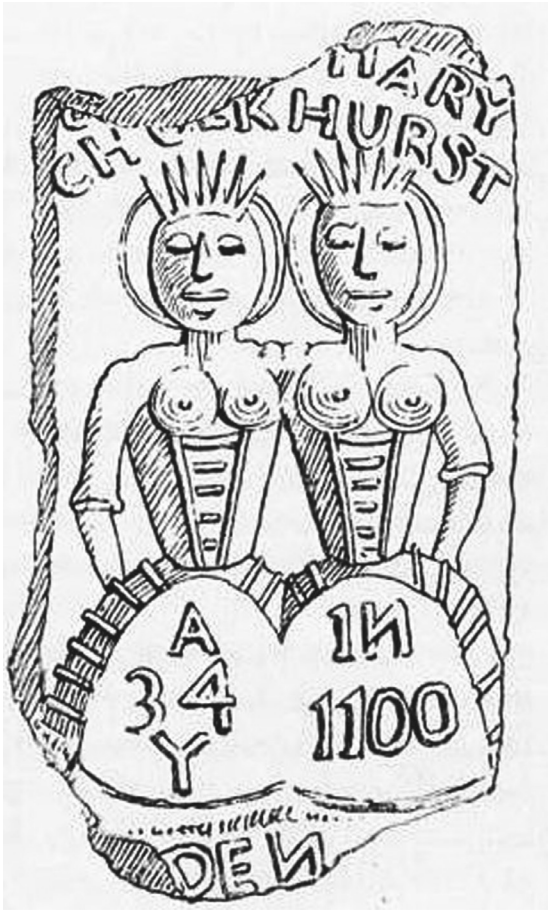


FIG. 6.1 A portrait of *Elisa and Mary Chulkhurst* (pygopagus conjoined twins) (1100–1135) on the Easter Wafers.

Plaster casts of wooden stamp for Biddenden cakes that were available in 1900.

Source: George M. Gould and Walter L. Pyle. Anomalies and Curiosities of Medicine. Philadelphia: W.B. Saunders. 1896. Fig. 39. p. 175. Biddenden Maid's cake (Ballantyne).

remarking: "As we came together, we will also go together." In their memory, the locals bake several hundred cakes stamped with their image and distribute them to the poor on Easter Sunday (Fig. 6.1).[7,8]

The term "Siamese twins" originated with Eng and Chang Bunker. The boys were joined from sternum to sternum by a three-inch cartilage that connected their livers. They were born in Siam (now Thailand) in 1811 and lived to age 63 (Fig. 6.2).[4,8] They were the longest-lived CT before the Galyon twins, who died in 2020 at the age of 68.

We cannot undertake the listing of all CT cases therefore the main characteristics of the most famous and longer-lived cases are presented in Table 6.1 (Figs. 6.3 and 6.4).[8–10]

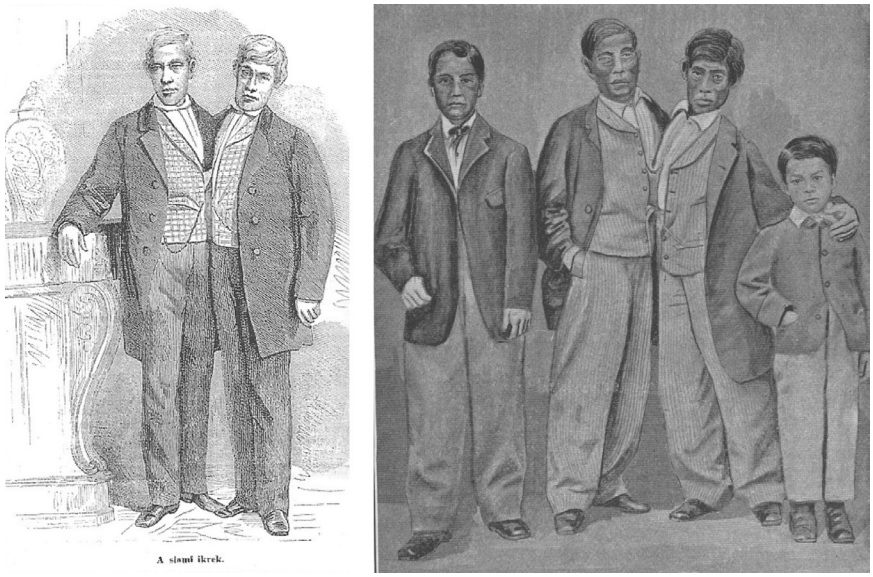


FIG. 6.2 ***Chang and Eng Bunker*** **(May 11, 1811 – January 17, 1874).**

The expression "Siamese twins" has become a synonymous of all conjoined twins. They were fused in the ligament (containing a part of their livers) connecting their sternums (xyphopagus). Up to 2012, both the Bunker and Tocci brothers had the longest known lifespan (63 years) of any conjoined twins in history.

Source: (left) The picture of Chang and Eng Bunker was published in a Hungarian newspaper called "Vasárnapi Újság" which means "Sunday news" in 1868, with a short, medically expert article, and, source (right). Representation of the Siamese twins in old age. On each side of them is a son. George M. Gould and Walter L. Pyle. Anomalies and Curiosities of Medicine. Philadelphia: W.B. Saunders. 1896. Fig. 31. p. 169. The original photograph is in the Mutter Museum, College of Physicians, Philadelphia.

Table 6.1 "Records" of conjoined twins.

Known conjoined twins from the oldest age
• Two-headed (dicephalic) boys born in the castle of Emmaus, 375 AD, they died 3 days apart
Longest-lived, male conjoined twins (deceased)
• Age 68 - *Ronnie and Donnie Galyon* – Born October 28, 1951, Dayton, Ohio, USA, died July 4, 2020. They were joined from the sternum to the to the groin and had separate hearts and stomatchs, but shared a set of lower organs. According to the 2009 Guinness World Records, the Galyons were the oldest living set of conjoined twins in the history.
• Age 63 - *Chang and Eng Bunker* – Born in Siam (now Thailand), May 11, 1811. The boys joined at sternum (xyphopagus). They were brought to the United States in 1829 and later settled in North Carolina. Both married and they fathered a total of 21 children. They died on January 7th, 1874, 3 hours apart (Fig. 6.2)
• Age 63 - *Giacomo and Giovanni-Batiso Tocci* - Born 1877 in Locana, Turin, Italy, died 1940. They had two interlocking upper bodies and a common lower body (parapagus dicephalus tetrabrachius dipus) (Fig. 6.3)

(Continued)

Table 6.1 "Records" of conjoined twins. *Continued*

Longest-lived, female conjoined twins (deceased)

• Age 61 - *Millie and Christine McKoy* Born in North Carolina, July 11, 1981, died October 12, 1912. They were joined at the inferior posterior parts of their bodies (joined at their buttocks) (pygopagus). The African-Americans girls traveled throughout the world performing song (one soprano and the other contralto voice) and dance for entertainment. They became known as "The Two-Headed Nightingale"

• Age 53 - *Masha and Dasha Krivoshlyapova*. Born in Moscow, Russia, on January 4, 1950, died in April 2003. They were fused at the pelvis and vertebrae; they had two heads, four arms and three legs (parapagus dicephalus tetrabrachius tripus). They were removed from their mother's custody at birth to be studied by Soviet physiologists. Their mother was told that her daughters had died soon after their birth

Currently the world's oldest living conjoined twins

* Reba, although born female, identifies as male and changed his name to George in 2007

• *Lori and Reba (George*) Schappell* (born 1961, in Reading, Pennsylvania, USA) - They are joined at the head (craniopagus). Lori works in a laundromat and is Reba's (George's) facilitator. Reba (George) has performed as a country singer and also designed support equipment for people with physical disabilities

Currently living conjoined twins without separation (including Schappell twins, see below)

• *Ganga and Jamuna Mondal* (their name were previously: Ayara and Jayara Ratun) (born 1970, in a Bengali family in Basirhat, West Bengal, India) - Joined at the abdomen and pelvis; they have a fused leg, share a stomach, liver, and reproductive tract (ischi-omphalopagus), known professionally as The Spider Girls or The Spider Sisters. They share a husband who loves them equally

• *Abigail and Brittany Hensel* (born March 7, 1990, Minnesota, USA) - They share a single body, but they have separate heads side by side (dicephalic parapagus tribrachius twins). Both graduated in 2012 from Bethel University, St. Paul and work as teachers

• *Carmen and Lupita Andrade* (born in June 2000, Veracruz, Mexico). They later moved to the United States for healthcare with their parents (dithoracicus tetrabrachius)

• *Anastasia and Tatiana Dogaru* (born in Lazio, Italy, January 13, 2004 - the top of Tatiana's head is attached to the back of Anastasias's head (craniopagus)

• *Krista and Tatiana Hogan* (Vancouver, British Columbia, Canada, born October 25, 2006) - They are joined at the head (craniopagus) and they share part of their brain; they can pass sensory information and thoughts between each other

• *Jesus and Emanuel de Nazaré* (born Pará, Brazil, December 19, 2011) - conjoined by the head

• *Marieme and Ndeye Ndiaye*, dicephalic parapagus twin girls born in Senegal in 2017, living in Cardiff, UK, in 2019

First successful separation surgery

• *The first record of separating conjoined twins* took place in the Byzantine Empire in 975 AD by a 30-year-old male omphalopagus twin born in Armenia. One of the conjoined twins had already died. The doctors attempted to separate the dead twin from the surviving twin, but the other also died three days later

• The *first recorded successful separation* was performed by Johannes Fatio in Basel in 1689 in the case of an xyphopagus twin girls who were connected to each other with a 12 cm connective tissue tape, (bundle). They both survived the intervention

(Continued)

Table 6.1 "Records" of conjoined twins. *Continued*
In 1955, neurosurgeon Dr. Harold Voris of Mercy Hospital in Chicago performed the first successful procedure separating conjoined twins joined at the head

Separation at the oldest age of conjoined twins
• 28-year-old *Laden and Laleh Bijani* - Iranian women born on January 17, 1975 (craniopagus). Their separation was attempted in Singapore on July 8, 2003, but they both lost their lives during the surgery
Conjoined twins who have given birth to a child
• *Rosa and Josepha Blazek* (pygopagus): In 1910, at the age of 32, Rosa gave birth to a boy who both women could breastfeed. At the age of 44, they died 12 min apart in the United States. This was the only known case in the world in which "siamese" twins had a healthy boy raised together (Fig. 6.4)
• *Ganga and Jamuna Mondal* (Jandal), also known as The Spider Girls (ischio-omphalopagus): At the age of 23, they gave birth to a daughter by Caesarean section in 1993, but the newborn lived only a few hours

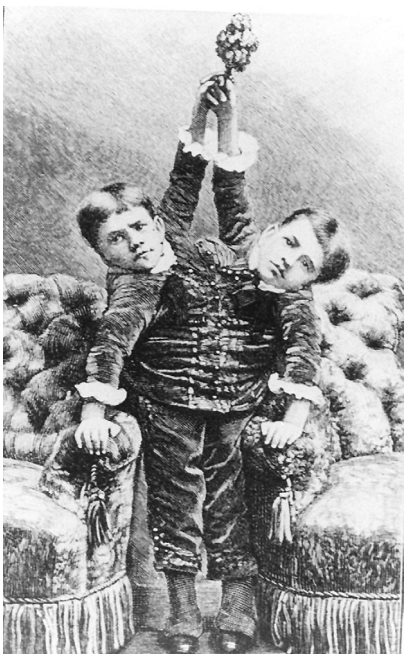


FIG. 6.3 The *Tocci brothers* photographed with their inner arms raised and clasped.

Born in Locana, Italy, *Giacomo and Giovanni Tocci* (1877–1941) have two interlocking upper bodies fused in a common lower body (parapagus dithoracius). They had common sexual organs, yet they married two women (sisters). They could not walk on their own. They lived to the age of 63. They were the inspiration for Mark Twain "Those extraordinary Twins."

Source: George M. Gould and Walter L. Pyle. Anomalies and Curiosities of Medicine. Philadelphia: W.B. Saunders, 1896. Figure 48. p. 186. The photo represents Giacomo and Giovanni Tocci as they were exhibited several years ago in Germany.

FIG. 6.4 ***Rosa and Josepha Blazek*** **touring music halls and theaters throughout England and Europe.**

The Czech Rosa and Josepha Blažek (Bohemian twins) (1878–1922) were joined in the lumbal region of the spine, they were pygopagus female twins. They had a single urethral opening and anus, but had two vaginas. They were attractive women, spoke many languages, sang beautifully, danced well, and played many musical instruments. In 1907, Rosa married. In 1910, at the age of 32, she gave birth to a boy who could both breastfeed and whom they brought up together.

Source: George M. Gould and Walter L. Pyle. Anomalies and Curiosities of Medicine. Philadelphia: W.B. Saunders, 1896. Fig. 43. (p. 181).

6.3 Conjoined status in plants and animals

CT specimens are not limited to human beings; they can also occur in plants and animals. In the former, the most common are the united fruits (e.g., plums, sour cherries, apples, and kiwi); sometimes even double-triple fusion can be detected. Doubling of vegetative parts of plants (leaf, tuber: potato, root: carrot) may be due to imperfect separation of the plant embryo.

In animals, the earliest known example is the fossil of a dicephalus embryo of an ancient reptile from the Cretaceous era, 125 million years ago, in China.[11]

Many vertebrates—fish, reptiles, amphibians, birds, primates, and other mammals—were observed to have different types of duplication with varying frequency by species. In domestic animals such as calves, lambs, goats, horses, dogs, cats, pigs, and chickens, such cases have also been found (Fig. 6.5). Among the wild animals, the two-headed snake is the most common.

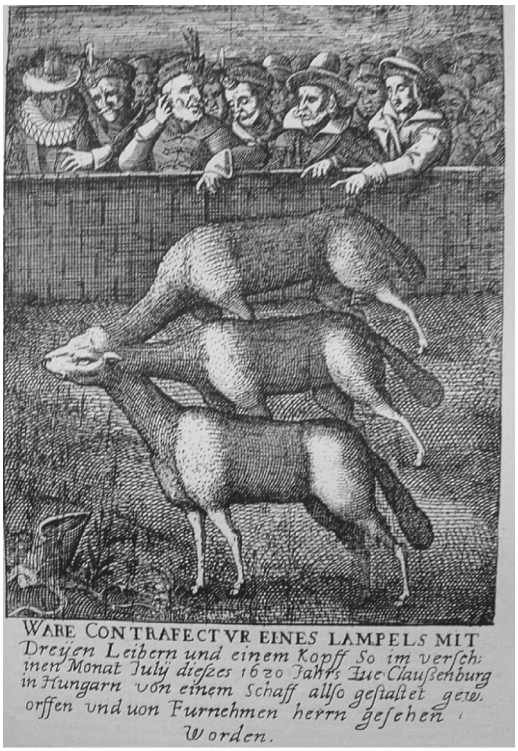


FIG. 6.5 The *one-headed and three-bodyed wonderland* (sheep) was born in Cluj-Napoca in 1620.

Source: Eugen Holländer: Wunder, Wundergeburt und Wundergestalt, Stuttgart, 1921, Fig. 33.

6.4 Development of conjoined twins

There are two contradictory theories on their formation, which are still debated today: *fission* and *fusion.*[12]

According to *fission theory*, CTs are formed due to imperfect splitting of the zygote after the formation of two embryonic plates. At days 14–15 after conception, the split begins, but the primitive strip and embryo shield do not separate perfectly. The process stops before perfect separation and the development of partially detached cells continues as a fused fetus. The twin formation is incomplete and a missed or incomplete doubling at the head or tail end of the body axis leads to the disorder. Many variations of fusion are observed depending on the location and extent of the defect.

Others, on the other hand, assume that CT is created by the *fusion of two embryos* that have previously been completely separated. In this case, the two embryo plates, which had previously been separated, grow to each other in secondary locations, at defined developmental sites. Nowadays, this theory is related to Rowena Spencer, who says the process is possible 14–25 days after conception.[12]

Parasitic twins result from embryonic death of one twin, leaving various viable body parts vascularized by the surviving autosite.

The reason for their formation is not completely clarified. It is not certain that each type of CT can only be created by one type of mechanism. It is also possible that each type may develop differently: the formation of parapagus would be explained by the theory of fission, whereas fusion theory, for example, would be more appropriate for the formation of cephalopagus or pygopagus.[12]

6.5 Embryology of conjoined twins, mechanism of their development

CT represent a special group of congenital anomalies occurring in genetically identical, monozygotic, monoamniotic and monochorionic twins with the same sex. CT are always of the same sex, even though several cases of pseudo-hermaphroditism have been documented.

The union is always homologous: head to head, side by side, etc. Fusion can occur only on certain areas of the body (affecting eight regions), the extent of which may vary by case and type: from a small connective tissue bridge to complete fusion of two bodies. In typical *symmetrical* cases, both have their own separate organs apart from their junction (Figs. 6.2 and 6.4). In the case of a small degree of fusion they can be viable individually after separation.[13]

In the case of *asymmetric, parasitic* (nonviable in itself) forms, the parasite may be associated with different regions of the intact (autosite) twin body. In such cases, often only a few supernumerary body parts (e.g., hands or feet) are detected on the body of autosite (Figs. 6.6 and 6.7).[12,13]

FIG. 6.6 ***Lazarus Colloredo – Joannes Baptista*** **1617–1640 (1650?) (epigastricus parasiticus).**

He was born in Genoa, in 1617, and exhibited himself all over Europe. From his epigastrium hung an imperfectly developed twin that had one thigh, hands, body, arms, and a well-formed head covered with hair. There were signs of independent existence in the parasite, movements of respiration, etc., but its eyes were closed. He was married and the father of several healthy children.

Source: George M. Gould and Walter L. Pyle. Anomalies and Curiosities of Medicine. Philadelpha: W.B. Saunders, 1898. (Fig. 55. p. 190).

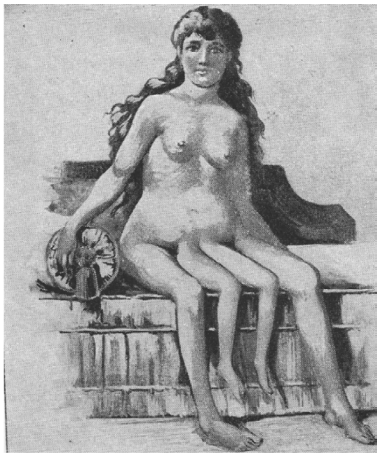


FIG. 6.7 ***Josephine Myrtle Corbin*** **(The Women from Texas with Four Legs) (1868–1927) was double-bodied from the waist down (including the perfectly functioning genitals) (dipygus parasitic conjoined twin, duplicatio caudalis.**

The growth of the two middle legs stopped in her childhood, so she could only walk with the two outer legs. She married and gave birth to five children, three from one uterus and two from the other.). After her marriage she was known as Madame B. or Mrs B.).

Source: George M. Gould and Walter L. Pyle. Anomalies and Curiosities of Medicine. Philadelpha: W.B. Saunders, 1898. Fig. 55. p. 190. 17. Fig. 59. p. 195.

One type of the parasitic form is the "*fetus in fetu*" (fetus in the fetus or endoparasite) where the vestigial (parietal) parasite is located inside a cavity of the autosite's body.[13,14]

6.6 Classification of conjoined twins

CT are typically classified by the point at which their bodies are joined. The major types of CT were described first in 1573 by the French renaissance surgeon, Ambroise Paré.[15] The classification of CT is based on Geoffroy Saint-Hilaire's 1830

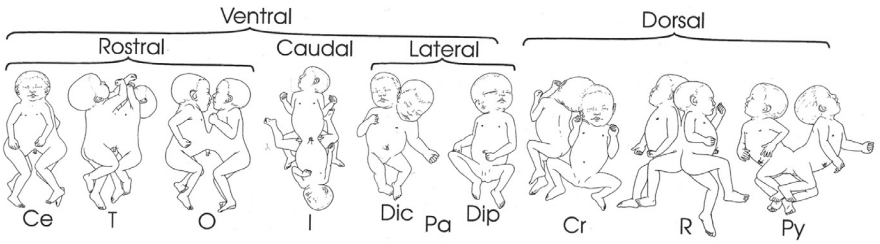


FIG. 6.8 The *eight types of symmetrical conjoined twins* grouped together according to the location of union.

Abbreviations: Cephalo: cephalopagus; Thoraco: thoracopagus; Omphalo: omphalopagus; Ischio: ischiopagus; Para: parapagus, dicephalus, diprosopus; Cranio: craniopagus; Rach: rachipagus; Pygo: pygopagus.

Source: Rowena Spencer: Conjoined twins. 2003. p. 13. Fig.2-2. Reprinted with the permission from W.B. Saunders or John Hopkins University Press.

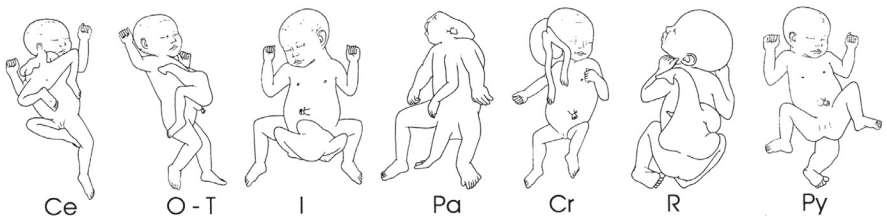


FIG 6.9 The *eight types of parasitic conjoined twins* grouped together according to the location of union.

Source: Rowena Spencer: Conjoined twins. 2003. p. 53. Fig.2-16. Reprinted with the permission from W.B. Saunders or John Hopkins University Press.

classification, their modified and improved version is still used today.[16] It is worth mentioning the classifications of Luigi Gedda[17] and Guttmacher and Nichols,[18] which were widespread until the mid-1990s. The latest and the most up-to-date developmental and pathological classification related to Rowena Spencer who distinguished 8 major types of both symmetrical and asymmetric (parasitic) CT twins[12] (Figs. 6.8 and 6.9). According to Spencer, the twins conjoined on the ventral and lateral sides, forms a continuum (Fig. 6.10). Besides that, the twins conjoined on the ventral and dorsal sides also show a continuum. The connection point between these two groups is the ischiopagus tetrapus type.

The definition of each type is shown in Table 6.2. The listed types do not always occur in a clear, distinct manner; they can often be mixed, with two or three types that may occur simultaneously in the same individual. The location and extent of union seen can be extremely variable. To illustrate the examples, some images of real, observed cases are presented in Figs. 6.11–6.20.

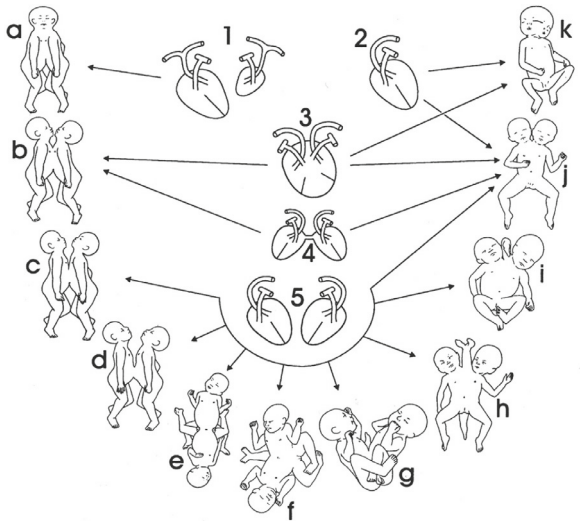


FIG 6.10 The *continuum of ventrally conjoined twins.*

In R. Spencer's opinion the twins conjoined on the ventral and lateral sides, forms a continuum. Besides that, the twins conjoined on the ventral and dorsal sides also show a continuum. The connection point between these two groups is the ischiopagus tetrapus type.

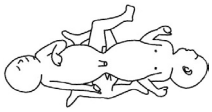
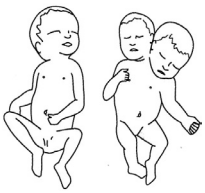
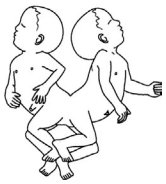
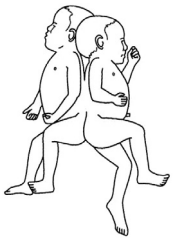
Source: Spencer R., 2001c, pt 1. fig.5. and in Rowena Spencer: Conjoined twins. 2003. p. 22. Fig.2-6. Reprinted with the permission from John Wiley and Sons.

Table 6.2 Definition of conjoined twins.

Site of union		Types	Definition	Figures
Ventral	**Rostral**	**Cephalopagus**	There are two faces and are joined from the top of the head to the umbilicus (Figs. 6.11 and 6.12)	
		Thoracopagus	Are joined face-to-face from the upper thorax to the uppert part of abdomen and always involved the heart (Fig. 6.13).	
		Omphalo-pagus	The fusion includes the umbilicus region frequently at the lower thorax, but never the heart	

(Continued)

Table 6.2 Definition of conjoined twins. *Continued*

Site of union		Types	Definition	Figures
	Caudal	**Ischiopagus**	The union usually includes the lower abdomen and duplicated fused pelvic bones, and external genitalia and anus are always involved (Figs. 6.14 and 6.15).	
	Lateral	**Parapagus**	Are laterally joined, regularly shared the pelvis. Varieties of parapagus conjoined twins are parapagus dithoracic (separated thoraces), **parapagus dicephalus** (Fig. 6.16) (one trunk two separate heads), and **parapagus diprosopus** (one trunk, one head, and two faces) (Fig. 6.18).	
Dorsal		**Craniopagus**	Joined by the skull, share meninges but rarely the brain surface and do not include the face and trunk (Fig. 6.19).	
		Pygopagus	Are dorsally fused, sharing perineal and sacrococcygeal areas, has only one anus but two rectums (Fig. 6.20).	
		Rachipagus	Dorsally fused, the defect may involve the dorsolumbal vertebral column and rarely the cervical vertebrae and the occipital bone	

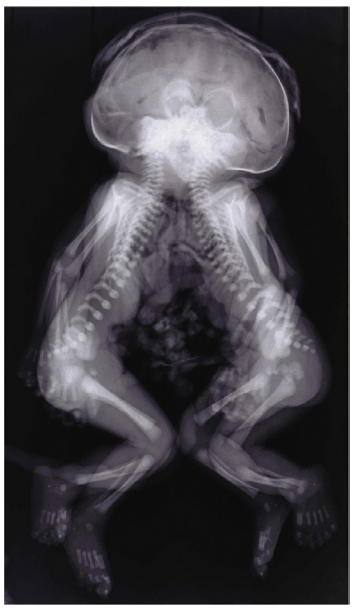


FIG 6.11 ***Male cephalopagus conjoined twins.***

The stillborn conjoined twin fetuses were born at 32 weeks of gestation.

(Photo taken by Prof. Dr. Gyula Lázár, permission with his consent).

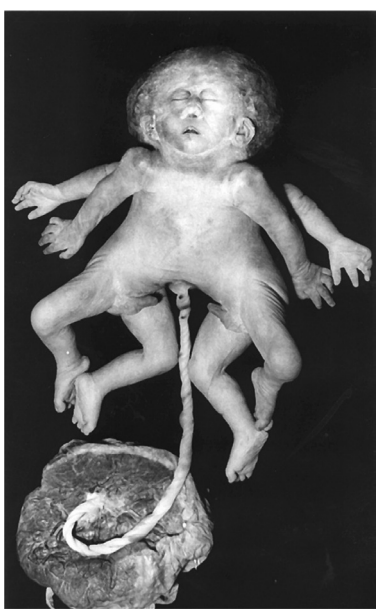


FIG. 6.12 Anterior–posterior X-ray image of *the cephalopagus fetuses* before autopsy. 1972. Pápa, Hungary.

(Photo taken by Prof. Dr. Gyula Lázár, permission with his consent).

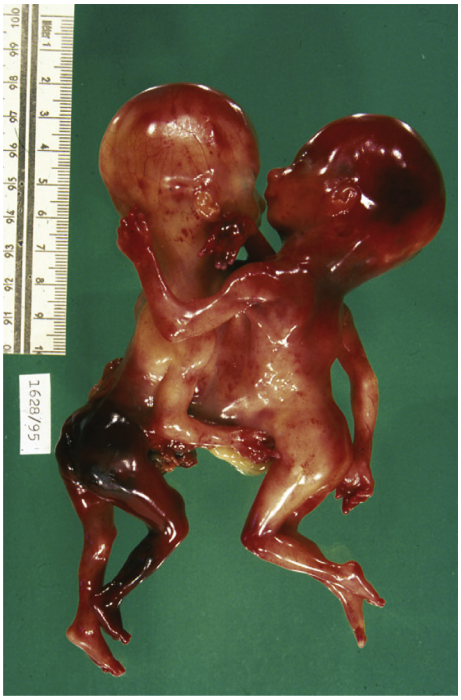


FIG. 6.13 Photo of *thoracopagus conjoined fetus*.

The diagnosis of conjoined twins was detected by ultrasound examination at the 19th week of pregnancy. Due to a developmental abnormality incompatible with life, pregnancy was terminated at the 20th week of gestation. 1983. Budapest, Hungary.

(Permission performed with the consents of Dr. Krisztina Hajdu and Prof. Dr. Miklós Török).

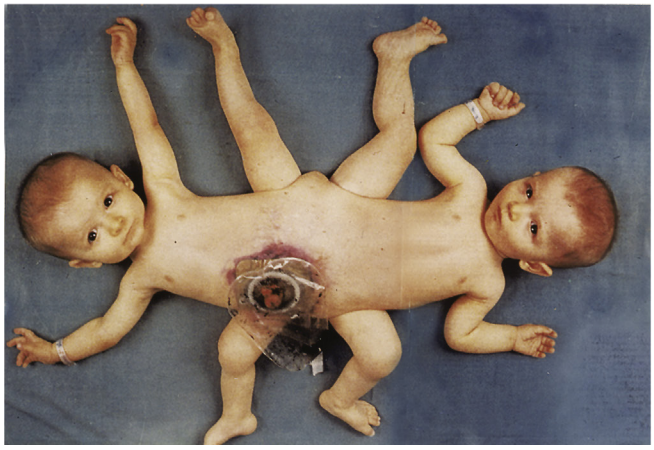


FIG. 6.14

Photo of *ischipagus conjoined twins* at the age of two months (1983. Pécs, Hungary).

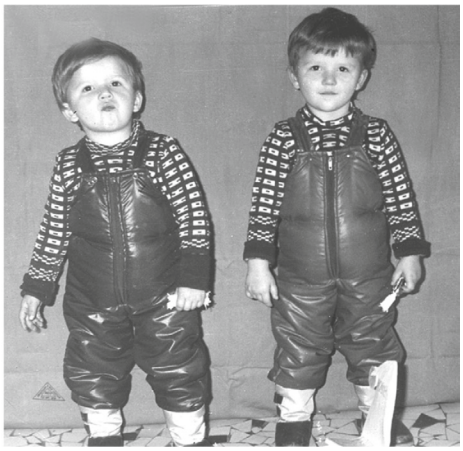


FIG. 6.15 Photo of the same pair of ischiopagus male twins at the age of two. Their surgical separation took place at the Pediatric Clinic of the Medical University of Munich at the age of nine months.

As a result of the successful intervention they are both healthy and well.

(Photos taken by Prof. Dr. Tamás Decsi, permission with his consent).

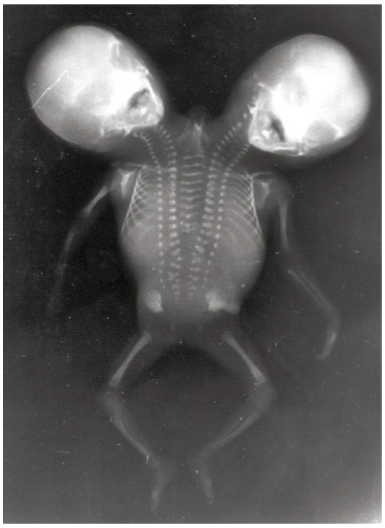


FIG. 6.16 Photo of X-ray examination of *parapagus dicephalus* conjoined twin fetus diagnosed at the 19th of pregnancy.

(Photos taken by Dr., László Hernádi, permission with his consent).

FIG. 6.17 ***Parapagus dicephalus*** **case born in 1980.**

Case 3. of Mihály Lenhossék, professor of anatomy.

Source: Lenhossék Mihály: Nachricht von einigen menschlichen Doppelmißgeburten, Wiener Medic. Jahrbücher, Wien, 1820, VI. B., II. St, pp. 155–156. Figure: 3.

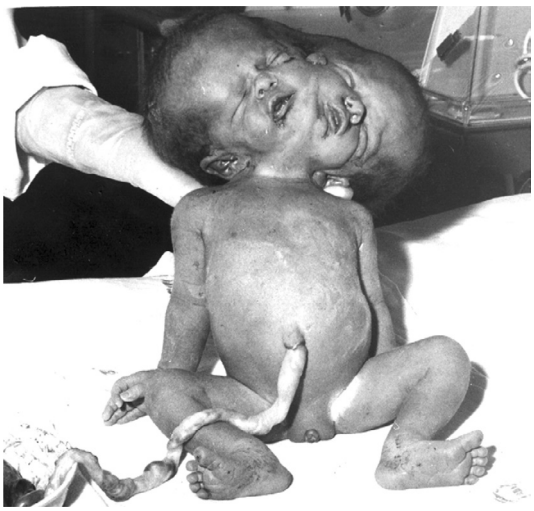


FIG. 6.18 Photo of parapagus diprosopus (dicephalus incomplet) conjoined twins after birth. 1978.

Debrecen, Hungary. The embriopathy (imperfect separation) is considered to be the consequence of untreated diabetes mellitus of the mother.

(Photo: taken by Dr. Károly Csécsei, permission with his consent).

FIG. 6.19 ***Dicephalus female conjoined twins*****, born at Worms in 1495.**

Paré's contemporary, Sebastien Munster, published in 1552, in his Cosmography presents an excellent example of the dire effect of prenatal influence or maternal impression. He comments the wonderful phenomenon as follows: "As the mother of this girls joined by the forehead was gossiping with another women upon the street, an unexpected thing happened and stuck the two foreheads of the women, thereupon, the pregnant women ill with fright, so that the fruit within her womb had to suffer for it."

Source: Reproduction of woodcut published in 1495. Brant, Sebastian, 1458–1521.

FIG. 6.20 ***Helen (Ilona) and Judith, Hungarian sisters*** **(pygopagus conjoined twins)- known as "monstrum hungaricum"—was born in Szőny, Hungary, on October 26, 1701 joined in the lumbal region of the spine, back to back.**

They were born three hours apart. They were locked up in a convent from the age of 9. They lived for 22 years without separation. It was believed that the reason for their junction was attributable to maternal admiration: "the mother looked closely at mating dogs in the first week of pregnancy …"

Source: Engraving drawing in Philisophical Transactions, London 1757. Vol. L. P. I. p. 316.

6.7 Etiology

Thousands of years ago, human imagination regarded the births of CT as supernatural phenomenon. In the absence of knowledge, various beliefs, among them irrational and mystical explanations, were born. Ambroise Paré, in his book of "Monsters and Prodigies," lists the presumed causes generally accepted concerning their etiology, such as "punishment" of God for the wickedness of men, the abundance or deficient of seed (sperms), maternal imagination (wondrous) (Fig. 6.19), too narrow uterus, too tight clothing, sitting cross-legged, etc.[15,18]

Although researchers have been able to artificially induce a fusion state in animal experiments by various physical and chemical effects (e.g., shaking, cooling, warming, inducing oxygen deprivation, UV radiation, embryonic cell attachment, and certain teratogenic substances), the etiopathology in the human is still not well known.[19–22] It can be assumed that the causes are essentially the same as that for MZ twins in general,[23] although it has also been suggested that secondary partial fusion of blastocysts may occur.[24]

Table 6.3 includes some suspected risk factors that could play a role in the formation of CT.[25–45] In most cases examined, *no specific environmental impact* can be found to justify the developmental disorder. In fact, it is difficult to prove that it would be causal relationship between a drug taken in early pregnancy, maternal illness, or suspected work-related injury and CT; however, these effects cannot be ruled out.

Table 6.3 Suspected risk factors playing role in the formation of conjoined twins.

Suspected risk factors	References
Gene mutation	25
Abnormal X-inactivation (it could also be related to the increased female predominance)	26
Oral contraceptive pills used in periconceptional period or long-term usage of contraceptive pills	27,28
Assisted reproduction techniques (ovulation induction, ART, ISCI)	27,29,30,31,32 33,34,35,36,37
Ovulatory dysfunctions and calcium depression in extremely underweight woman	28
Medicines (teratogen drugs) used in early pregnancy	
valproic acid,	38
prochlorperazine "Stemetil,"	39
Griseofulvin	40
(other research has not confirmed this latter effect)	41,42
Maternal diseases: diabetes	27,43,44
Possible occupational hazards (eg. heavy metals: lead, copper, chromium)	27,28
Chronic low-dose radiation	45

6.8 Epidemiology of conjoined twins, genetic and demographic risk factors

Studies on the epidemiological characteristics of CT have been conducted in several countries. To summarize diverse epidemiological aspects of CT, a worldwide collaborative epidemiological study was performed by the International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR). It was the latest and largest sample of CT ever studied before. The analysis included a total of 383 carefully reviewed sets of CT, which were obtained from 26,138,837 births reported by 21 Clearinghouse Surveillance Programs (SP).[29] Previously, a similar epidemiological study based on 312 CT cases was published by ICBDMS.[30]

Frequency – CT are very rare; their occurrence is estimated in 1% of MZ twins. Total prevalence, although variable, has been estimated to be 1:50,000 to 1:100,000 births[46–64] (Table 6.4). They are expected in every 40,000–60,000 pregnancies, but

Table 6.4 Prevalence of conjoined twins observed in diverse populations studied: 1930–2010.

References	Location	Prevalence	Value ranges
46	India	1:2800	Higher than 1:20,000 births
47	Uganda	1:4242	
48	Taiwan	1:6500	
49	Rhodesia-Africa	1:14,000	
50	Sweden	1:20,000	Between 1:20,000 and 1:50,000 births
51	Brazil	1:22,284	
52	Maltese Islands	1:25,000	
53	China	1:30,600	
54	China	1:35,100	
55	Australia	1:40,000	
56	USA-Los Angeles	1:50,000	Between 1:50,000 and 1:100,000 births
57	USA- Chicago	1:50,000	
29	Worldwide	1:68,027	
27	Hungary	1:68,500	
58	South America ECLAMC	1:74,626	
59	Sweden	1:75,000	
60	USA-Atlanta	1:97,560	
61	Japan	1:100,000	Between 1:100,000 and 1:200,000 births
62	Spain	1:151,500	
63	New York-USA	1:166,000	
64	USA	1:200,000	

many cases are spontaneously aborted or artificially interrupted. The frequency of live births is less (between 1: 100,000–1: 200,000).

Some studies reported prevalence of CT as high as 1 in 2800 livebirths in India,[46] to as low as 1 in 200,000 livebirths in the USA.[64] The very varying figures are most likely attributable to the population types and size monitored, the different methods of ascertainment (hospital or population records), inclusion or exclusion of stillbirths, spontaneous abortions, and elective termination of pregnancy, which resulted in significant under-registration among some populations studies.[30,55,56]

Geographical, temporal and seasonal clustering of CT has been reported, specifically in the State of New York,[65] South Africa,[66,67] Sweden,[59] Uganda,[47] West Africa,[68] Jerusalem,[69] Latin America,[58] Cardiff,[70] and in Maltese Islands.[52] Clear explanation (e.g. exogen influence) has not been found in any studies.

6.9 Ethnicity

CT are more frequent in Africa, India, and Taiwan than in the United States and Europe, suggesting an increased incidence in black and Asian populations. In the worldwide epidemiological study by ICBDSR, there were significant differences in prevalence by ethnicity: it was higher in Latin America than in Anglo-Saxon/Caucasian and Latin European ethnic groups.[30] This is a surprising finding because the occurrence of MZ twinning is nearly the same in all ethnic groups; ethnic variations are known only for dizygotic twinning.

Family studies have not revealed other CT in their families, and neither twins nor other congenital anomalies in siblings are more common than expected. Among more than 1500 descendants of Chang and Eng's 21 children, several pairs of twins including MZ twins were born, but no other CT was recorded.[71] There has been only one report of a second set of CT in the family.[72] First-degree consanguinity between parents was also not found. Chromosomal aberration characteristic of CT has not been detected; the possibility of inheritance can be almost excluded.

Occurrence of multiple twins—Their incidence is extremely rare. Schinzel thought that CT occur with unexpected frequency in triplet sets.[73] Some authors described the occurrence of CTs in triplet[26,74,75] and quadruplet[31,76] pregnancies, both spontaneously and artificially (due to IVF and ICSI) achieved. CT Info reports on a quadruplet pregnancy (Norway, 1953), in which two sets of the newborns were conjoined, all died at birth.[77]

Triplet CT—There are also reports of triple union: a boy was born with three heads in Sicily (1834) and another in Turkey (1955).[12,77]

Sex ratio—The higher proportion of females (70%–75%) among CT is a general characteristic. Female conceptions may be at higher risk of becoming conjoined, or spontaneous abortion of female conjoined conceptions may be less likely than male ones.[78] However, the ratio of males is higher among stillborn CT. Significant differences were observed in prevalence by sex and by type of CT. Thoracopagus type is almost four times more frequent in females than in males, while parapagus and parasitic types is significantly more frequent in males than in females.[29]

Distribution of CT types—Symmetric cases have an obvious predominance. Thoracopagus represent the largest number of cases (42%). The following most common types are parapagus dicephalus (11.5%), omphalopagus, and craniopagus with 5.5% each in the latest worldwide study.[29] Parasitic CT were reported to be 3.9% of all specified CT. It appears that in previous centuries, the pygopagus type of CT was more common than nowadays.[9]

Maternal age and birth order did not show any association with CT in epidemiological studies involving large numbers of cases. However, gestational age, birth weight, and previous spontaneous abortions showed a surprising correlation.[29] The proportion of preterm delivery was very high (60%–75%); CT babies are born significantly earlier and with less weight than normal twins due to their pathological development.

Previous spontaneous abortion was reported in a higher proportion of CT mothers than is usual in most healthy populations, as seen in both Hungarian (26.7% vs. 13.1%) and worldwide epidemiological studies (19.7%).[27,29] Maternal factors therefore may be of importance in the etiology of CT.[27,78]

Congenital anomalies unrelated to the site of union have been reported in 40%–60% of CT.[27,29,30,60] Among them the most common were musculoskeletal, gastrointestinal, nervous system and genital disorders, as well as VACTERL, schisis, and caudal regression abnormalities. The incidence of lip and cleft palate was also significantly higher than expected. Concordant occurrence of malformations is more common but cases discordant for disorders have been also reported. Some of the malformations seemed to be associated with a more fundamental disturbance of embryogenesis.[79] On the basis of the higher-than-expected incidence of unrelated congenital anomalies, it is presumable that the formation of CT can trigger a so-called "cascade" process, which can result in further developmental disorders of the fetus.[27]

Pregnancy outcome—In mothers of CT, livebirth rate is gradually decreasing with the widespread use of ultrasound scans in recent decades. Majority of CT fetus are stillborn or die soon after birth. Nowadays, CT can be diagnosed as early as the 9th week of gestation by ultrasonography (Figs. 6.21 and 6.22).

Life expectancy depends primarily on the severity of the associated disorders and the extent of fusion. In the Hungarian epidemiological study including 197 cases,[9] only one pygopagus female twin pair (the Szőnyi sisters born in 1701) lived to the adult age of 22 years without surgical separation. Only a few (around 20) sets of CT are living in the world without separation, of these, the well-known pairs are shown in Table 6.1. The oldest living CT are Lori and George Schappell born in 1961.

There is only one case in the world of CT twins giving birth to a healthy child: the Blazek sisters. While only Rosa experienced labor pains, both sisters were able to nurse the baby. The Indian Mandala (Jandal) twins—who became known as "Spider girls"—also gave birth to a daughter, but the newborn lived only a few hours.

Separation of conjoined twins—Conjoined twinning is one of the most challenging human malformations for surgeons. Moreover, these cases often raise religious, moral, ethical, and legal issues, for example, is it permissible to "sacrifice" one CT in the interest of saving the other's life? The surgeries often involve a moral dilemma. The first recorded attempt[80] and the first successful separation of CT were mentioned in Table 6.1.

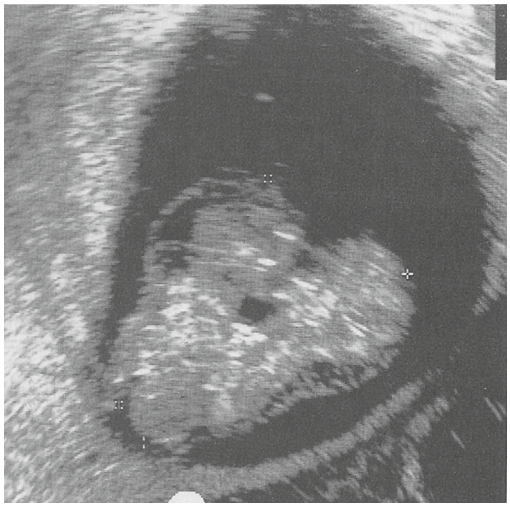


FIG. 6.21 Image of ultrasound examination of *thoracopagus conjoined twin fetus*.

The diagnosis of conjoined twin was detected by ultrasound examination at the 9th weeks of pregnancy. Due to a developmental abnormality incompatible with life, pregnancy was terminated at 10th week of gestation.

(Photos taken by Dr. András Tankó, permission with his consent).

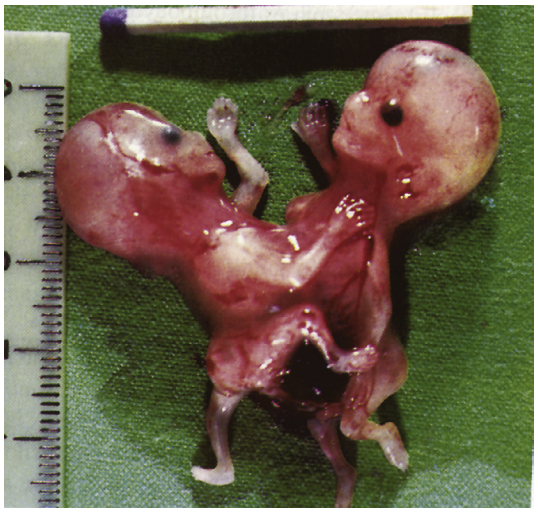


FIG. 6.22 *Thoracopagus fetus* after induced abortion.

(Photos taken by Dr. András Tankó, permission with his consent).

The surgical separation of CT depends on the location and size of fusion, and the vital common internal organs that are shared. Separation in most cases carries a serious risk and often threatens the lives of one or both twins. Success in the management of CT requires an experienced team functioning in a specialized center with a full range of medical, neurological, and surgical specialities. In risky cases, it may be more advantageous to disregard surgical separation. In some cases, they can have a better quality of life by remaining in a fused state, such as the Schappel or Hensel twins.

Surprisingly, most CT do not feel physically entrapped by this condition; they tend to readily accept the anatomy with which they were born.[81] Laleh and Ladan Bijani were the first and only CT in history who asked to be separated in 2003 at the age of 29, even at the risk of their lives. The Iranian women both lost their lives tragically during the surgery.

From the 1950s to the present day, more than 200 surgeries have been performed. Among them, separation of craniopagus twins remains a rarity, which requires extensive, highly complex surgeries. However, modern neurosurgical techniques have created opportunities for successful separation and brings hope for a more normal life for these children. The most recent world-sensational operation was performed by a Hungarian medical team in the case of craniopagus twin girls, Rabeya and Rukaya Islam, born in Bangladesh, in 2018–2019 (Figs. 6.23 and 6.24).[82]

FIG. 6.23 ***Rabeya and Rokaiya Islam*, born in Pabna, Bangladesh on July 16, 2016, joined at the head (craniopagus-type twins).**

A team of Hungarian doctors has successfully separated Rabeya and Rukaya, 3-year-old craniopagus twins. The 33-hour final separation took place in Dhaka, Bangladesh on August 1-2, 2019, as part of the surgery series called "Operation Freedom," started in 2018, organized by Action for Defenceless People Foundation, led by Dr. Gergely Pataki, chief coordinator and team leader general and plastic surgeon (left) and Dr. Andras Csokay, team leader neurosurgeon (right).

FIG. 6.24 The *craniopagus twins* 7.5 months after the successful separation surgery with their parents, during the IV phase of "Operation Freedom," which consists of their cranial reconstruction and rehabilitation, organized by Action for Defenceless People Foundation.

(Photo by Miklos Bemer, Action for Defenceless People Foundation. (Permission by Dr. Gergely Pataki).

6.10 Summary

CT are an extremely rare group of identical twins. In this chapter, we review the most important historical cases of CT and summarize the current knowledge regarding their past artistic representation, incidence, types, etiology, pregnancy outcome, antenatal diagnosis, and surgical separation.

References

1. Warkany J. Congenital Malformations. Notes and Comments. Year Book Medical Publisher, Chicago, IL, 1971.
2. Raffensperger J. Conjoined twins in Polynesia? *Rapa Nui J*. 2001;15:108.
3. Spitz L, Kiely E. Conjoined twins. *JAMA*. 2003;289:1307.
4. Kennedy GE. The 3000-year history of conjoined twins. *West J Med*. 2001;175:176–177.
5. Kocku A, Cetinkaya MB, Aydin O, Tosun M. Conjoined twins: historical perspective and report of a case. *J Matern Fetal Neonatal Med*. 2007;20:349–356.
6. Bondeson, J. The Two-headed Boy and Other Medical Marvels. Cornell University Press, Ithaca and London, 2000;152.
7. Bondeson J. The Biddenden Maids: a curious chapter in the history of conjoined twins. *J Roy Soc Med*. 1992;85:217–222.

8. Quigley Ch. Conjoined Twins: An Historical, Biological and Ethical Issues Encyclopedia. McFarland and Company Inc. Jefferson, NC and London, 2003.
9. Varjassy P, Métneki J. Egy vagy Kettő? Összenőtt ikrek Magyarországon. (In Hungarian) One or two? Conjoined twins in Hungary) Kossuth Kiadó, Budapest, 2008.
10. Gould, GM. and Pyle WL. Anomalies and Curiosities of Medicine. W. B. Saunders, 1898;194.
11. Buffetaut E. Two-headed lizard spied in a fossil. New Scientist. In: Daily News, 2006;20;12.
12. Spencer R. Conjoined twins: Developmental Malformations and Clinical Implications. The John Hopkins Press, Baltimore, 2003.
13. Spencer R. Anatomic description of conjoined twins: a plea for standardized terminology. *J Ped Surg*. 1996;31:941–944.
14. Mohini, GD. Pregnant man who gave birth to his brother after 36 years. Archived from the original on Dec 22, 2014. Original version: ABC News. Man With Twin Living Inside Him - A Medical Mystery Classic, August 23, 2006.
15. Pare A. From on Monsters and Marvels (orig, 1573), University of Chicago Press, Chicago, IL, 1982.
16. Saint-Hilaire G. Histoire Générale et Particulière des Anomalies de L'organization. Traite de Teratologie. Paris: 1832-1837;3:327-346.
17. Gedda L. Twins in History and Science. Charles C. Thomas, Publisher, Sprinfield, IL. 1961.
18. Guttmacher AF, Nichols BL. Teratology of conjoined twins. In: Bergsma D, (ed.). New York: National Foundation – March of Dimes; 1967. *Birth Defects Original Article Series*. 3:3–7.
19. Feron VH. Conjoined twins in mammalian teratology. *Arch Environ Health*. 1969;19:353.
20. Ingalls TH, Philbroak FR, Majiman A. Conjoined twins in Zebra fish. *Arch Environ Health*. 1969;19:344.
21. Spemann H. Entwicklungsphysiologische studien am triton-Ei. *Arch Enttw Mech*. 1901;12:224–264.
22. Kelly GM, Erezylmaz DF, Moon RT. Induction of a secondary embryonic axis in zebrafish following the overexpression of beta-catenin. *Mech Dev*. 1995;53:261–273.
23. Witschi E. Overripeness of the egg as a cause of twinning and teratogenesis: a review. *Cancer Res*. 1952;12:763–786.
24. Aird I. Conjoined twins – further observations. *Br Med J*. 1959;2:1313–1315.
25. Petzel MA, Erickson RP. Disorganisation: a possible cause of apparent conjoint twinning. *J Med Genet*. 1991;28(10):712–714 Oct.
26. Zeng SM, Yankowitz J, Murray JC. Conjoined twins in a monozygotic triplet pregnancy: prenatal diagnosis and X-inactivation. *Teratology*. 2002;66:278–281.
27. Métneki J, Czeizel A. Conjoined twins in Hungary, 1970-1986. *Acta Genet Med Gemellol*. 1989;38:285–299.
28. Steinman G. Mechanisms of twinning. V. conjoined twins, stem cells and the calcium model. *J Reprod Med*. 2002;47:313–321.
29. Mutchinick OM, Luna-Muñoz L, Amar E, et al. Conjoined twins: a worldwide collaborative epidemiological study of the International Clearinghouse for Birth Defects Surveillance and Research. *Am J Med Genet Part C Semin Med Genet*. 2011;157:274–287.
30. ICBDMS. Conjoined twins – an epidemiological study based on 312 cases. The International Clearinghouse for Birth Defects Monitoring Systems. *Acta Genet Med Gemellol*. 1991;40:325–335.

31. Maymon R, Mendelovic S, Schachter M, Ron-El R, Weinraub Z, Herman A. Diagnosis of conjoined twins before 16 week's gestation. The 4-year experience of one medical center. *Prenat Diagn*. 2005;25:839–843.
32. Hansen M, Bowe C, Milne E, de Klerk N, Kurinczuk JJ. Assisted reproductive technologies and the risk of birth defects – a systematic review. *Hum Reprod*. 2005;20:328–338.
33. Goldberg Y, Ben-Shlomo I, Weiner E, Shalev E. First trimester diagnosis of conjoined twins in a triplet pregnancy after IVF and ICSI: case report. *Hum Reprod*. 2000;6:1413–1415.
34. Rimm A, Katayama A, Katayama A. A meta-analísis of the impact of IVF and ICSI on major malformation after adjusting for the effect of subfertility. *J Assist Reprod Genet*. 2011;28:699–705.
35. Zhu JL, Basso O, Obel C, Bille C, Olsen J. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. *BMJ*. 2006;333:679.
36. Mendilcioglu I, Simsek M. Conjoined twins in a trichorionic quadruplet pregnancy after ovulation induction with clomiphene citrate. *Fetal Diagn Ther*. 2008;24(1):51–54.
37. Fujimori K, Shiroto T, Kuretake S, Gunji H, Sato A. An omphalopagus parasitic twin after intracytoplasmic sperm injection. *Fertil Steril*. 2004;82:1430–1432.
38. Tanaka H, Takeda A, Izumi M, Okada H, Ishikawa S. Effects of antiepileptic drugs on delivery and early childhood - comparison among mono-therapies of valproic acid, phenytoin, carbamazepine and phenobarbital. *Rinsho Shinkeigaku*. 1991;3:266–269.
39. Brambati B, Lanzani A, Sanchioni L, Tului L. Conjoined twins and in utero early exposure to prochlorperazine. *Reprod Toxicol*. 1990;4:331–332.
40. Rosa FW, Hernandez C, Carlo WA. Griseofulvin teratology, including two thoracopagus conjoined twins. *Lancet*. 1987;1:171.
41. Métneki J, Czeizel A. Griseofulvin teratology. *Lancet*. 1987;1:1042.
42. Czeizel AE, Métneki J, Kazy Z, Puho E. A population-based case-control study of oral griseofulvin treatment during pregnancy. *Acta Obstet Gynecol Scand*. 2004;83(9): 827–831.
43. Mills JL. Malformations in infants of diabetic mothers. *Teratology*. 1982;25:385–394.
44. Lampé L. A szülészet-nőgyógyászat aktuális kérdései. (In Hungarian) *(Current Issues in Obstetrics and Gynecology. I-III.) Debreceni Orv*. Tud. Egyetem (Debrecen, University of Medicine); 1981.
45. Werteleczki W. Malformations in a chernobyl-impacted region. *Pediatrics*. 2010;125: 836–843.
46. Mudaliar AL. Double monsters: a study of their circulatory system and some other anatomical abnormalities and the complications in labour. *BJOG*. 1930;37(4):753–768.
47. Zake EZN. Case reports of 16 sets of conjoined twins from an Uganda Hospital. *Acta Genet Med Gemellol*. 1984;33:75.
48. Emanuel I, Huang SW, Gutman LT, Yu FC, Lin CC. The incidence of congenital malformations in a Chinese population: the Taipei collaborative study. *Teratology*. 1972;5:159–169.
49. Bland KG, Hammar B. Xyphopagus twins. Report of obstetric and surgical management of a case. *Centr Afr J Med*. 1962;8:371–375.
50. Ryden AL. Kasuistischur beitrog zur kenntris der gerburtron thoracopagen ZBL. *Gynak*. 1934;58:972975.
51. Berezowski AT, Duarte G, Rodrigues R, et al. Conjoined twins: an experience of a tertiary hospital in Southeast Brazil. *Rev Bras Ginecol Obstet*. 2010;32:61–65.
52. Savona-Ventura C, Grima S, Buttigieg GG. Conjoined twinning int he Maltese Islands. *J Obstet Gynecol*. 2009;29:599–604.

53. Liang J, Xu CI, Wang Y. Epidemiological survey of conjoined twins in China. *Hua Xi Yi Ke Da Xue Xue Bao*. 1999;30:56–68.
54. Tang Y, Zhu J, Zhou GX, Dai L, Wang YP, Liang J. An epidemiological study on conjoined twins in China, from 1996 to 2004. *Zhonghua Yu Fang Yi Xue Za Zhi*. 2007;41:146–149.
55. Beischer NA, Fortune DW. Double monsters. *Obstet. Gynecol*. 1968;32:158–170.
56. Robertson EG. Craniopagus parietalis: report of a case. *Arch Neurol Psychiatry*. 1953;70:189–205.
57. Potter EL Pathology of the Fetus and Infant. Chicago: Chicago Year Book Medical Publishers; 1961. p. 217.
58. Castilla EE, Lopez-Camelo JS, Orioli IM, Sánchez O, Paz JE. The epidemiology of conjoined twins in Latin America. *Acta Genet Med Gemellol*. 1988;37:111–118.
59. Källén B, Rybo G. Conjoined twinning in Sweden. *Acta Obstet Gynecol Scand*. 1978;57:257–259.
60. Edmonds LD, Layde PM. Conjoined twins in the United States, 1970-1977. *Teratology*. 1982;25:301–308.
61. Imaizumi Y. Conjoined twins in Japan, 1979-1985. *Acta Genet Med Gemellol (Roma)*. 1988;37:339–345.
62. Martínez-Frías ML, Bermejo E, Mendioroz J, et al. Epidemiological and clinical analysis of a consecutive series of conjoined twins in Spain. *J Pediatr Surg*. 2009;44:811–820.
63. Milham S. Symmetrical conjoined twins: an analysis of the birth records of twenty-two sets. *J Pediatr*. 1966;69(4):643–647.
64. Bender C. Studies on symmetrically conjoined twins. *J Pediatr*. 1967;70:1010–1011.
65. Hanson JW. Letter: incidence of conjoined twinning. *Lancet*. 1975;2:1257.
66. Bhettay E, Nelson MM, Beighton P. Epidemic of conjoined twins in Southern Africa? *Lancet*. 1975;11:741–743.
67. Viljoen DL, Nelson NM, Beighton P. The epidemiology of conjoined twinning in Southtern Africa. *Clin Genet*. 1983;24:15–21.
68. Mabogunje OA, Lawrie JH. Conjoined twins in West Africa. *Arch Dis Child*. 1980;55(8):626–630.
69. Kaplan M, Eidelman AI. Clustering of conjoined twins in Jerusalem, Israel: an epidemiologic survey. *Amer J Obstet Gynec*. 1983;145(5):636–637. DOI: https://doi.org/10.1016/0002-9378(83)91211-5.
70. Rees AE, Vujanic GM, Williams WM. Epidemic of conjoined twins in Cardiff. *Br J Obstet Gynaecol*. 1993;100:388–391.
71. Newman C. Together forever. *Nat. Geo*. 2006;209:148–152.
72. Hamon A, Dinno N. Dicephalus dipus tribrachius conjoined twins in a female infant. *Birth Defects*. 1978;14:213–218.
73. Schinzel AAGL, Smith DW, Miller JR. Monozygotic twinning and structural defects. *J Pediatr*. 1979;95:921.
74. Sellami A, Chakroun N, Frikha R, Bouayed NA, Amouri H, Rebai T. Xipho-omphalopagus conjoined twins in a spontaneous triplet pregnancy: autopsy findings. *APSP J Case Rep*. 2013;18:49.
75. Talebian M, Rahimi-Sharbaf F, Shirazi M, Teimoori B, Izadi-mood N, Sarmadi S. Conjoined twins in a monochorionic triplet pregnancy after *in vitro* fertilization: a case report. *Iran J Reprod Med*. 2015;13(11):729–732.
76. Allegra A, Monni G, Zoppi MA, Curcio P, Marino A, Volpes A. Conjoined twins in a trichorionic quadruplet pregnancy after intracytoplasmic sperm injection and quarter laser-assisted zona thinning. *Am Soc Reprod Med*. 2007;87(1) 189.e9–189.e12.

77. Conjoined Twins Info. https://flamsterette.wordpress.com/2011/03/07/conjoined-twins-info-as-of-august-2007/
78. Little J, Bryan E. Congenital anomalies in twins. *Semin Perinat*. 1986;10(1):50–64.
79. Holländer E. Wunder, Wundergeburt und Wundergestallt, Stuttgart, 1921;109.
80. Skylitzes J. A Synopsis of Byzantine History 811–1057. Translation by John Wortley, with Introduction by Jean-Claude Cheynet and Bernard Flusin and Notes by Jean-Claude Cheynet. Cambridge University Press, Cambridge – New York 2010. XXXIII, pp. 491.
81. Dreger Alice D. One of Us. Conjoined Twins and The Future of Normal. Harvard Univesity Press. Cambridge, Massachusetts, London, England. 2004;7.
82. Pataki G, Hudak I, Valalik I, et al. Successful Multistaged Operative Separation of 3-Year-Old Craniopagus Twins in A Multidisciplinary, International Collaboration. Elsevier. 2020. (article in press)

SECTION

Twin Families 3

CHAPTER

Growing up as twins: the perspectives of twin researchers

7

Adam D. Tárnoki[a], David L. Tárnoki[a], Júlia Métneki[b], Nancy L. Segal[c]

[a]*Medical Imaging Centre, Semmelweis University, Hungarian Twin Registry, Budapest, Hungary*

[b]*Hungarian Twin Registry, Budapest, Hungary*

[c]*California State University, Fullerton and Director of the Twin Studies Center, CA, USA*

7.1 Introduction

The investigators who have contributed short papers to this chapter are all twins. In addition, four of the five board members of the Hungarian Twin Registry (HTR) are identical twins. The investigators include identical twins, Drs. Ádám Tárnoki and Dávid Tárnoki, the founding members of the HTR, as well as Dr. Júlia Métneki, one of the pioneers of Hungarian twin research. Some twin researchers are fraternal, such as Dr. Nancy L. Segal who has a fraternal twin sister. These individuals highlight the importance of twin research as twins, based on their personal perspectives.

7.2 Adam & David Tarnoki (MZ twins or Identical Twins)

The motivation to become a twin researcher arose during our academic years, when we gave a lecture on the diseases of twins and the heritability calculation using twin research design. This event took place in Sarasota, FL, USA, in 2007. The lecture caught the attention of Istvan Luczek M.D., a gynecologist of Hungarian descent practicing in Ohio, who recommended that we visit the Twins Days Festival in Ohio, in 2008, where we conducted our first questionnaire-based data collection. The rest is history.

When our mother learned that she would deliver twins, she was very surprised. This happened during her academic years when she was studying medicine. Adam was in a vertex position (head down), and David was in a breech position (feet down). Luckily, since our mother studied gynecology at that time, she was aware that her obstetrician preferred to deliver babies naturally. However, she was adamant that she have a Cesarean section as per textbook indications, which considered natural delivery as a contraindication in such cases due to its risk. Accordingly, Adam and David were born three minutes apart with a C-section, Adam was the firstborn twin (Fig. 7.1).

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00019-2

FIG. 7.1 Adam (L) and David (R) Tarnoki, at about age one year.

Courtesy: Drs. Adam and David Tarnoki.

We studied in the same class except for one semester, when the teachers tried to separate us. One semester later we were back in the same group, and our efficiency showed no difference due to being apart. According to psychologists, it is worth considering having twins in separate classes if they have very different abilities. Thus, the negative effects of constant comparison are less pronounced, and they are less likely to fail. However, our abilities were closely matched, and we enjoyed being together (Fig. 7.2).

It was not easy to prepare for our application to universities. We applied to the same specialties, and, after successful written and oral exams—with similar points—we were both admitted to medical university. During the first years, we wanted to start student scientific work. Due to our twinship, we chose the following topic: "Twins' diseases." We began looking for a mentor who was a twin researcher from Hungary, and thanks to the Internet, we found Dr. Júlia Métneki, who was a twin

FIG. 7.2 Adam (L) and David (R) Tarnoki in kindergarten.

Courtesy: Drs. Adam and David Tarnoki.

herself. Our first twin study was on the heritability of how weather changes affect Hungarian twins. Later, we gave our first scientific presentation in the United States. As previously mentioned, Dr. Luczek, a famous gynecologist from Ohio, attended the presentation, and subsequently invited us to the Twins Festival, which was very close to his home in Solon, OH. A few years later, we returned to Twinsburg with some Hungarian researchers, to conduct a comprehensive cardiovascular twin study on atherosclerosis. Since 2007, we have been working with Dr. Métneki. We suppose that, as twins, daily work as twin researchers is much more meaningful for them than itis for non-twins.[1]

We discussed everything with each other, and we spent the daytime mostly with each other, except when we had to work separately. Adam (the firstborn twin) was the "leader," while David, the second born twin, has always had more practical skills. Therefore, we can work very well together because we complement each other during the whole day (Fig. 7.3).

Twins have a constant companion from the very beginning, and they develop close relationships with each other. After marriage, the relationship between us naturally became a little less involved, as more attention was paid to the spouse at the expense of the twin brother. Finding a partner was not easy for us as we had spent a lot of time together during our childhood as well as our university years. Due to our similar taste, our choice fell on two ladies of the same occupation, who had graduated from the same law school but did not know each other. However, it tells a lot about the kind of relationship, including our taste in partners. We both met our wives online a few months apart because they look different, there was no problem distinguishing between them. The extant research presents a mixed picture regarding whether or not identical twins choose similar mates.[2,3] When Adam's little daughter

FIG. 7.3 Adam (L) and David (R) Tarnoki, at about age three years.

Courtesy: Drs. Adam and David Tarnoki.

was born, she often had trouble figuring out who her father was when we stood next to each other—this is a situation that many young twins confront. However, by the age of several months, she could differentiate between us.

As university teachers, we have worked with several twin medical students who were also interested in twin research. One of them became a pediatrician and another is a PhD student. A Japanese twin pair, who studied medicine in the English faculty of Semmelweis University in Hungary, wrote their theses on twin research under our supervision. They returned to Japan after graduation and are in contact with the Osaka Twin Registry (Fig. 4).

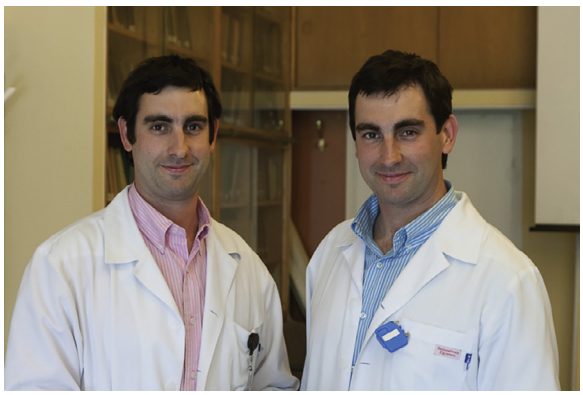


FIG. 7.4 Adam (L) and David (R) Tarnoki at Semmelweis University, Budapest.

Courtesy: Drs. Adam and David Tarnoki.

In Hungary, experience shows that it is much easier for twins to recruit twins for research, as it seems more credible for them to be invited to a twin study by twins. This way, it is easier to inform potential participants about how we the researchers saw the research, for example, what kind of examinations/tasks need to be done, how long they will take, and what the purpose/outcome of the study is.

As part of a cardiovascular twin study performed in 2009, we wanted to involve a dizygotic female twin pair; however, only the older sister's phone number was available. She was willing to come for the research but only alone. Surprisingly to us, she did not know her sister's phone number. The reason why she wanted to come alone was that she and her twin sister had quarreled before and had not talked to each other for many years. Shortly afterward, her sister's phone number was found and she asked to arrange for her sister to come on a separate day as she did not wish to meet her. Her sister also wanted to participate, but on a separate day. Several years later, fortunately, they reconciled thanks to the research and they participated in subsequent studies together.

The great advantage of being twins during research is that they can share tasks with each other. During the research, the brother or sister can always assist and help, thus the examination time can be reduced. We find that this is always true for us.

7.3 Julia Metneki (MZ twin)

I am working on twin research as a biologist, but I am also a twin myself. In elementary school, I took part in a twin study with my sister Esther, having been the subject of extremely exciting twin studies. This event made a great impact on me.

I was born after World War II, in 1946—it was only while giving birth, after I was born, that my mother found out that she had been pregnant with twins. My parents were flabbergasted, not knowing whether to be happy or worried about the double 'child-blessing.' We were born underweight at just 1700 g each. The incubator was replaced by hot water bottles in our cradle, and in the absence of infusion, we received mother's milk with an eyedropper every half hour, which we sometimes threw up. It was not until the age of 3 months when we reached the average birth weight of healthy babies. Our parents planned to name Esther for their future baby girl, but since I had a less favorable life expectancy as a firstborn, my second-born sister got the previously planned name (Fig. 5).

I have some visual memories from my childhood, and in those, I never see myself alone, but always with my sister. Usually, we referred to ourselves as "us," even when the other was not present. (The same thing happened with Adam and David.) As identical twins, we were extremely similar to each other, so much so, that even our father, relatives, and the family's best friends confused us quite often. The feeling of discomfort caused by the similarity was obviously increased by the fact that our parents dressed us in exactly the same way.

As the only pair of twins at our school, we undeniably aroused interest from our peers. In class, we often sat in the double desk, side by side, and had the same

FIG. 7.5 Julia Métneki (L) and her twin sister (R), Esther at age 4 years (1950) and 67 years later (2017).

Courtesy: Julia Métneki.

friends. We were anxious girls, but the constant presence of the other twin gave us a tremendous sense of security and reassurance. Therefore, if one of us was ill, the other one often simulated illness, so neither of us would have to go to school alone. Sometimes we changed roles, and when called to answer, we responded instead of the other, which initially seemed like fun, but it did not really make much sense, because whatever I knew, so did Esther, and vice versa.

While we were young, we did not recognize this high degree of similarity, but now we often cannot identify who is who in childhood photos. We were confused on several occasions and in certain situations. For example, as adults, at the first twin ball organized in Budapest, in 1983. Esther went to the restroom and saw me come face to face with her. Esther was just about to tell me something, when she hit the wall mirror. It was shocking for her not to recognize her own reflection.

What was so typical of our childhood was that we internalized each other's experiences as our own, as if those things had happened to us, as well. For example, when Esther was called to answer at school, I was almost more excited about her success than she was. If she got a bad grade, it hurt me even more, and when Esther cried, my eyes filled with tears, too. Our hope for each other's success was mutual.

Another family legend demonstrates the commitment of my sister. At the age of 10, Esther had warts on her hands that were to be burned off by a dermatologist. During the treatment, while she was quietly enduring the pain like a real hero, I was screaming outside the door, feeling Esther's pain. (Later, interviews with twins confirmed my earlier guess that empathy is much stronger for twins than for siblings and for singletons.) Not all researchers, such as Dr. Nancy Segal, find persuasive evidence that twins feel each other's physical pain at the same time without knowing that the brother of sister is in real pain. Of course, I knew that Esther was going through a difficult procedure which may explain my response.

We were used to the same things happening to us, whether we were good or bad. When I was unexpectedly operated on with appendicitis at the age of 14 years, Esther hardly found her place during my one-week absence. She felt it was "unfair" to miss out on something.

Beyond the similarities in our looks, our thoughts and interests were also the same. We both played the piano (there were several four-handed pieces in our repertoire), we liked the same dishes, and we were passionate about the same poems, actors, and music. Our father was a medical doctor who orientated our interest toward healthcare and medicine. In our most common childhood games, we played at being doctors, examining toy dolls, injecting them, and operating on them. Later in life, we both ended up working with diseases, just in different professional areas.

Esther and I understood each other almost without words. From each other's eyes and movements, we discerned what the other one wanted or needed. Of course, we sometimes quarreled and even hit each other, but after our mother separated us. Then, we started looking for each other's company within minutes. As I remember vividly, we laughed a lot and were often suffocating with laughter. Today, we spend time together much less frequently, but we cannot giggle with anyone as much as we used to with each other, and we sometimes relive this carefree part of our childhood.

Typically for most firstborns, I was the dominant twin, the one who initiated our games and activities, that is, the 'spokesperson'. The more peaceful and accepting Esther did not resent me in any way since it was convenient for her to have me handle everything, as if I were her secretary.

This close relationship was natural for us until our final exams and high school graduation, and we had a wonderful time in this symbiotic relationship. We mostly

enjoyed the benefits of twinning and noticed its drawbacks less that may result from constant comparisons. Despite our idyllic relationship, our parents clearly saw the disadvantages of this twin situation. They advised us to continue our studies at different schools, a decision that came to us unexpectedly. We simply could not imagine our lives without each other's constant presence. Sadly, we finally resigned to the situation. My more practical sister applied to the Faculty of Dentistry at the Medical University, and I started my studies at the Faculty of Biology at the University of Sciences. At that time, I was already thinking that this training in biology could give me a good foundation for realizing my dream: to carry out twin research in the future.

In time, I finally agreed with my parents' decision. It was the right time to start our independent lives. But then, at the age of 18, I felt as if I was one half of a human being cut in two. I felt the lack of my sister physically, too. She was not walking alongside with me on the street, and she was not there to confirm my decisions. Half a century has passed since then, but in a sense, I feel her absence even today.

After completing our university studies, Esther began practicing as a dentist in a small rural town, 120 km away from Budapest. Her decision scared me—I did not understand why my twin sister wanted to leave our common home to move to a strange city where she did not know anyone. "How will she do it alone?" I asked. I was also thinking that all of this may have happened due to my dominant nature. In the end, it was proven that our separation became beneficial in all respects.

It was only in the 1990s that—by studying the twin literature—that I could finally understand what must have been behind Esther's decision. Regine Billot, the French author of *Les Jumeaux* (1991), wrote about the difficulty of separating twins.[4] She supposed it was logical that the suppressed twin wanted to become independent, and therefore exited from the close twin relationship to avoid situations that were often disadvantageous. Paradoxically, twins would be looking for a partner earlier, would get married sooner than their twin sibling, perhaps because stronger emotions were at work to gain their freedom. I believe this refers mostly to identical twins.

Returning to my career choice, my childhood dream was finally fulfilled, and over the past nearly half-century, I have done a number of national and international twin studies with my mentor, Professor Andrew Czeizel. Initially, my research work was mostly theoretical, so, in fact, I rarely had any personal contact with twins—this gave rise to a strong feeling that something was missing. Change did not occur until the early 1980s when 100 twin pairs were involved in an international adult twin study concerning the heritability of lactose intolerance.[5] On the day of the examination, the twins showed an amazing amount of enthusiasm—in fact, the atmosphere resembled that of a folk celebration. Following up on the initiative of the participants, we created a "twin club," and the atmosphere of the monthly gatherings was intimate and family friendly from the start. The most successful and attractive events were the "twin festivals" and the "twin balls," where the stars of the party were, of course, twins. These successful events further increased interest in twin research (Figs. 6 and 7).

In 1989, at the Twin Congress in Rome, I met Dr. Elizabeth M. Bryan, a British pediatrician, and I purchased her book *Twins in the Family*.[6] While reading this book, I realized how important it would be to write a similar handbook in Hungarian that

FIG. 7.6 A group of identical twins at the foundation of the Budapest Twin Club. Budapest, (1982).

(Photo credit; Imre Benkő).

FIG. 7.7 Leaders of the Hungarian Twin Club (Ildikó Busi and Teresa) with the Presidents of the American Twins Association (Judy Stillwagon and Julie Kirk, and Lew and Lee Vaughn), at the Twin Ball held in Budapest, in 1983)

(Photo credit; Imre Benkő).

would provide theoretical and practical knowledge from conception to adulthood for parents expecting and raising twins. After a long "labor," my *Book of Twins* was published in 1997, and the revised version was released with up-to-date information in 2005.[7]

In 2006, I published another book about conjoined twins, in cooperation with a physician. In the book, nearly 200 such Hungarian cases detected from the 14th century until the early 2000s are described and evaluated. This work is an overview of the ethical, legal and religious aspects of conjoined twins, documented with interesting illustrations.[8]

Finally, one more thought about the twin situation. Most psychologists agree that the twins must eventually separate. However, since I met the radiologist twins, Ádám and Dávid Tárnoki, I am not sure of this advice, because they have been extremely successful in their field of expertise and in twin studies, following a common path, helping and complementing each other. "One and one is not always two, sometimes the double"—as their example shows.

7.4 Nancy L. Segal (DZ twin)

7.4.1 Personal background

I am passionate about twin research. Twin studies offer many elegant ways for examining the interplay between genetic and environmental influences as they affect human development. I am also intrigued by twinning as a phenomenon—what it is like to be an identical or a fraternal twin, how we can best raise and educate twins, and why there is universal interest in twins.

My fraternal twin sister, Anne, and I were born in Boston, Massachusetts, the only children in the family. My mother was shocked to discover that she was carrying twins when she went for her five-month pregnancy checkup. Part of her surprise came from the fact that there are no twins on the maternal side of our family. However, one of my father's uncles had been born a twin, although his twin brother died shortly after birth. Of course, the twin type of his singleton twin uncle is unknown, because zygosity testing was not routinely perfomed (and still is not done unless twins are enrolled in research) and DNA analysis had not been developed, given that. In addition, my father had first cousins who were identical female twins; they were considered to be identical based on their matching physcial appearance. Even today, the transmission of twinning in families has not been fully worked out by geneticists. We do know that fraternal twinning seems to run in families, and that identical twinning seems to run in some families.[9] Fraternal twinning has also been positively associated with factors such as older maternal age, heavier maternal weight, taller maternal height, African ancestry, and increased coital frequency.[10]

My family lived in Boston for a very short time before moving to Philadelphia when I was less than one year old. At age four, my sister and I were assigned to different kindergarten classes at the local elementary school—Anne adjusted easily,

while I was traumatized and missed her terribly. Today, at least in the United States, most educators believe that young twins should be placed apart in school or they will not develop separate identities. However, research does not support this claim and many studies indicate that young twins perform better together.[11] Given the foregoing and my own experience as a young twin, I believe that each pairs' school placement should be considered on a case-by-case-basis, and that parents deserve to contribute to this decision. A year later, when I was five, my family moved to New York City and remained there for the rest of my growing up years. My sister and I were placed together in kindergarten, first grade and second grade. We were then placed in separate classes from the third grade on and attended different schools from the seventh through twelfth grades, but we were ready to be apart (Fig. 8).

An assignment in a senior-level psychology class at Boston University drew my attention to twin studies. The professor asked for an essay on personal adjustment and I immediately thought about my experiences at school as a twin. The studies I read for this assignment were informative, insightful and enjoyable like no other

FIG. 7.8 Dr. Nancy L. Segal (R) and twin sister with their mother, at about age four years.

Photo Credit: Alfred M. Segal, Courtesy, Dr. Nancy L. Segal.

topic I had previously investigated. I knew that twin research would be the focus of my future academic career.

Upon graduating from Boston University, I completed a master's degree in Social Sciences at the University of Chicago, in 1974. My thesis was an overview of methods and findings in twin research. I went on to obtain my doctoral degree at the University of Chicago, in 1982, with a dissertation on cooperation and competition between young twins. Some summers and semesters during my graduate school years were devoted to twin studies. I spent the summer of 1974 at the National Institutes of Health, in Bethesda, Maryland, working on follow-up data from an earlier study of the identical Genain quadruplets—all four sisters suffered from schizophrenia.[12] I was a visiting student at Indiana University in Bloomington, Indiana in the spring of 1975, working on twin studies with Drs. Richard J. Rose and Walter E. Nance. In the summer of 1975, I attended a National Institute of Mental Health-sponsored program at the University of Colorado's Institute for Behavioral Genetics.

7.4.2 Professional history

After graduating from the University of Chicago I became a post-doctoral fellow and research associate at the University of Minnesota (1982–1991). During this time, I worked on the Minnesota Study of Twins Reared Apart (MISTRA), directed by Professor Thomas J. Bouchard, Jr. In 1985, I was appointed Assistant Director of the Minnesota Center for Twin and Adoption Research. Those 9 years were a highpoint of my career because I was so fortunate to meet the separated twins—I learned their personal stories at the same time that I helped gather their behavioral and physical data. In 1991, I joined the psychology department at California State University, Fullerton. One of my first tasks was establishing the Twin Studies Center to support student and faculty research with twins. Over the years, several individuals have donated books, journals, photographs and funds, making this center, especially its library, a unique resource.

My current twin studies address tacit coordination, social closeness, twin loss, personality and appearance, genetic and environmental influences on ability, personality, adjustment, and sexual orientation and identity. I study MZ and DZ twins, young Chinese twins reared apart, virtual twins (same-age unrelated children reared-together), twin-families, twins switched at birth, and unrelated look-alikes.

7.4.3 Professional activities

I have written seven books on twins[13] and have co-edited a conference volume.[14] My book, *Born Together-Reared Apart: The Landmark Minnesota Twin Study* (2012), describes the origins, methods, findings, and implications of the Minnesota Study of Twins Reared Apart. My seventh book about twins is titled, *Deliberately Divided: Inside the Controversial Study of Twins and Triplets Adopted Apart*.[15] This book is a detailed investigation of the 1960s and 1970s study conducted in New York City, in which twins and triplets were purposefully placed apart and studied until they turned

twelve. The twins' adoptive parents were never told that they were raising a singleton twin child. This study was featured in two documentary films, *The Twinning Reaction* (2017) and *Three Identical Strangers* (2018).[16]

I strongly believe that twin researchers have a responsibility to maintain close ties with the public, and not publish their findings solely in the scientific literature. It is for this reason that I write a regular column for the journal *Twin Research and Human Genetics*. Each of my contributions surveys an area of interest to twin studies, summarizes findings from several timely twin studies and reports a number of human interest stories about twins, of which there are many. These articles are sometimes adapted for publication in my *Psychology Today* magazine blog, *Twofold*. I have authored or co-authored three articles for the *New York Times's* Gray Matter column, two of them on twins. One of them concerned the social ties between twins and the other concerned the breastfeeding of twins in male-female pairs.[17]

One of the greatest pleasures of being in twin research is watching separated twins meet for the first time. Most memorable is my witnessing of reunions between two 6-year-old identical twin girls and two 78-year-old fraternal twin women. One of the young twins had been adopted by a family in the lively capital city of Sacramento, California, while her twin sister had been adopted by a family from the tiny village of Fresvik, Norway. Their adoptive parents met in China when they went to pick up their daughters. The mothers immediately recognized the physical resemblance between their babies and stayed in touch. They decided to have the girls' DNA tested and compared, and the results revealed that they were identical twins. One of the mothers contacted me and I arranged for the *BBC* to have the twins meet and to film their meeting. It was heartwarming to watch the twins jump up and down at the first sight of each other. Despite speaking different languages, the two girls got along beautifully with one another.

The older pair, which I wrote about in one of the Gray Matter columns, also lived in different countries—England and the United States. I was able to obtain funds to fly both twins to California where they met each other in a hotel room near my campus. It was lovely to see them recognize some common features, even though they looked fairly different. Unfortunately, one of the twins passed away approximately eight months after they had met.

A recent twin project of great fascination for me involved two sets of identical male twins, from Colombia, South America. It happened that one twin in each pair had been inadvertently exchanged with a twin in the other pair when the babies were less than one week old. Each pair of boys grew up thinking that they were fraternal twins when, in fact, they each had an identical twin brother they did not know about. The truth was revealed when the twins living in the country moved to the city and one of the twins was mistaken for his identical brother. They were twenty-five years of age at the time—the revelation was shocking and disturbing at first, but all four brothers and their families have come to terms with it. The twins now regard themselves as a group of four—one family. I traveled to Bogotá twice to test and interview the four young men. I wrote about my research and the story of their lives in my 2018 book, *Accidental Brothers*.[18]

7.4.4 Closing statement

Twin research has undergone significant change in that many researchers are turning their attention to the molecular bases of behavior. A question of great interest is why one identical twin may become affected with a disease and the other will not. Another question concerns how such information can be used to help the general public. However, twin research remains as vibrant today as it was in 1875 when Sir Francis Galton first recognized the power of twin research to tell us how we come to be the people that we are.[17]

I am very happy to be a twin for a number of reasons. At an early age, twinship gave me appreciation for genetic influences on development. Being a twin has also helped me invite twins as participants in research, as we come to the task knowing that we share something very important. Finally, twinship has given me my sister Anne, whose friendship and support I treasure above all others. This accident of birth has made me aware of what I have enjoyed and what has been lost by twins who did not grow up together.

References

1. Tarnoki AD, Tarnoki DL, Littvay L, Métneki J, Melicher D, Pari A. *Twin research and epigenetics*: *Medicina*. In Hungarian; 2020.
2. Lykken DT, Tellegen A. Is human mating adventitious or the result of lawful choice? A twin study of mate selection. *J Pers Soc Psychol*. 1993;65:56–68.
3. Rushton JP, Bons TA. Mate choice and friendship in twins: evidence for genetic similarity. *Psychol Sci*. 2005;16:555–559.
4. Billot R. *Les Jumeaux de la conception a l'école.* (In French) *Twins from the conception to school age*: Éditions Balland; 1991.
5. Métneki J, Czeizel A, Flatz SD, Flatz G. A study of lactose absorption capacity in twins. *Hum Genet*. 1984;67:296–300. https://pubmed.ncbi.nlm.nih.gov/6432676/.
6. Bryan EM. *Twins in the family—a parent's guide*: *Constable* London; 1984.
7. Métneki J, Fogamzástól a felnőtté válásig. (In Hungarian) (From the conception to adulthood). *Ikrek könyve*. Melánia. I. 1997. Second edition: 2005. https://www.antikvarium.hu/konyv/metneki-julia-ikrek-konyve-102838.
8. Varjassy P, Métneki J. Egy vagy kettő? Összenőtt ikrek Magyarországon. (In Hungarian) *One or two? Kossuth Kiadó*. 2008. https://moly.hu/konyvek/varjassy-peter-metneki-julia-egy-vagy-ketto.
9. Segal NL. *Twin Mythconceptions: False Beliefs, Fables, and Facts About Twins*. San Diego, CA: Elsevier; 2017.
10. Segal NL. *Entwined Lives: Twins and What They Tell Us About Human Behavior*. New York, NY: Plume; 2000.
11. Rosenthal D, ed. *The Genain Quadruplets*. New York, NY: Basic Books; 1963.
12. (a) Segal NL. *Indivisible by Two: Lives of Extraordinary Twins*. Cambridge, MA: Harvard University Press; 2005/2007; (b) Segal NL. *Someone Else's Twin: The True Story of Babies Switched at Birth*. Amherst, NY: Prometheus Books; 2011; (c) Segal NL. *Born Together-Reared Apart: The Landmark Minnesota Twin Study*. Cambridge, MA: Harvard University Press; 2012.

13. Segal NL, Weisfeld GE, Weisfeld CC, eds. *Uniting Psychology and Biology: Integrative Perspectives on Human Development*. Washington, D.C.: American Psychological Association Press; 1997.
14. Segal NL. *Deliberately Divided: Inside the Controversial Study of Twins and Triplets Adopted Apart*. Lanham, Maryland: Rowman & Littlefield; 2021.
15. (a) Shinseki L. *The Twinning Reaction*. U.S.: Fire Horse Pictures; 2017; (b) Wardle T. *Three Identical Strangers*. London, UK: Raw Films; 2018.
16. (a) Segal NL. *The Closest of Strangers*: New York Times, Sunday Review (Gray Matter), SR12; 25, May 2014; (b) Segal NL, Kanazawa S. *Does Breast Milk Have a Sex Bias?*: New York Times, Sunday Review (Gray Matter); 20, January 2017:8.
17. Segal NL, Montoya YS. *Accidental Brothers: The Story of Twins Exchanged at Birth and the Power of Nature and Nurture*. New York, NY: St. Martin's Press; 2018.
18. Galton F. The history of twins as a criterion of the relative powers of nature and nurture. *J Anthropol Inst*. 1875;5:391–406.

CHAPTER

Parenting twins, triplets, or more

8

Monica Rankin
International Council of Multiple Birth Organisations (ICOMBO)

8.1 Parenting twins, triplets, or more

Throughout this chapter, I will be referring to twins, but all comments relate equally to all multiple birth families.

Parenting twins is a unique experience, right from the time that the twin pregnancy is diagnosed and it brings challenges but also rewards. The first thing that parents are often told is that a multiple pregnancy is a high risk pregnancy. Many other thoughts will whirl through the minds of the parents-to-be, but overwhelmingly they will wonder how they will cope with more than one baby at once—emotionally and financially as well as practically. Parenting can be a daunting prospect, expectant parents are told that it is challenging to raise a baby so how much harder will it be to raise two, or even more? There may also be impacts on family dynamics if there are already other siblings and/or the mother has no partner available for support. Preparation and connections to the multiple birth community are the key to surviving parenting of twins—from pregnancy right through the school years and beyond.

8.2 Pregnancy

I won't comment on the medical issues of a twin pregnancy as they are covered in another chapter, I will address it from the parent's perspective and look at the various issues compared to a single pregnancy. Once a pregnancy is diagnosed as a multiple birth, there will be additional medical appointments and this often requires taking leave from work, there are often additional medical expenses due to the extra ultrasounds and tests that are required with a twin pregnancy, compared to a single pregnancy. Then there is the stress and worry that comes with being pregnant with twins. It is a high-risk pregnancy and the mother needs to take extra care during this pregnancy—so the twin parenting starts here. The risks and parental concerns may also be impacted by the type of twins being carried and if there are other risk factors (e.g., maternal age, health status, etc.).

How is a twin pregnancy different to a single pregnancy?

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00020-9

If the mother is working, it is almost certain that she will need to commence family leaves earlier and therefore the family income will decrease earlier than planned. Each country has its own family leave provisions, and some are more generous than others when it comes to multiple births—parents are advised to check their eligibility to understand their rights.

The additional medical appointments and tests, compared with single pregnancy, adds to the increased costs of having twins. The mother can expect to gain more weight when carrying twins compared to a single baby and of course, her stomach will be much larger! This has impacts in many areas—the ability to fit behind the steering wheel to drive a car, to be comfortable to sleep, and sometimes toward the end, even finding comfortable places to sit and rest. The mother needs to listen to her body and her doctors, and do what works for her and is appropriate for her babies.

During the pregnancy, parents will be physically preparing for the arrival of the babies. There are many items to organize—cots, prams, clothing to name a few. The list can be quite daunting and very expensive. It is not necessary to purchase new items for the babies; they will be just as contented in preloved furniture. Family and friends will make offers to donate or lend their baby items, so it can be a good opportunity to save some money. When purchasing clothing items remember that twins do not require twice as many items and triplets do not require three times as many. In addition, many people will want to welcome the new additions to the family with gifts. Parents may find that they have far more outfits than the babies will ever wear! Being pregnant with twins, triplets or more is a time when the parents begin to enjoy the well wishes that come with being parents of multiples—you are special!!

8.3 Birth

The pregnancy of multiples will be shorter than that of a single baby. The delivery of twins has a much higher chance of requiring intervention than the birth of a single baby. When delivering triplets or more, most specialists will want the mother to have a caesarean section rather than natural delivery. Parents should be prepared for all eventualities and try to "go with the flow." The doctors will recommend procedures that are in the best interest of both mothers and babies. The newborns are likely to be low birthweight and may be born prematurely. Some babies need to spend time in a special care nursery and a smaller number require more intervention and spend time in a neonatal intensive care unit. This can be a very scary place for a new parent and it is quite confronting to see your newborn hooked up to numerous machines and tubes. Staff in intensive care units and special care units will explain what is happening to the babies and how the parents can be involved in their care.

The early days are about recovering from the birth and learning how to feed and care for two or more tiny babies. Most mothers will want to breastfeed their babies and if this proves difficult, it can be a very stressful time. If the mother can be prepared by attending classes or reading about how to feed multiples, it will assist greatly at this time. In most countries, there are reputable groups such as La Leche League and breastfeeding associations that offer great support.

8.4 Early months

Coming home with two or more babies can be very daunting, particularly if one or more has health issues, or the mother has no support partner. The first weeks will be very tiring, and it will feel like it is 24 hours of feeding and nappy changing, day after day. However, things do improve as the babies grow, and especially when they begin to interact with their surroundings. There is nothing so precious as the smile of a young baby—just imagine receiving that smile two or three times over!! It is worth considering asking for assistance in the few weeks—grandparents and other relatives and friends often love to be asked to help. If there is no family nearby and it is affordable, then paying for some housekeeping can be a great idea.

It is not uncommon for the mother to be discharged from the hospital before the babies, particularly if they were born prematurely. They may need time for their lungs to develop, to learn how to feed, or just to grow and gain weight. Whatever the reason, it is difficult for the mother to go home without her babies. The hospitals are supportive of the parents and will allow generous visiting rights and also assist the mother with establishing feeding. The parents need to keep remembering that it is only for short while, that they will soon have their babies with them. If the babies are very ill, it may feel like a rollercoaster ride for the first few weeks with improvements and then setbacks in the babies' health. The parents can use this time to get to know each of their babies individually, as well as spend some time together as a couple before the babies come home.

With two or more babies in the home, life is very busy, but the parents need to self-care as well as caring for their infants. The incidence of postnatal depression is much higher in parents of multiples than it is in parents of single babies. It is important that parents are aware of this, and that health professionals working with the family are also aware so that early intervention can be offered if necessary.

Some mothers find breastfeeding two or more babies quite a challenge. It is advisable to seek the advice of a breastfeeding consultant who is experienced in feeding multiples. Talking to other mothers in the local multiple birth group can be beneficial, they have "been there, done that" so can offer advice built on their experiences. If the parents choose to formula feed their babies—they need to feel supported in their decision. It is not an easy decision and there are many instances when mothers are told they are not giving their babies a good start in life if they do not breastfeed. Mothering multiples are a challenge and sometimes breastfeeding is just too difficult for the mother. Once she has exhausted all reasonable attempts to breastfeed, her decision to use a formula needs to be respected by all who are working with her and the family. It is not helpful for her to hear that she is not a good mother because she is using formula.

Sleep can be a challenge for the new parents in the early months. It may seem like they are living on minutes of sleep each day, instead of hours of sleep. It can be helpful to have the babies in a routine as much as possible. Some parents find it better to allow each baby to independently wake and feed overnight; other parents prefer to wake the second twin when the first wakes. There is not right or wrong

decision—parents need to do whatever works for them. In many countries, residential sleep schools provide classes for the parents, to assist them in understanding the needs and wants of their babies, learn how to settle them and to establish routines.

8.5 Toddler

The toddler years are fun times! During this time in a person's life, their personality really becomes obvious. As a parent, it is great to see how your child is developing. It is amazing to see that in stereo. Of course, the toddler years can be challenging too. As the saying goes "double the trouble, twice as nice." And this sums up the toddler years. Two little ones can be very cute but the combined efforts of two when they are intent on destruction—it can seem like world war three is about to commence. Parenting can be very intense during these years, while the little ones learn to negotiate their way in the world, and take the first steps toward independence—feeding, toileting, and dressing are all times that can be challenging with a toddler. When you have twins, multiply that by two. The best advice is to take it one step at a time, sometimes one day at a time. Decide what is best for your family and work with that. There is not one solution that fits all families. Each solution is different, just as each family is different. Some parents have returned to the paid workforce by the time their babies have reached this stage, so they are juggling work, childcare, and home life. It is a balance and mistakes will inevitably occur. By learning from our mistakes, we learn and grow as parents.

8.6 School years

Twins are just like all other children—for some the school years are a breeze and for others it is a challenge that they cannot wait to get through. Of course, with twins there is the fact that they will always have someone there with them—sometimes this is good and sometimes not so good. How the children cope with this is often related to how well the parents cope. The parents need to remember that their concerns are often picked up by the children. The first question most parents of multiples ask when thinking of school is "should I separate them at school?" There is no definitive answer for this. As usual, what works for one family won't necessarily work for another. The best advice is to read what experts say, talk to parents in a similar situation and talk with the school—both teachers and principal if possible. For some children, it is important that they be in the same class, but for others, it is best to be in separate classes, sometimes even different schools. Look at what suits each child best and try to make it work for each individual child. And remember, that nothing is set in stone, these decisions can be changed each year. The question of separation should be considered each year to ensure that what is occurring is best for the children.

Another common concern of parents regarding the education of their children is what to do if one is performing at a much higher academic level than the other(s). This can be tricky to navigate with the children. It is often the case that they are

growing and developing at different rates, and that they will have different strengths and weaknesses. So, while one might outperform the cotwin this year, it might be reversed next year. Or perhaps one twin excels at sport and the other excels academically. Just because they are twins, even identical twins, one should not expect them to grow and develop at the same rate, be strong or weak at the same tasks, or even have the same interests. They are individuals and everyone needs to treat them as separate individual people.

Further information on the schooling of multiples can be found at www.icombo.org.

8.7 Adolescence

For some teenagers, adolescence is not easy—handling puberty, learning to be independent, making educational and career decisions, establishing one's own identity. Twins and multiples face the same issues, but there are a number of other issues that are unique to multiples. One multiple may begin puberty before their comultiple(s), one multiple may want to begin (or have the opportunity to begin) dating before the comultiple(s), and the multiples have to learn to be independent of their comultiple. Many of the challenges of adolescence are present in all teenagers, but the situation is more complex with twins and multiples. During this time, they are learning who they are as a person, as well as separating themselves from the twinship. They will compare themselves against their comultiples and wonder why they are at different stages. Parenting can be very intense at these times. It is not the same as having two or three teenagers of different ages—it is easy to explain why they are at different stages if they are at different ages. It is the fact that there are two or more teenagers of the same age, who may also look exactly the same, but are experiencing life differently.

Parents have a special role to play in these adolescent years—guiding their children through this time and watching independent adults come out the other end. Support and encouragement to pursue their individual passions will make this road easier for multiples. It can be difficult to explain to a 12 years old why her sister has developed breasts and experimenting with makeup, for example, while she is still enjoying being a child. Each person, even identical twins, will commence puberty in their own time, and often at different times. The second twin is not slower, less mature, developmentally behind, or any other label. It is just part of being human.

"Can I choose the same college or university as my cotwin, what about choosing the same career?" Multiples need to understand that by expressing their own passions and interests, they are being truly independent, even if that passion and interest is the same as their comultiple. It is OK for both of the twins to choose the same career path, if that is what each of them truly wants. Parents need to understand that at this time in their children's lives, they may seem to become distant from each other as they work to form their own individual personalities and lives. It is important to support the children and allow them to develop freely, without pressure to "be like your twin."

The Finnish Multiple Birth Association has an excellent publication—*Multiple Birth Siblings as Adolescents: A guide for parents of twins and higher order multiples.* The publication is available in Finnish and in English, from the International Council of Multiple Birth Organisations, www.icombo.org. I highly recommend it to parents of multiples, as it provides valuable insights to the growth and development of multiples from the teenage years right into adulthood.

8.8 Special situations

Sadly, some parents do not have the joy of seeing both their twins live long and healthy lives. Losing a child to death must be one of the hardest things that a parent can face. Losing one of a set of multiples must be incredibly difficult. Not only is the parent grieving the loss of a child, but they have a constant reminder of what could have been every time they look at the surviving twin. On all those special moments—birthdays, Christmas, and family celebrations—how do you celebrate with one child knowing that the cotwin is no longer with you? There are a number of support groups for parents who have experienced the death of one or more of their multiple birth children. These groups tend to be administered by parents who have experienced this too. They are able to offer support and comfort from a place of understanding what life is like for these families. The best way to find these groups is to do an internet search, most groups are online and able to offer online support no matter where the family lives.

Sometimes one of the twins will have some additional needs or health issues, that are not shared with the cotwin. For example, one twin may have cerebral palsy due to birth complications, may have a condition such as Down's Syndrome, or one twin may develop significant health problems. In these situations, it can be extremely isolating for the parents. They are parents of twins, but they may not feel that they fit in the local multiple birth group as their twins are "different." The support groups that assist with the issues may not understand what it is like to have one "healthy" twin and one twin with the condition. It is important that the parents seek treatment and assistance for the twin who suffers the condition, while at the same time, not ignoring the "healthy" twin. They also need time, love, and attention. The best advice is to reach out to the multiple birth community, because there will be other families facing the same situation, who can offer support.

8.9 Adult twins

Parenting is a life-long journey, so it doesn't end when the twins turn 21 years of age. There will always some unique opportunities and challenges for multiples for the whole of their lives. The role of parents is to support their children as best they can, through the journey of life. Some of the unique challenges for adult multiples may be:

- One twin is has a much more successful career, earning a much higher salary than the cotwin.
- One twin is in a very happy and stable relationship while the cotwin is struggling to find a partner.

- The twin's partner doesn't get along with the cotwin's partner; or worse, doesn't like the cotwin.
- The death of the cotwin.

All of these situations may occur with siblings, but the challenges are much greater when it is the cotwin and not a sibling. All through their lives, siblings have been able to do things independently, they are not expected to achieve the same things at the same time, whereas twins are generally expected to be the same, act the same, and have the same achievements at the same time.

Many twins have a very close bond to each other. The introduction of a partner into the relationship can create problems. It is only natural that there will be jealousy when a third person is introduced into the twin relationship. For some twins, the cotwin will always be "number one" and if the partner is unable to accept this, then the relationship is doomed. Some partners work hard to destroy the twin relationship, and this can lead to major disharmony in the family. Communication between the twins at this time is key to developing healthy relationships within the family.

Having strong, healthy relationships with family and friends will help the twins to navigate through the more difficult times.

CHAPTER

9

Reared apart twins: Background, research, case studies and what they reveal about human development

Nancy L. Segal, Francisca J. Niculae
Department of Psychology, California State University, Fullerton, CA, United States

9.1 Introduction

Twins reared apart from birth are an informative variant of the classic twin method. The classic twin method compares physical and behavioral trait resemblance between pairs of monozygotic (MZ or identical) twins and dizygotic (DZ or fraternal) twins. MZ twins share all their genes having resulted from the division of a single fertilized egg, or zygote, within the first two weeks after conception. DZ twins share half their genes, on average by descent, having resulted from the fertilization of two separate ova by two separate spermatozoa. Greater MZ than DZ twin resemblance is consistent with, although not proof of, genetic influence on the traits under study.[1] Complete proof is not provided because it is always possible that MZ twins' environments match more closely than those of DZ twins, enhancing their similarity. This concept, known as the equal environments assumption (EEA), must be met if findings from a twin study can be considered valid and representative. The vast majority of research on the EEA finds little evidence that this assumption has been violated.[2]

A way of circumventing the issue of matched environments is by studying MZ and DZ twins reared apart from birth (MZA and DZA pairs, respectively). Resemblance between MZA twins is explained by genetic factors, given that they did not grow up together. Reared-apart twin pairs are relatively rare, but the availability of online social platforms, personal genomic services, and various specialized registries have facilitated reunions between twins, even those who had no knowledge of their twinship.

The purpose of this chapter is fourfold. It begins with a historical overview of reared-apart twin studies, highlights key findings from classic twin studies, describes several unique cases of twins who have grown up apart, and concludes with a brief statement of what the findings imply for how we understand human development.

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00002-7

9.2 Twins raised apart: Past, present, and future

Many people will be surprised to learn that the first mention of twins reared apart was not in the scientific literature but in the world of comic drama. Separated twins first appeared in the play *Menachemi* (*The Brothers Menaechmus* or *The Two Menaechmuses),* written by the Roman playwright, Titus Maccius Plautus (c. 205 BCE - 184 BCE). Interestingly, Sir Francis Galton, the Father of the Twin Method, whose 1875 study laid the foundation for future twin research and who studied behavioral resemblance in adoptive families, did not conduct or mention reared-apart twins.[3] However, Galton was aware that twins could be reared apart, given that he had received a letter whose sender described such a case.[4] Credit for the first scientific study of reared-apart twins belongs to Paul Popenoe (1922) for his case study of twins, Bessie and Jessie.[5] This case was later expanded upon by Hermann J. Müller.[6]

The first formal study of reared-apart twins was conducted at the University of Chicago in 1937. The investigative team consisted of biologist Horatio H. Newman, educational psychologist Frank N. Freeman and statistician Karl J. Holzinger.[7] Their book reports statistical findings on many variables (e.g., intelligence, personality, height, and weight) for 19 pairs of MZA twins, as well as 50 pairs of MZ twins reared together (MZT) and 50 pairs of DZ twins reared together (DZT). A fascinating compendium of life history material was included for each MZA twin pair. Key findings were MZA IQ intraclass correlations of $r_i = 0.68–0.74$ (The intraclass correlation, or $r_{i,}$ presented here was recalculated from the IQ data included in their book in order to compare consistency across studies.[8] It expresses the degree of resemblance, or shared variance, between the twins.)

The three researchers also presented within-pair correlations between the separated twins' IQ differences and educational differences of $r = 0.55–0.79$ and within-pair correlations between the separated twins' IQ differences and social differences of $r = 0.51–0.53$. The last two sets of correlations have been misinterpreted by some in ways that undermine the role played by genetic factors. However, the IQ correlation showing that co-twins are more like each other than they are to the members of other pairs is evidence of genetic effects.

The next major reared-apart twin study was reported in book form by James Shields at the Institute of Psychiatry at the Maudsley Hospital, in London, England.[9] Shields studied 44 MZA twin pairs in great detail and provided brief data for 11 DZA twin pairs. He also included comparative data on 44 MZT twin pairs and 32 DZT twin pairs. Shields presented a range of behavioral and physical findings, as well as a section detailing the life history backgrounds of the reared-apart pairs.

Several of Shields's personality results are worth noting. He found that MZA twins were more alike than MZT twins in both extraversion ($r_i = 0.61$ and 0.42, respectively) and in neuroticism ($r_i = 0.53$ and 0.38, respectively). Shields suggested that when twins grow up apart, they express their genetically based traits more freely because they are not in a relationship with their co-twin. This same pattern of results was found by several other investigators when comparing similarity in

divergent thinking and in extraversion between MZ twins living together and living apart; however, this result was not found for psychosomatic complaints.[10,11] More recent reared-apart studies have failed to find this effect, instead reporting that MZA and MZT twin pairs show similar levels of resemblance across personality traits.[12] Reasons for this discrepancy are uncertain, but may be a partial function of the personality inventories administered. Anecdotally, a number of researchers have observed that when twins are together, one co-twin tends to dominate the conversation, and that MZ twins appear to interact more similarly with others when they are apart.

The third major reared-apart twin study was conducted by Niels Juel-Nielsen in Odense, Denmark.[13] A special feature of Juel-Nielsen's study of 12 MZA twin pairs is that he included not just a sample, but the entire population of reared-apart twins, born between 1870 and 1934 who satisfied the specified criteria. Like his predecessors, Juel-Nielsen included life history summaries of each twin pair at the end of his book. He found few co-twin differences in general health (e.g., height, EEG); both similarities (appearance of disorder) and differences (injuries, infections) in somatic disorders; differences in verbal areas, intellectual differences associated with educational differences, and personality similarities (e.g., gestures, facial expressions) associated with genetic factors; and personality differences in areas associated with interpersonal contact (e.g., cooperation, need for contact). Few of the twins had expressed psychiatric childhood symptoms, but Juel-Nielsen recognized a complex interplay between heredity and environment when it came to behaviors in this domain.

The fourth and last major investigation was the Minnesota Study of Twins Reared Apart, directed by Dr. Thomas J, Bouchard, Jr., at the University of Minnesota, in Minneapolis.[14,15] This study was ongoing for twenty years (1979–1999); the first author (NLS) worked on that project for nine of those years, first as a postdoctoral fellow before becoming a research associate and Director of the CSU Fullerton Twin Studies Center (1982–1991). *This study is the only one to have included both MZA and DZA twins in the full test battery.* This methodological aspect is important because it avoided possible exclusion of dissimilar looking and/or behaving MZA pairs.

Twins spent an entire week at the university undergoing extensive medical and psychological examination; however, only selected findings are reported. In the domain of general intelligence, the weighted average intraclass correlation, based on 162 MZA twin pairs from the original four studies plus an ongoing, large scale study from Sweden was $r_i = 0.75$.[16] Looking at the correlations across studies shows little variation, attesting to the robustness of this finding. This is impressive, given that the studies were done by different investigators from different countries using different tests. The Minnesota Study reported an IQ correlation of 0.69 (primary test) and 0.79 (mean of three tests) for 48 MZA twin pairs. In addition, the twins' IQ scores were not associated with their parents' education, home facilities, family achievement, or intellectual motivation. A later analysis from the study reported a heritability estimate of 0.77 for general intelligence.[17] Of course, intelligence is also affected by environmental factors. A recent study of reared-together young twins from low socioeconomic circumstances showed reduced genetic effects.[18]

In the personality domain, it was found that MZA twins were as similar as MZT twins across eleven personality traits (median = 0.48, 0.52, respectively).[19] This indicates that personality similarities between biological relatives living together are explained by their common genes, not by their common environment. Heritability estimates were also approximately 0.50 for religiosity, social attitudes, and periodontal characteristics. These three findings remain remarkable because it had been assumed that such traits were mostly affected by environmental factors. For example, prior research had shown that young MZ and DZ twins were equally alike in religiosity—however, studies of adult twins show that MZ twins are more alike than DZ twins. This suggests that as people get older and able to choose their own lifestyle choices, their genetic inclinations are more freely expressed. Research in all three areas was largely revamped in light of these analyses.

By way of contrast, it is informative to compare more currently reported intraclass correlations for MZA twins, MZT twins, DZT twins and nontwin siblings (SIB) for the phenotypes mentioned above. For IQ the r_is are 0.75 (MZa); 0.86 (MZT); 0.60 (DZT); 0.47 (SIB). For height and weight, respectively, the r_is are 0.86 and 0.73 (MZA); 0.93 and 0.83 (MZT); 0.55 and 45 (DZT); 0.47 and 0.36 (SIB). For the personality trait of extraversion, the r_is are 0.51 (MZA); 0.53 (MZT); 0.17 (DZT); 0.22 (SIB).[20]

In general, within-pair resemblance is larger for pairs with higher degrees of genetic relatedness, but there are exceptions. IQ resemblance is somewhat higher for MZA than MZT twins, variously reflecting different educational backgrounds, but also the fact that most MZT studies were conducted using young twins living at home when family effects are most potent—in contrast with the MZA studies that assessed adults. Intraclass correlations for height and weight are also higher for MZT than MZA pairs, likely due to the female MZA twins whose susceptibility to environmental effects (e.g., pregnancy, diet, exercise, and hormonal fluctuations), as well as possible rearing effects, that reduced their resemblance. This trend has been previously reported in other reared-apart twin investigations.[21] The correlations for extraversion are nearly identical for the MZA and MZT twin pairs, indicating that shared environments play a negligible role in resemblance among family members living together. Instead, environmental influences on personality traits appear to come from events that are not shared among family members.[22] Examples might be taking an exotic vacation, reading a great book, or experiencing emotional trauma. However, some researchers have challenged this conclusion, asserting that the causal mechanisms underlying nonshared environmental variability in outcome are unknown[23]

Other important and influential reared-apart twin studies have been completed or are ongoing. Their locations are Japan, China, Sweden, and Finland.[24] Findings from these studies have been presented in research articles, rather than in book form as in the four investigations detailed earlier.

9.3 Fullerton study of Chinese twins reared apart

The studies described above mostly included twins who met for the first time as adults. In contrast, the Twin Studies Center at California State University, Fullerton

(CSU Fullerton) is conducting the first prospective study of separated twins, known as the Fullerton Study of Chinese Twins Reared Apart. The twins, mostly female pairs, were separated indirectly because of China's One-Child Policy that was enacted in 1979 and remained in place until 2015.[25] The policy limited urban families to one child and rural families to two. Given that the Chinese culture prizes sons over daughters, the policy led to the abandonment of hundreds of thousands of baby girls, with twins among them. The current sample includes 22 reared-apart twin pairs (15 MZA and 7 DZA); some sets come from Taiwan and Vietnam. Two pairs are male, and one pair is opposite-sex.

All twins completed a general mental ability test, while their adoptive parents completed a series of inventories and questionnaires concerning their child's background, health history, educational history, personality traits, behavioral problems, and creative tendencies. Parental characteristics, such as education, religious affiliation, and occupation, are also recorded. A companion study of 50 pairs of Chinese twins adopted together is also ongoing at CSU Fullerton. The reared-apart twin study began in 2005, yet participant identification and data collection have required considerable time and effort.[26] Three papers from the project, one on twins' first meetings, one on early behavioral problems, and one on intellectual resemblance have so far been published. The first study found that reared-apart twins older than 18 months ($n = 7$ pairs) expressed greater emotion at the first meeting than twins younger than 18 months ($n = 3$ pairs).[27] The three categories of interaction upon first meeting were (1) *high*: intense attraction and interest in one another (e.g., smiling, hugging), (2) *moderate*: not immediately drawn to or focused on each other (e.g., somewhat withdrawn; generally quiet, but interested), and (3) *low*: little attraction or interest in one another (few verbal exchanges; no physical interactions).

In the second study, genetic effects were found for all developmental measures given (developmental delays at adoption, crying/clinging, initial adaptation to adoption, and refusal/avoidance), with shared environmental variance also affecting the first two.[28] The third paper compared the IQ resemblance of the Chinese twins reared apart to that of the Chinese twins reared together, and to virtual twins (VTs) (same-age unrelated children raised together since birth). A key finding was that the MZT twins were more alike in intelligence than both the MZA and VT pairs, evidence of both genetic and environmental influences. However, the differences only reached statistical significance between the MZT pairs and VT pairs, for overall IQ and verbal IQ; this result is likely due to the small sample of MZA twins. Interestingly, the MZA pairs outperformed the MZT pairs in verbal IQ. This finding supports the view that twins reared together engage in less verbal interaction with adults than twins reared apart, thereby reducing their linguistic skills. Lastly, the VT pairs composed of an adopted child and biological child scored higher in intelligence than the VT pairs composed of two adoptees. This may reflect the fact that the genotype of the biological child is correlated with the environment of the adoptee.[29]

9.4 Unique case studies

Case studies of reared-apart twins may not be representative. However, most pairs have unique features that stimulate thinking about questions and hypotheses that can be assessed in the future. Selected details about two such pairs are provided.

The first pair concerns MZA twins born in South Korea and raised apart in the United States and France. (The twin raised in the United States is labeled "US"; the twin raised in France is labeled "FR.") US was raised in New Jersey, while FR was raised in a Parisian suburb; neither knew that they had a twin. As a young woman seeking a career in acting, US moved from New Jersey to Los Angeles. She posted a video of herself that was seen by one of her sister's friends who was impressed with their remarkable physical resemblance. The friend notified FR, then a fashion student in London, and the two 25-year-old women connected over the Internet. Their matching looks, birthdays, voices, and health histories convinced them that they were identical twins. When the first author learned about them it was clear that they had to undergo DNA testing prior to celebrating their possible, albeit likely twinship. The insistence stemmed from an experience with a pair of young Chinese girls raised by different families who looked a lot alike but proved to be genetically unrelated, as indicated by DNA testing.[30] US and FR agreed to undergo the procedure, and both their twinship and their monozygosity were confirmed.

US and FR visited the Twin Studies Center at CSU Fullerton and completed a comprehensive assessment battery that included a general intelligence test (WAIS Adult Intelligence Scale-IV[31]), special mental ability tests, personality inventories and a job satisfaction questionnaire.[32] The twins showed both striking similarities and informative differences. Their IQ scores were 17 points apart, higher than the average six-point difference reported for MZ twins reared together.[33] A difference of this magnitude was not anticipated, but may have been partly linked to US's more diverse activities (her acting and waitressing both required memorization and processing speed). However, the twins' subtest profile, based on fifteen subtests of the WAIS-IV ($r_i = 0.993$, $P < 0.001$), was extremely similar; the intraclass correlation captures elevation, scatter and shape.[34] The profile shape measured separately was also similar ($r = 0.53$, $P < 0.05$). Examining their profiles according to the four main scales (verbal comprehension, perceptual reasoning, working memory, processing speed, and IQ) showed remarkable resemblance. The twins also showed similar special mental ability profiles ($r_i = 0.92$, $P < 0.001$) and profile shape ($r = 0.91$, $P < 0.001$).

US and FR also showed resemblance across the 21-dimensional scales of the Personality Profile for Professionals (PfPI).[35] This was true for the scales alone ($ri = 0.89$, $P < 0.001$) and the scale plus the Big Five personality traits (openness, conscientiousness, extraversion, agreeableness, and neuroticism) derived from them ($r_i = 0.98$, $P < 0.001$). This pattern was essentially repeated for the personality traits measured by the adjective checklist (ACL).[36] In contrast, the twins showed little resemblance across the Big Five scales of the NEO-PI-R for reasons that are unclear.

Interestingly, FR scored lower in extraversion, a possible consequence of her having been raised in a community with relatively few people of Asian ancestry, whereas US grew up in a more ethnically diverse New Jersey neighborhood. Finally, US and FR showed very similar profiles across the four scales of the Job Satisfaction inventory. Additional information about the twins can be found in their book, *Separated @ Birth*[37] and in their documentary film, *Twinsters*.[38]

A second case study involves the world's longest separated pair, a characteristic that merited mention in the *Guinness World Records*.[39] The twins, who are DZA females, met for the first time at the age of 78 years. They had been born in the UK to a single mother who could only afford to raise one child. One of the twins was adopted by a British family and only learned of her twinship after her own daughter traced her mother's genealogy. The twins' mother and the other twin whom she raised moved to the United States when the twin was in her twenties. This case came to attention when the son of the US twin contacted the first author. It was arranged for each twin and one of their children to visit our campus where their reunion took place.

Like the Korean twins, this older pair completed a comprehensive test battery from which selected findings will be presented.[40] The twins did not look physically alike; however, their three-pound weight difference and 1.72-inch height difference were more typical of MZ than DZ twins. Regardless, their dizygosity was confirmed by discordance for 5 of 15 short tandem repeat (STR) markers. Their WAIS-IV subtest profile ($r_i = 0.02$) and shape showed considerable discrepancy ($r = 0.07$). Their IQ difference was eleven points, consistent with the mean difference of ten points for DZ twins reared together.[41] Organizing their IQ data into the four major scales revealed similarities and differences in patterning.

In the domain of personality, the twins' ACL trait profiles showed considerable similarity overall ($r_i = 0.998$, $P < 0.001$) and in shape ($r = 0.98$, $P < 0.001$). In contrast, this level of resemblance was not observed for the Big Five personality measured by the NEO-PI-R[42] or personality mini-markers.[43] Reasons for these differences remain unclear, but it is important to note that the same effect was noted for the Korean-born twins. Both DZA twins showed high levels of satisfaction with the jobs they had held, as well as with their social support, although the US twin perceived greater availability of resources.

The final area to be discussed are the rare pairs of twins who are reared apart because one twin is switched soon after birth with an unrelated infant, due to presumed negligence on the part of hospital staff. There have been 11 such cases documented in the scientific literature and/or the media. The first switch took place in 1941 and the most recent switch took place in 2021. These cases come from different countries around the world. In two cases, there was a double switch, meaning that one twin in one pair was exchanged with one twin in another pair. (There are also two other switched-at-birth twin cases that have surfaced anecdotally, and a third such pair is mentioned briefly in a book about twins, but they are not included here.) The chronology of cases is presented in Table 9.1.

Table 9.1 Switched-at-birth twins.[44]

Location	Date of birth	Gender	Age at meeting
			(Years)
Switzerland	1941	male	5.0
Canada	1971	male	20.0
Poland	1983	female	16.0
Puerto Rico[a]	1985	female	1.5
Canary Islands	1973	female	28.0
Canary Islands	–	male	–
Colombia[a]	1988	male	25.0
Kenya	1999	female	19.0
Malaysia	2001	female	19.0

[a] *Doubly switched twins.*

The focus here is on a rare case recently studied by the first author and a research team. The book that resulted, referenced below, concerns two sets of identical male twins from Colombia, South America. The twins are shown in Fig. 9.1.

FIG. 9.1 Doubly switched Colombian twins.

Photo Credit: Dr. Nancy L. Segal.

The Colombian twins can be organized into (1) two MZA twin pairs, (2) two VT pairs (i.e., same-age unrelated siblings raised together—but believing they were DZ twins), and (3) two "replicas," or unrelated siblings who were *not* reared together, but who genetically replicate the unrelated reared-together pairs. Comparing replicas with VTs provides an index of shared environmental effects. One pair of unrelated twins lived in the culturally rich city of Bogotá and attended college. The other pair lived in a rural area far from a city and did not advance beyond the fifth grade (although one of the twins later completed a high school equivalency course and completed law school in 2022). Here, selected findings from this case study are described; additional information is available in the book, *Accidental Brothers* and published papers cited therein.[45]

Both reared-apart pairs showed similarities and differences in general mental abilities, as did the VTs; the profiles of one MZA pair closely aligned. One set of replicas showed considerable difference, whereas the other set showed resemblance in their profile contours. The twins raised in Bogotá either outperformed or performed similarly to their country-raised counterparts. Again, this is not surprising, given the extreme differences in their education. The twins also completed the Raven Advanced Progressive Matrices (APM), Set II.[46] Here, both twins from Bogotá obtained higher scores than their co-twins and scored closer to each other than to their co-twin. The biggest difference was between one of the replicated sets.[47]

These results are shown in Figs. 9.2 and 9.3. Intraclass correlations were not calculated for the mental ability profiles because only four indices were reported with the actual scores omitted in the interest of confidentiality. Note that with the other reared-apart pairs discussed in this chapter we included intraclass correlations, given that scores across all subtests could be reported. Intraclass correlations were not calculated for the Raven APM, Set II which yields a single score.

Finally, there has been considerable attention to the origins of myopia (nearsightedness). Our research team examined the hypothesis that spending longer outdoor time results in more normal vision and refractive status, as compared with spending more time indoors. The Colombian twins were an ideal case to use for testing this idea, given their differences in rearing circumstances and educational history.[50] It was discovered that uncorrected visual acuities were 20/160 and 20/200 for the city-raised twins and 20/20 and 20/30 for the country-raised twins. These differences could not be explained with reference to premature birth, low birth weight, computer use and reading time. Thus, time spent outdoors appeared to be a key factor in preventing the development of myopia. This finding underlines the additional importance of twin studies for identifying non-genetic causes of co-twin differences. Twin research is about both genetic and environmental sources of influence.

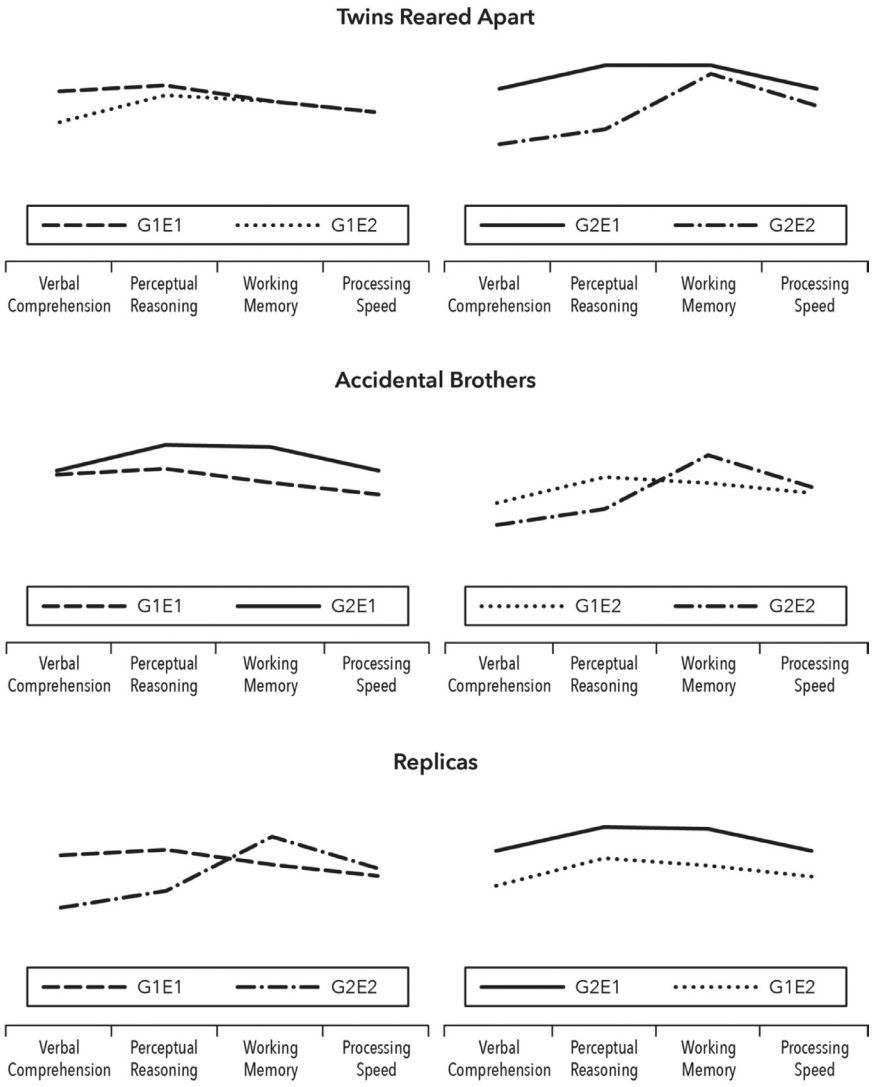


FIG. 9.2 Mental ability profiles: reared-apart twins, virtual twins and replicas.

Adapted from published source.[48]

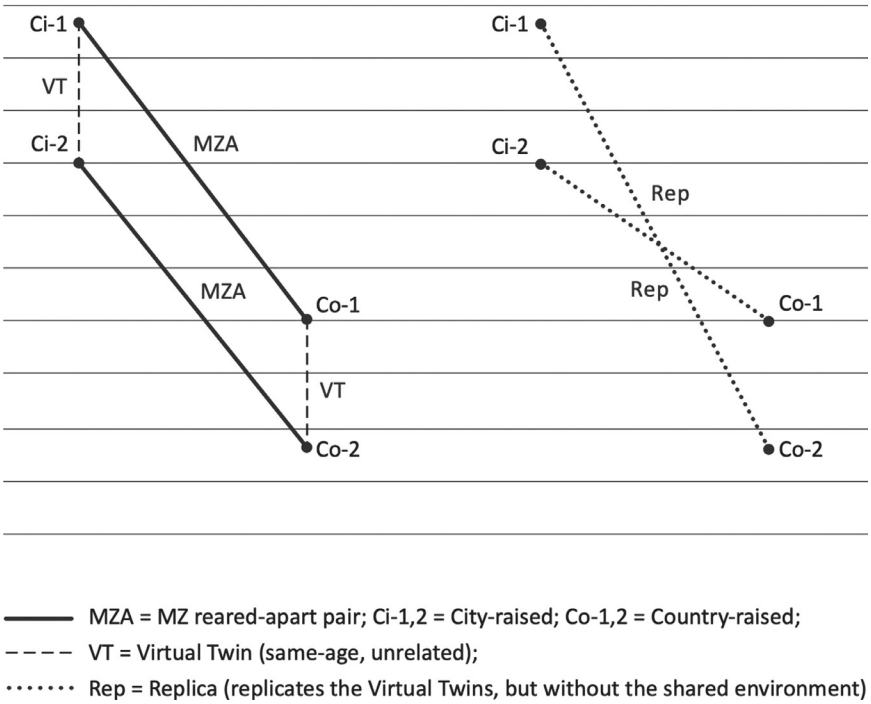


FIG. 9.3 Raven APM, Set II: reared-apart twins, virtual twins and replicas.

Adapted from published source.[49]

9.5 Twin study controversy

Twin research has produced a wealth of psychological and medical findings that have improved lives and enhanced understanding of human development. However, there are occasional examples of research that depart from conventional standards, and whose methods seriously challenge our sensibilities and ethical principles. A study undertaken in the 1960s and 1970s in New York City studied intentionally adopted-apart twins from birth to age twelve years, without informing their adoptive parents that they were raising a singleton twin. Dr. Viola W. Bernard, a psychiatric consultant to Louise Wise Services (LWS), formulated and implemented the separate rearing of twins for adoption. Bernard believed that twins growing up together could not development separate identities. She also believed that she would reduce the emotional challenges and financial constraints that -affect some families raising twins.

This study was the focus of two recent documentary films, *The Twinning Reaction*[51] and *Three Identical Strangers*.[52] These films were highly informative, but could not fully capture the characteristics of the principal investigators (the psychoanalytically-oriented psychiatrists, Drs. Peter B. Neubauer of New York's Child Development Center, and Viola W. Bernard of Columbia University and LWS), the research methodology, the twins' life histories and the legal and ethical implications of publishing their work as a book-length treatment.

Given the foregoing, the first author wrote *Deliberately Divided: Inside the Controversial Study of Twins and Triplets Adopted Apart*.[53] It is dedicated to the twins who were unwitting participants in this long-term twin study. The reunited twins have variously expressed outrage, sadness, and disappointment at having been deprived of their growing up years together. Their adoptive families, who had placed full confidence in the adoption agency that had separated the twins, feel emotionally devastated, and extremely angry at not having been told that their adoptive child had a twin. Very few publications resulted from their efforts—the few articles, single book chapter and book (that addresses a variety of developmental topics) are highly descriptive of selected pairs and include considerable overlapping content. The original data files are stored in Yale University's archives, not to be made available until 2065. Twin-related materials reside in Columbia University's archives, a portion of which can be accessed. The twin study's data records that had been sealed at Columbia University until 2021 can now be examined by the public.

9.6 Research directions

Twin research is rapidly expanding, partly due to the growing number of twins in western populations. The dramatic increase in twinning in the United States (from 1/60 birth in 1980 to 1/33 currently) has been largely attributed to the use of in vitro fertilization (IVF) and other assisted reproductive technologies that involve multiple ova or eggs. IVF involves extracting two or more eggs from the prospective mother, mixing them with spermatozoa from the father and implanting the resulting embryos in the mother's uterus. In addition, women are delaying the child-bearing years to complete their education and pursue their career goals. The association between fraternal twinning and older maternal age, beginning when women are in their mid-thirties, has been well documented.[54]

There are other explanations for why twin research is proliferating in institutions that, until recently, did not consider genetic perspectives in crafting projects. Specifically, scholars are interested in all sources of influence on religious interests and activities, political beliefs, social attitudes, social interaction and dentistry.[55] Clearly, there is growing appreciation for the role of genetic influences across academic disciplines. Twins tell us a lot about who we are, how we came to be and may provide clues as to where we might be headed.

There have also been numerous advances in identifying genes associated with various illnesses, such as schizophrenia,[56] educational attainment,[57] and

educational mobility that contribute to variation among people.[58] Twins who differ in serious disorders (e.g., diabetes; psychoses), with respect to expressing the condition and/or who differ in the number and severity of symptoms, are especially valuable research participants. That is because when MZ twins differ in certain ways, researchers can look for prenatal and postnatal factors that might have triggered the condition in one twin and silenced it in the other twin. This information can be applied to protect, manage and/or control illnesses in predisposed individuals in the general population.

Despite the redirecting of twin research, observing twins up close remains a scientifically fruitful undertaking. Studies that allow for such observation can generate a range of unique hypotheses and conclusions. At the same time, the increased twinning rate means that we need to pay attention to epigenetic factors and other influences in MZ and DZ twins' unique prenatal environments, such as fetal positioning and nutritional supply[59,60] and events in their shared environments, such as parenting quality[61] and socioeconomic status[62] that could affect their development. The many local, national, and international parents of twins clubs, such as Orange Coast Mothers of Twins, Mothers of Multiples Society, and the International Council of Multiple Birth Organizations, have helped draw attention to twins' special rearing and educational issues.

9.7 A quote that will endure

I will leave readers with this statement from Dr. Thomas J. Bouchard, Jr., the Director of the Minnesota Study of Twins Reared Apart:

"Twin studies . . . refute both biological and environmental determinism. They do not negate the effect of the environment on behavior, nor do they overglorify the role of genes. They account for the uniqueness of each of us."[63]

References

1. Segal NL. *Twin Mythconceptions: False Beliefs, Fables, and Facts About Twins*. London: Academic Press; 2017.
2. Barnes JC, Wright JP, Boutwell BB, Schwartz JA, Connolly EJ, Nedelec JL, Beaver KM. Demonstrating the validity of twin research in criminology. *Criminology*. 2014;52(4): 588–626.
3. Galton F. The history of twins, as a criterion of the relative powers of nature and nurture. *The Journal of the Anthropological Institute of Great Britain and Ireland*. 1876;5:391–406.
4. Segal NL. *Born Together-Reared Apart: The landmark Minnesota Twin Study*. Cambridge, MA: Harvard University Press; 2012.
5. Popenoe P. Twins reared apart. *J Hered*. 1922;13:142–144.
6. Müller HJ. Mental traits and heredity. *J Hered*. 1925;16:433–448.
7. Newman HH, Freeman FN, Holzinger KJ. *Twins: A Study of Heredity and Environment*. Chicago, IL: University of Chicago Press; 1937.

8. Bouchard TJ, Lykken DT, McGue M, Segal NL, Tellegen A. Sources of human psychological differences: the Minnesota Study of Twins Reared Apart. *Science*. 1990;250:223–228.
9. Shields J. *Monozygotic Twins Brought Up Apart and Together*. London: Oxford University Press; 1962.
10. Wilde GJ. Inheritance of personality traits: an investigation into the hereditary determination of neurotic instability, extraversion, and other personality traits by means of a questionnaire administered to twins. *Acta Psychol (Amst)*. 1964;22:37–51.
11. Claridge G, Canter S, Hume WI. *Personality Differences and Biological Variations: A Study of Twins*. London: Elsevier; 1973.
12. Tellegen A, Lykken DT, Bouchard TJ, Wilcox KJ, Segal NL, Rich S. Personality similarity in twins reared apart and together. *J Pers Soc Psychol*. 1988;54:1031–1039.
13. Juel-Nielsen N. *Individual and Environment: Monozygotic Twins Reared Apart*. New York, NY: International Universities Press; 1965 /1980.
14. Bouchard TJ, Lykken DT, McGue M, Segal NL, Tellegen A. Sources of human psychological differences: the Minnesota Study of Twins Reared Apart. *Science*. 1990;250:223–228.
15. Segal NL. *Born Together-Reared Apart: The Landmark Minnesota Twin Study*. Cambridge, MA: Harvard University Press; 2012.
16. Pedersen N, Plomin R, Nesselroade J, McClearn G. A quantitative genetic analysis of cognitive abilities during the second half of the life span. *Psychol Sci*. 1992;3:346–353.
17. Johnson W, Bouchard TJ, McGue M, Segal NL, Tellegen A, Keyes M, et al. Genetic and environmental influences on the Verbal-Perceptual-Image Rotation (VPR) model of the structure of mental abilities in the Minnesota study of twins reared apart. *Intelligence*. 2007;35:542–562.
18. Turkheimer E, Haley A, Waldron M, D'onofrio B, Gottesman II. Socioeconomic status modifies heritability of IQ in young children. *Psychol Sci*. 2003;14:623–628.
19. Tellegen A, Lykken DT, Bouchard TJ, Wilcox KJ, Segal NL, Rich S. Personality similarity in twins reared apart and together. *J Pers Soc Psychol*. 1988;54:1031–1039.
20. These data were drawn from multiple sources: Bouchard TJ, Lykken DT, McGue M, Segal NL, Tellegen A. Sources of human psychological differences: The Minnesota study of twins reared apart. *Science*. 1990;250:223–228; Bouchard TJ. Genetic and environmental influences on adult personality: Evaluating the evidence. In: J. Hettema J, Deary, I eds. Foundations of personality. Dordrecht: Springer; pp. 15–44. Dordrecht: Kluwer; Škarić-Jurić T, Ginsburg E, Kobyliansky E, Malkin I, Smolej Narančić N, Rudan P. Complex segregation analysis of body height, weight and BMI in pedigree data from Middle Dalmatia, Croatia. Collegium Antropologicum. 2000;27:135–49; Segal NL, Feng R, McGuire SA, Allison DB, Miller S. Genetic and environmental contributions to body mass index: comparative analysis of monozygotic twins, dizygotic twins and same-age unrelated siblings. Int. J. Obesity. 2009; 33:37–41; Knopik VS, Neiderhiser JM, DeFries JC, Plomin R. Behavioral genetics. New York: Worth Publishers; 2016; Anger S, Schnitzlein DD. Cognitive skills, non-cognitive skills, and family background: evidence from sibling correlations. J. Popul. Econ. 2017;30:591–620.
21. Shields J. *Monozygotic Twins Brought Up Apart and Together*. London: Oxford University Press; 1962.
22. Plomin R, Daniels D. Why are children in the same family so different from one another? *Behav Brain Sci*. 1987;10:1–6.

23. Turkheimer E, Waldron M. Nonshared environment: a theoretical, methodological, and quantitative review. *Psychol Bull*. 2000;126:78–108.
24. Segal NL. *Born Together-Reared Apart: The Landmark Minnesota Twin Study*. Cambridge, MA: Harvard University Press; 2012.
25. Evans K. *The Lost Daughters of China: Adopted Girls, Their Journey to AMERICA, and the Search for a Missing Past*. New York, NY: Jeremy P. Tarcher/Penguin; 2008.
26. Segal NL. *Indivisible by Two: Lives of Extraordinary Twins*. Cambridge, MA: Harvard University Press; 2007.
27. Segal NL, Stohs JH, Evans K. Chinese twin children reared apart and reunited: first prospective study of co-twin reunions. *Adoption Quarterly*. 2011;14:61–78.
28. Segal NL, Tan TX, Graham JL. Twins and virtual twins: Do genetic (as well as experiential) factors affect developmental risks? *J Exp Child Psychol*. 2015;136:55–69.
29. Segal NL, Niculae FJ, Becker EN, Shih EY. Reared-apart/reared-together Chinese twins and virtual twins: Evolving research program and general intelligence findings. *J Exp Child Psychol*. 2021;207:105106.
30. Segal NL. Laboratory findings: not twins, twins, not twins. Twin Research and Human Genetics. 2006;9:303.
31. Wechsler D. Wechsler Adult Intelligence Scale (WAIS IV)*The WAIS-IV was administered to each twin by local examiners in their communities*. Pearson: San Antonio, TX; 2008.
32. Segal NL, Cortez FA. Born in Korea-adopted apart: behavioral development of monozygotic twins raised in the United States and France. *Personality and Individual Differences*. 2014;70:97–104.
33. Plomin R, DeFries J. Genetics and intelligence: recent data. *Intelligence*. 1980;4:15–24.
34. Furr RM. The double-entry intraclass correlation as an index of profile similarity: meaning, limitations, and alternatives. *J Pers Assess*. 2010;92:1–15.
35. Rolland JP, de Fruyt F. *PfPI Questionnaire de Personnalite au Travail*. Paris: Editions du Centre de Psychologie Appliquée (ECPA); 2009.
36. Gough HG, Heilbrun AB. *The Adjective Checklist: Manual*. Palo Alto, CA: Consulting Psychologists; 1965.
37. Bordier A, Futerman S. *Separated @ birth: A True Love Story of Twin Sisters Reunited*. New York, NY: G.P. Putnam's Sons; 2014.
38. Futerman S, Miyamoto R. *Twinsters*. Los Angeles, CA: Small Package Films; 2015.
39. Segal NL. Humans: twins*Guinness World Records*. London: Guinness World Records Ltd.; 2016:62–63.
40. Segal NL, Cortez FA, Zettel-Watson L, Cherry BJ, Mechanic M, Munson JE, et al. Genetic and experiential influences on behavior: Twins reunited at seventy-eight years. *Personality and Individual Differences*. 2015;73:110–117.
41. Plomin R, DeFries J. Genetics and intelligence: recent data. *Intelligence*. 1980;4:15–24.
42. McCrae RR, Costa PT. A contemplated revision of the NEO five-factor inventory. *Personality and Individual Differences*. 2004;36:587–596.
43. Saucier G. Mini-markers: a brief version of Goldberg's unipolar big-five markers. *J Pers Assess*. 1994;63:506–516.
44. The Star (June 27, 2021). "Twin Sister Swapped at Birth: Truth Unfolds aAfter 19 Years." *The Star* (Nation), https://www.thestar.com.my/news/nation/2021/06/27/twin-sister-swapped-at-birth-truth-unfolds-after-19-years.
45. Segal NL, Montoya YS. *Accidental Brothers: The Story of Twins Exchanged at Birth and the Power of Nature and Nurture*. New York, NY: St. Martin's Press; 2018.

46. Raven JC. *Advanced Progressive Matrices, Set-II*. Eynsham, Oxford: Oxford Psychologists Press; 1965.
47. Segal NL. Montoya YS, Becker EN. Twins reared apart and twins in families: the findings behind the fascination. *Twin Research and Human Genetics*. 2018;*21*:295–301.
48. Segal NL. Montoya YS. *Accidental Brothers: The Story of Twins Exchanged at Birth and the Power of Nature and Nurture*. New York, NY: St. Martin's Press; 2018.
49. Segal NL. Montoya YS, Becker EN. Twins reared apart and twins in families: the findings behind the fascination. *Twin Research and Human Genetics*. 2018;21:295–301.
50. Segal NL. Montoya YS, Peña FY, Burgos S, Katz X. Eye refraction in doubly exchanged monozygotic twins. *Twin Research and Human Genetics*. 2019;22:177–182.
51. Shinseki L. *The Twinning Reaction*: Durham, NC: Fire Horse Films, Inc. 2017.
52. Wardle T. *Three Identical Strangers*. London: Raw Films; 2018.
53. Segal NL. Deliberately Divided: Inside the Controversial Study of Twins and Triplets Adopted Apart. Lanham, MD: Rowman & Littlefield; 2021.
54. Segal NL. *Twin Mythconceptions: False Beliefs, Fables, and Facts About Twins*. London: Academic Press; 2017.
55. Segal NL. *Born Together-Reared Apart: the Landmark Minnesota Twin Study*. Cambridge, MA: Harvard University Press; 2012.
56. Braff DL, Tamminga CA. Endophenotypes, epigenetics, polygenicity and more: Irv Gottesman's dynamic legacy. *Schizophr Bull*. 2016;43:10–16.
57. Smith-Woolley E, Selzam S, Plomin R. Polygenic score for educational attainment captures DNA variants shared between personality traits and educational achievement. *J Pers Soc Psychol*. 2019;117:1145–1163.
58. Plomin R. *Blueprint: How DNA Makes You Who We Are*. Cambridge, MA: MIT Press; 2018.
59. Knight AK, Craig JM, Theda C, Bækvad-Hansen M, Bybjerg-Grauholm J, Hansen CS. An epigenetic clock for gestational age at birth based on blood methylation data. *Genome Biol*. 2016;17:1–11 206.
60. Segal NL, Montoya YS, Loke YJ, Craig JM. Identical twins doubly exchanged at birth: a case report of genetic and environmental influences on the adult epigenome. *Epigenomics*. 2017;9:5–12.
61. McGuire S, Segal NL, Hershberger S. Parenting as phenotype: a behavioral genetic approach to understanding parenting. *parent*. 2012;12:192–201.
62. Turkheimer E, Haley A, Waldron M, D'onofrio B, Gottesman II. Socioeconomic status modifies heritability of IQ in young children. *Psychol Sci*. 2003;14:623–628.
63. Bouchard Jr TJ. Whenever the twain shall meet. *Sciences (New York)*. 1997;37:57.

CHAPTER

Opposite-sex twins in medical research

10

Lucas Calais-Ferreira[a,b], Sue Malta[a], John L. Hopper[a]

[a]*Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia*

[b]*Centre for Adolescent Health, Murdoch Children's Research Institute, Melbourne, VIC, Australia*

10.1 Introduction

The medical research literature on studies involving twins as participants has a rich, 150-year history[1, 2], though principally based on same-sex pairs. In 1876, before twin zygosity was fully understood, Galton was already convinced that extreme physical resemblance was more frequent in twin pairs of the same-sex and that genetics could somehow explain why some same-sex twin pairs were more similar than others[3].

Unlike monozygotic (MZ) twin pairs, dizygotic or "fraternal" (DZ) twin pairs can be of same-sex or opposite-sex. It is now established that DZ twin pairs originate from two fertilized eggs or zygotes and are born sharing around 50% of their genetic variants. MZ twin pairs originate from one fertilized egg that later splits into two zygotes, therefore, both twins are of the same-sex (with rare exceptions) and "identical" DNAs[4]. For that reason, any physical or mental dissimilarity between MZ twin pairs can only be explained by nongenetic (or environmental) differences between them[5].

When studies compare MZ twin pairs for a trait or condition, such as when using a within-pair case-control approach, they fully "control" for genes; which essentially means protecting from biased estimates due to genetic differences (or similarities) between individuals. In other words, this model provides confidence that genes are not the cause of any individual differences observed between members of the studied MZ twin pairs. By default, such differences, if and when they exist, can only be explained by either shared environmental factors such as those associated with cohabitation if they are reared together, as well as paternal characteristics or socioeconomic status, or other factors uniquely pertaining to each individual, such as smoking status or weight, for example.

Studying same-sex DZ twin pairs alone is not as straightforward because they only share on average half of their genetic variants, being only as genetically similar as any pair of siblings. But all within-twin pair studies, using MZ or DZ twin pairs, control perfectly for age and year of birth.

Studying opposite-sex DZ twin pairs, therefore, allows for studying differences between males and females for health outcomes while holding many other factors

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00017-9

constant, including age and year of birth. This design also controls for on average half of autosomal genetic factors as well as for shared environmental factors that make family members more similar than pairs of individuals chosen at random. Observed differences between males and females in opposite-sex pairs can shed light upon the aetiology of sex differences by ruling out confounding due to a range of potentially important familial factors.

In this brief chapter, we first present evidence for a potential effect on opposite-sex cotwins due to them sharing the mother's womb which contributes to greater dissimilarities between male and female twins from the same pair than would be expected for twins from same-sex pairs or for males and females in the general population. We then briefly explore study designs based on direct comparisons between males and females in opposite-sex pairs; and how these designs can help explore sex and gender differences related to how people experience health and disease.

10.2 Sharing more than a womb

As in any other scientific field, a good statistical model using twin data is only a sensible approximation of the phenomenon that researchers want to explain. Studying sex differences of opposite-sex twin pairs requires assuming that having an opposite-sex cotwin does not produce any substantial differences other than what would be reasonable between male and female non-twin siblings. If this assumption does not hold, then findings from studies of opposite-sex twin pairs would only be relevant to opposite-sex twins and not to same-sex twins or males and females in the general population.

Two major hypotheses guide scientific research investigating potential health effects of having an opposite-sex compared with a same-sex cotwin[6] which could determine the validity of the assumption above. The first is a biological one; that intrauterine effects originating from hormonal transfer between the male and female twin in the mother's womb are responsible for making opposite-sex twins different to same-sex twins. The second hypothesis relates to the possible effects on social relationships and other psychological outcomes related to growing up with an opposite-sex cotwin.

10.2.1 Biological effects

Evidence supporting the "biological" hypothesis shows that male twins with a female cotwin are born heavier and are longer at birth than male twins who are born with a male cotwin. However, the modest differences observed have been previously attributed to the longer gestational age of opposite-sex twin pairs[7]. Sharing the mother's womb with a female instead of a male cotwin can also be linked to better early life health outcomes for males[8], while simultaneously increasing the risk for early respiratory morbidity for their female cotwin[9].

In children, there have been reports of opposite-sex twins being more susceptible to myopia than same-sex twins, although the studies have been limited in terms of

sample size[10]. Sharing the womb with a male cotwin also appears to be associated with lower cognitive scores for females, with males from opposite-sex pairs doing better cognitively than males from same-sex pairs[6]. This difference in cognition has been linked to changes at the molecular level related to how genes are expressed; it also supports the intrauterine hormonal transfer hypothesis[11].

Despite findings that female cotwins might be cognitively disadvantaged due to exposure to their male cotwins either due to sharing the same womb or due to cohabiting effects, having a male cotwin can decrease the risk of dementia for females in opposite-sex twin pairs when compared with females in same-sex pairs[12]. Potential *in utero* hormonal transfer might not always result in noticeable biological differences for opposite-sex twins as they grow older. For example, the sex of a cotwin does not appear to be associated with height and body mass index in adulthood[13].

A recent systematic review showed that differences between opposite-sex and same-sex twins, when they exist, do not appear to be substantial at the population level for most traits or conditions[6]. Therefore, at least biologically, opposite-sex twins are largely comparable to same-sex twins for scientific purposes.

10.2.2 Socialisation effects

When it comes to potential "socialisation" effects related to growing up in an opposite-sex twin pair, the evidence is also not entirely conclusive. For example, one study found no major differences in social competence and friendship between same-sex and opposite-sex pairs[14]. Likewise, the same study found that twins had lower social competency scores than singletons.

A study comparing same-sex and opposite-sex twin pairs for eating disorders (ED) found an association between the sex of the cotwin and ED, with females in same-sex pairs having a higher prevalence of ED than females in opposite-sex pairs[15]. The study did not find support for a "social masculinisation" effect, suggesting that intrauterine effects might be a more plausible explanation for the observed sex differences in this condition. The study's small sample size means that interpretations and conclusions arising from the results should be treated with caution.

A study of male and female attitudes toward issues such as sexuality, religion, and politics found that female twins in opposite-sex pairs had more similar responses to male twins compared to female twins in same-sex pairs[16]. This approach did not, however, rule out genetic and shared environmental factors as possible causes of the observed sex differences in responses.

Ruling out familial factors when studying sex differences in health outcomes is essential, given substantial evidence that family members are typically more alike than unrelated individuals for a variety of conditions including heart diseases, cancers, and mental disorders, such as depression[17]. Individuals with a family history of these and many other chronic conditions are at a higher risk of having these conditions themselves[18]. Studying differences between males and females of opposite-sex twin pairs allows for comparisons between individuals with a similar family history for health or disease outcomes, which is a critical advantage when investigating sex differences.

10.3 What can sex differences in opposite-sex twin pairs tell us?

Studies of sex differences in opposite-sex twins resemble clinical or randomised control trials (RCT) to some degree. In an RCT, for instance, groups of similar individuals are randomly exposed to different treatments or risk factors (exposures) for a disease (commonly including a placebo group), followed over time and then later compared for their disease outcomes. Opposite-sex twin pairs fulfill the following criteria, in that they provide: (1) two individuals of the same age that are genetically similar, (2) who have shared environmental factors since conception, and (3) who differ in sex—a critical "exposure". Comparing health outcomes within opposite-sex twin pairs, especially over time, can therefore offer important insights into sex differences in a more accurate way, protected from aspects of familial cofounding, than from comparing groups of males and females who are unrelated.

For example, a study that compared male and females in opposite-sex twin pairs from birth found that males were about 60% (95% Confidence Interval: 39%–83%) more likely to die within the first year of life than their female cotwins, even after adjusting for birth weight and, birth order[19]. This finding suggests the existence of intrauterine effects that disproportionally affect males who share the mother's womb with a female cotwin, beyond what is normally expected from the well-recognized male early-life "disadvantage"[20]. Similarly, males in opposite-sex twin pairs are more likely to be born with a congenital disability than their female cotwins[21].

A Swedish study of opposite-sex twin pairs also found that male and female cotwins differed in the prevalence and severity of the health conditions they experienced throughout their lifetimes[22]. For example, the males were more vulnerable than their female cotwins to suffering severe cardiovascular conditions.

Direct comparisons between males and females from opposite-sex twin pairs for a trait or condition provide insight into sex differences, and studying associations between exposures and health outcomes in such pairs can generate important findings. A study of opposite-sex twin pairs found that higher quality interpersonal relationships protected the female twins from developing a major depressive disorder (MDD) more than it did their male cotwins[23]. Furthermore, males appeared to be more affected by stressors such as lowered self-worth and their perceived failure to achieve established goals. From an earlier study, social support also appeared to be a protective factor for MDD, the more so for females than for their male cotwins[24]. Differences in how males and females from opposite-sex twin pairs experience sleep, hypersomnia, and agitation[25] provide some evidence on possible pathways for these health-related conditions.

10.4 Conclusions

Despite the advantages of studies of opposite-sex twin pairs, they have rarely used in medical science. On the contrary, it is not uncommon for twin researchers to purposely choose not to recruit opposite-sex twin pairs for their studies. This can

be at least partially attributed to the perceived impact of confounding conferred by hormonal differences between males and females. These valuable twin pairs, however, provide possibly one of the most robust scientific methods to study sex and gender differences by protecting from bias due to uncontrolled from shared familial factors (both genetic and nongenetic) while controlling for age and year of birth.

It is important to acknowledge the potential limitations of this method. First, it relies on the assumption that opposite-sex twins are not substantially different from same-sex twins due to in utero hormone transfer effects and cohabitation-related factors. Second, while twin studies confer advantages not possible in studies of unrelated individuals, generalising findings from twin studies to the general population might require extra caution and possibly additional analytical steps depending on the availability of suitable data[26].

Several twins registries and cohorts worldwide are custodians of valuable datasets available for analysis in medical research projects. Some of these entities have collected data from twins of all sex and zygosity categories who volunteered for research over several decades. Prominent examples can be found in many parts of the world[27]. Making use of such valuable data resources collected from opposite-sex twin pairs presents a viable and rewarding way to conduct more reliable scientific research on sex and gender differences that can lead to more equitable public health outcomes.

References

1. Craig JM, Calais-Ferreira L, Umstad MP, Buchwald D. The value of twins for health and medical research: a third of a century of progress. *Twin Research and Human Genetics*. 2020;23:8–15.
2. Hrubec Z, Robinette CD. The study of human twins in medical research. *N Engl J Med*. 1984;310:435–441.
3. Galton F. The history of twins, as a criterion of the relative powers of nature and nurture. The Journal of the Anthropological Institute of Great Britain and Ireland. 1876;5:391–406
4. Umstad MP, Calais-Ferreira L, Scurrah KJ, Hall JG, Craig JM. 14 - Twins and Twinning editors. In: Pyeritz RE, Korf BR, Grody WW, eds. *Emery and Rimoin's Principles and Practice of Medical Genetics and Genomics*. Seventh Edition: Academic Press: London, U.K. 2019:387–414.
5. Hopper JL. The epidemiology of genetic epidemiology. *Acta Genet Med Gemellol (Roma)*. 1992;41:261–273.
6. Ahrenfeldt LJ, Christensen K, Segal NL, Hur Y-M. Opposite-sex and same-sex twin studies of physiological, cognitive and behavioral traits. *Neuroscience & Biobehavioral Reviews*. 2020;108:322–340.
7. Jelenkovic A, Sund R, Yokoyama Y, Hur Y-M, Ullemar V, Almqvist C, et al. Birth size and gestational age in opposite-sex twins as compared to same-sex twins: an individual-based pooled analysis of 21 cohorts. *Sci Rep*. 2018;8:6300.
8. Melamed N, Yogev Y, Glezerman M. Effect of fetal sex on pregnancy outcome in twin pregnancies. *Obstet Gynecol*. 2009;114:1085–1092.

9. Shinwell ES, Reichman B, Lerner-Geva L, Boyko V, Blickstein I. "Masculinizing" effect on respiratory morbidity in girls from unlike-sex preterm twins: a possible transchorionic paracrine effect. *Pediatrics*. 2007;120:e447.
10. Miller EM. Reported myopia in opposite sex twins: a hormonal hypothesis. *Optom Vis Sci*. 1995;72:34–36.
11. Kong S, Peng Y, Chen W, Ma X, Wei Y, Zhao Y, et al. Epigenetic consequences of hormonal interactions between opposite-sex twin fetuses. *Clin Transl Med*. 2020;10:e234.
12. Luo J, Beam CR, Karlsson IK, Pike CJ, Reynolds CA, Gatz M. Dementia risk in women higher in same-sex than opposite-sex twins. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*. 2020;12:e12049.
13. Bogl LH, Jelenkovic A, Vuoksimaa E, Ahrenfeldt L, Pietiläinen KH, Stazi MA, et al. Does the sex of one's co-twin affect height and BMI in adulthood? A study of dizygotic adult twins from 31 cohorts. *Biology of Sex Differences*. 2017;8:14.
14. Laffey-Ardley S, Thorpe K. Being opposite: is there advantage for social competence and friendships in being an opposite-sex twin? *Twin Research and Human Genetics*. 2006;9:131–140.
15. Culbert KM, Breedlove SM, Burt SA, Klump KL. Prenatal hormone exposure and risk for eating disorders: a comparison of opposite-sex and same-sex twins. *Arch Gen Psychiatry*. 2008;65:329–336.
16. Miller EM, Martin N. Analysis of the effect of hormones on opposite-sex twin attitudes. *Acta geneticae medicae et gemellologiae: Twin Research*. 1995;44:41–52.
17. Muñoz M, Pong-Wong R, Canela-Xandri O, Rawlik K, Haley CS, Tenesa A. Evaluating the contribution of genetics and familial shared environment to common disease using the UK Biobank. *Nat Genet*. 2016;48:980–983.
18. Yoon PW, Scheuner MT, Peterson-Oehlke KL, Gwinn M, Faucett A, Khoury MJ. Can family history be used as a tool for public health and preventive medicine? *Genet Med*. 2002;4:304–310.
19. Calais-Ferreira L, Barreto M, Mendonça E, et al. Birthweight, gestational age and familial confounding in sex differences in infant mortality: a matched co-twin control study of Brazilian male-female twin pairs identified by population data linkage. *Int J Epidemiol*. 2021;(dyab242):1–9.
20. Naeye RL, Burt LS, Wright DL, Blanc WA, Tatter D. Neonatal mortality, the male disadvantage. *Pediatrics*. 1971;48:902–906.
21. Cui W, Ma C-X, Tang Y, Chang V, Rao PV, Ariet M, et al. Sex differences in birth defects: a study of opposite-sex twins. *Birth Defects Research Part A: Clinical and Molecular Teratology*. 2005;73:876–880.
22. Gold CH, Malmberg B, McClearn GE, Pedersen NL, Berg S. Gender and health: a study of older unlike-sex twins. *J Gerontol Ser B*. 2002;57:S168–SS76.
23. Kendler KS, Gardner CO. Sex differences in the pathways to major depression: a study of opposite-sex twin pairs. *Am J Psychiatry*. 2014;171:426–435.
24. Kendler K, Myers J, Prescott C. Sex differences in the relationship between social support and risk for major depression: a longitudinal study of opposite-sex twin Pairs. *Am J Psychiatry*. 2005;162(2):250.
25. Khan AA, CO Gardner, Prescott CA, Kendler KS. Gender differences in the symptoms of major depression in opposite-sex dizygotic twin Pairs. *Am J Psychiatry*. 2002;159:1427–1429.
26. Morley R. Can we generalise from findings in twins? *Paediatr Perinat Epidemiol*. 2005;19(s1):54–59.
27. Hur Y-M, Bogl LH, Ordoñana JR, Taylor J, Hart SA, Tuvblad C, et al. Twin family registries worldwide: an important resource for scientific research. *Twin Research and Human Genetics*. 2019;22:427–437.

SECTION

Twin Methodologies 4

CHAPTER

11

Establishing a resource for genetic, epidemiological, and biomarker studies: The important role of twin registers

Veronika Odintsova[a,b], Gonneke Willemsen[a], Conor V. Dolan[a], Jouke-Jan Hottenga[a], Nicholas G. Martin[c], P. Eline Slagboom[d], Juan R. Ordoñana[e], Dorret I. Boomsma[a]

[a]Netherlands Twin Register, Department of Biological Psychology, Vrije Universiteit Amsterdam, and Amsterdam Public Health (APH), Amsterdam, The Netherlands
[b]National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I.Kulakov of Ministry of Healthcare of the Russian Federation, Moscow, Russia
[c]Genetic Epidemiology Group, QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia
[d]Leiden University Medical Centre, Leiden, The Netherlands
[e]Department of Human Anatomy and Psychobiology, University of Murcia, Murcia Institute for BioHealth Research (IMIB-Arrixaca-UMU), Murcia, Spain

Since the earliest times, it has been intuited that twins provide a window into the human condition, with numerous references in philosophical and literary texts, notably by Augustine of Hippo (400AD), Shakespeare, and others. The formalization of this intuition toward scientific study may best be attributed to Francis Galton in the late 19th century,[1] although at that time it was not yet established that there were two discrete types of twins. Researchers in the early 1900s[2,3] pointed to the scientific value of twin studies,[4] but in 1919 Sir Ronald Fisher still advocated that there was only one type of twins, stating that "…twins ordinarily share the hereditary nature of one gamete but not of the other".[2]

The first half of the 20th century saw a slow but steady development of twin research through the pioneering work of researchers as Poll,[5] Merriman,[6] Siemens,[7] and Holzinger[8] (see Mayo[9] for a detailed account of this development). In Russia, twin studies were initiated as early as 1900, with the first focus on psychological disturbances (see Box 11.1). Many twin studies were then undertaken and were essential

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00011-8

Box 11.1 Twin research in Russian science

Reviews on the history of twin research tend to focus on developments in Western European countries and the United States. However, early references to twin studies are also to be found in Russian science. Psychiatrists Sergey Sukhanov and Tihon Yudin studied the similarity of psychosis in twins from 1900,[79] and several small twin research of morphological, physiological, and psychological characteristics were conducted from 1900 to 1929.[78] The Russian Medical and Biological Institute, which was created in 1929 and continued as the Medical and Genetic Institute from 1935 onwards, conducted systematic and large-scale twin research where more than 700 twin pairs were studied. The research was conducted by medical doctors, psychologists, and pedagogues under the guidance of Solomon Levit. A special kindergarten for twins was created in the institute, in which motor functions, different forms of memory, level of psychic development, attention, and intellect features were studied. The method of twin-control design was used to study effectiveness of pedagogic, medical, and psychological interventions.[80] Unfortunately, these studies were limited and prohibited at the end of 1930s, and were restarted in Russia only in 1970ies in laboratory of genetic psychophysiology created by Irina Ravich-Shcherbo.[81]

in understanding the etiology of disorders. In France in the 1950s, Lejeune et al.[10], for example, became puzzled by the high concordance seen in Down syndrome in identical twins in comparison to the extremely low concordance in non-identical twins. This concordance pattern was inconsistent with single gene inheritance and was one of the observations which led to the discovery of trisomy-21 as the cause of Down syndrome. The history of twin studies, including the basic methodological insights and developments, has been described in multiple papers.[4,9,11,12] In the classical twin design, which includes mono- and dizygotic (MZ and DZ) twin pairs reared together, the resemblance for one or more human traits is compared between MZ and DZ twins to obtain estimates of uni- and multivariate heritability. A larger resemblance in MZ twins is consistent with genetic influences on the trait under study. In the classical twin design, the statistical power is the largest for detecting additive genetic influences (A). The two other variance components that contribute to resemblance of relatives, common or shared environmental variance (C), and dominance or non-additive genetic variance (D) require larger samples or the inclusion of additional family members to achieve reasonable power. Explorations of power of the classical twin study, first by simulation and later by direct analysis, showed that many thousands of pairs would be needed to separate these sources of variance.[13,14] This became the justification for the founding of large twin registries in a number of European countries, in the Scandinavian countries, the Netherlands, the United Kingdom, as well as in the United States and Australia. Many other countries in different parts of the world have followed suit. Compilations of twin registers across the world have been carried out periodically and published in the journal *Twin Research and Human Genetics* (2002, 2006, and 2013) and elsewhere.[15] Many of these twin registries are longitudinal, population based, and sufficiently large for epidemiological studies.

Twin registries have been a resource for thousands of studies, estimating the relative impact of genetic and environmental factors on trait variation across a wide range of biomedical and social science disciplines.[16,17] Their potential, however, for

disentangling the role of genetics in human traits goes much further, through different designs and investigative methods, from genetic epidemiology to molecular approaches.[18–20,70] In many of the large registers, data collection is undertaken by mailed questionnaire or by telephone interview and more recently, by online survey, and record linkage. Clinical twin studies were of necessity often smaller, requiring twins to visit research or medical facilities, or researchers visiting twins at home. In anticipating of the age of molecular genetics, many registers started DNA collections in the early 1980s, and these samples have become a highly valuable and much used resource for zygosity assessment, genetic linkage, and association studies. Collecting multiple sources of biological material has enabled twin studies of epigenetics, transcriptomics, metabolomics, proteomics, microbiome, and a wealth of biomarkers.

This chapter aims to discuss several aspects in establishing a twin register. We will attempt to cover what is important and why, and how to develop such a scientific resource. In this endeavor, we will also refer to when to start and who should participate. Our aim is not to offer a checklist or a complete step-by-step technical guide, but rather to discuss the issues that, in our experience, should be addressed at the launch and in the management of a twin register.

11.1 The first steps

The first question that should be addressed is "Do we really need to start a new twin register?" Establishing a twin register is a huge and long-lasting effort and, although it pays back in the long run, it is costly, both in terms of economic and personal investment. Hence, research objectives must be clearly defined. Working with twins has many compensations that go even beyond the classical methodological advantages (see Box 11.2), and establishing a register may appear to be the best

Box 11.2 Do it with twins

In 1982, David Lykken listed in his presidential address to the Society for Psychophysiological Research[82] several compelling reasons for doing research with twins, that are 'in addition to' the genetic analyses that the classical twin design allows:

- Twins are plentiful and easily recruited as experimental subjects.
- Twins are probably more representative of the general population than any other group.
- This representativeness is even more true of the families of twins.
- Twin data are invaluable to explore issues measurement. Any measure that shows high within-pair correlation among MZ twins deserves to be treated with respect.
- The method of co-twin control provides enhanced experimental power. Using one twin from each pair for the experimental group and the other for the control group provides a test of one's hypothesis that is as powerful as an experiment employing twice as many pairs of singletons.
- If one treats one's subjects properly, and keeps in touch, then it will be possible to bring many of them back repeatedly over the years to participate in additional experiments. This is useful not only for longitudinal research but as a method of enhancing each subsequent experiment with the information previously gathered on these same individuals.

choice. However, it may not be the only option. Already established twin registries with data, or willing to collect new information, may be open to collaboration. In fact, nearly always a twin researcher can be found with an interest in collaboration and in replication of results. Using this option will not only result in economy of effort, avoiding duplication, but the proposed project may also benefit from the experience of other twin researchers.

Still, there may be many good reasons to start a twin register, for instance, in specific countries or populations. In that case, some other focal questions arise, starting with the question of initial funding. This is, of course, a relevant question, and the available options will depend on many, often local, factors. When applying for initial funds, it may be practical to adapt the objectives to limited resources by focusing on a specific research topic rather than putting in a more general appeal to establish a research infrastructure. It is often wise to refine the research agenda in order to meet two complementary objectives: obtaining meaningful results in the short-term and looking for synergy with other research groups. Planning a long and complex research question will delay results that will be needed, since future funding will probably depend on early success. Hence, it is important to select a main phenotype to study based on its originality, the interest in the question within the scientific community, the uniqueness of the sample, or the available resources for data collection (see Box 11.3). Complementarily, it is important to seek out researchers not only within other twin registers around the world but also outside the twin community, who have an interest in the selected phenotype and/or have relevant data. Collaboration with experienced researchers in the field is of value for a new project, while researchers from different disciplines may be interested in the possibilities that collaboration with a twin cohort offers. Identifying possible topics of common interest to the newly starting twin register and existing groups, which can contribute specific knowledge or techniques, may open new perspectives and facilitate trade-offs.

Box 11.3 How many twins?

For most of the 20th century, until about 1970, there were only vague notions of how big twin studies needed to be to provide useful estimates of the degree of genetic influence (heritability), and many of the early studies, including small numbers, gave highly inconsistent results when complex human traits were analyzed. In retrospect, we can see this was mainly due to studies being underpowered, although inaccurate zygosity diagnosis also played a role. “Is there a genetic contribution to scholastic performance?” was the motivating question behind the first Australian twin study on school examination results from 1967.[83,88] While the study of 150 twin pairs was fairly large by the standards of that time, it soon became apparent that it was far too small to reliably estimate all genetic and environmental sources of variation, specifically the separation of additive genetic (A) and common or shared environmental variance (C). Multiple analytical and simulation studies now provide detailed tables with the required numbers of twin pairs for continuous and categorical traits, often distinguishing between uni and multivariate designs.[13,14,84]

11.2 Strategic planning

A twin register ideally is a longitudinal resource and, therefore, the first steps should be considered as the basis of a long-term effort. Decisions made during the first steps should facilitate the strategic planning of the register as a long-lasting organization. This involves setting the main goals and selecting the activities, in accordance with the available resources, which need to be undertaken to achieve the established objectives. Here, we first discuss human resources and adaptability to changing conditions. Human resources are, obviously, a core element of a twin register. A group of highly motivated and coordinated researchers is needed to start and develop a twin register. Thus, the question of identifying who may provide valuable help and be willing to participate in the endeavor becomes crucial. Two different kinds of human resources should be contemplated: established researchers, from inside and outside the twin research community, who can contribute with expertise, advice, and logistics in their respective fields; and researchers or support personnel, who will be in charge of developing and maintaining the register. While the former is important in providing support and visibility, the latter is essential, since they will take care of the multiple tasks involved in the daily running of the register, that is, from planning and conducting data collection or updating contacts, to analyzing data or writing papers. Therefore, human resources management (including selection, training, and career development) with the objective of forming a reliable and enduring core group is paramount if the register is to go ahead. Flexibility, adaptability, and keeping an eye on opportunities are also relevant issues. In a changing environment, where critical aspects such as funding or collaborations may change constantly, it seems wise to contemplate different horizons and be able to quickly adapt research objectives to different scenarios. This implies too the capacity to keep going with limited resources while being prepared for incoming opportunities. Focusing only on long-term and complex research objectives may represent a handicap for register development in case of funding shortage or operational obstacles. Keeping in mind and planning parallel sets of objectives adapted to different conditions may help to overcome temporary difficulties.

11.3 Basic elements

There are several key elements that are at the core of the development of a twin register and that will determine its endurance and scientific success.

11.3.1 Recruitment methods

One of the foremost questions of every researcher willing to start a twin register relates to which are the best practices for optimum recruitment and retention methods. There is not one clear answer and there may be as many methods as established registers. Recruitment strategies depend on a research protocol that can specify, for example, age at recruitment, inclusion and exclusion criteria, recruitment group (e.g., parents

of young twins, adolescent, and adult twins), and possibilities of the research team, which may be affiliated with an academic institute or a medical infrastructure.

Table 11.1 summarizes, in a nonexhaustive manner, some of the possibilities for recruitment of participants. They can be divided into four major groups: (1) existing databases managed by public (e.g., city council, educational or health systems) or private (e.g., hospitals or insurance companies) stakeholders; (2) institutions or organizations that have access to twins; (3) participants recruited through media, advertisement, and social events; (4) word-of-mouth and recruitment through enrolled participants of register. There are many ways to find and enlist twins and, within these categories, researchers should be creative in finding ways to invite participants to a register. Different citizen registers or records can provide information about twins (e.g., birth or military records). In some countries, sampling twin pairs are based on computerized population registers, either from direct information on multiple births or from applying algorithms based on sharing of date of birth, family name at birth, place of birth, and so on. A request to provide addresses of persons

Table 11.1 Recruitment methods of twin registries.

Using existing databases with information on twins managed publicly or privately
Previous twin studies
Population registries
Birth records
Immunization registries
Different patient registries
Voter records
Military records
Collaboration with institutions and organizations
State public health resources (e.g., healthcare departments)
Hospitals, maternal hospitals, and outpatient clinics
Insurance companies
Schools
Orphanages and adoption agencies
Multiple birth associations
Twin clubs and associations
Recruitment through media and social events
Media, newspapers, TV, and radio
Advertisement
Information brochures
Website
Social Media
Scientific and social events (e.g., twin festivals and annual gatherings)
Word-of-mouth and through enrolled participants in register

Note: The table does not attempt to be exhaustive.

born from the same mother with an identical date of birth can be done by municipalities. In all cases, "real" twins have to be distinguished from a larger subset of "possible" or "administrative" twins, as sharing the name of the mother and date of birth might occur by chance.[21] Next, parents of twins or twins need to be contacted, with an invitation to participate in the register. Population samples can also be obtained through collaboration with hospitals and schools. Records can be available at maternity hospitals, which may give an opportunity of direct recruitment of study participants. The recruitment through schools gives possibilities to obtain information on school achievements from teachers. Many registries collaborate with twins or parents of twins associations.

Other twin collections are gathered independently of centralized records or institutions and may depend more on the motivation of the twins or their parents. Recruitment through advertising has been used, as well as through mass media articles on twins and twin research in which information on major achievements is combined with continuing studies and contact information. Such approaches can be effective, and the possible effects of bias in non-randomly ascertained samples can be dealt with by statistical methods.[22,72] Twin pairs can be registered via completion of a registration form online by either the twins or their parents if they are under the age of legal consent. Other avenues of recruitment include offering of booklets to parents who expect twins. The exposure on twin research findings on general media also attracts new participants. Some registries organize social events (e.g., twin festivals, a range of exhibitions about twins, including photos and pictures). Common meetings of enrolled and new participants can benefit in the realization of a register and contribute to the strengthening of the role and value awareness in participants. A useful practice can be when participants give presentations about their own experiences during meetings or on social media or websites.

11.3.2 Informed consent

Twin registers are set up with the aim of conducting multiples studies across a long period of time and generally collect a wide variety of data in their participants. While participants upon registration may agree at the start of the study to the general aims provided in information brochures and will consent to be approached in years to come, the initial consent will not cover all the data to be collected in the future. Participants should be kept informed of the ways in which their data are used and be provided with the option of withdrawal at any stage of the research. Researchers need to establish how they will meet the participants' rights to know and to withdraw. Although the way this is laid down in law will not be the same across all countries in the world, it is always part of good scientific conduct. In the past, technology was not sufficient to provide individual feedback, and information on the use of collected data was often provided in a general manner via websites or mass-mailing of newsletters. As a result of technological advances, it is now possible to build portals or apps to provide much more personalized information, showing a person for which purpose his/her data were used, and allowing participants to indicate whether they

want to participate in specific projects or withdraw from the ongoing study. Such personalized platforms may require additional information from participants such as email address or phone number for verification purposes and provide new log-in information. The extent to which active informed consent requiring a handwritten signature is needed or it is sufficient to inform the participant and have an opt-out procedure needs to be discussed with an ethical committee any time new data collection takes place. Thinking of the different kinds of projects that will take place and the way information will be shared with participants and getting the tools ready before the start of the twin register will not only save valuable resources later on, but it may also show the participants you will protect their rights, leading to increased trust in the twin register.

11.3.3 Determination of zygosity in twin registries

For a twin register, a critical measurement point is the zygosity status of a twin pair, that is, MZ or DZ, as it is the basis for subsequent research that focus on heritability estimation and genetic covariance structure modeling. It is also one of the most frequently asked questions by the twins, as they are sometimes uncertain or misinformed about their zygosity status. Even when no genetic analyses are carried out and the large datasets are used for epidemiological studies, researchers may want to correct for clustering in the data, depending on zygosity status. Misclassification of zygosity status in MZ or DZ pairs generally results in the heritability estimate going down (Fig. 11.1). In extreme cases, it may even result in wrong conclusions to be drawn from variance components modeling.

Zygosity can be determined according to simple rules (see Box 11.4), but DNA testing will give the most conclusive zygosity assessment. A recent development is to genotype both twins with single nucleotide polymorphism (SNP) arrays such as the Illumina Infinium global screening array or the Affymetrix Axiom World Array.[23] These arrays allow for fast genotyping for over 600,000 SNPs, which is more than is required to determine twin zygosity. However, given the reductions in genotyping costs, and the possibilities for future genetic association studies, makes a genome-wide array a good investment. Of course, both twins need to provide their DNA. This can be collected by available prefabricated DNA kits for collection of buccal or saliva DNA at home, or blood can be provided at the study site. Once in the lab, DNA needs to be extracted, purified if needed, and diluted to the right concentration. The subsequent steps might be more array specific, but involve the fragmentation of the DNA into smaller pieces, then precipitation, and then hybridization to the chosen array. Here the sample fragments of DNA will ‘connect’ to the SNP alleles, variants of DNA sequence in humans, which are present on the array. This hybridization results in a fluorescent tag, which subsequently can be read from the array for all SNPs.

For zygosity assessment, a minimum number of typed SNPs needed is around 50; however, using between 20,000 and 30,000 typed SNPs is optimal. At the DNA level, MZ twins will share (close to) 100% of their alleles. DZ twins will share on average 50% of their alleles, similar to siblings. After using the factory standard

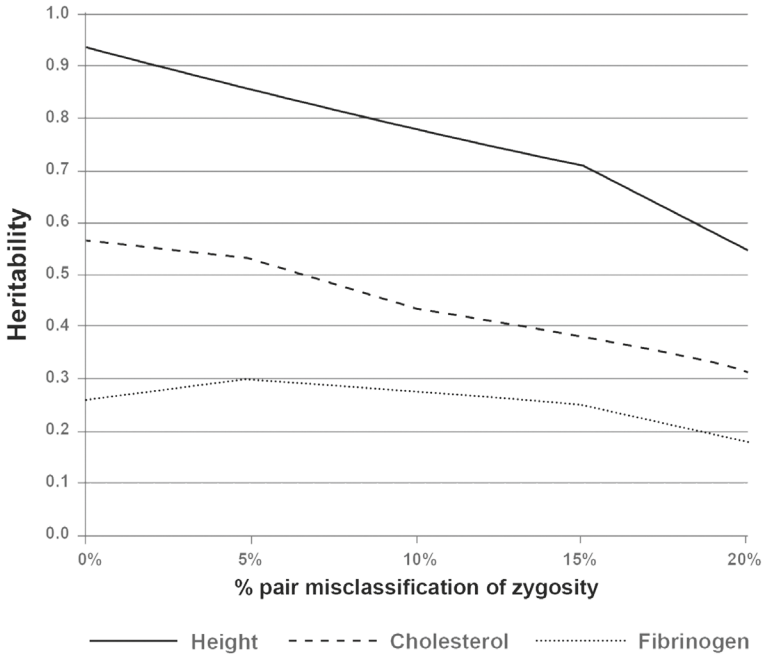


FIG. 11.1 The effect of zygosity misclassification on the heritability estimates within a twin study.

In this figure, heritability estimates for height, total cholesterol, and fibrinogen are given on the y-axis. These estimates were calculated from the phenotypic correlations "c" between the two individuals of 391 Dutch DNA-confirmed MZ and 391 DZ pairs, with the formula $(c_{MZ}\text{-}c_{DZ})/(1\text{-}c_{DZ})$. Subsequently, in 5%, 10%, 15%, and 20% of these pairs, the zygosity status was flipped from MZ to DZ, and from DZ to MZ, introducing misclassification (x-axis). Then, the heritability was recalculated and plotted in the figure. Depending on how strong the heritability of the phenotype is, the misclassification in general reduces the overall heritability estimate.

Box 11.4 Basic rules for zygosity determination

- Opposite-sex: DZ
- Different blood groups: DZ
- Large differences in eye, skin, and hair color: DZ
- One placenta: MZ (note that two placentas does not imply DZ)
- Alike as two peas in a pod; parents cannot tell the children apart: likely MZ
- Offspring and grandchildren cannot tell parents or grandparents and their twin apart: likely MZ
- Discordance for blood group or DNA markers: DZ

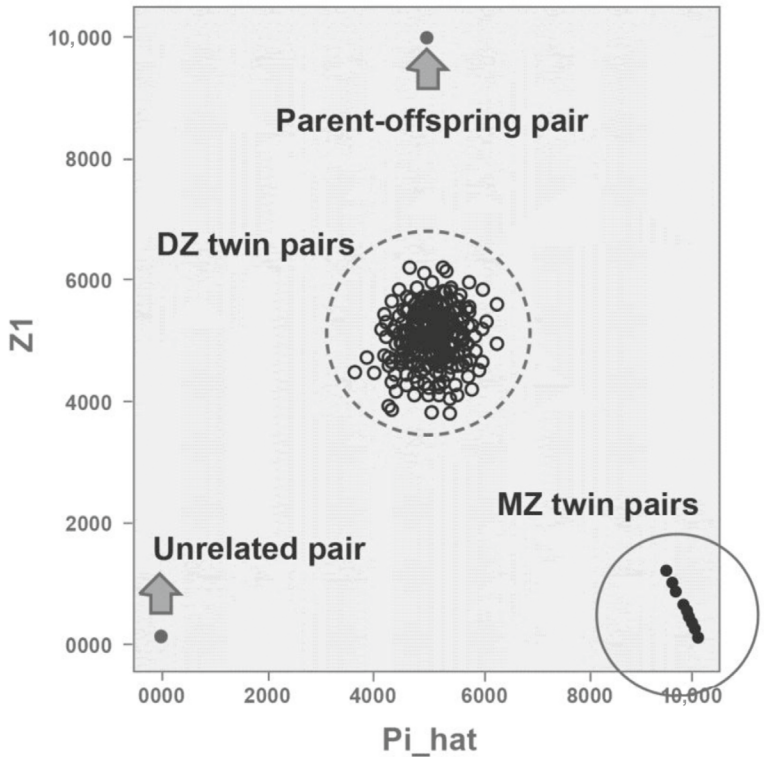


FIG. 11.2 Allele sharing of various family pairs plotting the sharing of one allele versus Pi-hat to identify monozygotic and dizygotic twin pairs.

tools for array genotyping (Beadstudio or APT-genotyper), a tool like Plink[24] can be employed to quality control the DNA data, select an optimal number of SNPs, and determine the allele sharing in all pairs (genome option). This sharing is then given by the percentage of markers for which a pair shares no alleles (Z0), one allele (Z1), and two alleles (Z2). From these proportions, the overall sharing is calculated, by π (pi), which equals 0.5 x Z1 + Z2. Then, MZ pairs can be identified from the results by finding pairs with a $\pi > 0.90$. The DZ pairs can likewise be selected, by finding pairs that have a π between ~0.30 and ~0.70, and a similar value for sharing 1 allele (Z1) (Fig. 11.2). For other values of π, researchers need to recheck which DNA sample was typed for the twins.

This approach has several more advantages. There is a useful genotyped dataset that allows for checking additional issues like genetic relatedness among participants, gender, heterozygosity, and if the study population is ethnically heterogeneous. As next steps, SNP sets can be imputed to, for example, the 1000 Genomes phase 3 or the Haplotype Reference Consortium genome reference panels.[25] These data can then be analyzed in genetic association studies and contribute through meta-analysis

in consortia to localization of genes for complex traits, to polygenic risk scores analyses and estimation of SNP heritability, or employing Mendelian randomization to find causative relations.

11.3.4 Phenotyping: from survey to record linkage

Twin registers have obtained a wide variety of phenotype data through various methods. The basic measurement method often is the survey, with registers sending out surveys at regular intervals. When deciding on what to include in a survey, the purpose of the current survey as well as the long-term goals need to be taken into account. For instance, a funded study may focus on alcohol use, but a long-term goal may be to determine how genes and lifestyle contribute to depression, so it would make sense to include a depression scale in the survey. Also, it is important to consider which data can still change over time and which data are fixed and do not need to be obtained again. This may of course be age-specific. For instance, in a middle-aged group questions regarding educational level may not need to be repeated. Questionnaires also need to keep a balance between the quantity of information gathered and the participants' needs, since they should not be burdened with too many questions, risking attrition, or incorrect/missing data. While devising the first survey may seem daunting, many twin registers will be happy to share information to help the new register use well-established procedures and avoid pitfalls in the survey set-up. While survey data can be obtained in all or at least large groups of participants, some data can only be collected in limited numbers. Laboratory procedures or specific phenotypes needing of complex settings, long assessment times, or expensive equipment are not easily applicable to large samples. Examples would be studies on brain imaging or extensive cognitive testing. In these cases, participants maybe invited based on specific inclusion criteria. New developments taking advantage of information technologies are modifying data collection procedures in epidemiological research and are also applied in twin studies. This includes computer-assisted surveys, and ambulatory assessment of objective (e.g., actimetry) and self-reported (e.g., mood and exercise) phenotypes through web or mobile applications.

Data collection is not, of course, limited to surveys or laboratory assessment. The assessment of environmental exposures linked to address or workplace information and the development of exposome-wide association studies represents novel approaches to gathering information for research purposes that do not need the direct involvement of the register participant.

Record linkage to external databases (e.g., hospital, primary care, or insurance and education records) also is an invaluable source of information that has been used by registers, in Scandinavian and other countries. For example, Van Beijsterveldt et al.[26] linked phenotype information from the Netherlands Twin Register to the database of the Dutch pathological anatomy national automated archive. Record linkage was successful for over 9000 twin pairs. The effect of chorion type was tested by comparing the within-pair similarity between monochorionic and dichorionic MZ twins

on 66 traits. They concluded that the influence of the intrauterine prenatal environment, as measured by sharing a chorion type, on MZ twin resemblance was small and limited to a few phenotypes, implying that the assumption of the equal prenatal environment of mono and dichorionic MZ twins, which characterizes the classical twin design, is largely tenable.

11.3.5 Possibilities for biobanking in twin registers

Many twin registries collect biological samples from their participants. Initially, the reason for the collection of blood samples often was to have a reliable measure of zygosity based on blood group or DNA typing, but biological sample collection can also extend the potential of genetic epidemiologic research into, for example, cardiovascular and late-life health and mortality, by allowing measurement of biomarkers. Combined with the twin design, this allows estimation of the contribution of genomic factors (genetic, epigenetic, and gene expression) and biochemical factors (metabolites and proteins) to intermediate phenotypes and risk factors of disease, such as lipid levels.[89] Designs involving MZ twin pairs allow discovery of variability genes, as demonstrated for lipid levels.[27] The development of laboratory technologies has dramatically increased opportunities to study collections of biospecimens and their related data. This allows for comprehensive studies of complex diseases and phenotypes, facilitates identification of predisposing genes and epigenetic factors, and provides support for a better understanding of disease etiology.

The organization of biobanks becomes an important element with the increase of biospecimens and the necessity to conserve them. For example, whereas germline variations in the DNA sequence of a person rarely depend on the age at which a sample was collected, this is different for somatic DNA variation, epigenetic, and telomeric variation, for which the subjects' age when the specimen was collected is an important determinant.[28–30] Other determinants of epigenetic profiles are tissue/cell type[31] and lifestyle factors such as smoking. Many types of samples (e.g., whole blood) contain a mixture of cell types with distinct epigenetic profiles. In epigenetic studies of such heterogeneous samples, assessment of cell counts allows to control for variation in cellular proportions between samples.

There are multiple strategies for collection, processing, and storage of biological samples. A wide variety of specimen types may be collected and in many molecular genetic studies more than one tissue is stored, including blood and blood fractions (plasma, serum, buffy coat, and red blood cells), RNA, saliva, buccal cells, urine, hair, fecal samples, or nails. Each of these specimen types needs to be collected, processed, and stored under conditions that preserve their stability with respect to the intended future analysis.[32,33,76,77]

Collection of blood specimens should be carried out by trained personnel. An evacuated tube system (vacutainers) or plastic tubes are commonly used to collect blood. Umbilical cord blood is a useful source for research purposes since the method of collection is not invasive. It can be obtained either through venous puncture of the umbilical cord or direct drainage to a sterile container immediately after delivery

(vaginal or caesarean). Blood is often fractionated in components (mononuclear leukocytes, neutrophils, erythrocytes, and plasma) before being analyzed or stored. When biobanking, blood should be aliquoted across series of tubes, as most assays use only a small amount of plasma or serum and this avoids thaw/refreeze. Serum or plasma allow for analyses of classical biomarker assays, antibodies, nutrients, lipids and lipoproteins, leptin, adiponectin, growth hormone axes, thyroid axis, inflammation, liver and kidney function, innate immunity, and metabolomic and proteomic analyses.

Metabolomics is the rapidly evolving field of the comprehensive measurement of ideally all endogenous metabolites in a biological fluid. The use of mass spectrometry and nuclear magnetic resonance spectroscopy provide novel biomarkers of metabolic health.[34] Depending on the biomarker of interest, it may be important to collect, and note, whether samples were taken after fasting, how long after a meal or on a particular day, and of the menstrual cycle in women.

Whole blood, saliva, or buccal cells are excellent sources of DNA. Self-collection of buccal cells is a safe, simple, and cheap method that can be used to reduce the cost of specimen collection and is often preferred over blood collection by participants. Several methods are used for collecting buccal cells, including swabs, cytobrushes, and a mouthwash protocol.[35,36,77] Other sources of DNA include, for example, toe nails.[37]

In contrast to DNA, RNA is very sensitive to degradation at room temperature. Transcriptomics studies require careful RNA collection, using the PAXgene Blood RNA System, which consists of a blood collection tube in which intracellular RNA is stabilized (PAXgene Blood RNA Tube) and can be isolated by using a nucleic acid purification kit (PAXgene Blood RNA Kit). Alternatively, the samples can also be by snap frozen in liquid nitrogen, or RNA can be isolated from PBMCs using Histopaque density gradients. Total RNA, including miRNA, can be isolated simultaneously from different biological sources. Plasma (300 μL) and serum isolations can be performed using miRNeasy Serum/Plasma kit from Qiagen. For isolation, after homogenization, from tissue biopsies (e.g., cartilage or adipose tissue), the miRNeasy Mini Kit from Qiagen can be used.

Many analytes, such as steroid hormones, pesticides, and a wide variety of drugs and their metabolites, can be measured in urine, making it a convenient specimen for a variety of studies. Urine collection can be performed under different conditions depending on the study goal: immediately upon rising in the morning, random urine specimens (for drug monitoring and cytology studies), fractional specimens after the last evening meal (to compare urine analyte levels with their concentration in blood), and timed urine collections (e.g., 12 and 24 h to allow comparison of excretion patterns). Urine specimens should be maintained on ice or refrigerated for the duration of the collection. Collection vessels are generally larger than for other liquid specimens (from 50 to 3000 mL). Due to the non-invasive method of collection and metabolic composition urine is widely used in research of metabolite biomarkers and a wide range of diseases.[38]

For microbiome investigation, fecal samples can be collected easily in a sealed container following simple instructions, and their processing can provide important information for classical twin analysis, such as in the studies estimating the heritability of gut microbiota,[39] and related epidemiological and molecular approaches.

11.3.6 Databases for twin registers

Both administrative processes and scientific applications require database systems that recognize the clustered structure of data collected in twin families. Administrative processes may consist of importing new participants, who may or may not be related to existing participants, address management, documenting the participation status of individuals (moved, not willing to participate, ill, and deceased), and storing information on contacts and mailings with, for example, invitations to take part in particular studies, the responses to mailings and invitations, and outcomes of approaching nonresponders. Any system that keeps track of personal information needs to adhere to guidelines concerning privacy. Identifying information, such as name, date of birth, and address, should be stored separately from other information collected on participants. Often, administrative and scientific processes will be supported by different database systems whose requirements depend on the dimensionality of the data. Phenotype data from surveys will require different systems than imputed genotype data which may contain as many as 50 million markers per person. Different databases may each work with separate anonymous IDs, and keys to link databases should be carefully kept.

Databases that contain multiple relatives, should consider how to store information on family relations,[40,41] especially when recruitment of participants not only involves twins, but also other relatives and multigeneration pedigrees – for example, parents or offspring of twins (see Box 11.5).

Box 11.5 Twin designs

The classical twin design encompasses MZ and DZ twin pairs but there are other designs. For instance, twin and adoption designs can be combined when twins reared apart are accessible. More often, twin registers may have the opportunity to incorporate other kind of relatives (extended twin family designs) that can be contemplated by a register even from the very beginning. These extended designs and possible combinations offer additional opportunities and statistical power to challenge research questions, such as the possibility to disentangling genetic from shared-environmental influences within family relationships.[14,20,85] The classical design may be enlarged around the twins by incorporating twins' parents (nuclear twin family design), twins' offspring (children-of-twins design), or parents, offspring, siblings, and spouses,[86,87] according to available information, to finally incorporate all different kind of relationships that can be found within a register dataset. An example of such broadening of sample scope is provided by the Netherlands Twin Registry,[41] which used an extended-twin pedigree, making use of all the relationship types available in their database (except teacher-student), to be able to estimate the contribution of shared household effects to neuroticism in the presence of non-additive genetic factors.

11.3.7 Data analyses issues in twin studies: batch effects and family clustering

Phenotyping in twins has often included biomarker assessments, such as lipids or hormone levels, and increasingly include assessments obtained by means of high-throughput technologies, such as genetic variants, gene expression data, and epigenetic modifications. These data are important to understand the nature of genetic variance components as established in twin and family studies,[42] and are themselves subject to such studies, for example, studies of the heritability of methylation and gene expression.[43–47]

Subtle differences in the processing of batches of biological samples are known to give rise to the batch effect. The registration of information relevant to batch (batch number, analyst, time, date) provides the means to correct for such effects, and various methods have been developed to this end.[48–51] Regardless of the methods to correct for batch effects, there is agreement that it is beneficial to randomize samples evenly over batches, and that this randomization should extend to case-control status and familial relatedness.[52,53] Furthermore, sample size per batch is an important factor: the larger the sample size per batch, the more accurate the batch correction.

Batch assignment of samples collected in family members raises the question of whether samples of family members should be processed together in the same batch or should be distributed—as far as possible—over distinct batches. We examined this question in two small simulation studies (for details, see Appendix). In the ideal situation of a balanced allocation design with relatively large batch sample sizes, accurate correction of batch effects is feasible, as we established in a simulation study (see Appendix). In the first simulation study, MZ twins were selected for concordance and discordance on phenotype X, which predicted phenotype Y, where Y (e.g., a biomarker) was subject to batch effects. Given the ideal scenario of random assignment and large batch sizes, we found that allocation regime (randomized as pairs or as individuals) had little effect on the results of either the regression of Y on X, or on the twin covariance matrix of Y conditional on X. The type of correction (random effects or fixed effect correction for batch) had no bearing on these results.

In the second simulation study, we considered the decomposition of phenotypic variance into additive genetic and shared and unshared variance components (ACE model) using linear mixed modeling.[54] The sample sizes (N_{MZ} and N_{DZ}) were relatively small: $N_{MZ} = N_{DZ} = 200$ (400 pairs) or $N_{MZ} = N_{DZ} = 120$ (240 pairs); the number of batches was 15 or 25. The batch assignment was random by pair (both twins share a batch) or by individual. Note that randomization by individual does not rule out batch sharing. Conditional on batch, the ACE components were 4 (A), 2 (C), and 4(E), and batch variance equaled 1 (i.e., 1/11 = 9.1% of the phenotypic variance). We conducted both one-step analyses and two-step analyses (correct for batch effects in step 1 and estimate variance components in step 2), and we treated the batch effects as fixed and random. The results suggested that in the one-step analyses, the estimates of the variance components were as good as those obtained in the standard ACE model (without batch effects). In the two-step analyses, we found that random assignment by individual resulted in slightly better estimates. Notably,

the C variance components were underestimated following random assignment by pairs (see Appendix).

Note that in the absence of batch effects, family clustering may still be an issue in statistical inference, based on the assumption that the data are independently and identically distributed. For instance, in genome-wide association studies (GWAS), family clustering violates the independence assumption. Happily, family clustering does not pose any statistical problems, as random effects modeling and generalized estimating equations can be used to either accommodate or to correct for the effects of family clustering, or more generally for genetic relatedness.[55–57] Regardless of randomization scheme (or not), detailed information should be recorded on batch (date and time of processing), operator (technician), plate number, and position (row and column).

11.3.8 Retaining the twins

To retain participants in a longitudinal study, it is important to remain in contact. Many twin registers have set up a website providing information of the latest study results, news on grants obtained, PhDs awarded, and more general information on twin meetings and such. However, these may not be the best ways to form an actual connection between the twin register and participants. Most twin registers therefore also contact their participants in a more personal manner, either by letter or e-mail, sending out a regular newsletter to make the participant aware that the register is still seeing them as a valuable contributor. A number of twin registers also organize events in which twins and their family members can meet each other but also can meet the researchers and ask any questions they may have in person. Worth mentioning here is the annual gathering of twins at the Twins Day Festival in Twinsburg Ohio, where researchers are welcome to recruit twins for specific studies. Unfortunately, financial limitations generally prevent the twin registers from organizing such large and regular gatherings, but when meetings are organized, they are generally judged as very valuable.

In addition to general information, personalized information may also be given out to participants. When participants take part in specific projects, information on test scores (e.g., the results of an IQ tests or the cholesterol levels obtained in a blood sample) may be returned to the participants, accompanied by an explanation of the results. However, often little feedback is provided to participants related to the surveys completed during the longitudinal follow-up, due to the material and personnel costs needed for sending personalized reports to the large number of participants generally included in a twin register. However, as technology advances new ways emerge of providing personalized information. Participants' portals may provide individual reports without needing to write and post separate reports. At the Netherlands Twin Register, such an effort is now well underway, with participants obtaining information on the survey results via the MyNTR portal. As with the informed consent, it is important to consider the requirements of such a portal in advance. Constructing a participant panel even before starting the actual twin register that includes a number

of twins who are willing to think about the various aspects involved in providing feedback would be helpful in setting this up in the best way possible for twins and the register support staff.

11.4 Conclusion

Twin registers have a long and successful history and a brilliant future as a research resource. The uniqueness of twin samples, the soundness and diversity of the methodological approaches, and the huge amount of data accumulated during the last decades characterize twin registers as invaluable contributors to the advancement of science, including social science. Their versatility to adapt to multiple scenarios and their orientation to collaborative work will preserve their value in the future as priceless instruments for the expansion of knowledge in the complexities of human phenotypes.

Although the global research agenda in the coming decades is difficult to forecast, twin registers can contribute to our understanding in virtually all areas related to human health and behavior. Population-based registers, especially when representative of the general population, are still cohorts of enormous epidemiological interest. The unique characteristics of twin studies, including the ability to control both genetic and shared environmental background, allow for addressing questions that are not easily solved in any other research design. These capacities make them extremely useful for gene-environment transaction research or causal inference studies.[58,59] Twin pairs—in particular those that are MZ—are remarkably informative in respect to variability of phenotypic expression, pathogenic mechanisms, epigenetics, and post-zygotic mutagenesis, and may serve as a model for research on genetic defects.[15,18,60,61] Participation of twins in co-twin, control-designed, and randomized controlled trials is an informative, albeit infrequently used, design.[62] The use of twin studies has been advocated for guiding post-GWAS studies on the effects associated with genetic variants,[63] enabling stronger tests of causal hypotheses,[64] formulating future strategies in pharmacogenomics research,[65] or refining phenotypic definitions and evaluating biomarkers for disease.[15] Furthermore, due to their amenability to numerous nonclassical study designs, data based on twin registers can integrate with other resources to boost research in virtually every field of human research. Probably the best example is provided by the participation of twin biobanks in many of the large association studies (GWAS and EWAS) that have been published in the last decade.

An additional feature empowering twin registers relies on their orientation to collaborative work. The community of twin registers has a long history of successful alliances. The very nature of their origin as research resources and their scientific environment implies, on the one hand, the existence of matching data across different registers and, on the other hand, the need for very large samples in order to find answers to some of the research questions investigators are interested in. In these circumstances, collaboration is not only practicable, but it is a must. Multiple consortia

and collaboration initiatives have seen the light as an answer to those needs. The GenomeEuTwin,[73] EuroDiscoTwin,[66] or the CODATwins (COllaborative project of Development of Anthropometrical measures in Twins)[67] consortia are just a few examples of associative efforts, joining together data from a large number of twin cohorts in order to advance in the analysis of the genetic and environmental underpinnings of human complex phenotypes. Other initiatives, such as the International Network of Twin Registries[68] have emerged from the International Society for Twin Studies, aiming to foster collaboration and serve as a platform for networking and establishing research relationships between twin registers and between them and the global scientific community.

These collaborative efforts have a parallel outcome on infrastructures related to the registers, such as biobanks. In the same way that registers multiply their scientific impact when joining efforts, the effective use of biobank resources depends on their accessibility. Building a centralized database for the research community allows storing of raw and processed data, reference data for case-control studies and imputation, and linking to clinical phenotypes, so that data can be effectively used not only by single research groups, but also in collaborative multicenter and consortium projects. For instance, the advent of the GWAS method took advantage of such multicenter collaborations in order to lead to the successful identification of thousands of variants that are robustly associated with complex disease phenotypes. The big databases permit research on genetic, methylation, expression level, available protein, lipid, metabolite level information, and on disease/phenotype level. In Europe, for instance, a range of biobanks joined in the Biobanking and BioMolecular Resources Research Infrastructure and national hubs (e.g., www.bbmri.nl) generated omics data by the same platforms and shared these combined with existing phenotype data.

Nowadays, the advancement of scientific knowledge requires such collaborations to gain explanatory power and optimize the invested resources. Twin registers, and associated biobanks, have an enormous potential that multiplies when joining efforts, and new or growing registers are always welcome to this endeavor. In this chapter, we have outlined what we feel are the main principles and recommendations for the establishment and management of a twin register, from its inception to its actual development. As pointed out before, our intention has not been to enumerate a detailed checklist of actions, or a complete step-by-step technical guide on this process, but rather to highlight the main aspects that, from our perspective, need to be taken into account for being able to make the difference between an isolated initiative and a successful long-lasting scientific resource.

Appendix

Processing biological material in batches may give rise to batch effects, that is, intrabatch correlation greater than zero. A question that is specific to the twin design (or any other design with naturally clustered observations) concerns the manner of allocation of twins to batches. One may allocate randomly by individual twin, or

randomly by twin pair. The latter implies that the twin pairs share the batch, the former does not rule out batch sharing. The following are the results of simulation studies carried out to answer this question in three situations.

How to allocate twins to batch in assay of metabolites in an extremely discordant and concordant (EDAC) twin design?

Discordant and concordant twin pairs are selected on the basis of phenotypic scores, for example aggression scores, for a biomarker study. Assays on the twins' urine samples are done to measure metabolites. The aim is to determine the association between metabolite levels and aggression. The metabolites are determined on plates (i.e., in batches). The present question concerns the allocation of twins to batch, given that plate is a source of systematic variation:

1. assign twin pairs randomly to batches
2. assign twin members (individuals) randomly to batches.

An additional question, specific to the EDAC design, is the choice of the independent variable. As the selection is on aggression scores it is statistically expedient to regress metabolite (predictor) on aggression (dependent). Selection on the predictor does not affect the regression, and if the selection is based on an EDAC scheme, the selection results in little loss of power. Alternatively, one may choose to regress metabolite on the binary aggression scores (e.g., 0=low, 1=high). Regression on the continuous score is expected to confer greater power.

We make the following assumptions concerning the analysis. We assume that the twin data are to be analyzed in a single statistical model, which will include the discordant and concordant twins. With respect to this model, in testing the association of metabolite and aggression, we have to accommodate 1) the inherent two-level structure (family clustering of twins in twin pairs), and 2) the batch effects. We consider two models:

1. Linear mixed model, in which effect of batch is accommodated by means of a random effect (variance component).
2. Fixed regression model with metabolite corrected for batch in one or two step procedure. Two step procedure: regress metabolite on plate first, use residuals in regression on predictor. One step procedure: regress metabolite on plate and on predictor at the same time.

The association between metabolite and aggression is accommodated by means of a fixed effect, that is, regression of metabolite on binary (0/1) or continuous aggression score.

Simulation 1: Random effects model.

The metabolite explains 5% of the variance in aggression. The heritability of metabolite is 0.6, the heritability of the residual of aggression is 0.5. The number of batches is 70, the number of twin pairs is 600. The true phenotypic variances of metabolite and aggression are both set to equal 1 and the variance is .25. All variables have zero mean. So, the metabolite variance is 1.25. The number of replications is 50.

Allocation regime (pairs vs individuals) has no effect on the estimate of the parameter of interest. We note that, as expected, regression on continuous predictors confers more power than regression on binary predictor (0/1). The variance components (additive genetic, environment, and batch) appear to be slightly downwards biased in the allocation by pair, but accurate in the allocation by individual condition.

Simulation 2: Fixed batch effects in two steps or one step.

It may be expedient to carry out analyses in two steps, that is, first correct for batch effects, and second carry out the analysis of actual interest. We compared one and two step analyses in simulation 2. We used linear mixed modeling in simulation 1 (estimating the twin covariance conditional on predictor and batch). Here we use GEE (generalized estimating equations), that is, we correct the standard errors after the analyses using a sandwich correction.

One-step: using GEE regress metabolite on predictor and batch simultaneously.

Two-steps: first correct metabolite for batch effect and then use GEE to analyze the residuals.

Conclusions are the same as those based on Simulation 1. Allocation regime (pairs vs. individuals) has little effect on the test of the parameter of interest. Again, as expected, regression of continuous predictors confers more power that regression on binary predictor (0/1). We see little differences between one and two step procedure.

Simulation 3: More extreme selection and fixed plate effects in two step or one step.

This simulation is the same as simulation 2. However here we employ a more extreme selection criterion rather than a mean split (Simulations 1 and 2), the selection of high and low scoring twins is on the basis of the criteria >0.5 std unit or < -0.5 std units. As in simulation 2, we carry out one and two step analyses using GEE. Given the selection, we set the total sample size to 5000 (random sample) and select from this sample based on the criteria mentioned.

The conclusions are consistent with those of simulations 1 and 2. The allocation regime, that is, pairs versus individuals, has little effect on the test of the parameter of interest. The regression of continuous predictors confers more power that regression on binary predictor (0/1), as expected. There is little difference between the results of the one and two step procedure.

How to allocate twins to batches in assay of metabolites in the classical twin design?

Simulation 4: estimating genetic and non-genetic variance components in the twin design.

In simulation 1, we noted that batch allocation by twin pair appeared to result in a slight bias in the estimates of the variance components. In simulation 4, we examined the effect on variance components by fitting an ACE model to twin data. Here we treat batch as a random and as a fixed effect, and we carry out both one step and two step analyses. We consider relatively small sample sizes. We use linear mixed modeling with REML (restricted maximum likelihood) estimation.

The results demonstrate that allocation regime has little effect in the one step analyses, regardless of whether this is based on random effects or fixed effects modeling of batch. In the two step procedures, we note a downward bias in the estimates of the A (additive genetic) and C (common environment) variance components. This bias is greater in given the allocation by twin pairs, and greater as the number of batches increases.

For details on the simulations and settings in the R code see: https://www.cambridge.org/core/journals/twin-research-and-human-genetics/article/establishing-a-twin-register-an-invaluable-resource-for-behavior-genetic-epidemiological-biomarker-and-omics-studies/A027C91A8B3EEBE4DE6AA5ADE49B2DA7#supplementary-materials

Acknowledgments

We gratefully acknowledge support by the Global Education Program (Russian Federation) to V.O.; the Royal Netherlands Academy of Science Professor Award (PAH/6635) to DIB; NWO grant 480-15-001/674: Netherlands Twin Registry Repository: researching the interplay between genome and environment and BBMRI-NL, a research infrastructure financed by the Dutch government (NWO, no. 184.021.007 and 184033111). The Murcia Twin Register is supported by the Seneca Foundation, the Regional Agency for Science and Technology, Murcia, Spain (19479/PI/2014) and the Ministry of Economy and Competitiveness, Spain (PSI2014-56680-R) including FEDER co-funding granted to JRO.

Disclosure of Interests

The authors declare no conflict of interest

References

1. Galton F. *The history of twins, as a criterion of the relative powers of nature and nurture*: Fraser's Magazine; 1875:566–576.
2. Fisher RA. The genesis of twins. *Genetics*. 1919;4:489–499.
3. Thorndike EL. *Measurements of Twins*. New York, NY: The Science Press; 1905.
4. Rende RD, Plomin R, Vandenberg SG. Who discovered the twin method? *Behav Genet*. 1990;20:277–285.
5. Poll H. Über Zwillingsforschung als Hilfsmittel menschlicher Erbkunde. *Zeitschrift für Ethnologie*. 1914;46:87–105.
6. Merriman C. *The Intellectual Resemblance of Twins*. Princeton, NJ: Psychological Review Co.; 1924.
7. Siemens HW. *Die Zwillingspathologie (Twin Pathology: Its Meaning, Its Method, Results So Far)*. Berlin: Springer Verlag; 1924.
8. Holzinger KJ. The relative effect of nature and nurture influences on twin differences. *J Educ Psychol*. 1929;20:241–248. http://doi.org/10.1037/h0072484.

9. Mayo O. Early research on human genetics using the twin method: who really invented the method? *Twin Research and Human Genetics*. 2009;12:237–245. http://doi.org/10.1375/twin.12.3.237.
10. Lejeune J, Turpin R, Gautier M. [Mongolism; a chromosomal disease (trisomy)]. *Bulletin de l'Academie Nationale de Medecine*. 1959;143:256–265.
11. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nature Rev Genetics*. 2002;3:872–882. http://doi.org/10.1038/nrg932.
12. Martin N, Boomsma D, Machin G. A twin-pronged attack on complex traits. *Nat Genet*. 1997;17:387–392. http://doi.org/10.1038/ng1297-387.
13. Martin NG, Eaves LJ, Kearsey MJ, Davies P. The power of the classical twin study. *Heredity (Edinb)*. 1978;40:97–116.
14. Posthuma D, Boomsma DI. A note on the statistical power in extended twin designs. *Behav Genet*. 2000;30:147–158.
15. van Dongen J, Slagboom PE, Draisma HH, Martin NG, Boomsma DI. The continuing value of twin studies in the omics era. *Nat Rev Genet*. 2012;13:640–653. http://doi.org/10.1038/nrg3243.
16. Ayorech Z, Selzam S, Smith-Woolley E, Knopik VS, Neiderhiser JM, DeFries JC, Plomin R. Publication trends over 55 years of behavioral genetic research. *Behav Genet*. 2016;46:603–607. http://doi.org/10.1007/s10519-016-9786-2.
17. Polderman TJ, Benyamin B, de Leeuw CA, Sullivan PF, van Bochoven A, Visscher PM, Posthuma D. Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nat Genet*. 2015;47:702–709. http://doi.org/10.1038/ng.3285.
18. Bell JT, Spector TD. A twin approach to unraveling epigenetics. *Trends Genet*. 2011;27:116–125. http://doi.org/10.1016/j.tig.2010.12.005.
19. Groen-Blokhuis MM, Middeldorp CM, van Beijsterveldt CE, Boomsma DI. Evidence for a causal association of low birth weight and attention problems. *Journal of American Academy of Child & Adolescent Psychiatry*. 2011;50:1247–1254.e1242. http://doi.org/10.1016/j.jaac.2011.09.007.
20. Knopick V, Neiderhiser J, DeFries J, Plomin R. *Behaviorla Genetics*. New York, NY: Worth Publishers; 2017.
21. Goldberg J, Miles TP, Furner S, Meyer JM, Hinds A, Ramakrishnan V, …, Levy PS. Identification of a cohort of male and female twins aged 65 years or more in the United States. *Am J Epidemiol*. 1997;145:175–183.
22. Bechger TM, Boomsma DI, Koning H. A limited dependent variable model for heritability estimation with non-random ascertained samples. *Behav Genet*. 2002;32:145–151.
23. Ehli EA, Abdellaoui A, Fedko IO, Grieser C, Nohzadeh-Malakshah S, Willemsen G, …, Hottenga JJ. A method to customize population-specific arrays for genome-wide association testing. *Eur J Hum Genet*. 2017;25:267–270. http://doi.org/10.1038/ejhg.2016.152.
24. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, …, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559–575. http://doi.org/10.1086/519795.
25. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, … HaplotypeReferenceConsortium. A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet*. 2016;48:1279–1283. http://doi.org/10.1038/ng.3643.
26. van Beijsterveldt CE, Overbeek LI, Rozendaal L, McMaster MT, Glasner TJ, Bartels M, …, Boomsma DI. Chorionicity and heritability estimates from twin studies: the prenatal environment of twins and their resemblance across a large number of traits. *Behav Genet*. 2016;46:304–314. http://doi.org/10.1007/s10519-015-9745-3.

27. Berg K. Variability gene effect on cholesterol at the Kidd blood group locus. *Clin Genet*. 1988;33:102–107.
28. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, …, Esteller M. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Nat Acad Sci USA*. 2005;102:10604–10609. http://doi.org/10.1073/pnas.0500398102.
29. Slagboom PE, Droog S, Boomsma DI. Genetic determination of telomere size in humans: a twin study of three age groups. *Am J Hum Genet*. 1994;55:876–882.
30. van Dongen J, Nivard MG, Willemsen G, Hottenga JJ, Helmer Q, Dolan CV, …, Boomsma DI. Genetic and environmental influences interact with age and sex in shaping the human methylome. *Nat Commun*. 2016;7:11115. http://doi.org/10.1038/ncomms11115.
31. Finnicum CT, Dolan CV, Willemsen G, Weber ZM, Petersen JL, Beck JJ, …, Ehli EA. Relative telomere repeat mass in buccal and leukocyte-derived DNA. *PLoS One*. 2017;12:e0170765. http://doi.org/10.1371/journal.pone.0170765.
32. Garcia-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, …, Rothman N. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. *Cancer Epidemiol Biomarkers Prev*. 2001;10:687–696.
33. Holland NT, Smith MT, Eskenazi B, Bastaki M. Biological sample collection and processing for molecular epidemiological studies. *Mutattion Research*. 2003;543:217–234.
34. Suhre K, Meisinger C, Doring A, Altmaier E, Belcredi P, Gieger C, …, Illig T. Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting. *PLoS One*. 2010;5:e13953. http://doi.org/10.1371/journal.pone.0013953.
35. Meulenbelt I, Droog S, Trommelen GJ, Boomsma DI, Slagboom PE. High-yield noninvasive human genomic DNA isolation method for genetic studies in geographically dispersed families and populations. *Am J Hum Genet*. 1995;57:1252–1254.
36. Min JL, Lakenberg N, Bakker-Verweij M, Suchiman E, Boomsma DI, Slagboom PE, Meulenbelt I. High microsatellite and SNP genotyping success rates established in a large number of genomic DNA samples extracted from mouth swabs and genotypes. *Twin Research and Human Genetics*. 2006;9:501–506. http://doi.org/10.1375/183242706778024973.
37. Hogervorst JG, Godschalk RW, van den Brandt PA, Weijenberg MP, Verhage BA, Jonkers L, …, Schouten LJ. DNA from nails for genetic analyses in large-scale epidemiologic studies. *Cancer Epidemiol Biomarkers Prev*. 2014;23(12):2703–2712. http://doi.org/10.1158/1055-9965.EPI-14-0552.
38. Duarte IF, Diaz SO, Gil AM. NMR metabolomics of human blood and urine in disease research. *J Pharm Biomed Anal*. 2014;93:17–26. http://doi.org/10.1016/j.jpba.2013.09.025.
39. Goodrich JK, Davenport ER, Beaumont M, Jackson MA, Knight R, Ober C, …, Ley RE. Genetic determinants of the gut microbiome in UK twins. *Cell Host & Microbe*. 2016;19:731–743. http://doi.org/10.1016/j.chom.2016.04.017.
40. Boomsma D, Willemsen G, Vink JM, Bartels M, Groot P, Hottenga JJ, …, van der Kleij F. Design and implementation of a twin-family database for behavior genetics and genomics studies. *Twin Research and Human Genetics*. 2008;11:342–348. http://doi.org/10.1375/twin.11.3.342.
41. Boomsma D, Helmer Q, Nieuwboer HA, Hottenga JJ, de Moor MH, van den Berg SM, …, de Geus EJ. An extended twin-pedigree study of neuroticism in the Netherlands twin register. *Behav Genet*. 2018;48:1–11. http://doi.org/10.1007/s10519-017-9872-0.
42. Schadt EE, Lamb J, Yang X, Zhu J, Edwards S, Guhathakurta D, …, Lusis AJ. An integrative genomics approach to infer causal associations between gene expression and disease. *Nat Genet*. 2005;37:710–717. http://doi.org/10.1038/ng1589.

43. Bell JT, Spector TD. DNA methylation studies using twins: what are they telling us? *Genome Biol*. 2012;13:172. http://doi.org/10.1186/gb-2012-13-10-172.
44. McRae AF, Powell JE, Henders AK, Bowdler L, Hemani G, Shah S, …, Montgomery GW. Contribution of genetic variation to transgenerational inheritance of DNA methylation. *Genome Biol*. 2014;15:R73. http://doi.org/10.1186/gb-2014-15-5-r73.
45. Petronis A. Epigenetics and twins: three variations on the theme. *Trends Genet*. 2006;22:347–350. http://doi.org/10.1016/j.tig.2006.04.010.
46. van Dongen J, BIOSconsortium B, M. J, Dekkers KF, Nivard MG, van Iterson M, …, Boomsma, D. I. DNA methylation signatures of educational attainment. *npj Science of Learning*. 2018;3:1–14. http://doi.org/10.1038/s41539-018-0020-2.
47. Wright FA, Sullivan PF, Brooks AI, Zou F, Sun W, Xia K, …, Boomsma DI. Heritability and genomics of gene expression in peripheral blood. *Nat Genet*. 2014;46:430–437. http://doi.org/10.1038/ng.2951.
48. Chen C, Grennan K, Badner J, Zhang D, Gershon E, Jin L, Liu C. Removing batch effects in analysis of expression microarray data: an evaluation of six batch adjustment methods. *PLoS One*. 2011;6:e17238. http://doi.org/10.1371/journal.pone.0017238.
49. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics*. 2007;8:118–127. http://doi.org/10.1093/biostatistics/kxj037.
50. Lazar C, Meganck S, Taminau J, Steenhoff D, Coletta A, Molter C, …, Nowe A. Batch effect removal methods for microarray gene expression data integration: a survey. *Briefings Bioinf*. 2013;14:469–490. http://doi.org/10.1093/bib/bbs037.
51. Sun Z, Chai HS, Wu Y, White WM, Donkena KV, Klein CJ, …, Kocher JP. Batch effect correction for genome-wide methylation data with Illumina Infinium platform. *BMC Med Genet*. 2011;4:84. http://doi.org/10.1186/1755-8794-4-84.
52. Leek JT, Scharpf RB, Bravo HC, Simcha D, Langmead B, Johnson WE, …, Irizarry RA. Tackling the widespread and critical impact of batch effects in high-throughput data. *Naturer Reviews Genetics*. 2010;11:733–739. http://doi.org/10.1038/nrg2825.
53. Nygaard V, Rodland EA, Hovig E. Methods that remove batch effects while retaining group differences may lead to exaggerated confidence in downstream analyses. *Biostatistics*. 2016;17:29–39. http://doi.org/10.1093/biostatistics/kxv027.
54. McArdle JJ, Prescott CA. Mixed-effects variance components models for biometric family analyses. *Behav Genet*. 2005;35:631–652. http://doi.org/10.1007/s10519-005-2868-1.
55. Li X, Basu S, Miller MB, Iacono WG, McGue M. A rapid generalized least squares model for a genome-wide quantitative trait association analysis in families. *Hum Hered*. 2011;71:67–82. http://doi.org/10.1159/000324839.
56. Lippert C, Listgarten J, Liu Y, Kadie CM, Davidson RI, Heckerman D. FaST linear mixed models for genome-wide association studies. *Nat Methods*. 2011;8:833–835. http://doi.org/10.1038/nmeth.1681.
57. Minica CC, Dolan CV, Kampert MM, Boomsma DI, Vink JM. Sandwich corrected standard errors in family-based genome-wide association studies. *Eur J Hum Genet*. 2015;23:388–394. http://doi.org/10.1038/ejhg.2014.94.
58. Johnson W, Turkheimer E, Gottesman II, Bouchard Jr TJ. Beyond heritability: twin studies in behavioral research. *Curr Dir Psychol Sci*. 2010;18:217–220. http://doi.org/10.1111/j.1467-8721.2009.01639.x.
59. McGue M. The end of behavioral genetics? 2008. *Behav Genet*. 2010;40:284–296. http://doi.org/10.1007/s10519-010-9354-0.
60. Castillo-Fernandez JE, Spector TD, Bell JT. Epigenetics of discordant monozygotic twins: implications for disease. *Genome Medicine*. 2014;6:60. http://doi.org/10.1186/s13073-014-0060-z.

61. Zwijnenburg PJ, Meijers-Heijboer H, Boomsma DI. Identical but not the same: the value of discordant monozygotic twins in genetic research. *American Journal Medical Genetics Part B: Neuropsychiatric Genetics*. 2010;153B:1134–1149. http://doi.org/10.1002/ajmg.b.31091.
62. Sumathipala A, Yelland L, Green D, Shepherd T, Jayaweera K, Ferreira P, Craig JM. Twins as participants in randomized controlled trials: a review of published literature. *Twin Research and Human Genetics*. 2018;21:51–56. http://doi.org/10.1017/thg.2017.67.
63. Dick DM, Barr PB, Cho SB, Cooke ME, Kuo SI, Lewis TJ, …, Su J. Post-GWAS in psychiatric genetics: a developmental perspective on the "other" next steps. *Genes, Brain and Behavior*. 2017. http://doi.org/10.1111/gbb.12447.
64. Iacono WG, Heath AC, Hewitt JK, Neale MC, Banich MT, Luciana MM, …, Bjork JM. The utility of twins in developmental cognitive neuroscience research: How twins strengthen the ABCD research design. *Developmental Cognitive Neuroscience*. 2017. http://doi.org/10.1016/j.dcn.2017.09.001.
65. Rahmioglu N, Ahmadi KR. Classical twin design in modern pharmacogenomics studies. *Pharmacogenomics*. 2010;11:215–226. http://doi.org/10.2217/pgs.09.171.
66. Willemsen G, Ward KJ, Bell CG, Christensen K, Bowden J, Dalgard C, …, Spector T. The concordance and heritability of Type 2 diabetes in 34,166 twin pairs from international twin registers: the discordant twin (DISCOTWIN) consortium. *Twin Research and Human Geneticss*. 2015;18:762–771. http://doi.org/10.1017/thg.2015.83.
67. Silventoinen K, Jelenkovic A, Sund R, Honda C, Aaltonen S, Yokoyama Y, …, Kaprio J. The CODATwins Project: the cohort description of collaborative project of development of anthropometrical measures in twins to study macro-environmental variation in genetic and environmental effects on anthropometric traits. *Twin Research and Human Genetics*. 2015;18:348–360. http://doi.org/10.1017/thg.2015.29.
68. Buchwald D, Kaprio J, Hopper JL, Sung J, Goldberg J, Fortier I, …, Harris JR. International network of twin registries (INTR): building a platform for international collaboration. *Twin Research and Human Genetics*. 2014;17:574–577. http://doi.org/10.1017/thg.2014.67.
69. Grigorenko EL, Ravich-Shcherbo IV. Russian psychogenetics: sketches for the portrait. In: Grigorenko PMREL, Sternberg RJ, eds. *Psychology in Russia: Past, Present, and Future*. Hauppauge, NY: Nova Science Publishers; 1997:84–121.
70. Kaprio J, Silventoinen K. Advanced methods in twin studies. *Methods Mol Biol*. 2011;713:143–152. http://doi.org/10.1007/978-1-60327-416-6_11.
71. Martin NG, Martin PG. The inheritance of scholastic abilities in a sample of twins. I. Ascertainment of the sample and diagnosis of zygosity. *Ann Hum Genet*. 1975;39:213–218.
72. Neale MC, Eaves LJ. Estimating and controlling for the effects of volunteer bias with pairs of relatives. *Behav Genet*. 1993;23:271–277.
73. Peltonen L, GenomEutwin. GenomEUtwin: a strategy to identify genetic influences on health and disease. *Twin Res*. 2003;6:354–360. http://doi.org/10.1375/136905203770326358.
74. Petronis A. Epigenetics as a unifying principle in the aetiology of complex traits and diseases. *Nature*. 2010;465(7299):721–727. http://doi.org/10.1038/nature09230.
75. Snieder H, van Doornen LJ, Boomsma DI. The age dependency of gene expression for plasma lipids, lipoproteins, and apolipoproteins. *Am J Hum Genet*. 1997;60:638–650.
76. Tworoger SS, Hankinson SE. Collection, processing, and storage of biological samples in epidemiologic studies: sex hormones, carotenoids, inflammatory markers, and proteomics as examples. *Cancer Epidemiol Biomarkers Prev*. 2006;15:1578–1581. http://doi.org/10.1158/1055-9965.EPI-06-0629.

77. Vaught JB, Henderson MK. Biological sample collection, processing, storage and information management. *IARC Sci Publ*. 2011:23–42.
78. Yudin TI. O shodstve psihoza u bratjev i sester. [On similarity of psychosis in brothers and sisters]. *Sovremennaya psihiatria*. 1907:337–342.
79. Sukhanov S. O psihozah u bliznetsov [On psychosis in twins]. *Klinicheskij jurnal*. 1900:341–352.
80. Levit SG. Twin investigations in the U.S.S.R. *J Pers*. 1935:188–193.
81. Malykh S, Egorova M, Meshkova T. *Osnovi povedencheskoj genetiki*. Moscow: Epidaurus; 1998.
82. Lykken DT. Presidential address, 1981. Research with twins: the concept of emergenesis. *Psychophysiology*. 1982;19:361–373.
83. Martin N. The inheritance of scholastic abilities in a sample of twins. II. Genetical analysis of examinations results. *Ann Hum Genet*. 1975;39(2):219–229. https://doi.org/10.1111/j.1469-1809.1975.tb00125.x.
84. Neale MC, Eaves LJ, Kendler KS. The power of the classical twin study to resolve variation in threshold traits. *Behav Genet*. 1994;24:239–258.
85. Keller MC, Medland SE, Duncan LE. Are extended twin family designs worth the trouble? A comparison of the bias, precision, and accuracy of parameters estimated in four twin family models. *Behav Genet*. 2010;40:377–393. http://doi.org/10.1007/s10519-009-9320-x.
86. Keller MC, Medland SE, Duncan LE, Hatemi PK, Neale MC, Maes HH, Eaves LJ. Modeling extended twin family data I: description of the Cascade model. *Twin Research and Human Genetics*. 2009;12(1):8–18. http://doi.org/10.1375/twin.12.1.8.
87. Maes HH, Neale MC, Eaves LJ. Genetic and environmental factors in relative body weight and human adiposity. *Behav Genet*. 1997;27:325–351.
88. Martin NG, Martin PG. The inheritance of scholastic abilities in a sample of twins. I. Ascertainments of the sample and diagnosis of zygosity. *Ann Hum Genet*. 1975;39(2):213–218. https://doi.org/10.1111/j.1469-1809.1975.tb00124.x.
89. Snieder H, van Doornen LJ, Boomsma DI. The age dependency of gene expression for plasma lipids, lipoproteins, and apolipoproteins. *Am J Hum Genet*. 1997;60(3):638–650.

CHAPTER

Methodology of twin studies

12

José J. Morosoli, Brittany L. Mitchell, Sarah E. Medland
Mental Health & Neuroscience Department, QIMR Berghofer Medical Research Institute, QLD, Australia

12.1 Introduction

Quantitative genetics is the discipline that studies the genetic and environmental factors that underlie phenotypic variation among individuals (e.g., height, cardiovascular disease, educational attainment, bone density, musical ability and so on) in a population. The archetypical study designs in quantitative genetic investigations of human anthropometrical, behavioral, and health traits are twin and adoption studies. Molecular genetics has a complementary focus on identifying genetic loci associated with a specific phenotype and the magnitude and mechanisms of these associations. Quantitative and molecular genetic methods can be combined to understand better the way in which genetic variation influences phenotypic distributions in a population. When the phenotype of interest is human behavior, either from a quantitative or a molecular perspective, we use the term behavioral genetics. Although behavioral genetic studies typically focus on the role of genetic factors, genetically informative designs, such as twin studies, provide a valuable tool to study the ways in which environmental factors influence behavior, as they take into account and correct for genetic factors.[1]

12.2 A brief note on the biometrical model

When a trait is described as heritable it implies that at least one gene has a measurable effect on the trait—though most often, many genes are involved. In this section, we briefly present some key concepts of the *biometrical model* which underlies the methods presented below. For a more detailed explanation of the biometrical model, we refer the reader to Neale[2] and Falconer and Mackay.[3] For any given continuously distributed trait, the value of any individual's phenotype (P) can be described as a linear composite of that individual's genotypic value (G) and the deviation from this value that is due the individual's environment (E):

$$P = G + E \tag{12.1}$$

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00035-0

FIG. 12.1 The genotypic values of a diallelic locus.

Because the effect of the environment is characterized as a deviation, the mean of this effect is zero. Thus, the mean phenotypic value of a population is equal to the mean of the genotypic values. In theory, the phenotypic value could be obtained by examining the trait in a series of genetically identical individuals, as in a series of sufficient size where the effects of the environmental deviations would be minimized. Assuming a single locus with two alleles (diallelic), A_1 and A_2, there are three possible genotypes an individual can have in the population: A_1A_1, A_1A_2 and A_2A_2. The frequencies at which the A_1 and A_2 allele occur in the population can be denoted using p and q respectively (note: $p + q = 1$).

In the case of the diallelic autosomal locus in Hardy-Weinberg equilibrium, shown in Fig. 12.1, where the two alleles are denoted A_1 and A_2, the genotypic values of the two homozygotes A_1A1 and A_2A_2 are given by $-a$ and $+a$ respectively. The mean of our phenotype for the entire population is donated using μ. The distance between the population mean and each homozygote genotype is then donated as $+a$ or $-a$ respectively, the distance between the two homozygotes as $2a$, and the deviation of the heterozygote from the midpoint of the two homozygotes as d. The genotypic value of the heterozygote A_1A_2, d, depends on the degree of dominance.

It is important to note that the parameter d is dependent on the mode of action of the locus in question. In the absence of dominance, $d = 0$, the two alleles act additively, and the genotypic value of the heterozygote is equal to the mean genotypic values of the two heterozygotes. With partial or incomplete dominance, when A_1 is dominant over A_2, d is greater than zero but less than $+a$, when A_2 is dominant over A_1, d is less than zero but greater than $-a$. In the case of complete dominance, the genotypic value of the heterozygote is equal to that of one of the homozygotes (d equals either $-a$ or $+a$). In overdominance, the genotypic value of the heterozygote is greater than the genotypic values of the homozygotes and cannot be explained by the additive effects of the alleles.[3]

If random mating is assumed, and the frequencies of A_1 and A_2 are given by p and q, then the frequencies of the genotypes A_1A_1, A_1A_2, and A_2A_2, are p^2, $2pq$, and q^2 respectively. The genotypic contribution to the population mean is given by the sum of the products of the genotypic frequencies by the genotypic values:[3]

$$\mu = ap^2 + 2pqd - aq^2 = a(p-q) + 2pqd \tag{12.2}$$

Complex traits, however, can involve the effects of thousands of genes. If we assume that multiple loci influence a trait and that the effects of the individual loci

combine in an additive manner, the population mean is given by the sum of the contributions of the individual loci:[3]

$$\mu = \sum a\left(p-q\right)+2\sum pqd \tag{12.3}$$

We can distinguish between *additive or linear* and *nonadditive or within local allelic interaction* genetic effects. Additive genetic effects refer to the sum of the individual contributions of single genes to the phenotype, while nonadditive genetic effects include allelic interactions within genes (i.e., dominance) and interaction between multiple genes (i.e., epistasis). The contribution of a specific allele to the population variance is given by:[3]

$$\theta = \left(a-\mu\right)^2 p^2 + \left(d-\mu\right)^2 2pq + \left(-a-\mu\right)^2 q^2 \tag{12.4}$$

The amount of variance explained by additive genetic effects is equal to $2{\cdot}pq[a+(q\text{-}p)d]^2$ which denotes the additive variance component (V_A), where p and q are the two allele frequencies, and a is the effect size of the allele in standard deviation units. That is, genetic variance is a function of the allele frequency and effect size. Using the example provided by Gibson (2018), a genetic variant with a frequency of 0.5 would contribute an average of 7 mm to a person's height (i.e., 0.1 standard deviation units) and accounts approximately for 0.5% of the variation in the population, while a genetic variant with a frequency of 0.1 adding 1 mm would explain 0.0037% of the variation.[4] See Fig. 12.2 adapted from Gibson (2018) for a graphical representation of the relationship between allele frequency, effect size, and variance explained, for three alleles with different effect sizes.

The variance explained by dominance effects is equal to $(2{\cdot}pqd)^2$ and denotes the dominance variance component (V_D). In the next section, we explain how these two variance components, plus the variance explained by environmental sources of variation, can be estimate using data obtained from families.

12.3 Classical twin study

From a quantitative genetics approach, the variance in a phenotype can be described as a function of both an individual's genotype and their environment. That is, phenotypic variance (V_P) is the result of two sources of variance: genetic (V_G) and environmental (V_E).

$$V_P = V_G + V_E \tag{12.6}$$

Genetic variance can be further decomposed into additive genetic variance (V_A) and nonadditive genetic variance (V_D).[5] Additive genetic variance is the sum of all the effects of independent genes on the phenotype. Nonadditive genetic variance results from gene interactions, either between the alleles of the same gene (i.e., dominance), or between the alleles of different genes (epistasis). In addition, environmental variance can be decomposed into shared environmental variance (V_C; i.e., common/family

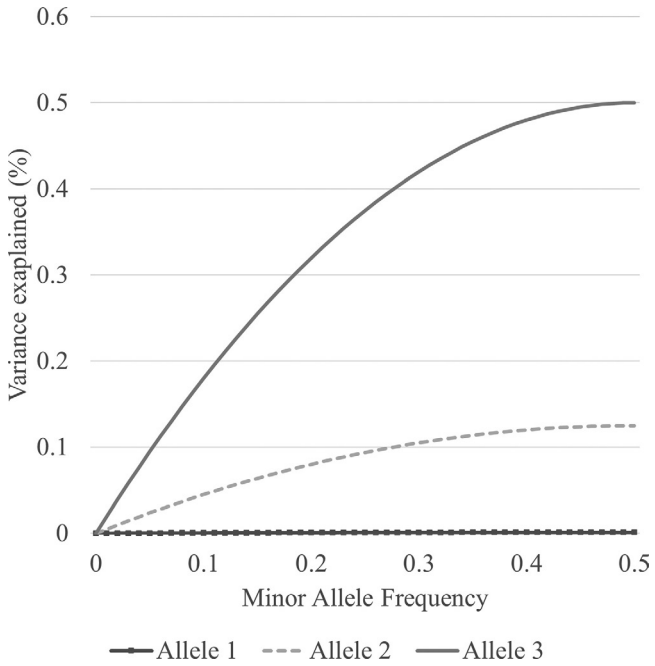


FIG. 12.2 Curve shows percentage of variance explained by three different alleles, all with the specified minor allele frequency, and each one additively contributed (*a*) 0.005, 0.05, and 0.1 of a standard deviation to the population mean, respectively.

environment) and individual or unique environmental variance (V_E; i.e., idiosyncratic experiences and measurement error). In the absence of gene–environment correlations or interactions, phenotypic variance can be expressed as follows:

$$V_P = V_A + V_D + V_C + V_E \tag{12.7}$$

A key concept in quantitative genetics is that of *heritability*. The term "heritability" (of a given trait) refers to the proportion of the variation we observe in a population that can be explained by genetic variation. Conversely, *environmentality* refers to the proportion of observed differences that can be explained by environmental sources. Broad-sense heritability (H^2) is defined as the proportion of variance explained by the sum of V_A and V_D (i.e., the sum divided by V_P), while narrow sense heritability (h^2) is equal to the proportion of V_P explained by V_A. Importantly, both heritability and environmentality estimates are specific to the specific sample under study.

The classical twin design (CTD) is the most common research tool to investigate the relative contribution of genetic and environmental factors to phenotypic variation in humans. When phenotypic data are available for identical or monozygotic (MZ) and nonidentical or dizygotic (DZ) twin pairs who have been raised together, the

total variance of the trait can be decomposed into variance due to genetic and environmental factors. These different variance components may be estimated using twin data because MZ twins share 100% of their segregating genes, given they originate from the same fertilized egg, while DZ twins share on average 50% of their segregating genes.[6] In contemporary studies, zygosity is usually determined using genotyping; however, standardized questionnaires may also be used.[7] The reason why models comparing the similarity of MZ and DZ twins is such a useful tool is because DZ twins are a nearly perfect control group for MZ twins: while it is possible to compare MZ twins to other relatives, only DZ twins are perfectly age matched, share prenatal conditions, and experience similar environmental factors due to being raised within the same family, in the same period of time, under the same socio-economic circumstances. These models assume that DZ twins, although genetically different, share the same intra-familiar influences that influence the trait being studied in the same way the MZ twins do. This is known as the equal environment assumption (see section 12.5.1). Therefore, the covariance between MZ co-twins (Cov_{MZ}) is due to both genetic and common environmental influences (note that by definition V_E is uncorrelated across co-twins)[5]:

$$Cov_{MZ} = V_A + V_D + V_C \tag{12.8}$$

While the covariance of DZ co-twins (Cov_{DZ}) reflects that, on average, DZ twins share only half of their segregating genes and therefore a ¼ of the variation due to the combination of two alleles (i.e., dominance or additive-by-additive epistasis). The expectation for the covariance within DZ pairs is:

$$Cov_{DZ} = \frac{1}{2}V_A + \frac{1}{4}V_D + V_C \tag{12.9}$$

Broadly speaking, greater phenotypic resemblance or correlation in MZ twin pairs (r_{MZ}) compared with DZ twin pairs (r_{DZ}) is consistent with the influence of genetic factors. Assuming that the environment has a similar effect on both MZ and DZ twin pairs (i.e., the equal environment assumption), if the within-pair correlation for DZ pairs is greater than half the MZ correlation, this suggests the influence of shared/family environmental factors. The contributions of unique experiences or measurement error act to reduce the similarity of MZ and DZ twins.[8]

Building upon this, when the association between two variables is analyzed, the proportion of covariance of the traits explained by genetic and environmental factors can be similarly estimated. For example, bivariate genetic models can estimate the extent to which genetic or environmental effects on one measure correlate with these effects on another measure. That is, these models estimate the degree to which the same genes or environmental factors contribute to the observed phenotypic correlation between two variables: a genetic correlation (r_G) of 1.0 indicates that the same genes influence the two variables, although their effects can differ, whereas $r_G = 0$ indicates that entirely different genes influence the two traits. The same logic applies to environmental correlations (r_C and r_E). In cases when no specific causal

order between variables is hypothesized, a correlated factors model (that does not assume ordering of the variables) is estimated.[9] However, decomposing the covariance between any two phenotypes only makes sense when: (1) cross-twin cross-trait for MZ twins is significantly different from zero, and (2) the cross-twin-cross-trait correlation for DZ twins is not greater than for MZ twins. These two restrictions ensure that there is a meaningful covariance to decompose and that the constructs are influenced by genetic or family factors to some extent, which is an underlying assumption of the classical twin design.

Unfortunately, it is not possible to estimate V_C and V_D simultaneously in a classical twin model, where only MZ and DZ twin pairs are included. In classical twin studies, the effects of dominant genetic and shared environmental influences are confounded, so that when using data from twins raised together, either V_D or V_C may be estimated.[10] Dominant genetic effects are suggested when the correlation between DZ twins is less than half of the MZ twin correlation and therefore V_D is estimated. Conversely, when the DZ co-twin correlation exceeds half the MZ correlation, a model including a common environmental effect is generally fitted to the data. The decision to model either V_C or V_D is usually based on the pattern of MZ and DZ correlations: V_C is estimated when r_{DZ} is higher than half the r_{MZ}, and V_D is estimated if r_{DZ} is less than half of r_{MZ}.[8,10] In an additive model, the genetic effect "is equal to the sum of the average effects of the genes [an individual] carries, the summation being made over the pair of alleles at each locus and over all loci" [3; p. 115]. An additive genetic model hypothesizes that correlation of DZ co-twins is predicted to be half the MZ co-twin correlation. Nonadditive genetic effects act to further increase the discrepancy between MZ and DZ co-twin correlations. Nonadditive genetic effects include dominant effects, which result from interactions between alleles within a single locus, and epistasis, which results from interactions between alleles at different loci. Dominant genetic effects reduce the DZ co-twin correlation to approximately a quarter of that observed in MZ twins, and the DZ correlation reduces even further in the presence of epistasis.[3] The classical twin design cannot estimate V_C and V_D simultaneously, thus for a model which includes shared environmental effects (i.e., ACE model), and fixes nonadditive genetic effects to zero the decomposition of the total variance becomes:

$$V_P = V_A + V_C + V_E \tag{12.10}$$

It should be noted that the same covariance structure[a] can result from different combinations of environmental factors and assortative mating (i.e., the tendency for individuals to choose partners who are more similar or dissimilar to themselves based on phenotypic features more than we would expect by chance), as well as

[a] A specific pattern in the MZ twin and DZ twin covariance matrices. A covariance matrix is a symmetric square matrix that stores the covariance between each pair of variables under analysis. Its main diagonal contains variances (i.e., the covariance of each variable with itself).

unknown ratios of additive and nonadditive sources of genetic variation. This problem is known as the parameter indeterminacy problem in the CTD.[11] Best strategies to address this include: (1) adding more indicators of each phenotype included in the model (i.e., multiple observations, different scales or instruments, etc.); (2) using multivariate models that include multiple phenotypes that are significantly correlated; (3) including information from other relatives (see *Extension of the Classical Twin Model* section); and (4) representing the parameter indeterminacy using the appropriate graphs and confidence intervals. For more information, we recommend accessing the materials made freely available by the International Statistical Genetics Workshop.[12]

12.4 Methodological assumptions

The CTD is based upon two main assumptions: (1) MZ and DZ twins' environments with respect to the trait of interest are equally correlated, and (2) twins are representative of the general population with respect to the traits under study. Two further presuppositions are often made to simplify twin modeling: (3) there is no assortative mating, and (4) the environmental and genetic influences are independent of one another. When these assumptions are violated, the estimates of genetic and environment effects may be biased. Next, we will discuss the first three assumptions. The fourth assumption, that is, that environmental and genetic influences are independent, will be covered in the section *Gene–environment correlation vs interaction*.

12.4.1 The equal environments assumption

The first assumption is known as the equal environment assumption (EEA) and has been the focus of much debate and research for many decades. The EEA implies that MZ and DZ twins experience the same sources of environmental variation, that is, environmental sources relevant to the trait under study are equally correlated in MZ and DZ pairs, and twins do not experience different treatment based on their zygosity, or degree of genetic relatedness, with respect to the trait of interest. Violation of this assumption could lead to inflated estimates of heritability, due to unmeasured sources of additional similarity between MZ twins (e.g., assigning them to the same school classroom, greater degree of sharing the same extra-curricular activities, etc.). Although it has been shown that MZ twins are treated more similarly than DZ twins, it is not clear whether increased environmental similarity results in increased phenotypic similarity.[13] In addition, inequality of environments would only result in bias if the environmental factors that differ between the MZ and DZ pairs affect the trait of interest.[14] The equal environment assumption has repeatedly been tested and shown to hold in most cases.[15,16] Additionally, the EEA assumption could also be violated if, despite their genetic similarity, members of MZ pairs experienced different environments, for example intra-uterine differences in growth due to

competition for resources during pregnancy, which would lead to an underestimation of the heritability.

12.4.2 The representativeness assumption

Although this assumption is key for any study, for some phenotypes there might exist more skepticism regarding the ability to generalize results from twin studies to singletons and nontwins. The main approach to assess representativeness of twin samples has been to compare the twin sample to a nontwin population of reference, either by evaluating the differences in fetal development between twin and nontwins for the phenotype under study, or by comparing the prevalence, means, or variances of the phenotype in the twin sample with those of the population of reference (e.g., national surveys). Extending the twin design to include sibling data is a very useful method of assessing the generalizability of results from twin studies to singleton populations.[17] For example, comparing the prevalence of a trait in twins with that of their non-twin siblings allows one to examine firstly whether the experience of being a twin (including the sharing of limited space and resources and the differences in the birth process) is associated with an increased predisposition towards the trait in twins. Similarly, comparing the DZ co-twin correlation with twin-sibling correlations allows an examination of the role of pre- or perinatal interaction between the twins that might influence the trait. One of the advantages of comparing twins with their nontwin siblings is that by using siblings as the control group we can, at least in part, control for variance in maternal size (i.e. intrauterine size and body shape which may influence the length of gestation and ease of delivery) and the effects of genetic transmission (as both DZ twins and their full siblings share, on average, 50% of their genetic material).

12.4.3 The assumption of random mating

An assumption often used in applying the CTD is that there is no assortative mating. This is the basis upon which the degree of genetic sharing between DZ twins is derived: we assume that DZ twins share, on average, 50% of their genetic make-up if parents choose their partners randomly for the trait of interest.[18] However, in the presence of assortative mating, there is an increased chance of inheriting the same genetic variants from both parents, thus inflating the estimates of the proportion of variance due to V_A. Two well-known examples of assortative mating in humans are height and educational attainment where individuals tend to choose partners who are similar to themselves on these traits.[19] If assortative mating does occur then this would affect the covariance structure the event that individuals chose their partners based on heritable phenotypes, the DZ co-twin correlation for the trait on which parents are assorting, and on traits that are genetically correlated with this trait, would increase, which would result in spuriously increased estimates of shared environmental factors (V_C) and reduced estimates of heritability. Extending the twin design to include parental data allow for a test of the assumption of random or nonassortative mating.[1]

12.5 Use of structural equation modeling in twin analysis

Structural equation modeling (SEM) is commonly used in quantitative genetics to estimate the variance that is explained by each of the latent components V_A, V_C, V_D, and V_E. While originally SEM models were fitted to covariance matrices, currently it is common to fit these models to raw data. With the SEM approach one can take into account covariates (e.g., correcting the phenotype for age, sex, or level of education effects) by introducing them as fixed effects, compare the fit of various submodels, obtain confidence intervals for the estimates, and in some cases, it allows imputation of missing data via full-information maximum likelihood. In its simplest form, the CTD is analyzed using a multigroup SEM approach, with one group corresponding to MZ twins and the other group to DZ twins. Following the principles described above (see 12.2 *Classical Twin Study*), we formulate different expected variance-covariance matrices for each group (in SEM terms, the structural model):

$$\Sigma_x = \begin{pmatrix} V_A + V_C + V_D + V_E & \alpha \cdot V_A + V_C + \beta \cdot V_D \\ \alpha \cdot V_A + V_C + \beta \cdot V_D & V_A + V_C + \beta \cdot V_D + V_E \end{pmatrix} \tag{12.11}$$

Where Σ_x is the expected variance-covariance matrix for the variables measured in twin 1 and twin 2; V_A, V_C, and V_D, are the genetic and environmental variance components; α is a scalar equal to 1 for MZ twins and to 0.5 for DZ twins, which captures the sharing of additive genetic effects across twins; and β is a scalar equal to 0.5 for MZ twins and 0.25 for DZ twins, which captures the sharing of dominance or additive-by-additive epistasis effects across twins. In addition, we must include a measurement error, V_E, term in our model. In the twin modeling space, E subsumes all sources of that contribute to differences between co-twins, and includes unshared (unique) experiences and measurement error. In SEM, it is common to represent relationships between variables and regression coefficients using *path diagrams*. A path diagram is a convenient way to represent structural equations because when used correctly, it allows one to obtain the expected variance-covariance matrix from the graphic representation using *path tracing rules*. See below Fig. 12.3A and B for a path diagram of a multigroup SEM for MZ and DZ twins.

The model depicted in Fig. 12.3 is parameterized in accordance with the equal environment assumption, stipulating that the shared environments are equally correlated with respect to the trait of interest in the MZ and DZ twins. This is reflected by fixing the variances to be the same across twin 1 and 2, and across MZ twins and DZ twins. These assumptions are tested prior to model fitting using log-likelihood ratio tests between a model that freely estimates all variances, covariances, and means, and a series of nested models where each set of parameters is set to be the same for either the co-twins, or across zygosity. Other assumptions commonly made and reflected in this model are that: there is no assortative mating, environmental and genetic influences are not correlated (i.e., there are no correlations between the variance components V_A-V_C, V_A-V_E, etc.), and the coefficients associated with the variance components are not moderated by any other variable, observed or latent (e.g., G × E

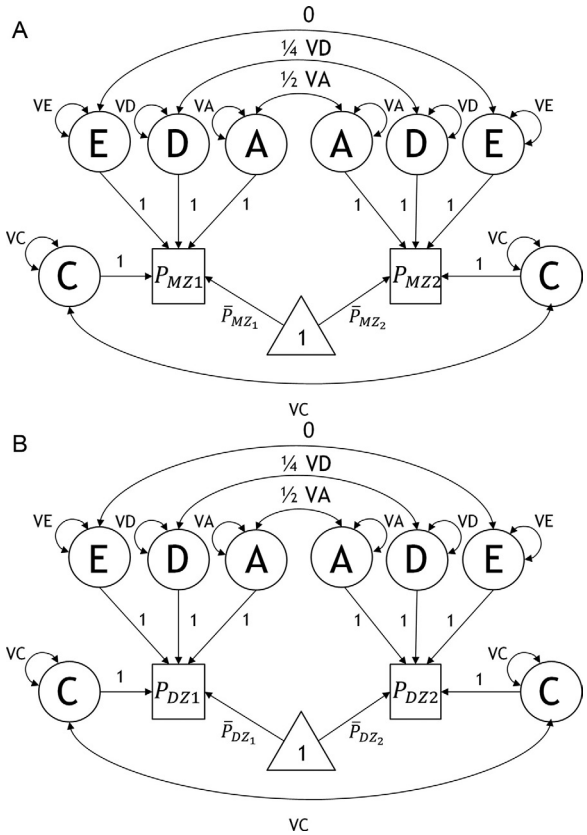


FIG. 12.3 Path diagram for MZ (A) and DZ (B) twins in a CTD where shared environment is modeled. Circles indicate latent variables, squares indicate observed variables, and triangles with a 1 inside indicate fixed-effect variables in the means model. Plots for models with a mean structure typically include a 1 in a triangle with the paths indicating the means/intercepts. Single-headed arrows indicate regression coefficients and double-headed arrows indicate covariances. In this figure, variances and covariances of a single phenotype measured in MZ twins and DZ twins is decomposed in four latent variables: A, C, D, and E. Note that in practice C and D cannot be modeled simultaneously.

interaction). It should be noted that both the ACE and ADE models have zero degrees of freedom. This is common practice in twin modeling but less common in other areas. For this reason, the goodness of fit is evaluated by comparing the fit of nested submodels (i.e. an AE or CE model) against that of the saturated model (i.e. ACE) using the log-likelihood ratio test and, in some cases, by evaluating other indices of how well the ACE or ADE model accounts for the observed variance-covariance structure in the data.[20,21] Additionally, the accuracy of the model estimates is assessed using likelihood-based 95% confidence intervals.[22] Test of the assumption of

independence between genetic and environmental influences can be conducted with specific models that will be presented in section *Structural Equation Modeling for corGE and G × E interaction* at the end of this chapter. For more information, we refer the reader to reference.[12]

Finally, very recently twin models have started to use the direct variance method, rather than the traditional path analysis method.[23] In direct variance specification, V_A, V_C, and V_E, are estimated directly with no imposed boundaries and therefore may take negative values. Negative variance estimates are implausible and indicate that the model is misspecified. For example, negative estimates of V_C are expected when r_{DZ} are less than ½ rMZ, which implies an ADE model.[23] Also, negative estimates of V_A but positive V_D are expected when r_{DZ} is less than ¼ r_{MZ}.[11] An alternative approach might be to collapse the two genetic variance parameters (V_A and V_D) into one (V_G), thereby modeling the total genetic effect or broad-sense heritability. A main advantage of the GE model is that the confidence intervals for the total genetic influences on the variance of the traits will be much tighter than for either V_A or V_D, and this allows for more powerful tests. This is of particular interest when there is insufficient power to distinguish between A and D, resulting in nonsignificant pathways for both types of genetic influences. In addition, a GE model does not assume that V_D is solely due to the interaction between only two alleles (i.e., dominance or additive-by-additive epistasis) and the genetic covariation between DZ twins is free to range between 0 and 0.5 rather than fixed to either 0.5 (V_A) or 0.25 (V_D). More specifically, when we estimate a GE model, we do not fix the correlation between DZ twins for parameter V_D to 0.25 (which implies V_D is solely due to dominance and/or additive-by-additive epistasis). Instead, the degree of genetic covariation is estimated (parameter *q*). This new parameter can range from almost zero (when both V_A and $V_D \sim 0$) to ½ (when $V_D = 0$). Therefore, when the ratio between DZ and MZ correlations is very low and only V_D, but no V_A is estimated, a GE model would explain the covariance pattern better than an ADE model. Lastly, although significant dominance genetic variance in the absence of additive genetic variance is theoretically possible, it is highly unlikely. Consequently, behavioral geneticists do not usually fit a DE model.[24] More in depth explanation about modeling broad-sense heritability can be found in Keller and Coventry,[11] Boomsma, Princen,[25] Do Ha, Lee,[26] and Mosing, Magnusson.[27] We refer the reader to Grasby, Verweij[28] for a guide on how to conduct the preliminary steps of a heritability analysis in OpenMx.

12.6 Analysis of discrete traits

The methods described above were designed to analyze continuous data. However, there are occasions when it is not possible to measure a trait using a continuous metric or the data do not meet the assumptions of normality. In these instances, methods designed to analyze discrete traits that vary in a discontinuous way should be used.[29]

TABLE 12.1 Summary of affection status for a sample of related pairs.

		Individual 2		
		Affected	**Unaffected**	**Totals**
Individual 1	Affected	a	b	E
	Unaffected	c	d	F
Totals		G	H	n

12.6.1 Binary data

For many traits, the most common type of measurement is binary. For example, having a particular diagnosis or not. Sometimes, a binary variable is created by imposing an arbitrary cut-off to a continuous trait, such as categorizing a score from an aggressive behavior scales as indicative of normal *vs* clinical behavior. In more general terms, the two trait values are usually described as affected or unaffected, with the probability of affection being unity for an affected individual. Data for a sample of related pairs may summarized by the following table (Table 12.1):

Where lowercase letters indicate cell counts, uppercase letters indicate marginal totals, and n indicates grand total. From an epidemiological point of view, binary data gathered from related individuals may be described in terms of conditional probability. For example, if the data were collected from pairs of siblings the question becomes: what is the probability that an individual will be affected if they have an affected sibling? This is known as the recurrence risk. Assuming random sampling and that there is no effect of birth-order, the recurrence risk is estimated as the probandwise concordance[b] rate (PC) and can be found using the following equation:

$$\mathrm{PC} = \frac{2a}{2a + c + b} \tag{12.12}$$

In the literature many studies report related statistics, such as the pairwise concordance rates (PWC), concordance or agreement, or phi-coefficients (binary correlations):

$$\mathrm{PWC} = \frac{a}{a + b + c} = \frac{\mathrm{PC}}{2 - \mathrm{PC}} \tag{12.13}$$

$$\mathrm{Concordance} = \frac{a + d}{a + b + c + d} \tag{12.14}$$

$$\text{Phi coefficient } (\Phi) = \frac{ad - bc}{\sqrt{efgh}} \tag{12.15}$$

[b] Probandwise concordance rate is the percentage of pairs of twins who exhibit a particular phenotype. Also called concordance ratio.

TABLE 12.2 Genetic hypothesis testing for twin studies. PRR is the population risk ratio, which equals probandwise concordance divided by population prevalence, from Risch and Rao.[30]

Relationship	Interpretation
$PRR_{MZ} > 4\ PRR_{DZ}$	Epistasis - must be polygenic.
$PRR_{MZ}\text{-}1 > 2(PRR_{DZ}\text{-}1)$	Genetic dominance (or epistasis).
$PRR_{MZ}\text{-}1 = 2(PRR_{DZ}\text{-}1)$	Additive genetic effect.
$PRR_{MZ} = PRR_{DZ} > 1$	No genetic contribution - effects of shared environment.
$PRR_{MZ} = PRR_{DZ} = 1$	No familial aggregation.

As the prior probability of a proband is equal to the prevalence of the disorder, it is useful to compare population risk ratios (PRRs), which compares the risk of an event (e.g., disease, injury, risk factor, etc.) among one group with the risk among another group. PRRs are given by:

$$\text{PRR} = \frac{\text{PC}}{\text{prevalence}} \tag{12.16}$$

Where the prevalence is the proportion of affected individuals in the population. Broadly, the interpretation of the comparison of PRR in MZ and DZ twins can be found in Table 12.2.

When binary data from twins are available, comparison of MZ and DZ PRR, or tetrachoric-correlations (correlations between binary variables), can provide information about the sources of co-twin covariation in a trait, similar to that derived for continuous traits. Specifically, when both variables are categorical, numerical integration can be used to estimate the expected proportion of observations in each cell of the multivariate contingency table and the correlation between the underlying liabilities is computed.[29] For example, in an additive genetic model, it is hypothesized that the PRR_{DZ} will be half the PRR_{MZ}. Nonadditive genetic effects act to further increase the discrepancy between MZ and DZ PRR so that $(PRR_{MZ} - 1) > 2\ (PRR_{DZ} - 1)$. In the presence of epistasis, the PRR_{DZ} falls even further in relation to the MZ PRR, $PRR_{MZ} > 4 \cdot PRR_{DZ}$.[3] Although the PRR can provide insight into the relative importance of genetic factors, the most common approach to the estimation of genetic and environmental effects in categorical data uses threshold models. The threshold model approach was selected because of the flexibility of model specification, and the ease with which these approaches can be extended to the multivariate case.

12.6.2 Threshold approaches

Threshold models describe discrete traits as reflecting an underlying normal distribution of liability (the vulnerability, susceptibility, or predisposition) that has not been, or cannot be, measured precisely. Instead, liability is measured as a series or ordered categories, characterized by phenotypic discontinuities that occur when

the liability reaches a given threshold. Liability, which represents the sum of all the multifactorial effects, is assumed to reflect the combined effects of a large number of genes and environmental factors each of small effect.[10]

To illustrate this using an example from laterality, many different methods have been used to study handedness, each with its own characteristic distribution. Hand skill as measured by a test with low practice bias, such as the Annett peg board task[31] yields a relatively normal distribution. A biased test, such as a box crossing task[32] yields a bimodal distribution. The distribution of hand preference assessed for multiple items is J-shaped, and at its simplest level of measurement, such as self-classification or writing hand, the distribution is ternary or binary. Yet, despite the lack of agreement regarding the most appropriate method of assessment, the methods described are all recognized as measures of the latent variable, handedness.[33] If hand preference is considered as a coarse and biased measure of hand skill, and we assume that the distributions of handedness and unbiased hand skill are similar, it is not difficult to conceptualize hand preference as being an imprecise measurement of a continuously distributed normal variable.

For a dichotomous variable, such as hand used for writing, only one threshold is required to discriminate between phenotypes. As the standard deviation is the unit of liability, it is possible to express the position of the threshold as a z-score, so that the proportion of individuals in each liability class matches the proportion of individuals in each category of the ordinal variable.[33] For example, if ten percent of a sample reported writing with their left hand (and left- and right-handedness were coded as 0 and 1 respectively) then a threshold at a z-value of –1.28 would partition the distribution of liability as required. This method easily generalizes to variables with more than two categories by viewing successive categories in terms of their cumulative prevalence as shown in Fig. 12.4.

The goodness-of-fit of a liability model can be tested if the data have three or more categories. However, this is not possible in the binary case, as there are no degrees of freedom associated with the model.[10] Genetic models such as the one described in Fig. 12.3 may be fit either to contingency tables, or tetrachoric (or polychoric) correlations and asymptotic covariance matrices. The thresholds may be influenced by various factors, including year of birth and sex. These factors can be estimated as fixed effects in a threshold model. The correlation between liabilities can be estimated as a random effect, while estimating the fixed effects in the thresholds. The procedure is readily extended to multiple groups, to enable the testing of hypotheses about equality of thresholds or correlation between studies, or zygosity groups.[35]

A limitation of the use of ordinal data is loss of power associated with categorical compared to continuous measurement.[29,36] As shown in Fig. 12.5, the number of twin pairs required to detect the presence of additive genetic effect in traits with low prevalence can be prohibitive. A similar problem is observed when we wish to detect common environmental effects (Fig. 12.6). The addition of sibling data helps ameliorate this problem.[17] Power also increases if the data can be modeled as more than two categories, but not if the addition of an extra category involves subdividing the smaller binary category.[29]

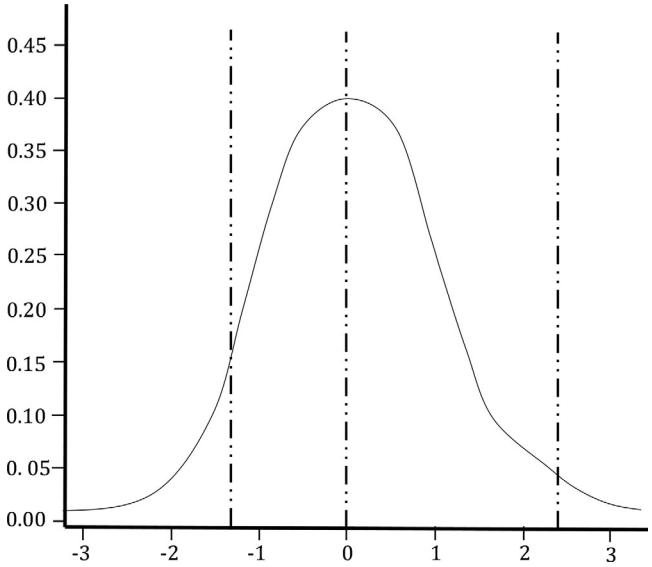


FIG. 12.4 Univariate normal distribution with thresholds distinguishing ordered response categories.

Three thresholds are shown (at z-values of -1.28, 0, and 2.33) corresponding to 4 categories with the frequencies, 10%, 40%, 40%, and 1%.[34]

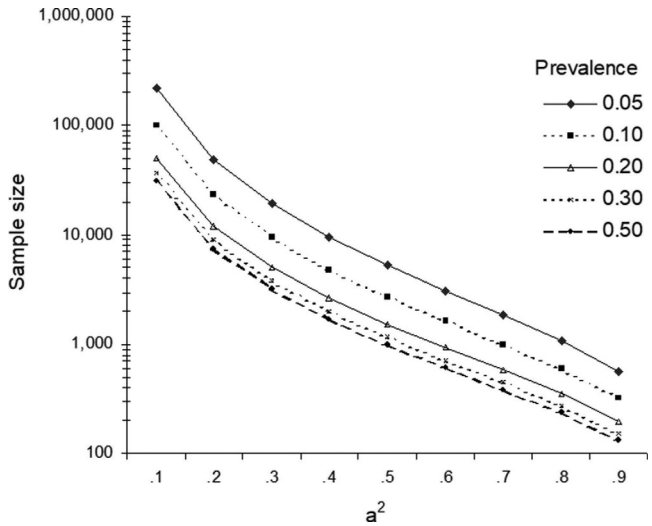


FIG. 12.5 An illustration of the effects of prevalence and true heritability on the power to reject the common and unique environmental (CE) model at the 0.05 level of significance and 80% power.

The required sample size is plotted on a logarithmic scale. Equal numbers of MZ and DZ pairs are assumed. Note: Sample size indicates number of pairs.

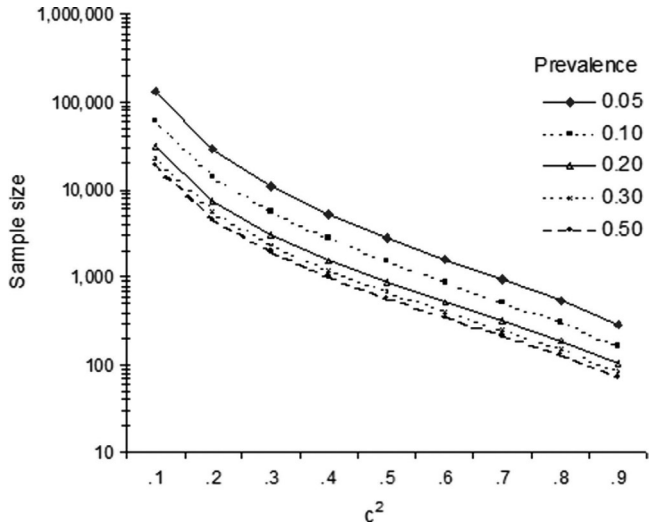


FIG. 12.6 An illustration of the effects of prevalence and true common environmental variance on the power to reject the additive genetic and unique environmental (AE) model at the 0.05 level of significance and 80% power.

Sample size is plotted on a logarithmic scale. Equal numbers of MZ and DZ pairs are assumed. Note: Sample size indicates number of pairs.

The growing interest in using SEM for twin modeling led to the development of Mx, a dedicated software that catered to the needs of quantitative geneticists.[35] Mx provided a flexible syntax that streamlined the estimation of genetic models, as well as facilitate global accessibility. Mx received a major upgrade when it was integrated into the R environment as a standalone R-package, called OpenMx.[37] OpenMx allows the analysis of both continuous and categorical variables as well as multigroup analysis. OpenMx also implements a number of estimation methods (including full information maximum likelihood) and allows both path analysis and matrix algebra styles for model specification. More importantly, the development team is constantly working on improving the software and they actively participate in the official OpenMx forum, where they reply to both technical and theoretical questions raised by the community.[38] All the models introduced in this chapter can be estimated using any SEM software, however, given the historical link between the Mx community and behavior geneticists, most work published in the space of behavior genetics has been published using Mx or OpenMx.

12.7 Extension of the classical twin model

The classical twin model can be easily extended to incorporate the data from additional relatives. For example, to incorporate the data from additional siblings the

means/threshold and covariance models are simply extended to include the additional information and the genetic and environmental relationships of the siblings are specified within the covariance structure. For example, the ACE covariance model for a pair of MZ twins can be easily extended to incorporate data from an extra sibling.

Thus:

$$\begin{pmatrix} V_A + V_C + V_E & V_A + V_C \\ V_A + V_C & V_A + V_C + V_E \end{pmatrix}$$

becomes:

$$\begin{pmatrix} V_A + V_C + V_E & V_A + V_C & \frac{1}{2} V_A + V_C \\ V_A + V_C & V_A + V_C + V_E & \frac{1}{2} V_A + V_C \\ \frac{1}{2} V_A + V_C & \frac{1}{2} V_A + V_C & V_A + V_C + V_E \end{pmatrix}$$

12.8 Gene–environment correlation vs interaction

Two phenomena further complicate the interpretation of the results from the CTD: gene–environment correlation (rGE) and gene–environment interaction (G × E). We say that rGE is influencing the trait of interest when the selection of environments that an individual experiences is linked to their genetic make-up.[18,39] An example of rGE could be that someone with a higher genetic predisposition towards musical proficiency is also more likely to engage in music activities because they receive positive feedback (therefore increasing their music skills further). In contrast, G × E interaction pertains to specific genes responding differently to the same environment. An example of this might be that two people with different genetic predispositions towards developing emotional disorders experienced the same traumatic event, leading to two different probabilities of them developing such disorders. Moreover, G × E interaction refers to the mechanisms through which genes and environment co-act (not to be confused with epigenetics, which involves the modification of gene expression at a molecular level). One of the assumptions of the CTD is that the genetic and environmental components do not correlate or interact. However, gene by environment interaction may be common and this assumption should be tested. The study of gene by environmental interactions has become an important area of scientific enquiry in itself[40,41] and in the next section, we will introduce models that can be used to test for the presence of rGE and G × E.

12.8.1 Genotype–environment correlation and assortative mating

Genotype–environment correlation occurs when an individual's environment is influenced by either their own genotype, or that of a genetic relative.[18,39] If rGE is positive, then the result may be an increase in the total phenotypic variance of the trait. Continuing with our laterality example, an example of a positive rGE would be

if children who have a higher genetic liability for left-handedness are more likely to grow up in an environment where left-handedness was encouraged through modeling or instruction. Alternatively, when a negative rGE exists, the total phenotypic variance is decreased. It is probable that traditional biases against left-handedness had such an effect, where individuals who might have been left-handed, and many individuals who were left-handed, were made to write with their right hand under threat of disapproval or corporal punishment. Genotypes and environment can be, however, correlated in different ways. One of the most extended taxonomies of rGE was developed by Eaves, Last[42] which proposes three different types of rGE:

a. **GE autocorrelation** includes active rGE and evocative (or reactive) rGE. Active rGE refers to the situation where an individual seeks or creates environments related to their genetic make-up. Evocative rGE refers to the situation where the environment reacts in a particular way depending on the individual's genetic make-up, modifying the type of context they experience. The cultural coercion towards right-handedness described above is an example of a GE autocorrelation, an environmental response evoked by an individual's genotype. It is difficult to resolve the correlated effects of genotypes and environments. However, in this case, stratification of the sample by birth cohort or a regression correction for the year of birth might correct some of the bias due to autocorrelation.
b. **Cultural transmission** refers to the environmental effect of the parental phenotype on the offspring's phenotype.[1] The positive rGE described above are examples of cultural transmission, whereby the modeling or instruction from the parents might influence the handedness of the child. The effects of cultural transmission may be examined by extending the twin design to include parental data. Such a design also allows for a test of the assumption of random or nonassortative mating[1] which may be based on phenotypic similarity (positive assortment) or dissimilarity (negative assortment).
c. **Sibling interactions** may be either co-operative, increasing the trait value of the co-twin, or competitive, decreasing the trait value in the co-twin. Co-operation effects increase the variance and decrease the covariance of MZ twins in relation to DZs, while competition produces the opposite effects. For a binary trait, cooperation will increase the rate of affection in twins when compared to singletons, while competition would have the opposite effect. Finding significant differences between means and variance across co-twins in the assumptions testing step of a twin model could point towards the existence of sibling interactions.

Finally, all three types of rGE result in a nonrandom association of genotypes across all possible environments under study. In particular, unmodeled rGE leads to the overestimation of shared-environmental factors, when rGE occurs between additive genetic factors and shared-environmental factors, and to the overestimation of additive genetic factors, when the rGE occurs between additive genetic factors and unique environmental factors.[43]

12.8.2 Gene–environment interaction

In terms of G × E interaction, there are two main theories used to predict potential consequences of G × E interactions:

- The *diathesis-stress model* predicts that, in the presence of an environmental stressor associated with the development of a specific problem, individuals with higher genetic predisposition for that problem will be more likely to develop it than those with a lower genetic risk.[44] Therefore, heritability estimates will be higher in a high-risk environment (i.e., with a higher prevalence of environmental stressors). Vice versa, the presence of protective factors in an environment will mask the influence of genetic factors in the development of the condition, leading to lower heritability estimates.
- The *bioecological model* predicts that enriched environments will amplify pre-existent genetic differences in a population, by means of what Bronfenbrenner and Ceci[45] called "proximal processes," which are interactions between the individual and their immediate social context (e.g., family, teachers, health professionals, etc.). Such interactions can increase competency or buffer dysfunction.

12.9 Structural equation modeling for rGE and G × E interaction

G × E interaction is closely link to the statistical concept of heteroscedasticity, that is, the case when the variability of a specific trait or measure is unequal across the range of values of a second variable. In the same way, in the face of G × E interaction, we expect to observe differences in variance between groups of individuals that have been exposed or not to risk or protective factors, or meaningful proximal processes. This second variable, across which values we find differences in the relative contribution of genetic and environmental factors to the phenotypic variation in our trait of interest, is commonly known as *moderator*. When we want to evaluate heteroscedasticity across populations generated by a binary moderator (e.g., exposed/unexposed, rural/urban, etc.) it is common to use a multigroup approach.[1] That is, we evaluate the invariance of the parameters associated with genetic and environmental variance components across the two groups created by the moderator by equating the coefficients associated with each variance components to be the same in both groups and then evaluating change in model fit. For example, we could be interested in assessing if V_A, V_C, and V_E can be assumed to be equal in men and women for a particular phenotype, such as smoker status. We first estimate these parameters freely for each gender and then proceed to equate and test for changes in goodness of fit in our model. When we only have MZ and DZ twin pairs, and both members of the pair are concordant in the moderator (gender in this example), we can evaluate if there are quantitative differences in the relative contribution of genetic and environmental factors to individual differences in the phenotype

(e.g., smoking) for each level of the moderator. However, if we also have available data from discordant twin pairs in the moderator (e.g., opposite-sex dizygotic twins, or one twin is exposed to a protective intervention but not the other) we can also evaluate if there are qualitative differences in the variance components. That is, if new sources of genetic or environmental variation appear in one of the levels of the moderator. The most common example of this approach are sex-limitation models[1] which refers to the phenomenon in which the expression of some genes, despite their presence in both sexes, is limited to only one sex.[46]

12.9.1 Analysis of sex differences

Effects such as sex limitation or sex differences in gene or environmental expression, may also be incorporated in twin modeling. If data from both MZ and DZ males and females, and opposite-sex dizygotic (OSDZ) twins, are collected, it is possible to test several hypotheses about sex differences (or sex-limitation effects) in the proportion of phenotypic variance explained by genetic and environmental factors.[47] Sex limitation or sex moderation effects may be quantitative or qualitative in nature.[10,47] The quantitative effects model tests the hypothesis that while the same genes are expressed in both males and females, the magnitude of these effects across the loci involved differs between males and females. In this case, quantitative sex differences can be modeled by specifying the sets of variance components (V_A, V_C or V_D, and V_E) separately for male and females. However, qualitative sex-limitation models require having data collected from OSDZ and can be used to detect differences in the magnitude of genetic and environmental influences between males and females, and to determine whether the same sets of genetic and environmental factors influence a trait in males and females. When qualitative sex differences are present, the correlation of OSDZ twins is expected to be significantly lower than that of same-sex DZ twins. A higher same-sex DZ than OSDZ correlation would suggest that there is not complete overlap in the sets of genes or shared environmental factors that explain trait variation in males and females.[48] These sex-limitation hypotheses can be tested by comparing the fit of different SEM. Here we will discuss three models (for a more detailed description of these models, see Neale and Maes[1]):

a. Full Sex-Limitation model: this model specifies a unique variance component associated with either sex-specific additive genetic effects, *VA′j*, or dominance genetic effects, *VA′j*, in the ADE model, or the common environment effect, *VC′j*, in the ACE model, is freely estimated, as are different sets of parameters (VA, VC or VD and VE) for males and females. Both sex-specific effects and quantitative sex differences are modeled. A depiction of this model is presented in Fig. 12.7.

Due to space limitations, only the OSDZ twin group is presented; however, it contains all the idiosyncratic parameters of the sex-limitation models family. Same-sex twins' models are identical, with the exception that members of a pair share the same set of sex-specific parameters (e.g., VA_m, VC_m or VD_m and VE_m).

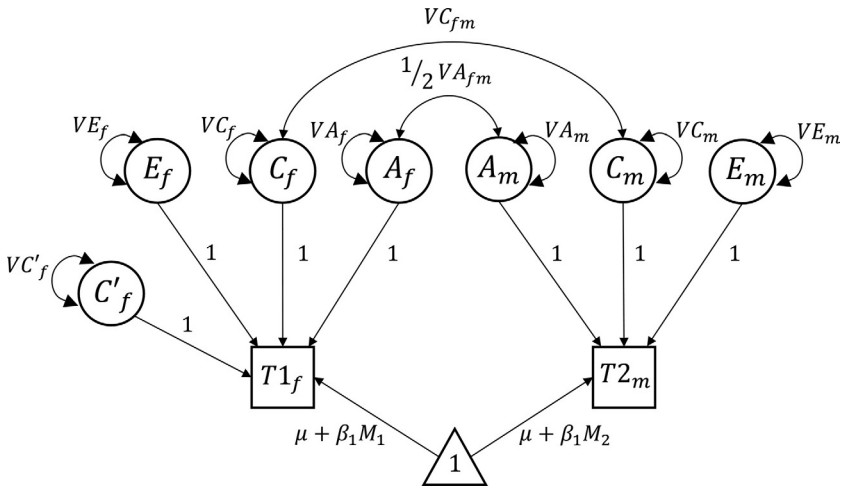


FIG. 12.7 Path diagram of a full sex-limitation ACE model for one phenotype, measured in pairs of twins (*T*1 and *T*2) moderated by a variable M, measured for each twin (M1 and M2).

A sex-specific variance component for shared environment is represented by *VC′f*. The weights of the latent variables (A, D, and E) on the phenotype can differ by sex as indicated by VA_f, VA_m, VC_f, VC_m, VE_f, and VE_m. In addition, two coefficients are specified among the opposite-sex twins, for modeling the covariation between the genetic (VA_{fm}) and shared environmental (VC_{fm}) factors.

b. Common Effects Sex-Limitation model: this model fixes the sex-specific variance component to zero, but allows the for sex differences in the parameters that estimate the genetic and environmental variance components. In this model, no sex-specific effects are present, only quantitative sex differences are modeled. The fit of this model is tested against that of the full sex limitation model to determine the importance of sex-specific effects.

c. Scalar Sex-Limitation model: this model specifies that the proportions of variance accounted for by V_A, V_C, V_D and V_E are the same for males and females, but sex differences in the total variance are allowed. This model is nested in the common effects sex limitation model, and tests of the comparative fits of these two models can be conducted to determine which model best explains the nature of the sex differences.

12.9.2 G × E with continuous moderators

Due to its burden on sample size, the multigroup approach presented above (section 12.8.1) is only feasible for moderators with only a few levels of response (i.e., because we need to split the sample for each level of the moderator). In addition, combining a multigroup approach with the variance component approach of the CTD means

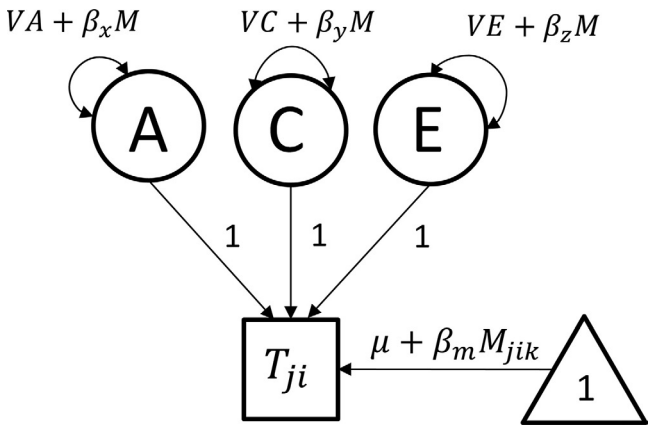


FIG. 12.8 Path diagram for an ACE model where each variance component and the phenotypic mean is modified by a moderator *M*.

It represents a different relationship between the moderator and each variance component (βx, βy, and βz) and the mean (βm) across zygosity (j), twin order (i), and participant number (k).

that it is not possible to evaluate if there are differences in variance across levels of the moderator (i.e., more or less variation for certain values of the moderator), nor can we take into account ordering in the values of the moderator.[43,49] The use of Full Information Maximum Likelihood, and its capacity to model individual likelihoods, accounts for this. It allows for the introduction of covariates measured at an individual level, which makes it possible to correct for the effect of this covariate on the phenotype, and also can be used to modify the weight of the coefficients associated with the variance components as a function of the levels of the moderator (Fig. 12.8). By including covariates (also known as *definition variables*) in the model, we can correct for the effect of extraneous variables on the phenotype, meaning that we will model the residuals obtained from the regression in our subsequent SEM. Please note that this is equivalent to directly introducing the residuals obtained from generalized estimating equations, or a mixed-effects model, where clustering of observations is accounted for.

The use of definition variables to model G × E interactions was proposed by Purcell.[43] His work included examples on how to apply this method to binary and continuous moderators that could have different mathematical relationships with the phenotype (i.e., linear, quadratic, etc.). Since then, his model has been extensively applied to twin data and several revisions of his approach have been published, including alternative models for testing gene–environment interaction in the presence of gene–environment correlation[50] the extension of the univariate moderation model to prevent an elevation of false positive moderator effects when the moderator is also correlated between twins[49] as well as the re-parameterization of these models for use with ordinal and binary outcome data.[51]

12.10 Final remarks

Twin modeling is experiencing a resurgence in the genome-wide association (GWAS) era: the possibility of introducing polygenic scores into twin models now allows the covariance between additive genetic and common environmental factors to be estimated.[52] The same multivariate genetic models that were applied to twin data are now applied to GWAS summary statistics via genomic structural equation modeling.[53] Twin modeling is also used to screen phenotypes for heritability prior to GWAS studies. Contemporary analysts will likely find that training in structural equation modeling, analysis of genetically informative datasets, and an understanding of the biometrical model and its psychometric development, will significantly increase their insight into future findings in the field of human genetics. Furthermore, heritability and environmental estimates are, by definition, specific to the population in which they are obtained. Understanding how genetic and environmental factors influence human traits will require continuing investigations across time and, especially, across populations, as most studies have been conducted in western, educated, industrialized, rich, and democratic populations and more information is needed from other populations. Finally, in the present chapter, we introduced the basic concepts of twin analysis and provided some references for the reader to explore further. There are many topics we have not included in this chapter: direction of causation and comorbidity models, developmental, longitudinal and survival analysis of twin data, use of genetic scores in twin models, or power calculation in classical twin design. Some of these topics are covered elsewhere in this book. For the rest, we refer the reader to the International Statistical Genetics Workshop website[12] and the OpenMx forum.[38]

References

1. Neale MC, Maes HHM. *Methodology for Genetic Studies of Twins and Families*. 3rd ed. Dordrecht, the Netherlands: Kluwer Academic Publishers; 2002.
2. Neale MC. Biometrical models in behavioral genetics*Handbook of Behavior Genetics*: Springer; 2009:15–33.
3. Falconer D, Mackay T. Introduction to Quantitative Genetics. 4th ed. D.S. Falconer and T F. C. Mackay. eds. Burnt Mill, England: Longman; 1996.
4. Gibson G. Population genetics and GWAS: a primer. *Plos Biology*. 2018;16(3):e2005485.
5. Fisher RA. The correlation between relatives on the supposition of Mendelian inheritance. *Trans R Soc Edinburgh*. 1918;2(52):399–433.
6. Boomsma DI, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nature Reviews Genetics*. 2002;3(11):872–882.
7. Heath AC, Nyholt DR, Neuman R, et al. Zygosity diagnosis in the absence of genotypic data: an approach using latent class analysis. *Twin Research*. 2003;6(1):22–26.
8. Verweij KJ, Mosing MA, Zietsch BP, et al. Estimating heritability from twin studies. *Methods in Molecular Biology*. 2012;850:151–170.
9. Loehlin JC. The Cholesky approach: a cautionary note. *Behavior Genetics*. 1996;26(1): 65–69.

10. Neale MC, Cardon LR. *Methodology for Genetic Studies of Twins and Families*. 3rd ed. Dordrecht, the Netherlands: Kluwer Academic Publishers; 1992.
11. Keller M, Coventry W. Quantifying and addressing parameter indeterminacy in the classical twin design. *Twin Res Hum Genet*. 2005;8(3):201–213.
12. Institute for Behavioral Genetics. International statistical genetics workshop 2021. Available from: https://www.colorado.edu/ibg/workshop-2021.
13. Evans DM, Martin NG. The validity of twin studies. *Genescreen*. 2000;1:77–80.
14. Heath AC, Neale MC, Hewitt JK, et al. Testing structural equation models for twin data using LISREL. *Behavior Genetics*. 1989;19:9–35.
15. Bouchard TJ, McGue M. Genetic and environmental influences on human psychological differences. *Journal of Neurobiology*. 2003;54(1):4–45.
16. Martin NG, Eaves LJ, Heath AC, et al. Transmission of social attitudes. *Proc Natl Acad Sci PNAS*. 1986;83(12):4364–4368.
17. Posthuma D, Boomsma D. A note on the statistical power in extended twin designs. *Behavior Genetics*. 2000;30:147–158.
18. Falconer D, Mackay T. *Introduction to Quantitative Genetics*. 4th ed. London: Logman; 1996.
19. Robinson MR, Kleinman A, Graff M, et al. Genetic evidence of assortative mating in humans. *Nat Hum Behav*. 2017;1(1):1–13.
20. Akaike H. Factor analysis and AIC. *Psychometrika*. 1987;52(3):317–332.
21. Hu L-t, Bentler PM. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. *Struct Equat Model*. 1999;6(1):1–55.
22. Neale MC, Miller MB. The use of likelihood-based confidence intervals in genetic models. *Behavior Genetics*. 1997;27(2):113–120.
23. Verhulst B, Prom-Wormley E, Keller M, et al. Type I error rates and parameter bias in multivariate behavioral genetic models. *Behavior Genetics*. 2019;49(1):99–111.
24. McGue M, Christensen K. Genetic and environmental contributions to depression symptomatology: evidence from Danish twins 75 years of age and older. *Journal of Abnormal Psychology*. 1997;106(3):439.
25. Boomsma DI, Princen HM, Frants RR, et al. Genetic analysis of indicators of cholesterol synthesis and absorption: lathosterol and phytosterols in Dutch twins and their parents. *Twin Res Hum Genet*. 2003;6(4):307–314.
26. Do Ha I, Lee Y, Pawitan Y. Genetic mixed linear models for twin survival data. *Behavior Genetics*. 2007;37(4):621–630.
27. Mosing MA, Magnusson PK, Pedersen NL, et al. Heritability of proneness for psychological flow experiences. *Personality and Individual Difference*. 2012;53(5): 699–704.
28. Verweij KJ, Mosing MA, Zietsch BP, Medland SE. Estimating heritability from twin studies. *In Statistical human genetics*. 2012, pp. 151–170.
29. Verhulst B, Neale MC. Best practices for binary and ordinal data analyses. *Behavior Genetics*. 2021;51(3):204–214.
30. Risch N, Rao D. Genetic linkage and complex diseases, with special reference to psychiatric disorders. *Genetic Epidemiology*. 1990;7(1):3–16.
31. Left AM. *Right, Hand and Brain: the Right Shift Theory*. London: Lawrence Erlbaum Associates; 1985.
32. Hand McMCR, Hand L. *The Origins of Asymmetry in Brains, Bodies, Atoms and Cultures*. London: Weidenfeld & Nicolson; 2002.
33. Holder M. *Hand Preference Questionnaires: One Gets What One Asks For*. New Brunswick, New Jersey: Rutgers University; 1992.

34. Neale MC, Cardon L. *Methodology for Genetic Studies of Twins and Families*: Springer; 1992.
35. Neale MC, Boker SM, Xie G, et al. Mx: Statistical Modeling. 6th ed. Richmond, VA. http://www.vcu.edu/mx/: Department of Psychiatry; 2002.
36. MC N, Eaves LJ, Kendler KS. The power of the classical twin study to resolve variation in threshold traits. *Behavior Genetics*. 1994;24(3):239–258.
37. Neale MC, Hunter MD, Pritikin JN, et al. OpenMx 2.0: extended structural equation and statistical modeling. *Psychometrika*. 2016;81(2):535–549.
38. OpenMx Project. Forums 2021 [Available from: https://openmx.ssri.psu.edu/forums.
39. Plomin R, DeFries JC, Loehlin JC. Genotype-environment interaction and correlation in the analysis of human behavior. *Psychological Bulletin*. 1977;84(2):309.
40. Keller MC. Gene× environment interaction studies have not properly controlled for potential confounders: the problem and the (simple) solution. *Biological Psychiatry*. 2014;75(1):18–24.
41. Dick DM, Barr PB, Cho SB, et al. Post-GWAS in psychiatric genetics: a developmental perspective on the "other" next steps. *Genes, Brain, and Behavior*. 2018;17(3):e12447.
42. Eaves LJ, Last K, Martin NG, et al. A progressive approach to non-additivity and genotype-environmental covariance in the analysis of human differences. *British Journal of Mathematical and Statistical Psychology*. 1977;30(1):1–42.
43. Purcell S. Variance components models for gene–environment interaction in twin analysis. *Twin Res Hum Genet*. 2002;5(6):554–571.
44. Rende R, Plomin R. Diathesis-stress models of psychopathology: a quantitative genetic perspective. *Appl Prevent Psychol*. 1992;1(4):177–182.
45. Bronfenbrenner U, Ceci SJ. Nature-nuture reconceptualized in developmental perspective: a bioecological model. *Psychological Review*. 1994;101(4):568.
46. Harris H. On sex limitation in human genetics. *The Eugenics Review*. 1948;40(2):70.
47. Eaves LJ. Twins as a basis for the causal analysis of human personality. *Progress in Clinical and Biological Research*. 1978;24A:151–174.
48. Vink JM, Bartels M, Van Beijsterveldt TC, et al. Sex differences in genetic architecture of complex phenotypes? *PLoS One*. 2012;7(12):e47371.
49. Van der Sluis S, Posthuma D, Dolan CV. A note on false positives and power in G× E modelling of twin data. *Behavior Genetics*. 2012;42(1):170–186.
50. Rathouz PJ, Van Hulle CA, Rodgers JL, et al. Specification, testing, and interpretation of gene-by-measured-environment interaction models in the presence of gene–environment correlation. *Behavior Genetics*. 2008;38(3):301–315.
51. Medland SE, MC N, Eaves LJ, et al. A note on the parameterization of Purcell's G× E model for ordinal and binary data. *Behavior Genetics*. 2009;39(2):220–229.
52. Dolan CV, Huijskens RC, Minică CC, et al. Incorporating polygenic risk scores in the ACE twin model to estimate A–C covariance. *Behavior Genetics*. 2021;51(3):237–249.
53. Grotzinger AD, Rhemtulla M, de Vlaming R, et al. Genomic structural equation modelling provides insights into the multivariate genetic architecture of complex traits. *Nat Hum Behav*. 2019;3(5):513–525.

CHAPTER

Twin studies of complex traits and diseases

13

Christopher R. Beam[a], Alice J. Kim[a], Tinca J.C. Polderman[b,c]

[a]*University of Southern California, Department of Psychology*

[b]*Amsterdam UMC, Vrije Universiteit Amsterdam, Child and Adolescent, Psychiatry & Psychosocial Care, Amsterdam Public Health*

[c]*Vrije Universiteit Amsterdam, Clinical and Developmental Psychology, Amsterdam, The Netherlands*

Quantitative geneticists and social scientists declare time and again that the "nature-nurture debate" is over[1], citing years of genetically informed (twin) studies showing that nature *and* nurture account for variability in traits and disease.[2] Indeed, twin studies in the 21st century largely focus on how genes and environments correlate and interact to account for individual differences in traits and diseases.[3–5] Yet, when undergraduate students are asked, "Which matters more for intelligence, empathy, depressive symptomatology, relationships problems, and so forth? Nature? Or nurture?" two factions invariably form: the hereditarians and the environmentalists. To be sure, at the time of this writing—months into the COVID-19 pandemic—the science section of a reputable media outlet addressed genetic versus environmental causes of the mortality risk associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[6] Many people, thus, still care a great deal about the relative importance of genes versus environments for understanding trait outcomes and disease risk.

As a field of study, behavioral genetics clarified that all human complex traits are the product of people's genetic composition *and* their environmental exposure. Termed *The Three Law of Behavior Genetics*, Turkheimer[2] summarizes the major themes of behavioral genetics. The first two laws state that all traits are heritable (Law 1) whereas effects of environments two people share (i.e., rearing environments, like being raised in the same household) are smaller than effects of genotype (Law 2). Finally, unique life experiences, something quantitative geneticists term the "nonshared environment," account for equal if not more variance than genotype (Law 3). Although we do not go so far as to say that these observations are "laws," the reliability of these three findings is so well accepted that many consider them beyond scientific criticism.

That genotype and environment shape human complex traits and diseases come as no surprise; phenotypes (e.g., cognitive ability, height, depression) are the product of the interaction between genotype and environmental influences. Quantitative genetic studies provide information about the sources of variation in populations of interest. The question, thus, is not *which* matters more, genotype or environment,

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-128-21514-2.00013-1

but rather *how* do genotype and environments come together to produce observable differences in traits and diseases?[7,8] Our goal in this chapter is to first present an overview of the genetic and environmental mechanisms that cause differences in human complex traits and diseases. In this section, we focus on findings that demonstrate that all traits are heritable, whether and how heritability of traits and diseases differ between men and women, and whether the same genetic factors account for covariation between two traits (e.g., intelligence and achievement) or two diseases (e.g., autism and ADHD). We then present findings of how environmental factors—both measured and unmeasured—augment, moderate, and correlate with genotype to maximize (or minimize) genetic expression of traits and diseases. Here, we focus on the three most common domains of study in the quantitative genetics literature: cognitive ability, personality, and psychopathology. Finally, we cover the ways in which behavior genetics will be important in future research for clarifying the role of genotype and environment in understanding the etiology of traits and diseases.

13.1 All traits are heritable

It is widely accepted that all human complex traits are heritable. So, what do behavioral geneticists mean when they report that traits and diseases are "heritable"? The general meaning of heritability refers to the proportion of variance in a phenotype attributed to genetic differences in the population in a particular context. Heritability can be a confusing concept, as there is not just one measure of heritability even though all of them provide estimates of the proportion of observed variance in a phenotype attributed to genetic variance in the population. Broad sense heritability refers to the ratio of total genetic variance to phenotypic variance whereas narrow sense heritability refers to the ratio of additive genetic variance to phenotypic variance. Simply put, heritability refers to how much of the variation in a trait under study is explained by genetic differences in the people being studied. Twin studies most often report narrow sense heritability in which genetic influences consist of additive genetic factors only (i.e., sum of the mean effects of alleles). Less often reported, broad-sense heritability contains the *total* effect of genotype that includes additive genetic and nonadditive genetic (dominance, i.e., interactions between alleles within a specific locus, and epistasis, which refers to interactions between alleles at different loci) effects.

13.2 Landmark study in twin research: MATCH

In 2015, a landmark meta-analytic study on twin studies and heritability estimates was published: Meta-Analysis of Twin Correlations and Heritability (MATCH).[9] With over 2,700 publications of twin studies on human traits and disease, virtually all twin studies from the past 50 years, MATCH included data from millions of twin pairs to provide an overview of twin correlations and heritability estimates, across age and sex, of each human trait that had been investigated so far. When analyzing all traits together, the reported relative contribution of genetic and environmental

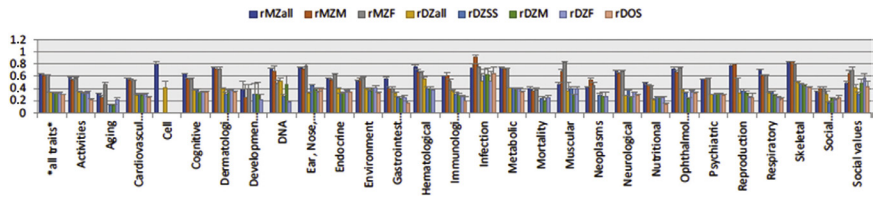


FIG. 13.1 Overview of twin correlations for 28 trait domains (based on 17,804 included traits) as investigated in a meta-analysis of 50 years of twins studies.[9]

Monozygotic twin correlations (r_{MZ}) were for all domains higher than dizygotic twin correlations (r_{DZ}), in male (M), female (F), same sex (SS), and opposite sex (OS) pairs, suggesting all domains are heritable to some extent. Of note, the majority of studies were conducted in western countries whereas twin data of the African, South American, and Asian continent were mostly absent.

influences was more or less "fifty-fifty": genes were just as important as environments. However, heritability estimates differed across specific traits, suggesting that the variance attributed to genotype depends on the trait under scrutiny. All results of MATCH can be visualized in the webtool http://match.ctglab.nl/ (Fig. 13.1).

The highest heritability estimates were for opthalmological phenotypes [71%] (i.e., characteristics of the eye), whereas one of the lowest heritability estimates was noted for social values and social interactions [~25%]. For some traits, heritability varied across age, that is, the degree to which genetic differences explained trait variation varied depending on the age of participants. For example, cognitive abilities show high heritability estimates in adulthood, with almost no variation explained by environmental differences between people, while in childhood heritability estimates are lower—about equal to environmental variance. Conversely, age effects on heritability estimates have been observed for neurodevelopmental disorders such as Attention Deficit/Hyperactivity Disorder (ADHD) and autism spectrum disorder (ASD), in which heritability estimates in childhood range between 60% and 80% while estimates in adulthood are much lower (~35%). Rater effects, that is, the switch from parental reports of childhood cognition to self-reports in adolescence and adulthood, may partly explain the difference in heritability estimates. The pattern of twin correlations based on self-reports of ADHD and ASD typically show lower monozygotic and dizygotic twin correlations, resulting in lower heritability estimates. Interestingly, a study using a more optimal design, namely that examined the shared view of multiple informants longitudinally, reported that ADHD symptoms over time were highly heritable throughout development.[10]

13.3 Sex differences in heritability

Sex differences in the etiology of traits are important to investigate as these differences may have implications for gene-finding studies and could also imply that differential strategies may be important for intervention and treatment approaches in males and females. A common example in the psychiatric literature is schizophrenia (SCZ),

whereby symptoms, drug-dosage, side effects, treatment response, and compliance differ between men and women.[11] Also, within the health, medical, social, and psychological sciences, sex differences are well known, and, therefore, studies investigating the relative contribution of genetic or environmental influences to trait variation are highly relevant. The rich dataset of the Netherlands Twin Register was utilized to investigate sex differences in heritability across a variety of behavioral, psychiatric and health related traits.[12] Significant sex differences in heritability were found for only 4% of the traits studied. In 2017, a systematic analysis of sex differences in the MATCH database was conducted based on a sample of more than 2,000 twin pairs for which sufficient data were available.[13] Among all the investigated traits ($N = 2{,}608$), only 1% demonstrated sex differences in heritability estimates; and when traits were clustered into trait categories ($N = 50$), none showed significant sex differences in the magnitude of genetic or environmental influences. The investigation also analyzed whether twin correlations within opposite-sex twin pairs (i.e., male-female pair) differed from same-sex DZ twin pairs, as that might indicate sex-specific genetic variance contributing to trait variance. Of 1,922 traits for which these particular data were available, only 3% of the twin correlations of opposite-sex twin pairs differed (i.e., were lower) compared to same-sex DZ twin pairs. However, for 25% of the 50 trait categories sex-specific genetic factors were indicated, and these included categories such as Eating Disorders, Specific Personality Disorders, Weight Maintenance Functions, Height, and Disorders of Puberty. In line with the results on weight maintenance functions and height, a large study on body mass index (BMI) in 37,000 twin pairs from eight countries showed convincing evidence for sex-specific genetic factors for BMI variance.[14] In sum, the paucity of studies so far suggests that sex-specific genetic variance on traits and diseases are found in traits in which biological differences generally are expected in the first place, such as sex-specific genetic factors that contribute to variation in disorders of puberty, and BMI.

13.4 Are twin designs the holy grail in heritability studies?

Although twin studies have been the "workhorses" of heritability studies for the last century, some criticism has arisen as well. For instance, twins differ in their prenatal circumstances (i.e., distribution of nutrition), are more often born preterm, and hence, often have lower birth weights compared to singletons. Thus, are results based on twin studies generalizable to the general population? Some believe so,[1] but the leap from "we observed it in twins" to "we will observe the same in the general population" rests on a set of assumptions some believe might be too strong.[15] Although the assumption that dizygotic and full-siblings share half as much of their genotype, on average, as monozygotic twins is generally accepted, as is the assumption that much of twins' and siblings' unique experiences are, in fact, unshared, some assumptions of the twin design have often been criticized. These assumptions are: exposure to common (i.e., family) environments is the same for MZ and DZ twins; parents do not assortatively (i.e., nonrandomly) mate; and genetic and environmental effects

are uncorrelated. (For a complete discussion on strengths and limitations of the twin design, we refer to chapter XX of this book and ref. Røysamb & Tambs.[16]) As a result of the contention over the validity of twin designs, others have used different research designs to infer heritability of traits and diseases.

In an aim to compare heritability estimates between the twin design and other genetically informative designs, ref. Pettersson et al.[17] examined heritability estimates of eight common psychiatric disorders in a family based design and a genetic design. For the family design, data on clinical diagnoses of full and half-siblings were analyzed. Like in the twin design, this design capitalizes on data of genetically related family members. In this case, the resemblance in clinical diagnoses between full siblings, who share on average half of their genetic material, was compared to the resemblance between half-siblings, who share on average 25% of their genetic material. The psychiatric disorders investigated in the study by ref. Pettersson et al.[17] were (1) alcohol dependence, (2) anorexia nervosa (AN), (3) ADHD, (4) ASD, (5) bipolar disorder (BIP), (6) major depressive disorder (MDD), (7) obsessive-compulsive disorder (OCD), and (8) SCZ. Heritability estimates based on the family design varied substantially per disorder, from 35% for MDD to 80% for ADHD, and highlights the large differences in genetic and environmental influences across disorders. Of note, the heritability estimates mirrored results of the MATCH (twin) findings. The genetic data used in this study were single nucleotide polymorphisms (SNPs) that were collected in large clinical samples of the Psychiatric Genomics Consortium (PGC). Heritability estimates based on this design were expectedly lower but correlated positively with the family-based estimates. Thus, different study designs (twin, family, genetic data), with different limitations, strengths, and assumptions converge in their findings of substantial heritability estimates.

13.5 Psychiatric disorders, comorbidity, and genetic overlap

A common finding in psychiatric research is the co-occurrence of psychiatric traits, or the observation that comorbidity is the rule rather than the exception. For instance, ADHD and ASD, often co-occur,[18] and the multivariate twin design has been utilized to examine if shared genetic factors might explain this co-occurrence. Indeed, genetic overlap between ADHD and ASD has been established,[19] also at sub-dimension level (e.g., inattention (ADHD), or social problems (ASD)) in children,[20,21] in adolescents,[22] young adults,[23] and older adults.[24,25] Also, recent large genome-wide association studies (GWAS) confirmed the genetic association between ADHD and ASD. For instance, the first adequately powered GWAS of ASD[26] reported a genetic correlation of 0.36 between ADHD and ASD.

The cross-disorder group of the PGC presented in 2019 an analysis across eight psychiatric and neurodevelopmental disorders, namely AN, ADHD, ASD, bipolar disorder (BIP), MDD, OCD, Tourette syndrome (TS), and SCZ. This study showed significant genetic correlations across virtually all pairs of included disorders, indicating

widespread genetic pleiotropy (i.e., the same genetic factor (or genotypes) influence more than one disorder). The highest genetic correlations were observed for BIP and SCZ (r_g 0.70), and for AN and OCD (r_g 0.50). Of note, MDD, considered an adult disorder, was correlated genetically with ADHD (r_g =0.44) and ASD (r_g 0.45). These findings suggest that genetic variance is even shared across early and late-onset disorders. Follow-up analyses suggested that pleiotropic genetic loci were predominantly expressed in genes that are involved in neurodevelopmental processes.[27]

In sum, genetic factors influence all traits and diseases that have been investigated so far to some extent, and the same genetic factors appear to influence multiple traits and diseases within certain domains. Sex differences, also, may be observed less often than previously assumed for traits in which biological differences are not an inherent part of the trait. Finally, heritability estimates are not devoid of environmental influences and context; indeed, heritability estimates always are interpreted in a population given the environmental context. This final point raises the question: How do environmental factors correlate and interact with genetic factors underlying complex traits and diseases?

13.6 Gene-environment interplay

13.6.1 Gene-environment correlation and gene-environment interaction

Although heritability estimates index the expression of genetic variance in a population, they always do so within an environmental context. In this way, heritability estimates oftentimes depend on environmental conditions or correlate with them in systematic ways. As Bronfenbrenner and Ceci[8] concluded: "h^2 cannot be interpreted as an estimate of the proportion of variance in a given developmental outcome that is completely free of environmental influence" (p. 583). Heritability, thus, always occurs in an environmental context and, therefore, can differ in one population of persons compared to another because of environmental contexts. Heritability of achievement outcomes, like grade point average, for example, is higher when children are given more educational opportunities earlier in life than not. There are two phenomena often examined in twin studies that attempt to seat heritability in an environmental context: gene-environment correlation and gene-environment interaction.

13.6.2 Gene-environment correlation (*r*GE)

A general research question among behavior geneticists is: Are genetic factors systematically correlated with environmental exposure? This question allows behavior geneticists to answer questions such as: Do people with a genetic propensity for antisocial behavior problems gravitate toward peers who are more likely to bend the rules than peers who follow the rules? Admittedly, the first question is broad and abstract, but twin designs allow researchers to test the processes through which genetic variance comes to be correlated with environment influences for all kinds of

human complex traits and diseases.[28] This is known as gene-environment correlation (*r*GE),[29] which is a statistical parameter that tests the extent to which variation in genotype (G) differentially exposes people to certain kinds of environments (E) that further encourage or discourage trait and disease development. Practically, *r*GE can be thought of as representing a systematic relationship between a measure of a person's genotype, like a child's ability to exert effortful control, and a measure of their surrounding environments, like level of chaos in the home environment. Essentially, *r*GE occurs when "genetic variants influence environmental exposure via behavior",[4] p. 433). The direction of the influence between genotype and environment is not specified, meaning that *r*GE also can occur when people's exposure to certain environments (e.g., good early education teachers) influences genetic expression of behavior (e.g., performing well on exams).

Gene-environment correlations can be positive and negative in sign. Positive *r*GE occurs when highly heritable traits are associated with environments favorable for trait development.[30, 29, 31] For example, children with higher effortful control tend to experience more organized home environments whereas children with less control experience more disorganized home environments.[32] Negative *r*GE occurs in cases when heritable traits are associated with environments unfavorable for the development of this trait; children experience environments that discourage phenotypic expression of existing genotype in pursuit of more effective functioning. Here, as an example, children with impoverished social skills may experience environments that provide more social coaching and opportunities for prosocial behavior that encourage genetic expression of enriched social skills.[30] Less discussed cases of negative *r*GE are ones in which children with desirable traits experience environments that discourage further trait development.

Gene-environment correlation can occur in three ways: passive, evocative, and active.[29,31] Passive *r*GE occurs when biological relatives pass down both their genes and home environment to children, creating an association between children's heritable characteristics and their environments. The child is passive in the process because the association occurs without any influence on the part of children. In this scenario, it is important to note that the home environment created by biological relatives is also influenced by their own heritable characteristics. Children who are genetically predisposed to be socially inhibited and have quiet demeanors may continue to be so because their parents provide them with a solitary home environment with few guests and visitors. In this example, parents pass down genes for introversion, and through their own inherited introversion, facilitate a home environment that supports their children's genetic expression of introversion.

Evocative *r*GE occurs when children elicit certain kinds of responses from others in the environment due to heritable behaviors or characteristics, creating an association between genotype and certain environments. Children who are genetically predisposed to introversion may elicit certain reactions from their parents and school teachers. A teacher, for example, may call on introverted children less often than extroverted children, thereby reinforcing these children's tendency to stay quiet. Teachers (and parents) may also provide shy children an outlet for personal expression and

participation through the written word, creating an environment favorable for further introversion.

Active *r*GE occurs when people seek certain environments over others based on their genetically influenced traits. Active *r*GE can be thought of as niche selection. Back to our introverted group of children, these children may seek social connection with other introverted peers who understand and appreciate solitude and quiet activities, like choosing solitary activities and socializing in small groups over large gatherings.

Gene-environment correlation shows how environments come to be correlated with genetic variance as people are provided, select into, and elicit experiences in life.[33] Gene-environment correlation is a fluid, dynamic process that changes over the lifespan. Passive *r*GE is most likely to occur in childhood (although it can occur in adulthood, too) whereas the presence of active *r*GE is likely nil in infancy, but increases as people age.[31]

13.6.3 Gene-environment interaction (GxE)

Whereas *r*GE explains how genotype comes to be correlated with environments, gene-environment interaction (GxE) addresses questions about the environmental conditions under which the expression of a genotype is more (or less) likely to occur. Gene-environment interaction, in other words, helps to clarify environmental conditions under which the genetic expression of cognitive ability or depressive symptomatology, for example, is maximized. Gene-environment interaction is a standard moderation scenario in which the effect of environment on behavior depends on genotype or the effect of genotype on behavior depends on environment. Although these conceptualizations are statistically equivalent, the chosen moderator—genotype or environment—depends on the scope and features of a given study. Furthermore, GxE studies can be carried out in twin studies and in nontwin samples. The latter, however, requires measured genotype in the form of candidate genes (i.e., a SNP) or polygenic scores (i.e., the weighted effect of multiple alleles on a phenotype).

One commonly researched GxE scenario is the Scarr–Rowe hypothesis, which states that heritability of cognitive ability is greater in higher socio-economic status (SES) environments than lower SES environments.[34] Using twin designs,[35,36] the Scarr–Rowe hypothesis has been important for clarifying child and adult environmental conditions under which genetic expression of cognitive ability is maximized.[37,38] GxE studies have continued to be useful and have been applied to phenotypes ranging from psychiatric disorders[39] to interpersonal factors like marital quality.[40] This has been especially true in the postgenomic era, in which much of the GxE studies that included candidate genes have failed to replicate,[41] like the finding that early stressful life events correlated with greater risk of depression only in those with a short allele in the 5-HTT gene-linked polymorphic region.[42,43]

Several GxE models are prominent in the literature. The first is the diathesis-stress model, which suggests that genetic factors may predict adverse behavioral outcomes for people who experience stressful environments.[44] In this conceptualization,

people with genetic susceptibility must experience riskier environments for expression of the adverse behavior; genetic susceptibility is a necessary but insufficient condition and must be triggered by an environmental stressor. Heritability of a trait or disease, thus, remains low unless one is exposed to the stressor. For example, under this model, genetic risk for depression will only materialize in the context of life stress. The diathesis-stress model has been criticized for focusing too much on risky environments at the expense of positive environments and for assuming a general vulnerability orientation for genotype. For example, Boyce[45] presents a series of studies that show that children who are genetically predisposed to be more sensitive and reactive (i.e., high risk) may benefit the greatest in low-stress environments but suffer the greatest consequences in high-stress environments.

Research like in ref. Boyce's[45] has led to a second model known as the differential susceptibility model.[46,47] Accordingly, people with certain genotypes may be more susceptible to the effects of the environment in both desirable and undesirable ways. These people are likely to experience the most positive outcomes in the most supportive environments whereas in the most negative environments, they will experience the worst outcomes. Ref. Belsky and Pluess[48] give the example of children with difficult temperaments (e.g., high negative emotionality) who experience problematic outcomes when born into high-risk environments (e.g., low parent responsiveness) but thrive when born into low risk, supportive environments (e.g., high parent responsiveness). These individuals, referred to as "orchids" because of their sensitivity to environmental conditions, are contrasted with individuals who neither suffer nor profit from negative or positive environmental exposures, referred to as "dandelions" because of their ability to thrive in nearly any environmental condition. Differential susceptibility models, thus, are considered better alternatives to diathesis-stress models for demonstrating the range in which genetic expression of a trait can occur.

13.7 Mechanisms that lead to *r*GE and GxE

In this section, we provide examples of *r*GE and GxE in commonly studied phenotypes. Cognitive ability is, by far, the most studied phenotype in both literatures to demonstrate that the environment in gene-environment correlations was as principal in the developmental system as genotype.[29] We then provide examples from the personality and externalizing behavior literature.

13.7.1 Cognitive ability

Genetic mechanisms mediate the correlation between environmental conditions in children's homes and their cognitive ability, suggesting that children actively employ their environments in their cognitive development.[49] Despite wide recognition that people fashion and evoke environments that either amplify or diminish genetic variance underlying cognitive ability,[50–52] few studies have explored the mechanisms

through which genetic and environmental influences on cognitive ability come to be correlated. We note two. First, ref. Tucker-Drob & Harden[53] showed that children differentially evoke parental learning behaviors because of genetically influenced characteristics. Conversely, quality of parenting, a genetically influenced characteristic, differentially predicted the kinds of learning environments parents provided their children. Heritability of early child cognition, thus, is not just a matter of which genes are inherited but of the environments that are given to and evoked by children.

Our second example is given from ref. de Kort et al.[54] who was the first to model gene-environment correlation explicitly in twin models via direct effects of phenotypes on environmental effects at a later point in time. This work showed that genetic and environmental factors partly explain increases in the population means of environmental influences underlying cognitive ability. Her study was the first of its kind, with only one subsequent replication in the Louisville Twin Study.[55] Ref. de Kort et al.'s[54] twin study of cognitive ability is special because it inserts observed ability as the mechanism through which genetic variance comes to be correlated with people's environments.

Studies like in ref. de Kort et al.'s[54] stand out from GxE models of cognitive ability, which quantify differences in heritability of cognitive ability as a function of environmental exposure. We noted the Scarr–Rowe hypothesis above—the hypothesis that socially disadvantaged populations are not as likely to realize genetic potential underlying cognition[56]—which has been replicated in numerous studies and summarized in a meta-analysis.[37] The economic disparity of the country in which twin population samples are studied matters quite a bit. Heritability-by-SES effects are observed more often in the United States than in Australia, Germany, The Netherlands, Sweden, and the United Kingdom. Greater inequality is observed in the United States than in these countries, at least as indexed by each country's respective level of income inequality (i.e., Gini coefficient). Economic inequality, in other words, may have an effect on the expression of cognitive ability.

13.7.2 Personality

Although several studies have investigated the relative influence of genetic and environmental factors on personality, most notably in the National Merit Study[57] and the Nonshared Environment in Adolescent Development,[58] fewer studies have investigated the environmental correlates of the heritability of personality. *r*GE is at play in the correlation between measured family environments and children's personality development, as measured the Big 5 factors of personality (i.e., neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness).[59] Similarly, in adult twins, ref. Kandler et al.[60] showed that genetic sources of variance mediated effects of personality on life event outcomes (although not vice versa), which provides evidence for active and evocative *r*GE in personality. Genetic factors positively mediated effects of neuroticism on negative life events whereas they positively mediated effects of extraversion and openness to experience on positive

life events. Genetic factors also mediated effects of openness to experience on greater number of life events. Taken together, Kandler's studies support the conventional understanding that parents are integral in the provision of environments that maximize genetic potential for personality development whereas in adulthood, people nonrandomly select and attract life events based on their genetic propensities.

As with cognitive ability, environmental factors moderate heritability of personality and temperament traits, particularly in childhood and adolescence.[61,62] Optimal parenting, like that of authoritative parents, has been found to be critical for the genetic expression of negative emotionality, a feature of the personality trait neuroticism. Although somewhat counterintuitive, the message here is that authoritative parenting may give children the flexibility to differentially express their personalities whereas authoritarian parenting potentially restricts children's flexibility to interact with their home environments in ways that allow them to maximize their genetic potential for their temperaments. Authoritarian parenting, unlike authoritative parenting, may require children to behave in accordance with their parents' wishes, irrespective of their genetically influenced strengths and weaknesses. Similarly, Lemery-Chalfant et al.[32] found that genetic variance underlying effort control, a measure of attention of self-control similar to the self-discipline facet of conscientiousness, and negative affectivity, a measure of neuroticism, was greater in children reared in chaotic homes than nonchaotic homes.

Adult social relationships, particularly romantic relationship satisfaction, moderate the heritability of the following traits: well-being (i.e., tending to be cheerful and feeling good), social potency (i.e., tending to influence and be forceful with others), alienation (i.e., tending to be suspicious that others have bad, harmful intentions), aggression (i.e., tending to take advantage and feel spiteful toward others), constraint (i.e., tending to be restrained, reserved, and traditional), and traditionalism (i.e., tending to have conservative values).[63] For the former three traits, genetic variance was highest when relationship satisfaction was low. Ref. South et al.[63] interpreted this pattern of results to be "evidence of, and very much in line with, the diathesis-stress theory, which suggests that in context of an environmental 'trigger'—here, an unsatisfying and/or distressed romantic relationship—genetic influences on personality will be expressed" (p.138). For the latter three traits, genetic variance was highest when relationship satisfaction was high. They interpreted this finding to be consistent with bioecological models,[8] such that a genetic predisposition to self-control (or lack thereof) is maximized in better environmental conditions.

13.7.3 Externalizing behaviors

Like all human traits, externalizing problem behaviors such as aggressive, rule-breaking, hyperactive, and impulsive behaviors are heritable (~50%) but environmental risk factors such as physical or emotional abuse, low socioeconomic status, and delinquent peer affiliations have also been suggested to play a significant role.[64,9] More recently, maternal smoking during pregnancy (MSDP) has been hypothesized as a prenatal environmental risk factor for externalizing

problem behaviors. The association between MSDP and externalizing problem behaviors, among which also clinical manifestations of externalizing problems such as conduct disorder, has been studied extensively. A meta-analysis observed a significant association between MSDP and conduct problems in offspring based on six studies.[65] The majority of included studies could not control for genetic variance, however, so the authors warn that results must be interpreted with caution, as genetic variance could potentially explain part of the observed statistical association with MSDP.[66] MSDP is an environmental factor that correlated with the genetic factor for externalizing problems.[67] In this study, using a highly informative family design, MSDP significantly predicted the development of conduct problems (in boys and girls) and interacted with genetic variance underlying conduct problems (in boys only), which suggests passive *r*GE. Several additional studies in genetically informative designs confirmed that the effects of MSDP on externalizing problem behaviors are at best moderate, or decrease when controlling for genetic associations.[68–73]

Other environmental factors that have been investigated in the context of externalizing behaviors are familial and social relationships. The Minnesota Twin Family Study published longitudinal results on the role of adolescent parent–child relationships and peer affiliations in the context of externalizing behaviors, and their potential interplay with genetic factors. A study of 1,382 same-sex twin pairs reported a GxE effect at age 17 (more genetic variance for externalizing behaviors when the parent-child relation showed more problems) but not at age 24. The authors, therefore, concluded that a GxE effect is "developmentally limited".[74] However, a shared genetic influence, (i.e., *r*GE), might explain the association between parent-child relation and externalizing behaviors in young adulthood. In the same sample, but this time using data at ages 17, 20, 24, and 29, it was investigated if antisocial peer affiliations would interact with genetic variance, cross-sectionally and over time. A significant GxE effect was found at age 17, but not after this age, again suggesting that these interaction effects are limited to the adolescent period but not to externalizing problems some years later.[75]

In a much younger sample of 5-year-old twins of the Southern Illinois Twins/Triplets and Siblings Study, Ref. DiLalla & DiLalla[76] investigated *r*GE in aggressive behaviors by matching each twin of one pair to an unfamiliar, same-age, same-sex playmate. By using twins, they could perfectly control, for example, parental style and genetic factors that might influence aggressive behaviors. Their observations revealed that the twins who were rated as more aggressive by their parents were more likely to be matched with children showing more aggressive, but not assertive behaviors. The authors suggest that evocative *r*GE is at play here: by behaving aggressively, the "aggressive twins" evoked aggressive behavior in their playmates. As playing is fundamental for child development, further research into the mechanisms underlying gene-environment interplay in aggressive behavior still needs to be understood.

In sum, GxE seems to play a minor role in externalizing behaviors and is limited mostly to late adolescence, whereas *r*GE, whether passive, or evocative, is observed at several ages.

13.8 Future directions of twin studies of traits and diseases

Quantitative genetic studies have been integral in clarifying genetic and environmental mechanisms that contribute to human complex traits and diseases. Just as ref. Meehl[77] predicted in 1978 that behavior genetics would persist in importance for the next half to full century, we also, expect that behavior genetics will be important for understanding the etiology of human development and health for the next century. What does the future of quantitative genetics hold for complex traits and diseases, especially 20 years into the postgenomic era?

The ability to measure genotype directly has opened up new opportunities for genetic research on traits and diseases. What we have learned from prior candidate gene studies and genome-wide association studies is that human complex traits are polygenic, meaning that traits are influenced by the cumulative small effects of many genes. These small effects can be combined into polygenic scores (or polygenic risk scores) that can be used in studies like any other random variable. The post-genomic era, once thought to retire the use of twin studies to infer genetic and environmental etiology of traits and diseases, has, instead, marshalled new opportunities for the use of twin designs, including the use of genotype data measured directly with DNA extracted from blood and saliva. Polygenic score estimation, holds promise as a tool for clarifying genetic influences on human complex traits. One of the more compelling areas is estimating ACE models that statistically adjust for effects of polygenic scores on phenotypes while simultaneously quantifying heritability.[78] Such approaches can help index the proportion of variance attributed to measured *and* unmeasured genotype. Although it probably is unrealistic to delineate all alleles associated with human complex traits like depression, cognition, and personality, understanding which genes are predictive of traits and behavior has appeal to both basic and clinical researchers alike.

Quantitative genetics likely will continue to be important for clarifying how genetic variation underlying complex traits and disease depend on environmental exposure. We expect research to continue to elucidate the mechanisms through which genetic and environmental factors cause individual differences in outcomes that range from normal developmental outcomes (e.g., temperament, personality, and cognition) and abnormal outcomes (e.g., major depression, SCZ, and Alzheimer's disease). Ref. Bronfenbrenner & Ceci[8] were right when they posited that heritability cannot be understood free of environmental context. Gene–environment interaction studies have been useful for demonstrating that heritability estimates depend on proximal and distal environments to which people have access. In a recent evaluation of behavioral genetics in the postgenomic era, Harden[79] writes that "future psychological research on G × E should seek to surmount its reliance on endogenous environmental variation and instead integrate experimental and econometric methods that allow for more rigorous inferences about causality" (p. 53) We agree. The econometric approaches that ref. Harden[79] describes emphasize exogenous environmental variables, like socioeconomic measures (e.g., military service, neighborhood), policy reform, and differential exposure to health and education programs.[80]

Twin studies also should continue to clarify the causal associations among risk and protective factors and complex traits and diseases. A strength of twin and sibling studies is that unobserved genetic and environmental selection factors can be ruled out as confounding factors in the association between two heritable phenotypes. Known as co-twin control studies[81] or quasi-causal modeling,[82] twin studies will continue to be useful for uncovering causal associations in studies where random assignment simply is impossible or unethical. The reason is that twins and siblings, especially MZ twins, share their genotype and experience many of their rearing environments in like ways. Take a pair of MZ twins, one who is diagnosed with SCZ, the other who is not. The causes of the twin's diagnosis cannot be attributed to genetic influences, as both twins share the same genotype, nor shared environmental influences. Some unique aspect of the twin's environment (e.g., substance use or traumatic brain injury), thus, must be causally related to the SCZ diagnosis. In this way, we believe that twin studies will continue to aid social, psychological, medical, and health sciences in testing causal effects against competing hypotheses.

Quantitative genetic studies also have the potential to clarify people's behavior that cause genetic variance and environmental factors to become correlated over time such that one twin (or sibling) becomes the more intelligent, more personable, or less depressed twin than his or her cotwin. Epigenetic research has much to offer here (see Chapter XX in the current volume). Studies of change in epigenetic profiles may clarify how selection and exposure to certain environments correlate with DNA methylation that, in turn, affects the role of genotype in development.

Finally, one area we are particularly enthusiastic about is integrating experimental approaches into genetically informed research designs.[83,84] One of us (CRB) is currently engaged in implementing an experimental approach to understand whether and how random assignment to exogenous environments can cause heritability of cognitive performance to change. Twin studies that include random assignment achieve two aims: greater understanding of the role of specific environmental conditions that maximize (or minimize) heritability of complex traits and diseases as well as the causal role of specific exogenous environments.

References

1. Conley D, Fletcher J. The Genome Factor What the Social Genomics Revolution Reveals about Ourselves.*Our History, and the Future*; 2017.
2. Turkheimer E. Three laws of behavior genetics and what they mean. *Curr Dir Psychol Sci*. 2000;9:160–164.
3. Dick DM, Agrawal A, Keller MC, Adkins A, Aliev F, Monroe S, …, Sher KJ. Candidate gene–environment interaction research: reflections and recommendations. *Perspectives on Psychological Science*. 2015;10:37–59.
4. Jaffee SR, Price TS. Gene–environment correlations: a review of the evidence and implications for prevention of mental illness. *Mol Psychiatry*. 2007;12:432–442.
5. Jaffee SR, Price TS, Reyes TM. Behavior genetics: past, present, future. *Dev Psychopathol*. 2013;25:1225–1242.

6. Sample I. Genetics in focus after coronavirus deaths of siblings and twins [Internet]. London (UK): The Guardian; 2020 ; [reviewed 2020 Oct 30; cited 2020 Oct 30]. Available from: https://www.theguardian.com/world/2020/may/05/genetics-in-focus-after-coronavirus-deaths-of-siblings-and-twins.
7. Anastasi A. Heredity, environment, and the question How? *Psychol Rev*. 1958;65:197.
8. Bronfenbrenner U, Ceci SJ. Nature-nuture reconceptualized in developmental perspective: A bioecological model. *Psychol Rev*. 1994;101:568.
9. Polderman TJ, Benyamin B, De Leeuw CA, Sullivan PF, Van Bochoven A, Visscher PM, Posthuma D. Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nat Genet*. 2015;47:702–709.
10. Chang Z, Lichtenstein P, Asherson PJ, Larsson H. Developmental twin study of attention problems: high heritabilities throughout development. *JAMA psychiatry*. 2013;70:311–318.
11. Riecher-Rössler A, Butler S, Kulkarni J. Sex and gender differences in schizophrenic psychoses—a critical review. *Archives of Women's Mental Health*. 2018;21:627–648.
12. Vink JM, Bartels M, Van Beijsterveldt TC, Van Dongen J, Van Beek JH, Distel MA, …, Ligthart L. Sex differences in genetic architecture of complex phenotypes? *PLoS One*. 2012;7:e47371.
13. Stringer S, Polderman TJ, Posthuma D. Majority of human traits do not show evidence for sex-specific genetic and environmental effects. *Sci Rep*. 2017;7:1–7.
14. Schousboe K, Willemsen G, Kyvik KO, Mortensen J, Boomsma DI, Cornes BK, …, Harris JR. Sex differences in heritability of BMI: a comparative study of results from twin studies in eight countries. *Twin Research and Human Genetics*. 2003;6:409–421.
15. Joseph J. *The Trouble with Twin Studies: A Reassessment of Twin Research in the Social and Behavioral Sciences*: Routledge; 2014.
16. Røysamb, E, & Tambs, K (2016). The beauty, logic and limitations of twin studies.
17. Pettersson E, Lichtenstein P, Larsson H, Song J, Agrawal A, Børglum A, …, Demontis D. Genetic influences on eight psychiatric disorders based on family data of 4 408 646 full and half-siblings, and genetic data of 333 748 cases and controls. *Psychol Med*. 2019;49:1166–1173.
18. Rommelse NN, Franke B, Geurts HM, Hartman CA, Buitelaar JK. Shared heritability of attention-deficit/hyperactivity disorder and autism spectrum disorder. *Eur Child Adolesc Psychiatry*. 2010;19:281–295.
19. Ronald A, Simonoff E, Kuntsi J, Asherson P, Plomin R. Evidence for overlapping genetic influences on autistic and ADHD behaviours in a community twin sample. *Journal of Child Psychology and Psychiatry*. 2008;49:535–542.
20. Ronald A, Larsson H, Anckarsäter H, Lichtenstein P. Symptoms of autism and ADHD: a Swedish twin study examining their overlap. *J Abnorm Psychol*. 2014;123:440.
21. Taylor M, Charman T, Robinson E, Plomin R, Happé F, Asherson P, Ronald A. Developmental associations between traits of autism spectrum disorder and attention deficit hyperactivity disorder: a genetically informative, longitudinal twin study. *Psychol Med*. 2013;43:1735–1746.
22. Pinto R, Rijsdijk F, Ronald A, Asherson P, Kuntsi J. The genetic overlap of attention-deficit/hyperactivity disorder and autistic-like traits: an investigation of individual symptom scales and cognitive markers. *J Abnorm Child Psychol*. 2016;44:335–345.
23. Ghirardi L, Pettersson E, Taylor MJ, Freitag CM, Franke B, Asherson P, …, Kuja-Halkola R. Genetic and environmental contribution to the overlap between ADHD and ASD trait dimensions in young adults: a twin study. *Psychol Med*. 2019;49:1713–1721.

24. Polderman T, Hoekstra R, Posthuma D, Larsson H. The co-occurrence of autistic and ADHD dimensions in adults: an etiological study in 17 770 twins. *Translational Psychiatry*. 2014;4:e435 -e435.
25. Polderman T, Hoekstra R, Vinkhuyzen A, Sullivan P, van der Sluis S, Posthuma D. Attentional switching forms a genetic link between attention problems and autistic traits in adults. *Psychol Med*. 2013;43:1985–1996.
26. Grove J, Ripke S, Als TD, Mattheisen M, Walters RK, Won H, …, Anney R. Identification of common genetic risk variants for autism spectrum disorder. *Nat Genet*. 2019;51:431–444.
27. Lee PH, Anttila V, Won H, Feng Y-CA, Rosenthal J, Zhu Z, …, Posthuma D. Genomic relationships, novel loci, and pleiotropic mechanisms across eight psychiatric disorders. *Cell*. 2019;179:1469–1482 e1411.
28. Rutter M, Dunn J, Plomin R, Simonoff E, Pickles A, Maughan B, …, Eaves L. Integrating nature and nurture: Implications of person–environment correlations and interactions for developmental psychopathology. *Dev Psychopathol*. 1997;9:335–364.
29. Plomin R, DeFries JC, Loehlin JC. Genotype-environment interaction and correlation in the analysis of human behavior. *Psychol Bull*. 1977;84:309.
30. Goldsmith HH. Behavior genetics. In: Benson MMHJB, ed.. Oxford: Elsevier; 2008. *Encyclopedia of INFANT and Early Childhood Development*. 1:165–172.
31. Scarr S, McCartney K. How people make their own environments: a theory of genotype→ environment effects. *Child Dev*. 1983;54(2):424–435.
32. Lemery-Chalfant K, Kao K, Swann G, Goldsmith HH. Childhood temperament: passive gene-environment correlation, gene-environment interaction, and the hidden importance of the family environment. *Dev Psychopathol*. 2013;25:51.
33. Plomin R, Asbury K. Nature and nurture: genetic and environmental influences on behavior. *Ann Am Acad Pol Soc Sci*. 2005;600:86–98.
34. Turkheimer E, Haley A, Waldron M, d'Onofrio B, Gottesman II. Socioeconomic status modifies heritability of IQ in young children. *Psychol Sci*. 2003;14:623–628.
35. Purcell S. Variance components models for gene–environment interaction in twin analysis. *Twin Research and Human Genetics*. 2002;5:554–571.
36. Van der Sluis S, Posthuma D, Dolan CV. A note on false positives and power in G × E modelling of twin data. *Behav Genet*. 2012;42:170–186.
37. Tucker-Drob EM, Bates TC. Large cross-national differences in gene× socioeconomic status interaction on intelligence. *Psychol Sci*. 2016;27:138–149.
38. Zavala C, Beam CR, Finch BK, Gatz M, Johnson W, Kremen WS, …, Reynolds CA. Attained SES as a moderator of adult cognitive performance: Testing gene–environment interaction in various cognitive domains. *Dev Psychol*. 2018;54:2356.
39. Kendler KS, Prescott CA. *Genes, Environment, and Psychopathology: Understanding the Causes of Psychiatric and Substance Use Disorders*: Guilford Press; 2007.
40. South SC, Krueger RF. Marital satisfaction and physical health: evidence for an orchid effect. *Psychol Sci*. 2013;24:373–378.
41. Hart AB, De Wit H, Palmer AA. Candidate gene studies of a promising intermediate phenotype: failure to replicate. *Neuropsychopharmacology*. 2013;38:802–816.
42. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, …, Braithwaite A. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*. 2003;301:386–389.
43. Risch N, Herrell R, Lehner T, Liang K-Y, Eaves L, Hoh J, …, Merikangas KR. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. *JAMA*. 2009;301:2462–2471.

44. Shanahan MJ, Hofer SM. Social context in gene–environment interactions: Retrospect and prospect. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*. 2005;60(Special_Issue_1):65–76.
45. Boyce WT. *The Orchid and the Dandelion: Why Sensitive People Struggle and How All Can Thrive*: Pan MacMillan; 2019.
46. Belsky J, Bakermans-Kranenburg MJ, Van IJzendoorn MH. For better and for worse: differential susceptibility to environmental influences. *Curr Dir Psychol Sci*. 2007;16:300–304.
47. Belsky J, Pluess M. Beyond diathesis stress: differential susceptibility to environmental influences. *Psychol Bull*. 2009;135:885.
48. Belsky J, Pluess M. Beyond risk, resilience, and dysregulation: phenotypic plasticity and human development. *Dev Psychopathol*. 2013;25:1243–1261.
49. Plomin R, Spinath FM. Intelligence: genetics, genes, and genomics. *J Pers Soc Psychol*. 2004;86:112.
50. Deater-Deckard K, Mayr U. Cognitive change in aging: identifying gene–environment correlation and nonshared environment mechanisms. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*. 2005;60(Special_Issue_1):24–31.
51. Dickens WT, Flynn JR. Heritability estimates versus large environmental effects: the IQ paradox resolved. *Psychol Rev*. 2001;108:346.
52. Plomin R, DeFries JC. The origins of individual differences in infancy; the Colorado adoption project. *Science*. 1985;230:1369–1371.
53. Tucker-Drob EM, Harden KP. Early childhood cognitive development and parental cognitive stimulation: evidence for reciprocal gene–environment transactions. *Dev Sci*. 2012;15:250–259.
54. De Kort JM, Dolan CV, Kan K-J, Van Beijsterveldt CE, Bartels M, Boomsma DI. Can GE-covariance originating in phenotype to environment transmission account for the Flynn Effect? *Journal of Intelligence*. 2014;2:82–105.
55. Beam CR, Turkheimer E, Dickens WT, Davis DW. Twin differentiation of cognitive ability through phenotype to environment transmission: The Louisville Twin Study. *Behav Genet*. 2015;45:622–634.
56. Rowe DC, Jacobson KC, Van den Oord EJ. Genetic and environmental influences on vocabulary IQ: parental education level as moderator. *Child Dev*. 1999;70:1151–1162.
57. Loehlin J, Nichols R. *Heredity Environment, and Personality*: Austin University of Texas Press; 1976.
58. Neiderhiser JM, Reiss D, Hetherington EM. The nonshared environment in adolescent development (NEAD) project: a longitudinal family study of twins and siblings from adolescence to young adulthood. *Twin Research and Human Genetics*. 2007;10:74–83.
59. Kandler C, Riemann R, Kämpfe N. Genetic and environmental mediation between measures of personality and family environment in twins reared together. *Behav Genet*. 2009;39:24–35.
60. Kandler C, Bleidorn W, Riemann R, Angleitner A, Spinath FM. Life events as environmental states and genetic traits and the role of personality: a longitudinal twin study. *Behav Genet*. 2012;42:57–72.
61. Bratko D, Butković A, Vukasović Hlupić T. Heritability of personality. *Psihologijske teme*. 2017;26(1):1–24.
62. Burt SA. Gene–environment interactions and their impact on the development of personality traits. *Psychiatry*. 2008;7:507–510.

63. South SC, Krueger RF, Elkins IJ, Iacono WG, McGue M. Romantic relationship satisfaction moderates the etiology of adult personality. *Behav Genet*. 2016;46:124–142.
64. Jonson-Reid M, Presnall N, Drake B, Fox L, Bierut L, Reich W, …, Constantino JN. Effects of child maltreatment and inherited liability on antisocial development: an official records study. *Journal of the American Academy of Child & Adolescent Psychiatry*. 2010;49:321–332.
65. Ruisch IH, Dietrich A, Glennon JC, Buitelaar JK, Hoekstra PJ. Maternal substance use during pregnancy and offspring conduct problems: a meta-analysis. *Neuroscience & Biobehavioral Reviews*. 2018;84:325–336.
66. Knopik VS. Maternal smoking during pregnancy and child outcomes: real or spurious effect? *Dev Neuropsychol*. 2009;34:1–36.
67. Marceau K, Rolan E, Leve LD, Ganiban JM, Reiss D, Shaw DS, …, Neiderhiser JM. Parenting and prenatal risk as moderators of genetic influences on conduct problems during middle childhood. *Dev Psychol*. 2019;55:1164.
68. Dolan C, Geels L, Vink J, van Beijsterveldt C, Neale M, Bartels M, Boomsma DI. Testing causal effects of maternal smoking during pregnancy on offspring's externalizing and internalizing behavior. *Behav Genet*. 2016;46:378–388.
69. D'Onofrio BM, Van Hulle CA, Waldman ID, Rodgers JL, Harden KP, Rathouz PJ, Lahey BB. Smoking during pregnancy and offspring externalizing problems: an exploration of genetic and environmental confounds. *Dev Psychopathol*. 2008;20(1):139–164.
70. Kuja-Halkola R, D'Onofrio BM, Larsson H, Lichtenstein P. Maternal smoking during pregnancy and adverse outcomes in offspring: genetic and environmental sources of covariance. *Behav Genet*. 2014;44:456–467.
71. Obel C, Zhu JL, Olsen J, Breining S, Li J, Grønborg TK, …, Rutter M. The risk of attention deficit hyperactivity disorder in children exposed to maternal smoking during pregnancy–a re-examination using a sibling design. *Journal of Child Psychology and Psychiatry*. 2016;57:532–537.
72. Skoglund C, Chen Q, D' Onofrio BM, Lichtenstein P, Larsson H. Familial confounding of the association between maternal smoking during pregnancy and ADHD in offspring. *Journal of Child Psychology and Psychiatry*. 2014;55:61–68.
73. Quinn PD, Rickert ME, Weibull CE, Johansson AL, Lichtenstein P, Almqvist C, …, D'Onofrio BM. Association between maternal smoking during pregnancy and severe mental illness in offspring. *JAMA Psychiatry*. 2017;74:589–596.
74. Samek DR, Hicks BM, Keyes MA, Bailey J, McGue M, Iacono WG. Gene-environment interplay between parent-child relationship problems and externalizing disorders in adolescence and young adulthood. *Psychol Med*. 2015;45:333.
75. Samek DR, Hicks BM, Keyes MA, Iacono WG, McGue M. Antisocial peer affiliation and externalizing disorders: Evidence for Gene × Environment × Development interaction. *Dev Psychopathol*. 2017;29:155.
76. DiLalla LF, DiLalla DL. Gene–environment correlations affecting children's early rule-breaking and aggressive play behaviors. *Twin Research and Human Genetics*. 2018;21:285–288.
77. Meehl PE. Theoretical risks and tabular asterisks: Sir Karl, Sir Ronald, and the slow progress of soft psychology. *J Consult Clin Psychol*. 1978;46:806.
78. Dolan CV, Huijskens RC, Minica CC, Neale MC, Boomsma DI. Incorporating polygenic scores in the twin model to estimate genotype-environment covariance: exploration of statistical power. *bioRxiv*. 2019:702738.

79. Harden KP. "Reports of My Death Were Greatly Exaggerated": behavior genetics in the postgenomic era. *Annu Rev Psychol*. 2020;72:37–60.
80. Gross RT, Spiker D, Haynes CW. *Helping Low Birth Weight, Premature Babies: The Infant Health and Development Program*: Stanford University Press; 1997.
81. McGue M, Osler M, Christensen K. Causal inference and observational research: the utility of twins. *Perspectives on Psychological Science*. 2010;*5*:546–556.
82. Turkheimer E, Harden KP. Behavior genetic research methods. In: Reis HT, Judd CM, eds. Handbook of Research Methods in Social and Personality. New York: Cambridge University Press; 2014:159–187.
83. Burt SA, Plaisance KS, Hambrick DZ. Understanding "What Could Be": a call for 'experimental behavioral genetics. *Behav Genet*. 2019;49:235–243.
84. Gross RT. Enhancing the outcomes of low-birth-weight premature infants: a multisite randomized trial. *JAMA*. 1990;263:3035–3042.

CHAPTER

14

Use of twin studies to make inference about causation for measured exposures by examining familial confounding

Vivienne F.C. Esser, Shuai Li, Minh Bui, John L. Hopper
Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia

14.1 Introduction

This chapter considers the use of twin studies to make inference about (1) causation *per se* for (2) putative causes that are measured for both members of the pair.

This is in contrast to the long history of twin studies being used to try to make inference about unmeasured familial "causes of variation," both genetic and nongenetic, where "causal" inference is based on a comparison of measures of similarity between monozygotic (MZ) and dizygotic (DZ) twin pairs under strong assumptions about the role of nongenetic factors that are difficult to address; see 14.1.4.

14.1.1 The importance of understanding causation

Understanding the causal relationships between measured risk factors and outcomes is at the core of epidemiological, medical and for that matter, almost all scientific research. Epidemiological research often considers the relationship between a measured exposure (in the broad sense) and a given health outcome, with the ultimate aim of contributing to what is currently known about the causes of the outcome.

Knowing if an exposure is causal is essential for understanding the etiology of diseases and for prevention. Conducting prevention based on misinterpreting associations can be disastrous, as exemplified by the α-Tocopherol, β-Carotene Cancer Prevention (ATBC) Study[1] and the β-Carotene and Retinol Efficacy Trial (CARET).[2] In these studies, sadly the incidence of lung cancer was increased in the treatment groups, highlighting the importance of conducting rigorous studies of causation

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00040-4

(versus association), such as randomized controlled studies, prior to specifying health-related guidelines.[3]

As an example which we will come back to, we might want to know whether body mass index (BMI), a measure of adiposity, causes changes in DNA methylation levels. This could lead to a better understanding of the impact of fat mass on gene expression and downstream consequences for health and disease, with implications for prevention based on interventions. But if instead DNA methylation changes cause changes in BMI, or if there are other factors that cause changes to both DNA methylation and BMI, research or interventions based on the "BMI causes methylation" hypothesis would be doomed to failure with serious consequences.

14.1.2 Association is not necessarily evidence for causation

An association between two traits X and Y can occur because X causes Y, or because Y causes X (referred to as reverse causation in the context where X and Y are *a priori* considered to be the exposure and outcome, respectively). But an association can also occur in the absence of causality if X and Y have a common cause, which is referred to as a confounder (U). For example, drinking more coffee (X) might be associated with a higher risk of lung cancer (Y) but drinking coffee is also associated with tobacco smoking (U).[4,5] In this instance smoking, which for the point of argument we assume is a cause of the outcome, is a confounding variable—a measure associated with both drinking coffee and developing lung cancer—and the association between the exposure and outcome is not causal.

Identification of and accounting for confounders is therefore critical in order to make inference about causation. Traditionally epidemiologists have tried to address this problem by measuring known or putative confounders and taking them into account in the design (e.g., by matching or stratifying potential confounders) and/or in the statistical analysis by assessing associations subject to the influences of a confounder through regressing putative confounders and exposures together. But we can never be sure that all confounders have been measured, even when epidemiologists introduce unmeasured confounders—based on their contextual knowledge—into their deliberations (e.g.,[6,7]). We consequently must consider the impacts of uncontrolled confounding.

14.1.3 Proof of causation

It is important to note that we cannot prove causation. The philosopher David Hume asserted that we cannot simply observe causation, it must be logically induced.[8,9] In other words, we can never be definite in the claim that an exposure is the cause of an outcome.[9] While we can build models based on thinking about causation and fit them to data to find evidence "consistent with causation," there can always be other interpretations. We can, however, take a Popperian approach by continually assessing whether the data are consistent with causation—or with other explanations—by conducting different and varied tests.[10]

14.1.4 A implies B does not imply B implies A

Logically, the fact that an observation leads to a specific outcome does not mean that observing that outcome implies that specific observation is the cause. Observing that the correlation in a trait for MZ twin pairs is greater than it is for DZ pairs (of the same sex) does not imply that genetic factors "cause" variation in the trait. The observation is simply "consistent with" the existence of genetic causes and should be viewed as hypothesis-generating. It does not prove that genetic causes exist. Other nongenetic causes of familial aggregation could exist, and these might be more than the simplistic (e.g., yes/no) approach of the classic twin model.[11,12]

14.1.5 Bradford Hill's so-called criteria for causation

In 1965, Bradford Hill created a set of guidelines that listed aspects of an association that occur if an exposure is causal as a way to help make decisions about causation.[13] These have been mislabeled as the "Bradford Hill criteria," something that Bradford Hill was careful to avoid.[14] All he did was to ask: "If an exposure X causes a disease Y, what must be true?". The exposure must occur before the disease starts (which is not the same as being diagnosed, a point glossed over). There must be a biological mechanism. But not knowing what the biological mechanism is does not imply the factor is not causal (which is a false argument used by the tobacco industry,[15] and by destructive reviewers of papers!).

The so-called "criteria" attributed to Bradford Hill have become widely used in epidemiological textbooks and studies.[9] However all but one of the guidelines proposed by Bradford Hill are not necessary, let alone sufficient, criteria for causation. The only guideline required for causation is that the exposure must occur before the outcome. This condition highlights the value of prospective studies, but the associations found from cohort studies are not necessarily causal even if the exposure predates the outcome.

14.1.6 Randomized controlled trials

The gold standard for providing evidence of causality is considered to be the randomized controlled trial (RCT).[16] An RCT is a prospective study that uses randomization to create the exposure groups and can thus examine causality between an exposure and an outcome.[16] Randomization means that results are less vulnerable to the effects of uncontrolled confounders as all confounders have the same population distribution for the exposed and unexposed samples,[17] though this does not necessarily mean the actual sample distributions are the same. However, RCTs are often not feasible due to ethical, practical, or financial reasons.

There is, therefore, a great need to make inference about causation from observational studies given they are more feasible even though the exposure-outcome association is vulnerable to the effects of uncontrolled confounders.

14.1.7 Mendelian randomization

New approaches to assessing causation have emerged. Mendelian randomization (MR) uses one or many genetic variant(s) associated with the exposure—so that the exposure need not even be measured in the given study—and assumes they are "instrumental variables," which means they perfectly satisfy several strong assumptions. Various justifications are given for this labeling based on a presumed genetic knowledge about the action of the variants on the totality of causes of the outcome (even though genetic knowledge is generally in its early stage) to hopefully account for unmeasured confounding when examining exposure-outcome associations.[18–20] These genetic variants must: (i) have an incontrovertible (though not necessarily causal) association with the exposure; (ii) be independent of all confounders between the exposure and outcome; and (iii) affect the outcome only via the exposure variable.[21] Because many genetic variants are pleiotropic (i.e., have an effect on multiple outcomes), statistical methods have been developed to test assumption (iii) (e.g.,[22]) but these empirical approaches always have limited statistical power and in any case, can never prove that the assumption is perfectly true. False confidence can be generated by a lack of statistical significance of the test.

There are also many environmental and social factors arising from assortative mating, dynastic (i.e., nongenetic familial) effects, and population structure, which can influence the outcome as well as the genetic variants and thereby violate assumption (ii) and give false conclusions from consideration of the association between the variants for the exposure and the outcome.[23–26] This problem can be addressed by combining MR with the within-family design.

MR studies of within-family differences have found some established associations that are greatly attenuated when using this design which naturally controls to some extent for unmeasured familial factors. For example, the Within-family Consortium considered whether height and BMI cause educational attainment and found that the associations identified using unrelated participants were attenuated when using the within-family method.[26] These findings suggest that these environmental and social factors can confound results relating to socioeconomic traits such as educational attainment.

14.2 Previous twin and family study approaches to address causation

14.2.1 Within-family designs: differences versus differences

Within-family designs analyze the within-family difference in an outcome as a function of the within-family difference in the exposure and thereby control for aspects of potential confounders shared by members in the same family. Within-twin-pair designs are a special, and perhaps optimal, type of within-family designs. The outcomes of these studies can be continuous, binary, or of other types.[27] The studies can be also conducted by considering, or over-sampling, pairs specifically chosen for being (most) discordant for the exposure (e.g.,[28]). The findings of these

studies are therefore more "compelling," given the natural control for unmeasured familial confounding, but cannot be used to imply causation because they cannot discount reverse causation or unmeasured individual-specific confounding.

The extent to which familial confounding explains an association between an exposure and outcome can be derived from comparing the within-individual association with the within-pair association. The former can be derived from the twin data itself, taking into account the correlation between pairs using an appropriate statistical analysis (such as using generalized estimating equations (GEE)[29] or the FISHER software[30] for a continuous outcome).

14.2.2 RCTs involving twins

Twin pairs can also be used in RCTs, where randomization is used to assign each member of a twin pair to a different intervention, or if using a cross-over design, to assign both members to each intervention at differing times. The twin pair RCT design is in theory more powerful than a traditional RCT of unrelated participants. Each twin is perfectly matched to their co-twin in terms of age, sex if necessary, and, depending on zygosity, their genetic background. Given that age, sex and genetic factors are potential confounders of almost all exposure-outcome relationships, the within-pair matching naturally increases the role of randomization alone in creating comparable exposure groups.

14.2.3 Classic multivariate twin model (CMTM) and components of covariance

The univariate classic twin model considers a single trait and makes inference about the genetic and nongenetic "causes of variation," typically under the equal environment assumption, a strong and controversial claim that MZ and DZ pairs share equal amounts of non-genetic variability.[31] Consequently, the model estimates "components of variance" attributed to genetic, shared nongenetic, and individual-specific nongenetic factors.

This model has been extended to consider multiple traits by first applying the univariate model to each trait to estimate their trait-specific components of variance. It then allows these trait-specific components of variance to be correlated. Modeling techniques can then be applied to estimate these correlations and determine the most parsimonious fits.

Therefore, the model estimates the "components of covariation" between the traits. This allows for conclusions to be made about the extent to which an exposure-outcome association is due to familial confounders, both genetic and nongenetic.

But the classic multivariate twin model (CMTM) does not allow for the possibility that there are causal relationships between the measured traits. Consequently, it is not possible to make strong inference about causation. Finding that the association between two traits is statistically explained by a set of genetic factors that predispose to both traits does not imply that there is no causal relationship between the two traits.

14.2.4 The direction of causation (DoC) model and the MR-DoC model

The direction of causation (DoC) model[32] assumes that there is causation between two traits and that the classic twin model applies separately to each trait. The DoC model in effect predicts the expected cross-twin cross-trait correlation for each direction that causation is modelled (i.e., from X to Y or from Y to X)[34] and then tests if this is consistent with what is observed. In this way, it can rule out causation in one or both directions. But to do so, it assumes that there is no familial confounding. That is, it is really a "lack of causation" model in that it can, in some instances, provide evidence against causation in one or both directions.[33]

The DoC model has the most power to assess the direction of causation, under the assumption that there is no familial confounding, when the mode of inheritance for each trait differs substantially.[34] Assume we have two traits, X and Y, and that each trait's genetic and environmental variation is correctly partitioned, another important caveat.

Suppose, for illustration, the variation in X is mainly due to genetic variation with no shared environmental variation and the variation in Y is mainly due to shared environmental variation with no genetic variation. In this example, when X causes Y, the cross-twin cross-trait correlation is a function of the mode of inheritance of X and the within-individual correlation between X and Y. When Y causes X, the cross-twin cross-trait correlation is a function of the mode of inheritance of Y and the within-individual correlation between Y and X. It is clear that, in this example, the expected cross-twin cross-trait correlations differ substantially between the scenarios of X causes Y and Y causes X.[34]

If the modes of inheritance for X and Y are similar, however, the same cross-twin cross-trait correlation will be produced and it will not be possible to identify whether X causes Y or Y causes X.[34]

The DoC model considers four possible models: (i) X causes Y; (ii) Y causes X; (iii) reciprocal causation – X causes Y and Y causes X; or (iv) the association between X and Y is due to an external factor (e.g., a pleiotropic genetic factor and/or underlying environmental factor), that is, familial confounding only.

These models can be compared using goodness-of-fit tests based on the likelihood ratio if the models are nested within one another. If models (i)–(iii) fit significantly worse than the familial confounding only model (iv), then rather than causation, the observed association is concluded to be due to familial confounding arising from shared genetic or environmental factors influencing both traits.[34]

However, as mentioned above, the unidirectional and reciprocal DoC models do not allow for familial confounding; the within-twin cross-trait and cross-twin cross-trait genetic and (shared and unique) environmental correlations are constrained to zero.[35] Consequently, while models assuming causation in one or the other direction can be compared with one another, these models assume causation is the *only* reason for an observed association and there is no way to determine whether this association is due to both familial confounding and causation.[36]

An extension of the DoC model is the MR-DoC twin model. This was developed by including genetic variant(s) associated with the exposure and restricting parameters of the DoC model to consider only unidirectional models.[35] While an assumption of MR is that the genetic variant(s) only influences the outcome through the exposure, the MR-DoC model allows the genetic variant(s) to be associated with the outcome (i.e., horizontal pleiotropy is considered). In other words, the MR-DoC model considers pleiotropy by including a parameter measuring the influence of the genetic variant(s) on the outcome and has higher power than the DoC model to detect causal effects when the mode of inheritance of the exposure and outcome are similar. In order to achieve model identification, the MR-DoC model typically assumes that there is no unique environmental confounding.[35]

14.3 Inference about causation from examination of familial confounding (ICE FALCON)

14.3.1 Model description

Inference about Causation from Examination of Familial Confounding (ICE FALCON) is a regression-based method for assessing causation using paired observational data from related individuals.[37] ICE FALCON assesses evidence for both causality, causal direction, and familial confounding between a measured familial exposure and outcome. Unlike the classic twin and DoC models, this allows for a more realistic scenario in which both bidirectional causation and familial confounding are possible. It does this by studying the pattern of changes in risk associations that would be generated by causation regardless of whether there is familial confounding.

ICE FALCON has many of the advantages that have made MR a popular method for assessing evidence consistent with causation. Both methods use an instrumental variable. In the case of MR, this instrumental variable is a measured genetic variant(s) associated only with the exposure variable. In ICE FALCON, an individual's co-twin is used as a proxy for the unmeasured truly instrumental variable consisting of all the genetic and nongenetic causes that influence the exposure alone, i.e., all the familial determinants. In ICE FALCON, the association between this proxy for the instrumental variable and the outcome is assessed.

ICE FALCON is analogous to bidirectional MR as the scenarios in which the exposure causes the outcome, and/or the outcome causes the exposure, are both assessed. However, a major difference between ICE FALCON and MR is that ICE FALCON does not make the strict assumptions of MR and allows for familial confounders to exist. In addition, rather than estimating a single parameter, ICE FALCON fits several regression models, and assessment of evidence about causality is performed by assessing how pairs of regression coefficients change between their marginal and conditional associations.

14.3.2 Formal model description

As previously described[37] let there be two traits, X and Y, measured for two related individuals. For simplicity, and without loss of generality, assume that these related individuals are a twin pair and denote twin 1 as "self" and twin 2 as "co-twin," although these labels can be interchanged. Both twins are considered for analysis. Assume that: X is positively associated with Y within an individual, X is the exposure variable, and Y is the outcome variable. In this scenario, we consider one exposure variable, but inclusion of multiple is possible.

ICE FALCON fits three regression models:

Model 1: $E(Y_{self}) = \alpha_1 + \beta_{self}X_{self}$
Model 2: $E(Y_{self}) = \alpha_2 + \beta_{co\text{-}twin}X_{co\text{-}twin}$
Model 3: $E(Y_{self}) = \alpha_3 + \beta'_{self}X_{self} + \beta'_{co\text{-}twin}X_{co\text{-}twin}$

where α_i is the model intercept (i = 1, 2 or 3) and β_{self}, $\beta_{co\text{-}twin}$, β'_{self}, and $\beta'_{co\text{-}twin}$ are the regression coefficients for the marginal and conditional associations of X_{self} and $X_{co\text{-}twin}$ with Y_{self}, respectively.

We consider four scenarios: (i) the effects of familial confounders S_{XY} (Fig. 14.1A); (ii) X causing Y (Fig. 14.1B); (iii) Y causing X (Fig. 14.1C); or (iv) a mixture of familial confounding and causation. For each scenario, we expect the patterns of *changes* in the *pairs* of regression coefficients when comparing the conditional estimates (β'_{self}, $\beta'_{co\text{-}twin}$) from Model 3 with the marginal estimates (β_{self}, $\beta_{co\text{-}twin}$) from Models 1 and 2 to differ.

14.3.3 Interpretation of changes in regression coefficients

As shown in Table 14.1, if there is only familial confounding (Fig. 14.1A), then the marginal associations between X_{self} and Y_{self} (β_{self} in Model 1) and $X_{co\text{-}twin}$ and Y_{self} ($\beta_{co\text{-}twin}$ in Model 2) will be nonzero. When X_{self} and $X_{co\text{-}twin}$ are fitted together in Model 3, the associations between X_{self} and Y_{self} (β'_{self} in Model 3) will be adjusted for $X_{co\text{-}twin}$ and the associations between $X_{co\text{-}twin}$ and Y_{self} ($\beta'_{co\text{-}twin}$ in Model 3) will be adjusted for X_{self}. Both β'_{self} and $\beta'_{co\text{-}twin}$ will attenuate toward the null (compared with β_{self} in Model 1 and $\beta_{co\text{-}twin}$ in Model 2, respectively).

If there is only a causal effect from X to Y only (Fig. 14.1B), then the marginal association between X_{self} and Y_{self} (β_{self} in Model 1) will be non-zero. There will be an association between $X_{co\text{-}twin}$ and Y_{self} ($\beta_{co\text{-}twin}$ in Model 2): through S_X and through conditioning on $Y_{co\text{-}twin}$ as it is a collider (which is done so as to account for the correlation between Y_{self} and $Y_{co\text{-}twin}$, e.g., using a GEE analysis which effectively conditions on $Y_{co\text{-}twin}$). Through conditioning on $Y_{co\text{-}twin}$, $X_{co\text{-}twin}$ and Y_{self} will be negatively correlated, meaning that $\beta_{co\text{-}twin}$ will depend on the within-pair correlation in X (ρ_X) and Y (ρ_Y): if $\rho_X > \rho_Y$, $\beta_{co\text{-}twin}$ will be positive, otherwise negative. When X_{self} and $X_{co\text{-}twin}$ are fitted together in Model 3, the association between X_{self} and Y_{self} is unaffected by conditioning on $X_{co\text{-}twin}$, and so the conditional association (β'_{self} in Model 3) is expected to be similar to β_{self} in Model 1. However, when the association between $X_{co\text{-}twin}$ and Y_{self} is conditioned on X_{self}, the pathways

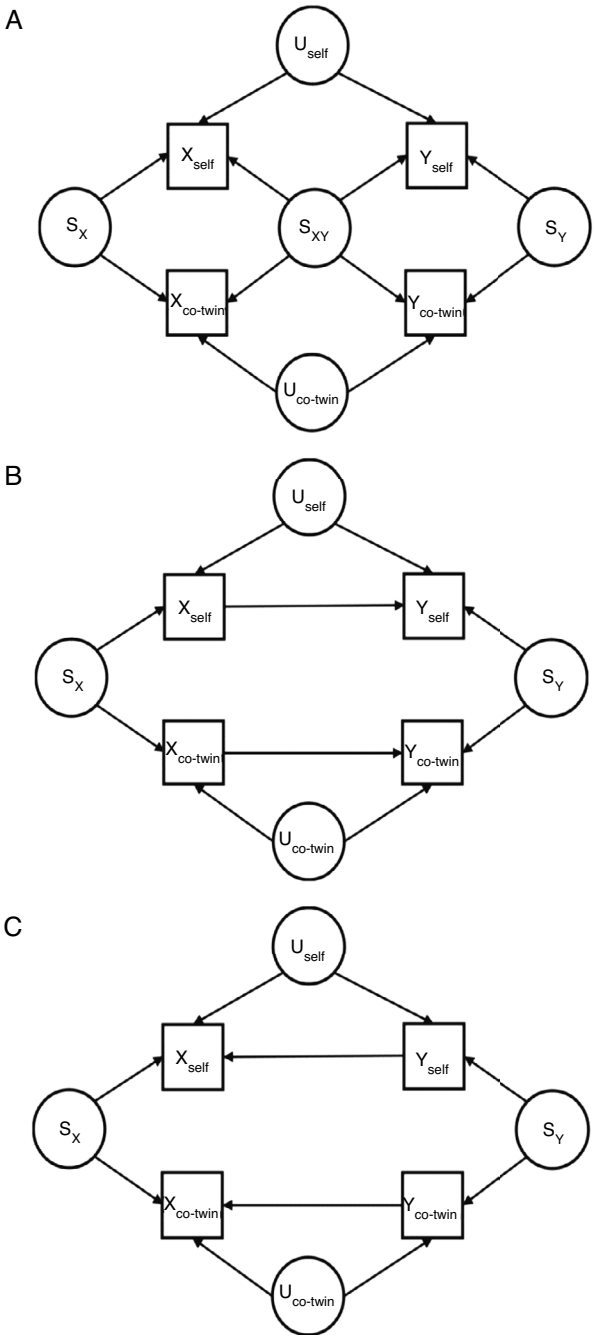


FIG. 14.1

Possible causal diagrams for traits X and Y measured in a twin pair. S_X, S_Y, and S_{XY} represent the unmeasured causes that influence X, Y and both X and Y, respectively. U represents the unmeasured individual-specific confounders influencing both X and Y. Reproduced from Li et al.[37].

TABLE 14.1 The expected ICE FALCON results for different causal scenarios.

Model	Familial confounding	X causes Y	Y causes X
1	$\beta_{self} \neq 0$	$\beta_{self} \neq 0$	$\beta_{self} \neq 0$
2	$\beta_{co\text{-}twin} \neq 0$	$\beta_{co\text{-}twin} \neq 0$	$\beta_{co\text{-}twin} = 0$
3	$\beta'_{self} \neq 0$ & $\beta'_{self} < \beta_{self}$	$\beta'_{self} \neq 0$ & $\beta'_{self} \approx \beta_{self}$	$\beta'_{self} \neq 0$ & $\beta'_{self} \approx \beta_{self}$
	$\beta'_{co\text{-}twin} \neq 0$ & $\beta'_{co\text{-}twin} < \beta_{co\text{-}twin}$	$\beta'_{co\text{-}twin} = 0$	$\beta'_{co\text{-}twin} \neq 0$

Reproduced from Li et al.[37]

through S_X and $Y_{co\text{-}twin}$ are both blocked, so the conditional association will be null ($\beta'_{co\text{-}twin}$ in Model 3).

If there is only a causal effect from Y to X (Fig. 14.1C), then the marginal association between X_{self} and Y_{self} (β_{self} in Model 1) will be non-zero. The marginal association between $X_{co\text{-}twin}$ and Y_{self} ($\beta_{co\text{-}twin}$ in Model 2) will be null as both paths between $X_{co\text{-}twin}$ and Y_{self} are closed – the path through X_{self} as it is a collider and the path through S_Y as $Y_{co\text{-}twin}$ is conditioned on. When X_{self} and $X_{co\text{-}twin}$ are fitted together in Model 3, both paths are open after conditioning on X_{self} so the conditional association between $X_{co\text{-}twin}$ and Y_{self} ($\beta'_{co\text{-}twin}$ in Model 3) will be non-zero, although this depends on ρ_X and ρ_Y: if $\rho_X > \rho_Y$, $\beta'_{co\text{-}twin}$ will be negative, otherwise positive. Conditioning on $X_{co\text{-}twin}$ will not affect the association between X_{self} and Y_{self}, so we expect β'_{self} in Model 3 to be similar to β_{self} in Model 1.

The cross-twin cross-trait associations may be due to a combination of causation and familial confounding, and in this case, we expect the results to be a combination of the results for each causal scenario. We expect to still observe changes in the pairs of regression coefficients and assessment of the evidence for causation will be possible. That is, inference about causation can be made while allowing for familial confounding.

14.3.4 Statistical inference for ICE FALCON estimates

We need to first formally test if the changes in regression coefficients are consistent with chance using statistical inference such as bootstrapping or simulation methods, see[37] for more detail. In addition, simulation studies can be a powerful tool to assist in making inference about causation by providing a visual assessment of the significance of the changes in regression coefficient estimates.

For example, if we simulate three causal scenarios based on the correlational structure observed in a dataset and then perform ICE FALCON, we can display the distribution of the simulated parameter estimates and the observed parameter estimate produced by ICE FALCON.

Fig. 14.2 shows the distributions of the simulated parameter estimates, changes in these estimates, and the observed parameter estimate produced by ICE FALCON (red line). Fig. 14.2A is under the assumption that there is no causation but there is familial confounding. Fig. 14.2B is under the assumption that X causes Y, while Fig. 14.2C is under the assumption that Y causes X. The first two distributions

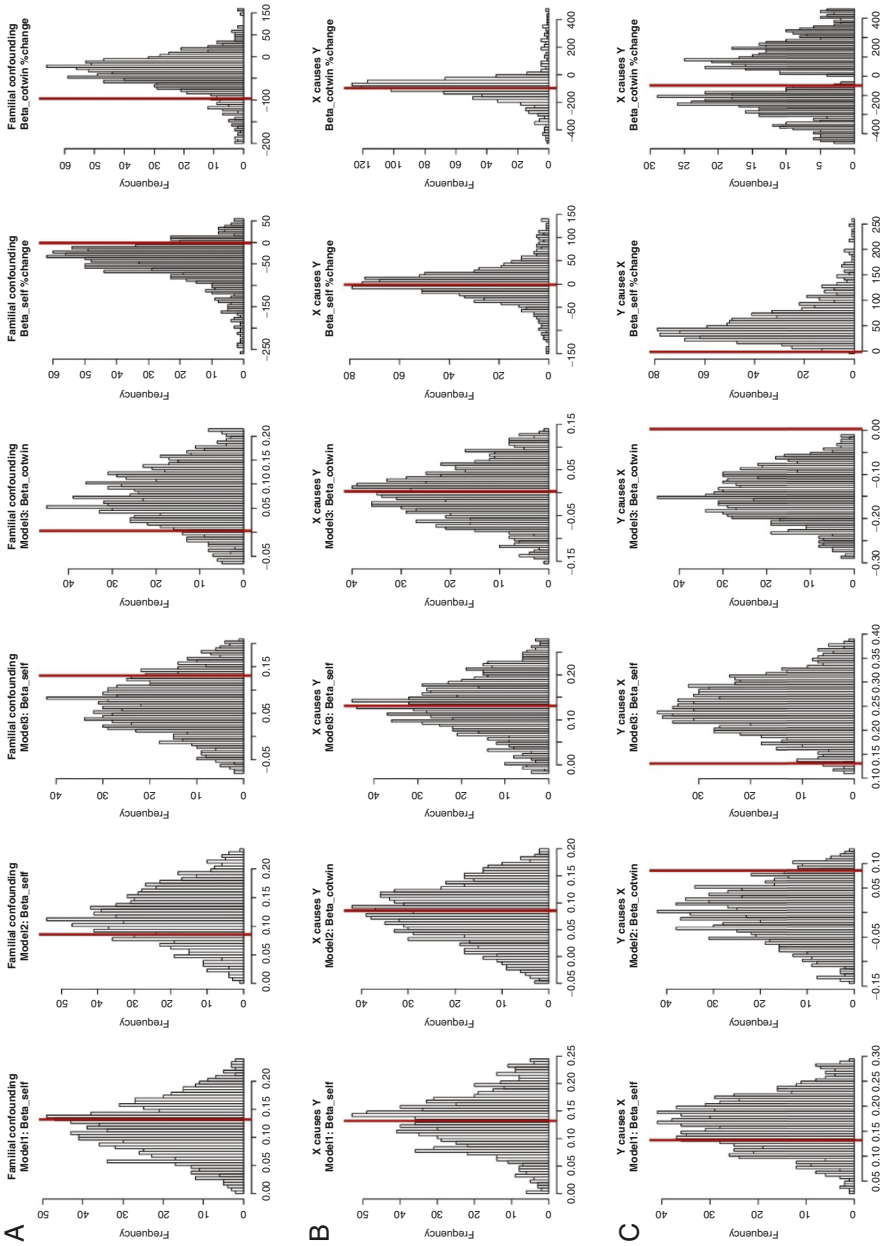


FIG. 14.2

The distribution of the simulated parameter estimates for three causal scenarios where (A) familial confounding, (B) X causes Y, (C) Y causes X. The red line represents the observed parameter estimate from conducting ICE FALCON on empirical data. Reproduced from Li et al.[37]

(columns 1 and 2) refer to the regression coefficients for the marginal associations β_{self} and $\beta_{co\text{-}twin}$ and the second two distributions (columns 3 and 4) refer to the regression coefficients for the conditional associations β'_{self} and $\beta'_{co\text{-}twin}$. The last two distributions (columns 5 and 6) refer to the changes in regression coefficients, $\beta_{self} - \beta'_{self}$ and $\beta_{co\text{-}twin} - \beta'_{co\text{-}twin}$.

We can clearly see that the red lines are closer to the mean of the parameter distributions in Fig. 14.2B compared with Fig. 14.2A and even more so when compared with Fig. 14.2C. Formal tests of statistical significance can be applied to quantify this decision making; see Li et al.[37] We conclude that X causes Y is the best fitting causal scenario among the three alternatives.

14.4 Comparison of the CMTM, DoC model, and ICE FALCON

Table 14.2 summarizes the main points of difference between the different approaches to association and causation discussed above.

Compared with ICE FALCON, both the CMTM and the DoC and MR-DoC models make stronger assumptions. In the case of the CMTM, the assumption that associations are only due to familial confounding (i.e., no causation) greatly limits its ability to understand the true relationship between the measured traits. The DoC model assumes the absence of familial confounding between the exposure and outcome when considering causal mechanisms. Once again, this presents a limited view of the true relationship and we cannot assume that results are evidence for causality, let alone the direction of causation. ICE FALCON, however, makes inference about causation and familial confounding at the same time.

Furthermore, DoC and MR-DoC make inference on the causes of familial similarity through assessing the genetic and nongenetic components of variance. While ICE FALCON uses the shared familial similarity of traits, it does not require the specificity of the DoC or MR-DoC models. ICE FALCON does, however, require

TABLE 14.2 Comparisons of the assumptions and inferential statistics for the within-twin pair model, classic multivariate twin model, the direction of causation model, and inference about causation from examination of familial confounding (ICE FALCON).

Model	Assumptions	Inferential statistics
Within-twin pair model		Associations between within-pair differences
CMTM	Family confounding only No causation	Components of covariance
DoC	Causation only No familial confounding	Goodness-of-fit statistics
ICE FALCON	Familial confounding and causation	Changes in pairs of regression coefficients

that data from related individuals contains variables of interest that have been measured the same way for both members of a pair. In comparison with MR-DoC, where the genetic instrumental variables will typically be minimally impacted by measurement error, the measured variables used in ICE FALCON analyses can be subject to substantial measurement error.

The CMTM, DoC models, and ICE FALCON all require the exposure to be correlated among members of the same families. ICE FALCON is less sensitive to assumptions because it makes inference based on changes in estimates, rather than the estimate itself. But in doing so, it requires there to be strong and highly significant within-pair correlations and associations, and this could limit applications, depending on sample size.

In addition, the CMTM, DoC, and MR-DoC models consider only the marginal associations between the exposure and the outcome. ICE FALCON performs inference about causation in a different way by comparing the *changes* between *pairs* of marginal and conditional regression coefficients. This is more comprehensive as ICE FALCON can be applied to different simulated causal scenarios using the correlation matrix observed in a dataset. Furthermore, ICE FALCON provides a simple and logical method for interpreting results through visualizing how the resultant changes in parameter estimates fit within a simulated distribution of changes in parameter estimates (see e.g., Fig. 14.2 and the Supplemental Material in[37]).

14.5 Applications of ICE FALCON

ICE FALCON has previously been applied to investigate causes of mammographic density,[38,39] allergic conditions,[40] bone architecture, bone density and markers of bone remodeling,[41,42] psychological disorders[43–45] and epigenetic modifications.[46,47] Recently, we published the most comprehensive and up-to-date description of ICE FALCON and its applications[37].

When ICE FALCON was first proposed, it was used to examine the potentially causal relationships on mammographic density of body weight, age at menarche, and height.[38] The relationships between these measures and both the total dense and nondense breast tissue areas were assessed. Findings were consistent with causation between weight and mammographic density measures. Age at menarche and height (adjusted for weight) were both associated with dense and nondense areas; however, the patterns of these associations were inconsistent with causation. Formal statistical inference on the changes in regression coefficients was not yet implemented at the time of this analysis.

Statistical inference was introduced by Bui and colleagues,[41] when the relationship between a summary measure of bone structure and a summary measure of bone remodeling and deterioration was assessed. Results were consistent with bone structure having a causal effect on remodeling and deterioration, but not with remodeling or deterioration having a causal effect on bone structure. This finding is of considerable importance for understanding the mechanisms, and therefore potential prevention, of osteoporosis.

Recently, the causal relationships between BMI and DNA methylation in blood,[46] and smoking and DNA methylation in blood,[47] have been assessed. DNA methylation influences the expression of genes without changing DNA sequence and is known to be affected by exposures and health-related lifestyle factors.[48] Findings were consistent with a causal effect from BMI to DNA methylation, but not with a causal effect from DNA methylation to BMI.[46] Specifically, an association was observed between a woman's DNA methylation level and the BMI of her co-twin, but after conditioning on the woman's own BMI, this attenuated to the null. When the predictor and outcome were reversed, an association was not observed between a woman's BMI and the DNA methylation level of her co-twin. Similar findings were found consistent with a causal effect from smoking to DNA methylation, but not with a causal effect from DNA methylation to smoking.[47] Specifically, an association was observed between a woman's DNA methylation score and the smoking status of her co-twin, but this disappeared after conditioning on the woman's own smoking status. Reversing the predictor and outcome to assess the causal effect of DNA methylation level on smoking status, an association was not observed between a woman's smoking status and the DNA methylation level of her co-twin.

The direction of causation findings above for ICE FALCON were consistent with those from previous MR analyses that assessed the relationships between BMI and DNA methylation[49,50] and smoking and DNA methylation.[51] Our ICE FALCON analyses used substantially smaller sample sizes. The studies using MR by Wahl et al.[49] and Mendelson et al.[50] used sample sizes of $n = 4034$ and $n = 2170$, respectively, and the study by Jhun et al. had $n = 822$.[51] Both studies by Li et al. using ICE FALCON had $n = 479$.[46,47]

We have previously shown[37] that we can compare the amount of causal information obtained from MR and ICE FALCON using the test statistic for the association between the polygenic risk score and the outcome (Z_{MR}) and the test statistic for the change in cross-trait cross-pair regression coefficient (Z_{IF}), divided by the square root of the sample size (n). We showed that studies by Wahl et al.[49] and Mendelson et al.[50] found $Z_{MR} = 4.00$ and 2.69, resulting in $Z_{MR}/n^{1/2} = 0.063$ and 0.058, respectively. A comparative ICE FALCON analysis[37] that had $n = 130$ found $Z_{IF} = 1.75$, so that $Z_{IF}/n^{1/2} = 0.153$. This shows that, for this example, ICE FALCON appears to have about 2.5 times as much information on causation per subject than does MR.[37] We will, however, conduct further research comparing the power per subject of ICE FALCON and MR.

14.6 Further developments

Further developments to the theoretical, analytical, and translational aspects of ICE FALCON are being undertaken. In order to provide a more comprehensive analysis of the causal relationships being studied, we will use simulation studies to conduct statistical power calculations of ICE FALCON. We will also examine what can be learnt from DoC by taking into account the zygosity of the twin pairs. Furthermore, if

additional family data is available (e.g., multiple siblings or siblings as well as twins), there is the potential for extracting more information than from only considering pairs of related individuals. Extensions will be made to the model that will allow for inclusion of more than two family members. Updates to ICE FALCON with methods for inclusion of other relevant information, such as family history, will also be developed. We also plan to make ICE FALCON more accessible to the wider research community, through production of user-friendly software packages in Stata and R, as well as the creation of a user guide. Within this user guide will be statistical power calculations, example datasets and demonstrations.

Funding

S.L. is a Victorian Cancer Agency Early Career Research Fellow (ECRF19020). J.L.H. is a NHMRC Senior Principal Research Fellow. V.F.C.E. is supported by an Australian Government Research Training Program Scholarship. This project was supported by grant 1187896 awarded through the 2019 Priority-driven Collaborative Cancer Research Scheme and funded by Cure Cancer with the support of Cancer Australia, and by grant 2004702 awarded through the 2020 Priority-driven Collaborative Cancer Research Scheme and funded by Cancer Australia.

References

1. Albanes D, Heinonen OP, Taylor PR, et al. Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst*. 1996;88:1560–1570. doi:10.1093/jnci/88.21.1560.
2. Omenn GS, Goodman GE, Thornquist MD, et al. Risk factors for lung cancer and for intervention effects in CARET, the beta-carotene and retinol efficacy trial. *J Natl Cancer Inst*. 1996;88:1550–1559. doi:10.1093/jnci/88.21.1550.
3. Greenwald P. Beta-carotene and lung cancer: a lesson for future chemoprevention investigations? *J Natl Cancer Inst*. 2003;95:E1. doi:10.1093/jnci/95.1.e1.
4. Galarraga V, Boffetta P. Coffee drinking and risk of lung cancer - a meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2016;25:951–957. doi:10.1158/1055-9965.EPI-15-0727.
5. Klesges RC, Ray JW, Klesges LM. Caffeinated coffee and tea intake and its relationship to cigarette smoking: an analysis of the Second National Health and Nutrition Examination Survey (NHANES II). *J Subst Abuse*. 1994;6:407–418. doi:10.1016/s0899-3289(94)90334-4.
6. VanderWeele TJ. Principles of confounder selection. *Eur J Epidemiol*. 2019;34:211–219. doi:10.1007/s10654-019-00494-6.
7. Zhang X, Faries DE, Li H, Stamey JD, Imbens GW. Addressing unmeasured confounding in comparative observational research. *Pharmacoepidemiol Drug Saf*. 2018;27:373–382. doi:10.1002/pds.4394.
8. Hume D. A treatise of human nature. Norton D, Norton M, Eds. Oxford and New York, NY: Oxford University Press; 2000.

9. Lucas RM, McMichael AJ. Association or causation: evaluating links between "environment and disease". *Bull World Health Organ*. 2005;83:792–795. doi:10.1590/S0042-96862005001000017.
10. Popper KR. *The logic of scientific discovery*. New York, NY: Basic Books Inc; 1959.
11. Li S, Wong EM, Dugue PA, et al. Genome-wide average DNA methylation is determined in utero. *Int J Epidemiol*. 2018;47:908–916. doi:10.1093/ije/dyy028.
12. Li S, Nguyen TL, Wong EM, et al. Genetic and environmental causes of variation in epigenetic aging across the lifespan. *Clin Epigenetics*. 2020;12:158. doi:10.1186/s13148-020-00950-1.
13. Bradford Hill A. The environment and disease: association or causation? *Proc R Soc Med*. 1965;58:295–300.
14. Doll R. Fisher and Bradford Hill: their personal impact. *Int J Epidemiol*. 2003;32:929–931. doi:10.1093/ije/dyg287 discussion 45–8.
15. Francis JA, Shea AK, Samet JM. Challenging the epidemiologic evidence on passive smoking: tactics of tobacco industry expert witnesses. *Tob Control*. 2006;15(Suppl 4):iv68–iv76. doi:10.1136/tc.2005.014241.
16. Hariton E, Locascio JJ. Randomised controlled trials—the gold standard for effectiveness research: study design: randomised controlled trials. *BJOG*. 2018;125:1716. doi:10.1111/1471-0528.15199.
17. Jepsen P, Johnsen SP, Gillman MW, Sorensen HT. Interpretation of observational studies. *Heart*. 2004;90:956–960. doi:10.1136/hrt.2003.017269.
18. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ*. 2018;362:k601. doi:10.1136/bmj.k601.
19. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. 2003;32:1–22. doi:10.1093/ije/dyg070.
20. Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. *Stat Methods Med Res*. 2017;26:2333–2355. doi:10.1177/0962280215597579.
21. Minica CC, Boomsma DI, Dolan CV, de Geus E, Neale MC. Empirical comparisons of multiple Mendelian randomization approaches in the presence of assortative mating. *Int J Epidemiol*. 2020;49:1185–1193. doi:10.1093/ije/dyaa013.
22. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. 2015;44:512–525. doi:10.1093/ije/dyv080.
23. Haworth S, Mitchell R, Corbin L, et al. Apparent latent structure within the UK Biobank sample has implications for epidemiological analysis. *Nat Commun*. 2019;10:333. doi:10.1038/s41467-018-08219-1.
24. Kong A, Thorleifsson G, Frigge ML, et al. The nature of nurture: effects of parental genotypes. *Science*. 2018;359:424–428. doi:10.1126/science.aan6877.
25. Robinson MR, Kleinman A, Graff M, et al. Genetic evidence of assortative mating in humans. *Nat Hum Behav*. 2017;1. doi:10.1038/s41562-016-0016.
26. Brumpton B, Sanderson E, Heilbron K, et al. Avoiding dynastic, assortative mating, and population stratification biases in Mendelian randomization through within-family analyses. *Nat Commun*. 2020;11:3519. doi:10.1038/s41467-020-17117-4.
27. Li S, Wong EM, Southey MC, Hopper JL. Association between DNA methylation at SOCS3 gene and body mass index might be due to familial confounding. *Int J Obes (Lond)*. 2017;41:995–996. doi:10.1038/ijo.2017.56.

28. Hopper JL, Seeman E. The bone density of female twins discordant for tobacco use. *N Engl J Med*. 1994;330:387–392. doi:10.1056/NEJM199402103300603.
29. Liang K-Y, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika*. 1986;73:13–22. doi:10.1093/biomet/73.1.13.
30. Lange K, Weeks D, Boehnke M. Programs for pedigree analysis: MENDEL, FISHER, and dGENE. *Genet Epidemiol*. 1988;5:471–472. doi:10.1002/gepi.1370050611.
31. Hopper JL. Variance components for statistical genetics: applications in medical research to characteristics related to human diseases and health. *Stat Methods Med Res*. 1993;2(3):199–223. doi: 10.1177/096228029300200302.
32. Heath AC, Kessler RC, Neale MC, Hewitt JK, Eaves LJ, Kendler KS. Testing hypotheses about direction of causation using cross-sectional family data. *Behav Genet*. 1993;23:29–50. doi:10.1007/BF01067552.
33. Gillespie NA, Martin NG. Direction of causation models. In: Everitt BS, Howell DC, eds. *Encyclopedia of statistics in behavioral science*. Chichester: John Wiley & Sons, Ltd; 2005:496–499.
34. Verhulst B, Estabrook R. Using genetic information to test causal relationships in cross-sectional data. *J Theor Polit*. 2012;24:328–344. doi:10.1177/0951629812439348.
35. Minica CC, Dolan CV, Boomsma DI, de Geus E, Neale MC. Extending causality tests with genetic instruments: an integration of Mendelian randomization with the classical twin design. *Behav Genet*. 2018;48:337–349. doi:10.1007/s10519-018-9904-4.
36. Posthuma D. Multivariate genetic analysis. In: Kim Y-K, ed. *Handbook of behavior genetics*. New York, NY:: Springer New York; 2009:47–59.
37. Li S, Bui M, Hopper JL. Inference about causation from examination of familial confounding (ICE FALCON): a model for assessing causation analogous to Mendelian randomization. *Int J Epidemiol*. 2020;49:1259–1269. doi:10.1093/ije/dyaa065.
38. Dite GS, Gurrin LC, Byrnes GB, et al. Predictors of mammographic density: insights gained from a novel regression analysis of a twin study. *Cancer Epidemiol Biomarkers Prev*. 2008;17:3474–3481. doi:10.1158/1055-9965.EPI-07-2636.
39. Stone J, Dite GS, Giles GG, Cawson J, English DR, Hopper JL. Inference about causation from examination of familial confounding: application to longitudinal twin data on mammographic density measures that predict breast cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2012;21:1149–1155. doi:10.1158/1055-9965.EPI-12-0051.
40. Hopper JL, Bui QM, Erbas B, et al. Does eczema in infancy cause hay fever, asthma, or both in childhood? Insights from a novel regression model of sibling data. *J Allergy Clin Immunol*. 2012;130:1117-22 e1. doi:10.1016/j.jaci.2012.08.003.
41. Bui M, Bjornerem A, Ghasem-Zadeh A, Dite GS, Hopper JL, Seeman E. Architecture of cortical bone determines in part its remodelling and structural decay. *Bone*. 2013;55:353–358. doi:10.1016/j.bone.2013.04.020.
42. Bui M, Zebaze R, Li S, Hopper JL, Bjornerem A. Are the relationships of lean mass and fat mass with bone microarchitecture causal or due to familial confounders? A novel study of adult female twin pairs. *JBMR Plus*. 2020;4:e10386. doi:10.1002/jbm4.10386.
43. Lopez-Sola C, Fontenelle LF, Bui M, et al. Aetiological overlap between obsessive-compulsive related and anxiety disorder symptoms: multivariate twin study. *Br J Psychiatry*. 2016;208:26–33. doi:10.1192/bjp.bp.114.156281.
44. Davey CG, Lopez-Sola C, Bui M, et al. The effects of stress-tension on depression and anxiety symptoms: evidence from a novel twin modelling analysis. *Psychol Med*. 2016;46:3213–3218. doi:10.1017/S0033291716001884.

45. Lopez-Sola C, Bui M, Hopper JL, et al. Predictors and consequences of health anxiety symptoms: a novel twin modeling study. *Acta Psychiatr Scand*. 2018;137:241–251. doi:10.1111/acps.12850.
46. Li S, Wong EM, Bui M, et al. Inference about causation between body mass index and DNA methylation in blood from a twin family study. *Int J Obes (Lond)*. 2019;43:243–252. doi:10.1038/s41366-018-0103-4.
47. Li S, Wong EM, Bui M, et al. Causal effect of smoking on DNA methylation in peripheral blood: a twin and family study. *Clin Epigenetics*. 2018;10:18. doi:10.1186/s13148-018-0452-9.
48. Alegría-Torres JA, Baccarelli A, Bollati V. Epigenetics and lifestyle. *Epigenomics*. 2011;3:267–277. doi:10.2217/epi.11.22.
49. Wahl S, Drong A, Lehne B, et al. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. *Nature*. 2017;541:81–86. doi:10.1038/nature20784.
50. Mendelson MM, Marioni RE, Joehanes R, et al. Association of body mass index with DNA methylation and gene expression in blood cells and relations to cardiometabolic disease: a Mendelian randomization approach. *PLoS Med*. 2017;14:e1002215. doi:10.1371/journal.pmed.1002215.
51. Jhun MA, Smith JA, Ware EB, et al. Modeling the causal role of DNA methylation in the association between cigarette smoking and inflammation in African Americans: a 2-step epigenetic Mendelian randomization study. *Am J Epidemiol*. 2017;186:1149–1158. doi:10.1093/aje/kwx181.

CHAPTER

Twins in clinical trials 15

Athula Sumathipala[a,b], **Kaushalya Jayaweera**[a]
[a]*Institute for Research & Development in Health & Social Care*
[b]*School of Medicine, Faculty of Medicine & Health Sciences, Keele University*

15.1 What is a randomized controlled trial?

Randomized controlled trials (RCTs) are the gold standard for testing interventions and "the most rigorous way of determining whether a cause-effect relationship exists between treatment and outcome and for assessing the cost-effectiveness of a treatment".[1] Random allocation provides participants the same chance of being assigned to each of the treatment groups.[2] The purpose of random allocation is to ensure that the characteristics of participants are as similar as possible across treatment groups prior to the initiation of an intervention (baseline). If randomization is done properly, it reduces the risk of a serious imbalance in known and unknown factors that could influence the clinical course of the participants. Therefore, any significant differences between treatment groups in the outcome of interest can be attributed to the intervention and not to any unidentified factor(s). Evidence-based practice in healthcare requires unbiased results from clinical trials to guide clinicians for better patient outcomes. RCTs are the gold standard to test interventions such as behavioral interventions or pharmaceuticals.

Genetic confounding of identified associations is often a very real possibility but is frequently overlooked by researchers. An advantage of involving twins pairs in intervention studies is that they share both genetic and familial factors. Such data can be used in statistical models within RCTs to allow for the controlling of familial confounding between interventions and outcomes. Such additional benefits provide greater accuracy when investigating the association between interventions and outcomes.

Generally, twins have been recruited mostly for studies using the "classical twin design." This method compares the similarity of monozygotic (MZ) and dizygotic (DZ) twin pairs to estimate genetic and environmental variance components, predominantly using observational study designs.[3,4] Hundreds of thousands of twins have provided a valuable resource for studying complex genetic phenotypes and their underlying biology.[5]

Different ways of using data from twins for research are explained in other chapters of this book. This chapter aims to explain how twins can be involved in RCTs.

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00003-9

15.2 Role of twins in RCTs

Assigning MZ twins to a cotwin control designed RCT is different from the traditional RCT involving unrelated individuals. The cotwin control design can increase statistical power due to the increased comparability between trial arms and control for confounding (Yelland et al., 2017). It can also provide perfect control for many of the potential confounding factors that could be imbalanced between treatment groups by chance, especially genetic makeup and age. The latter is particularly relevant to RCTs in children, where age matching is often a challenge.[6]

Studying DZ twins may also allow for matching due to the shared environment as well as 50% of their genetic variants and age, which can potentially justify the choice for recruiting DZ twins rather than siblings for a study. However, challenges such as teasing out individual factors in the context of complex interacting contributors may arise when involving DZ twins in RCTs.

In one of the earlier intervention studies involving twins which tested the effect of Vitamin C intake on common cold symptoms, the authors demonstrate the efficiency of using twins over randomly selected individuals.[7,8]

The full advantages and the rationale for involving twins in RCTs have not been adequately discussed or explored.[6] A review undertaken by Sumathipala et al. (2018) was the first step to identifying studies using twins as participants for RCTs. However, in-depth analyses of the quality of individual studies and methodological issues of these studies or meta-analysis were not reported in this review. According to the authors, a meta-analysis was not possible due to the heterogeneity of such studies and the varying interventions tested, resulting in no single treatment effect to be estimated. Instead, the authors reported all published material up to 2015, including RCTs involving only twins as participants. They reported basic trial characteristics including sample size, inclusion criteria, whether trials include only MZ, DZ, or both, and randomization method (i.e., whether same pair twins were randomly assigned to the same treatment group independent of each other, or to different treatment groups in a cotwin control design).

There is evidence from Sumathipala et al. (2018) that only a limited number of RCTs with twins had been carried out. There have been 90 clinical trials carried out, according to the US clinical trial database.[9] For these, 50 studies were registered with twins as study participants, and 40 studies were registered with mothers who were pregnant with twins as study participants. However, only 29 of the total studies used the RCT study design. Of these, 23 had recruited mothers pregnant with twins as the study participants and only six studies had recruited twins as the study participants. Worldwide, another 50 RCTs had been conducted recruiting twins as participants (Sumathipala et al. 2018).

The majority of the studies reviewed by Sumathipala et al. (2018) were conducted in the United States. The remaining studies were from Canada, Australia, UK, Finland, Germany, Greece, Bangladesh, Belgium, Dominican Republic, France, Hawaii, Hong Kong, India, Iran, Norway, Switzerland, Taiwan, and Thailand (Sumathipala et al. 2018). There was a great variation of sample sizes

across these studies, with the majority of studies having 100 twin pairs or fewer as participants.

15.3 Zygosity and twin assignment across the randomized controlled trials

Both MZ and DZ twins have been used in RCTs. However, on a majority of instances they have been MZ twins randomized to opposite arms of a RCT. As illustrated in Table 15.1, 13 RCTs assigned both twins in each pair to the same study arm,

TABLE 15.1 Characteristics of randomized controlled trials with twin participants.

Characteristic	Number (percentage) of trials (n = 50)
Number of participants recruited	
<10	12 (24)
10–100	21 (42)
101–250	14 (28)
>250	3 (6)
Sex	
Males only	7 (14)
Females only	9 (18)
Males and females	34 (68)
Age group	
Infants only	11 (22)
Child and adolescents only	16 (32)
Adolescents and adults	3 (6)
Adults only	20 (40)
Twin assignment	
Same treatment groups	13 (26)
Different treatment groups	33 (66)
Independent allocation	3 (6)
Unclear	1 (2)
Location	
United States	21 (42)
Canada	5 (10)
Europe	10 (20)
LMIC	2 (4)
Other	12 (24)
Twin recruitment method	
Twin registry	8 (16)
Other	42 (84)

10 of which included both MZ and DZ twins, two included only MZ twins and one included only DZ twins. This is in contrast to 33 RCTs where twin pairs were assigned to different study arms, of which six included both MZ and DZ twins, 24 included only MZ twins and three included only DZ twins. In most instances (33/50) the pair of twins had been assigned to different study arms, and most of these studies (24/33) had been with MZ twins.

Therefore, twins within a pair irrespective of their zygosity can be assigned to the same arm of a RCT, or separately to either the intervention or control arm.

When twins participate in a clinical trial, they may be randomized to the same treatment group, independent of each other, or to different treatment groups as in the cotwin control design. Most clinical trials involving twins have used the cotwin control design with MZ twins (Sumathipala et al., 2018). Allocating MZ twins are a perfect control of genetic variation between the treatment groups. This has important implications for future RCTs conducted in twins, since recruitment may be more successful if both twins in a pair will receive the same treatment, although the impact of different methods of randomizing twins on the sample size must also be considered.

15.3.1 The impact of twins on sample size and power

One of the advantages of conducting RCTs in twins is that the sample sizes can be smaller compared to using nontwin RCTs.[7,8,10]

This is especially true when using the cotwin control RCT approach which does not reduce the statistical power.[11]

However, if twins from the same pair are randomly assigned to the same treatment group or independently of each other, rather than to different treatment groups as in the cotwin control design, the benefits in sample size for an RCT involving twins may be lost.[12]

However, this will depend on how twins from the same pair are randomized. If the cotwin control design is used, such that one twin from each pair receives the intervention and the other acts as their control, the trial will have more power than a trial in singletons, and hence the sample size can be reduced. In contrast, if both twins are assigned to the same treatment group, the trial will have less power than a trial in singletons, thus requiring a larger sample size. This is due to the fact that comparisons of the intervention and control conditions must be made across twin pairs, rather than within twin pairs as in the cotwin control design. If twins from the same pair are randomized independently (ignoring that they are twins and treating as individuals), the trial will likely have similar power to a trial in singletons. Methods for calculating the sample size for trials involving twins only or a combination of singletons and twins have been discussed by Yelland et al. (2017).

The review by Sumathipala et al. (2018) demonstrates the variation and range of sample sizes used in the different studies (Table 15.1). Although the authors did not attempt to assess whether the sample size was adequate for addressing the specific

research question of each trial, this does raise the issue of whether small RCTs involving twins are adequately powered to detect meaningful treatment effects.

15.3.2 Implications for future work and directions

To understand the potential benefits of the cotwin control design, it would be useful to compare the sample sizes of twin RCTs and nontwin RCTs required to detect the same effect size. The advantages and disadvantages of the inclusion of both MZ and DZ twins in RCTs need more in-depth discussion and are areas for future methodological research.

Contamination between intervention and control groups is major challenge particularly in psychological interventions especially if twin pairs living together are assigned to either arm of a RCT.

The continuous development and implementation of innovative twin designs in intervention studies, especially RCTs, indicates that twin research can extend beyond the more widely recognized heritability estimates toward the possibility of inference on causation.

Involving twins in RCTs comes with its own advantages, and limitations such as conducting disease-specific clinical trials for cancer which may require pairs concordant or discordant for the outcome. However, such comparisons are arguably more efficient in intervention cotwin control studies using phenotypically concordant pairs, where one twin is randomly assigned to receive the intervention and the other twin acts as their control. A comparison between the cotwin control design in intervention and nonintervention studies, along with other novel utilities of this design have been discussed in detail previously.[11] Recruiting children in RCTs has ethical implications, and needs especially strict ethical oversight.

The relatively low sample size required when using twin pairs plays an important role in reducing the overall budget of trials. And as most twin registries worldwide are population based it provides and added value and advantage.[13]

Recruitment and traceability of participants would be faster as twin registries maintain contact details of a large number of potentially eligible participants, many of which engage with members through newsletters and gatherings. The authors' own personal experience in prospective studies have shown that traceability of twins is much better than nontwins, as tracing one twin in a pair allows easier and faster tracing of the second and reducing attrition. Members of a twin registry would also be more open to participate in clinical trials as they would have had some experience participating in twin research. This also gives the added benefit of forming patient and public involvement and engagement groups to enhance the quality of clinical trials.

It is evident that twins in RCTs are an underused methodology in testing interventions. This may be due to various reasons such as lack of infrastructure (twin registers) or expertise in twin methodology. However, it is interesting and reassuring to note that there is a growing interest in twin methodology globally with development

of new twin registries in many parts of the world through collaborations from experts with established twin registries. Therefore, RCTs with twins may come to play an important role in interventions studies worldwide in the future.

Acknowledgments

Prof Nick Martin for granting permission for reproducing some of the original material from the paper published in Twin Research and Human Genetics 2018 doi:10.1017/thg.2017.67. (Full reference: Sumathipala A, Yelland L, Green D, Shepherd T, Jayaweera K, Ferreira P, Craig. Twins as participants in RCTs: a review of published literature. Twin and Genetic research 2018 doi:10.1017/thg.2017.67. We acknowledge the contribution of all the authors of the original review.

References

1. Sibbald B, Roland M. Understanding controlled trials. Why are randomised controlled trials important? *BMJ (Clinical Research Ed.)*. 1998;316:201.
2. Altman DG. *Practical Statistics for Medical Research*. London: Chapman & Hall; 1991.
3. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nature Reviews. Genetics*. 2002;3(11):872–882.
4. van Dongen J, Slagboom PE, Draisma HHM, Martin NG, Boomsma DI. The continuing value of twin studies in the omics era. *Nature Reviews. Genetics*. 2012;13:640–653.
5. Hur Y-M, Craig JM. Twin registries worldwide: an important resource for scientific research. *Twin Research and Human Genetics : The Official Journal of the International Society for Twin Studies,*. 2013;16:1–12.
6. Sumathipala A, Lisa Y, Green D, et al. Twins as participants in randomized controlled trials: a review of published literature. *Twin Research and Human Genetics*. 2018;21:51–56.
7. Carr A, Martin N, Whitfield J. Usefulness of the co-twin control design in investigations as exemplified in a study of effects of ascorbic acid on laboratory test results. *Clinical Chemistry*. 1981;27:1469–1470.
8. Martin N, Carr A, Oakeshott J, Clark P. Co-twin control studies: vitamin C and the common cold. *Progress in Clinical and Biological Research*. 1982;103(A):365–373.
9. U.S. National Institutes of Health. (2021) About ClinicalTrials.gov. http://www.clinicaltrials.gov/ct2/info/about. (accessed October 17, 2021).
10. Miller PW, Mulvey C, Martin N, Miller P, Mulvey C, Martin N. What do twins studies reveal about the economic returns to education? a comparison Of Australian and U.S. findings. *American Economic Review*. 1995;85:586–599.
11. Plomin R, Haworth CMA. Genetics and intervention research. *Perspectives on Psychological Science*. 2010;5:557–563.
12. Yelland LN, Sullivan TR, Price DJ, Lee KJ. Sample size calculations for randomised trials including both independent and paired data. *Statistics Med*. 2017;36(8):1227–1239.
13. Buchwald D, Kaprio J, Hopper JL, et al. International network of twin registries (INTR): building a platform for international collaboration. *Twin Research and Human Genetics*. 2014;17:574–577.

SECTION

5 Behavior

CHAPTER

Twin studies in social science

16

Elena Cristina Mitrea[a], **Levente Littvay**[b]
[a]*Lucian Blaga University of Sibiu*
[b]*Central European University*

16.1 Introduction

For a century, the orthodoxy in most social science fields was that parent–child attitudinal and behavioral similarities are fashioned through socialization processes and environmental factors. It was reasoned that parents influence their children directly, through the explicit and implicit learning that takes place within the family, especially during childhood and adolescence, and indirectly, through their social structural position (class, ethnicity, or religion). In 2005, this status-quo was upended by Alford, Funk, and Hibbing's study published in the lead political science journal, the *American Political Science Review*[1] which showed that "political attitudes are influenced much more heavily by genetics than by parental socialization" (p.164). By comparing the resemblance in identical (monozygotic, or MZ) and fraternal (dizygotic, or DZ) twins, they reported that genes explain half of the variance in political conservatism. These findings undermined a century of consensus in political science research and sent a shock wave through the broader discipline, showing that the effect of socialization had been overestimated in previous studies.

The proposition that political orientations are genetically influenced was not novel. Breakthrough findings of significant genetic effects on political traits were published thirty years earlier in the journal *Nature*[2] and in 1986 in the *Proceedings of the National Academy of Sciences* by Martin et al.[3] While other studies followed,[4,5] they went largely unnoticed by social scientists due to a systematic lack of awareness and contact with research in the related, yet separate field of behavioral genetics, which had been studying attitudes and interests for decades. Consequently, genetic factors were not incorporated into the dominant paradigms that explain the origin of attitudes and various social behaviors.

With the publication of Alford, Funk, and Hibbing's article[1] in a flagship journal, the social sciences could not ignore evidence for the heritability of social traits. The article gained visibility in both the academic arenas and in the wider public sphere.[6] Not unexpectedly, it received mixed reviews and invited controversy and criticism,[7,8] although the concerns raised had been extensively explored and rebutted in previous decades in the field of behavior genetics.[9–11] Since 2005, articles addressing

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00001-5

questions about genetic influences in the social sciences appeared at an exponential rate in leading social science journals. This includes several special issues devoted to the topic, published in *Political Research Quarterly,*[12] *Social Science Quarterly,*[13] *Political Psychology,*[14] and *Journal of Theoretical Politics.*[15] A special issue on the genetic bases of politics was also published in the journal *Twin Research and Human Genetics,*[16] while other studies on this topic made it into prestigious journals such as *Science*[17] and *PNAS.*[18]

Despite the avalanche of publications exploring these topics, findings on heritability had a difficult time going mainstream in social science scholarship. One fallacy responsible for the reluctance to embrace opportunities offered by behavioral genetic research is the misguided view that genes have a direct impact on social and political traits. However, given the complex nature of the traits that are of interest to social scientists, such as ideology or participation, no specific gene was discovered to directly and substantially impact these outcomes. A second fallacy is the idea that the impact of genes is not context-dependent, which is the same as saying that their effects are universal. However, empirical studies[19] have shown that heritability varies considerably across contexts, and we know very little about what is it about a specific context that makes a trait more or less heritable. Therefore, generalization beyond the context of the population representative of that analyzed in twin studies would be misguided.

This chapter offers an overview of how genetics was incorporated as an explanatory factor in explanations of the origins of attitudes, interests, and other social behaviors. It begins with a literature review of studies employing the twin design to explore the heritability of complex traits relevant to the social sciences. Next, it outlines how twin studies are conducted, with a discussion of the main assumptions and criticism that the classical twin design received from social scientists. This chapter also touches on variations of the twin design that use extended family data and molecular analyses that link specific segments of the genome to phenotypes of interest. It concludes with a discussion of the ways in which behavior genetic methods can advance our understanding of the mechanisms underlying social traits and the challenges that lie ahead.

16.2 Findings from the literature

In 2005, political scientists John Alford, Carolyn Funk, and John Hibbing[1] employed the twin design to analyze the genetic basis of party affiliation and conservatism using the 28-item Wilson–Patterson scale. They found a large variation in the heritability of issue positions, ranging from 18% to 41% (for school prayer and property tax), with a mean of 32% for individual items and 43% for the overall conservatism index. The heritability estimates for partisanship were much more modest than expected, at 14%. These results indicate that genetic factors—although we do not know which genes, how many and through what mechanisms—play a more important role than parental influence in political conservatism.

Despite engaging later than other disciplines, such as psychology[20] and sociology,[21] with the proposition that genes play a role in human attitudes and interests,

political science is arguably the social science discipline (not counting psychology, of course) that most utilized twin studies in the past decade. Over the past fifteen years, a wide range of political attitudes and related behaviors received social science scrutiny in the form of the twin design. They include political knowledge,[22] political interest and efficacy,[23] individual voting decision strategy,[24] political sophistication,[25] ethnocentrism,[26] foreign policy preferences,[27,28] social trust,[29] cooperative behavior,[18] and survey response behavior.[30]

The extent of genetic influence that studies report varies greatly with the type of attitude or behavior, with most findings showing moderate levels of heritability (e.g., 50%). From a social scientific point of view, it is also interesting to see whether there is a common environmental component, which indicates the presence of socialized causes, and in fact, in a surprising number of cases, socialization does not seem to play a role. Genetic factors explain more than half of the variation in political participation,[31] political knowledge, authoritarianism, and social trust,[32] and have a more modest role in ethnocentric attitudes, sense of civic duty, and moral foundations.[33] For most of these traits, the environmental component is not only affected by the shared family environment as a socializing factor, but also by unique personal experiences that result from having different friends, teachers, colleagues, and, later in life, spouses and families. This indicates that parental influence is more limited than previously thought and in some cases nonexistent.

The low heritability estimates for political party identification reported by Alford, Funk, and Hibbing[1] were later supported by results from Australia[34] and the United States.[35,36] However, when reassessed in a sample from about the time of Obama's election, heritability was high.[19,37] This raises questions about the generalizability of the results and reinforces the idea that they are somewhat context-dependent. Similarly, a meta-analysis yielded great variation in the heritability of ideology, ranging from close to zero in Hungary to over 50% in the United States in 2008.[38] Of course, different methodologies also affect the findings. Bell et al.[39] found that a large number of ideological traits are heritable in a Canadian twin sample, with the notable exceptions of state activism on social issues and environmentalism. These results show that when a phenotype receives more rigorous scrutiny across both time and space, there is a great deal of variation in the results, which may be a cause for concern. Heritability also changes over the life course,[40] which indicates that no particular gene is responsible for a complex trait and that more focus should be devoted to the environmental stimuli that influence gene expression (what is referred to as gene-by-environment interactions).

The twin approach was also extended to more complex models in the social sciences that allow the multivariate assessment of relationships. Examples of such studies focused on psychological traits associated with social trust,[41] ideology,[42] in-group identification and favoritism,[43] participation,[44] civic duty,[45] and political interest.[46] Additional tested relationships included efficacy and participation,[47] social fear and out-group attitudes,[48] aggression and foreign policy attitudes,[49] the relationships of need for cognition and closure with political ideology,[50] religious and political beliefs,[51] and the impact of the need to evaluate on political ideology and extremity.[52] Verhulst and colleagues proposed the use of longitudinal, and even cross-sectional,

twin data to disentangle causal mechanisms[42,53,54]; and several studies have explored gene-by-environment interactions.[55,56]

16.3 The classical twin design

Twin studies are the most commonly used method for estimating the extent to which attitudes and behaviors have a genetic component. Some studies extend this design by including twins' families and use molecular genetic data for trying to identify the specific genes responsible for given traits. MZ twins reared apart from birth offer the most ideal data for the analysis of genetic effects, since they share 100% of their genes and none of their environment. Therefore, barring resemblances in environment, such as school climate or parental attitudes (which can be accounted for), any similarity between them can be largely attributed to the effects of genes. Although cases of twins reared apart are relatively rare and challenging to identify, such studies have been done.[57–59]

To overcome the scarcity problem associated with the twins reared apart design, most studies employ twins reared together in order to partition genetic and environmental effects. This approach is based on the difference in genetic makeup between MZ and DZ twins. One way to achieve this is through the Falconer method,[60] as employed by Alford, Funk, and Hibbing[1] which relies on bivariate cotwin correlations of the phenotype. This approach was outdated as of 2005[61,62,64] and for this reason, the article received criticism from twin researchers and geneticists. Later works that followed applied more current methods, including Hatemi's PhD dissertation, written under the supervision of John Hibbing, that arrived largely at the same conclusions as the original article after reanalysis with the contemporary structural equation modeling approach.[63] In fact, Medland and Hatemi's article in *Political Analysis*[64] offers one of the best reviews of twin heritability estimation methods (not only within the social sciences). The modeling presented includes the description of some common multivariate models that allow for the decomposition of not only the variance of a phenotype into its genetic and environmental components but also the covariance between phenotypes. The models also enable the simultaneous estimate of complex measurement models and multivariate structures of relationships. However, while the classical twin design allows researchers to assess whether genes have an effect, it cannot indicate the specific genes responsible for the phenotype, the number of genes, or the mechanism linking genes to the phenotype.

16.4 Assumptions of the twin model

Like any other method of analysis, the classical twin design rests on a number of assumptions. While the behavior genetics literature discussed and defended these assumptions over the past decades, they were more recently reiterated and brought forward in social science by researchers reluctant to engage with the findings of

twin studies. If violated, some of the assumptions bias heritability estimates upward (increasing the chances of a type I error) and others bias heritability downward (increasing the chances of a type II error). Given the central role they played in the twin studies scholarship in the social science, here we review both the assumptions and the specific arguments laid out in the social science, twin studies, and behavioral genetic literature.

16.4.1 Type I error for heritability

All scientists pay considerable attention to mitigating the risk of type I error, which refers to reporting as significant findings that only occur by chance. This is, arguably, a serious error that can occur in empirical research. For this reason, assumptions, if violated, increase the risk of type I error, an issue that has received considerable the attention. Of course, findings of no significant effect can sometimes provide useful knowledge.

The classical twin design decomposes variance into three distinct sources: additive genetic effects (a), common environmental influences (c), and unique environmental influences (e). This is what's referred to as the ACE model. One criticism is that there could be other sources of variance, such as genetic dominance or epistasis (forms of gene-by-gene interaction) and gene × environment interaction, which the model cannot easily account for. However, by assuming that these sources of variation do not exist, the additive genetic estimate of the model is biased upward. Since dominance and epistasis are also genetic effects (nonadditive), the statistical model indeed might overestimate the additive genetic component but only at the expense of these other nonadditive genetic components. For this reason, misspecifying the mechanism of genetic influence of the phenotype may overestimate additive genetic effects, but not the impact of genetics in general.

Gene-by-environment interactions occur when people with the same genotypes respond differently to different environments or the effects of a particular environment differ depending on someone's genotype. The omission of this (or anything else relevant) from the analysis can introduce bias, though social scientists bringing this critique often forget that this is a problem of all quantitative empirical studies, not just twin designs. Purcell[65] developed an extension of the ACE model that tackles this problem. This extension produces conditional effects of a, c, and e, moderated by a specific, measured "environmental" factor explicitly modeling the gene by environment interaction.

Another assumption that garnered substantial attention and criticism is the equal environment assumption (EEA). This was addressed in two debates in *Perspectives on Politics*.[7–11] This assumption holds that the contribution of the shared environmental influence for the trait of interest is the same for MZ and DZ twins. In the case of political views, parents most certainly expose both MZ and DZ twins to the same perspectives while they are living at home. The main point of criticism in this regard is that the shared environment may have a larger influence in the case of MZ than DZ twins, which would cause the heritability estimate to be

overestimated and the common environment estimate to be underestimated. Some anecdotal arguments against this assumption include evidence that MZ twins are more likely than DZ to spend time with each other and share the same friends,[8,66] which results in them sharing a larger proportion of their environment than DZ pairs. These studies do forget that the mechanism through which this could also occur is what is called gene–environment correlation, namely when genetics influence environments people select into (a convenient example of this mechanism is how people with fair skin, a highly heritable trait, are less likely to sit in the sun because they easily burn), though studies modeling this mechanism are still scarce in the social sciences.

The main issue surrounding the EEA assumption is whether the possibly larger part of the environment shared by MZ twins influences the specific trait under analysis. Even if we expect for this violation of the EEA assumption to occur, there are ways of measuring and correcting for this specific effect. One extension of the twin model includes an EEA correction when there is information on the specific environmental component believed to violate the assumption.[67–71] Derks et al.[72] proposed an alternative model that accounts for EEA violations without having to measure the environment. An empirical test using equal environment measures such as shared bedroom, friends, classes, and dressing alike found no EEA violations that would bias heritability estimates for issue positions.[73] In fact, most specific tests of the equal environment assumption suggest that it is not present at all. For example, it is unlikely that parents would offer their children more consistent political cues in families with MZ twins than in those with DZ twins. Exceptions are so rare, they are well below the probability of statistical errors we agree to tolerate. In other words, when it happens, most likely a positive estimate of EEA's presence is also due to just random chance. Additionally, even if there is an EEA violation in some environmental factor, that environmental factor has to have a strong impact on the phenotype to produce bias in the estimates of twin models not taking EEA violations into account. In the social sciences, EEA has been used as a blanket critique of all twin studies, but no critic attempted to test specific propositions empirically. People who did found no EEA violation.

16.4.2 Type II error for heritability

The classical twin design also makes several assumptions that, if violated, bias heritability estimates downward and increase the risk of type II error. While these assumptions receive less attention from critics in the social sciences, they are important in ensuring that estimates are unbiased. Some of these assumptions, especially for social traits, are often violated. One of these is the assumption of no measurement error, which is important in any survey-based study. In the twin design, measurement error is subsumed within the unique environmental influences (e), leading the estimates for nonshared environment to be biased upwards and those for heritability (a) and shared environment (c) to be biased downwards proportionally, since the (a), (c), and (e) estimates need to sum up to 1.

Another assumption of the classical twin design is random mating with respect to the phenotype under analysis. This assumption is violated when people choose partners who are similar to themselves in the trait of interest (also known as assortative mating), which biases heritability estimates downward. This occurs frequently when it comes to social traits, as spouses tend to be similar in ideological leaning and religiosity, though is certainly less the case for cancer susceptibility, other forms of disease, or even physical characteristics like height and body mass index where one could easily imagine assortment. For instance, Eaves and Hatemi[74] found strong assortative mating on attitudes toward gay rights and abortion. Moreover, political attitudes ($r = 0.64$) and party affiliation ($r = 0.59$) are the second and third most correlated traits among spouses, slightly lagging behind religiosity ($r = 0.71$) and surpassing education ($r = 0.50$), height ($r = 0.22$), weight ($r = 0.15$), physique ($r = 0.12$), neuroticism ($r = 0.08$), and extraversion ($r = 0.01$).[73]

Violations of the assumptions discussed in this section can be tested and mitigated in several ways in order to reduce the bias in estimates. Measurement error can be minimized through careful measurement and using the more complex statistical models covered by the aforementioned Medland and Hatemi article.[75,29] Assortative mating bias can be explicitly modeled if data are gathered from the parents of the twins or the spouses of adult twins.[76] The inclusion of opposite-sex twins[22,77] and nontwin siblings can bring additional analytical power and reduce bias associated with generalizing to the general population only based on twins.

16.5 The future of twin research in the social sciences

Researchers in the social sciences are used to having access to cross-national data with large sample sizes (e.g., thousands of participants) provided by studies such as the Comparative Studies of Election Systems, the World Values Survey, the European Values Survey, the European Social Survey, or the Eurobarometer. Unfortunately, openly accessible data to conduct behavioral genetic studies within the social science are still scarce, although behavioral geneticists have made great strides in collecting social and political twin data. Many of the early studies relied on a proprietary US sample, the Virginia 30k. While most twin data are proprietary, there are some freely available or easily accessible options. *The Minnesota Twins Political Survey* data, at the University of Nebraska's Political Psychology Lab, collected through the efforts of John Hibbing, is freely available.[78] The *Midlife in the United States (MIDUS) National Study of Health and Well-Being* is a relatively easily accessible dataset that contains a twin sample with many social characteristics.[43,47,79] The *National Longitudinal Study of Adolescent Health (Add Health)* twin data can be purchased for a modest fee.[31]

Unfortunately, these data sources suffer from various limitations of small sample sizes or restricted geographical and demographic reach in *the Minnesota Twins Political Survey* data. Others are volunteer registries where only those interested in study participation become participants. While less of an issue for medical research,

this interest often correlates with other social traits. This is somewhat mitigated by population registries in the Scandinavian countries, though social and behavioral traits are scarce in registry data and if these are used as a starting point for surveys, selection bias becomes a problem. Moreover, since collecting twin or genotyped data is significantly more difficult than survey data, the discipline of behavioral genetics has fewer incentives to encourage, enable or reward the sharing of these data. Consequently, genetically informative data rarely travel beyond the labs that collected the data or the consortiums they are a part of which rarely include social scientists in numbers to have an impact on the social science publication output. This limited culture of data sharing puts a break on scientific progress, keeps research costs inhibitively high, limits opportunities for additional publications through collaboration and the amount and visibility of research output.

Another factor that limits research on the heritability of social traits is the lack of know-how to conduct this type of research at the highest level. This stems from the separation between the fields of social science and behavior genetics and the lack of training in genetic methods in social science programs that would stimulate collaboration between the two fields.[80] Christopher Zorn and Peter Hatemi have made a significant step toward narrowing this know-how gap by convincing the National Science Foundation to fund political science participants in behavioral genetics workshops, funding that both the authors of this study and most of the social scientists cited here have directly benefited from.

However, even with such programs designed to democratize the field for young scholars, the twin studies line of research garners but a limited amount of interest among social scientists. This is due to the failure of the discipline to fully accept, engage with, and contribute to the conversation surrounding the genetic bases of attitudes and behaviors and to a lack of incentives to cross disciplinary boundaries, especially in the direction of natural sciences, and engage in interdisciplinary training and knowledge production. For example, a part of the research community in political science has remained unconvinced by the relevance of the heritability findings[81] and by the entire research program in behavior genetics, remaining entrenched in the conventional social approaches and explanations for the origins of attitudes and behaviors. To garner the interest and engagement of this part of the research community, the implications of the importance of biology for social phenotypes need to be better articulated. For it to develop and thrive in the future, the research program in the genetic bases of social traits requires a new afflux of data, interdisciplinary cooperation between socialization and behavior genetics researchers, and more theoretical development to better spell out the contributions of behavioral genetic findings to the social sciences.

Another type of genetic research relies on genotyping related or unrelated individuals in order to identify specific genes associated with a trait. However, for complex human traits including social or political ones, past decades of research in behavior genetics have failed to identify specific genes that are significantly associated with a trait and that stand up to rigorous replication. As it seems, no "politics gene" exists. As Hatemi argues, "[w]ith incredibly rare exceptions, individual

"genes" do not have a direct or even modest causal role in behavior (social or otherwise)."[82] Genome-wide studies have shown that no matter the heritability of a complex social phenotype, individual genes (or single nucleotide polymorphisms—which includes both protein-coding genes and other regulating regions of the DNA) have no direct or significant impact.[83] It could be tempting to seek simplistic explanations especially with findings that attribute the majority of variance in key political variables (like turnout) to genetic effects,[31] but giving into such temptations is not only misguided, it is simply incorrect. There is no and at this point, we are very certain there can never be a political gene. Complex behaviors are affected by many (probably thousands of) genes with tiny influences. This said, genetics does have a sizable overall impact on political behaviors, as well as on many complex human traits.

The interest of the popular media in such findings makes it that much more difficult to communicate the complexities of the processes at play when the headlines offer broad oversimplifications. Seeking to avoid this problem, Fowler and Dawes[84] titled the first political science study identifying specific genes linked to a political variable "Two Genes Predict Voter Turnout," to highlight that there is no single political gene. While more studies in this line of research followed, such as those by Dawes and Fowler[85] and Settle et al.,[86] the results of such candidate gene studies did not hold up to rigorous scrutiny. Therefore, the discipline, for the most part, considers most findings using this method wrong or misleading. In 2012, the leading field journal *Behavior Genetics* issued an editorial policy on the matter[87] and the *American Political Science Review,*[88] a flagship political science journal, quickly followed suit. The results of such studies have relied on limited samples of several hundred participants and were not successfully replicated in larger and well-powered samples. Therefore, researchers now have to employ genetically informative sample sizes never before seen in the social sciences even in generally easy-to-collect surveys, which severely limits research output.

Despite the sizable heritability in ideology and other social phenotypes, no studies that meet the standards of genetic research have so far found a single nucleotide polymorphism to significantly correlate with social characteristics.[83,38] This discrepancy between the high heritability estimates found in twin studies and the lack of findings on genes that have a significant and substantive contribution to a phenotype has been dubbed the "missing heritability problem."[89] How come genes have such a strong cumulative effect, yet they cannot be identified? The main reasons for the failure to discover a significant contribution of any locus on the genome to complex traits such as attitudes are the lack of power in existing studies and the lack of full-genome sequences.[90] To overcome these limitations, there is a need for sample sizes in the millions, which are still far off in the future, if not unobtainable, given the current limited potential for large-scale cooperation and pooling of several samples with information on social traits of interest. In fact, highly heritable phenotypes like height or BMI also suffer from the missing heritability problem. The difference is that while almost all studies around the world that included some form of genotyping collected data on these characteristics—through the merging of hundreds if not

thousands of dataset—the missing heritability is starting to become less missing.[91] However, social phenotypes are not even comparable outside of local specific social and political contexts[19] and, therefore, the sample sizes needed are practically impossible to achieve even through the hypothetical study of the entire population. It is no wonder that the most successful attempts for identifying the missing heritability of a social trait were conducted on educational attainment, a characteristic that spans social contexts; many studies with a genotyping component would include it in the data collection.[92–94]

16.6 Conclusions

Twin studies have only gained in popularity in the social sciences in the last 10–15 years, although the field of behavior genetics has studied various behaviors, attitudes, and dispositional traits pertaining to these fields for almost 50 years. Following initial surprise to the behavioral geneticists, genetic influences on political traits have become more widely accepted by political scientists as well, after a slow and uneven process. Currently, there is little surprise in finding that anything social turns out to also be partly heritable. People are not blank slates on which parents and society write codes only through socialization. A substantial amount of research has shown that genes play a significant role in attitudes, social traits, and interests, and that genetic explanations can complement and enhance the explanatory power of models that rely exclusively on environmental approaches. However, the complexities of the mechanisms that link genes to social traits are far from being clarified. Therefore, the next avenue for research in this field is to move beyond estimates of heritability and identify the mechanisms at work.

What is clear from the research conducted so far is that there is a little chance for any single gene to meaningfully affect complex behaviors such as voting, partisanship, or any other social trait. No specific locus on the genome has been identified as responsible for anything but minute, indirect effects. Additive effects of any single gene summing to explain more than 1% of the variation in social phenotypes is considered a huge finding. Barring a limited number of phenotypes, usually diseases, no single gene exerts a sizeable effect for complex traits like attitudes and behaviors. Hence, any fears of social scientists that genetics is a deterministic mechanism with a universal effect should be laid to rest. Context is important and it can modify outcomes. However, even if the quest for the "politics gene" is futile, twin studies still have valuable insights to offer to the social scientifically inclined.

Complex traits of interest to the social sciences arise from an intricate pathway of interactions between physiological and environmental influences. The exploration of such gene × environment interactions (or gene–environment correlations) is one of the research avenues to be pursued further, which will contribute to a better assessment of both genetic and environmental influences. If some environmental stimulus changes the proportional impact of genetic factors in a phenotype, we can consider these environmental changes important. If a social trait is highly heritable

in one place and not at all in another, as it is the case for ideology in Hungary,[38] then something really interesting is going on in how the social context influences the heritability estimates and this certainly warrants further exploration. The real challenge will, of course, always be to understand the mechanisms of this change.

In the future, twin studies will likely be more fruitful in assessing the extent of environmental influences on various phenomena and helping specify the mechanism of socialization. Variation in the presence or absence of socialized phenomena and the power of extended family designs, incorporating the parents, siblings, spouses, and even children of twins,[95,96] to dissect the shared family environment into its subcomponents will allow us to learn new facts about familial transmission processes for various social traits. Such extended family designs have been employed more often in genetics books and studies published in genetics publications[97–99] than in social science journals.[76]

The new way of thinking about attitudes and social traits offered by behavior genetics has predictably faced resistance in the social sciences. For a hundred years, the field was tributary to environment-dominated explanations of the origins of social outcomes of interest and this assumption continues to hold considerable sway. While resistance to new ways of thinking is natural, twin studies have merit in contributing to our understanding of how social traits and attitudes come about and synergies between researchers of socialization and genetics will, hopefully, continue to add to that understanding. With new theories and good quality data more widely available to the next generations of researchers, twin studies can develop further into multivariate analyses with the inclusion of extended pedigree and studies of gene-by-environment interactions which will shed new light on the origins of social phenotypes.

References

1. Alford JR, Funk CL, Hibbing JR. Are political orientations genetically transmitted?. *American Political Science Review*. 2005;99:153–167.
2. Eaves LJ, Eysenck HJ. Genetics and the development of social attitudes. *Nature*. 1974;249:288–289.
3. Martin NG, Eaves LJ, Heath AC, Jardine R, Feingold LM, Eysenck HJ. Transmission of social attitudes. *Proceedings of the National Academy of Sciences of the United States of America*. 1986;83:4364–4368.
4. Bouchard TJ, McGue M. Genetic and environmental influences on human psychological differences. *Journal of Neurobiology*. 2003;54:4–45.
5. Oniszczenko W, Jakubowska U. Genetic determinants and personality correlates of sociopolitical attitudes in a Polish sample. *Twin Research and Human Genetics*. 2005;1:47–52.
6. Carey B. Some politics may be etched in the genes. *New York Times*. 2005. https://www.nytimes.com/2005/06/21/science/some-politics-may-be-etched-in-the-genes.html?smid=url-share (Accessed August 30, 2021).
7. Beckwith J, Morris CA. Twin studies of political behavior: untenable assumptions?. *Perspectives on Politics*. 2008;6:785–791.
8. Charney E. Genes and ideologies. *Perspectives on Politics*. 2008;6:299–319.

9. Alford JR, Funk CL, Hibbing JR. Beyond liberals and conservatives to political genotypes and phenotypes. *Perspectives on Politics*. 2008;6:321–328.
10. Alford JR, Funk CL, Hibbing JR. Twin studies, molecular genetics, politics, and tolerance: a response to Beckwith and Morris. *Perspectives on Politics*. 2008;6:793–797.
11. Hannagan RJ, Hatemi PK. The threat of genes: a comment on Evan Charney's 'Genes and Ideologies.'. *Perspectives on Politics*. 2008;6:329–335.
12. McDermott R, Renwick Monroe K. The scientific analysis of politics: important contributions from some overlooked sources. *Political Research Quarterly*. 2009;62:568–570.
13. Lineberry RL. Letter from the Former Editor. *Social Science Quarterly*. 2011;92:1133.
14 Hatemi PK, McDermott R. The political psychology of biology, genetics, and behavior. *Political Psychology*. 2012;33:307–312.
15. Hatemi PK, Byrne E, McDermott R. Introduction: what is a 'gene' and why does it matter for political science?. *Journal of Theoretical Politics*. 2012;24:305–327.
16. Hatemi PK. The intersection of behavioral genetics and political science: introduction to the special issue. *Twin Research and Human Genetics*. 2012;15:1–5.
17. Fowler JH, Schreiber D. Biology, politics, and the emerging science of human nature. *Science*. 2008;322:912–914.
18. Cesarini D, Dawes CT, Fowler JH, Johannesson M, Lichtenstein P, Wallace B. Heritability of cooperative behavior in the trust game. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;105:3721–3726.
19. Fazekas Z, Littvay L. The importance of context in the genetic transmission of U.S. party identification. *Political Psychology*. 2015;36:361–377.
20. Joseph J. Twin studies in psychiatry and psychology: science or pseudoscience?. *Psychiatric Quarterly*. 2002;73:71–82.
21. Guo G. Introduction to the special issue on society and genetics. *Sociological Methods & Research*. 2008;37:159–163.
22. Hannagan RJ, Littvay L, Popa SA. Theorizing sex differences in political knowledge: insights from a twin study. *Politics and Gender*. 2014;10:89–114.
23. Klemmensen R, Hatemi PK, Hobolt SB, Skytthe A, Nørgaard AS. Heritability in political interest and efficacy across cultures: Denmark and the United States. *Twin Research and Human Genetics*. 2012;15:15–20.
24. Fazekas Z, Littvay L. Choosing sides: the genetics of why we go with the loudest. *Journal of Theoretical Politics*. 2012;24:389–408.
25. Arceneaux K, Johnson M, Maes HM. The genetic basis of political sophistication. *Twin Research and Human Genetics*. 2012;15:34–41.
26. D'Andra Orey B, Park H. Nature, nurture, and ethnocentrism in the minnesota twin study. *Twin Research and Human Genetics*. 2012;15:71–73.
27. Cranmer SJ, ChT Dawes. The heritability of foreign policy preferences. *Twin Research and Human Genetics*. 2012;15:52–59.
28. Stam AC, Von Hagen-Jamar A, Worthington ABH. Fear and attitudes towards torture and preventive war. *Twin Research and Human Genetics*. 2012;15:60–70.
29. Sturgis P, Read S, Hatemi PK, et al. A genetic basis for social trust?. *Political Behavior*. 2010;32:205–230.
30. Littvay L, Popa SA, Fazekas Z. Validity of survey response propensity indicators: a behavior genetics approach. *Social Science Quarterly*. 2013;94:569–589.
31. Fowler JH, Baker LA, Dawes CT. Genetic variation in political participation. *American Political Science Review*. 2008;102:233–248.

32. Hatemi PK, McDermott R. The genetics of politics: discovery, challenges, and progress. *Trends in Genetics*. 2012;28:525–533.
33. Smith KB, Alford JR, Hibbing JR, Martin NG, Hatemi PK. Intuitive ethics and political orientations: testing moral foundations as a theory of political ideology. *American Journal of Political Science*. 2017;61:424–437.
34. Hatemi PK, Medland SE, Morley KI, Heath AC, Martin NG. The genetics of voting: an Australian twin study. *Behavior Genetics*. 2007;37:435–448.
35. Hatemi PK, Alford JR, Hibbing JR, Martin NG, Eaves LJ. Is there a 'party' in your genes?. *Political Research Quarterly*. 2009;62:584–600.
36. Settle JE, ChT Dawes, Fowler JH. The heritability of partisan attachment. *Political Research Quarterly*. 2009;62:601–613.
37. Bell E, Kandler C. The origins of party identification and its relationship to political orientations. *Personality and Individual Differences*. 2015;83:136–141.
38. Hatemi PK, Medland SE, Klemmensen R, et al. Genetic influences on political ideologies: twin analyses of 19 measures of political ideologies from five democracies and genome-wide findings from three populations. *Behavior Genetics*. 2014;44:282–294.
39. Bell E, Schermer JA, Vernon PA. The origins of political attitudes and behaviours: an analysis using twins. *Canadian Journal of Political Science*. 2009;42:855–879.
40. Hatemi PK, Funk CL, Medland SE, et al. Genetic and environmental transmission of political attitudes over a life time. *Journal of Politics*. 2009;71:1141–1156.
41. Oskarsson S, Dawes CT, Johannesson M, Magnusson PKE. The genetic origins of the relationship between psychological traits and social trust. *Twin Research and Human Genetics*. 2012;15:21–33.
42. Verhulst B, Hatemi PK, Eaves LJ. Disentangling the importance of psychological predispositions and social constructions in the organization of American Political Ideology. *Political Psychology*. 2012;3:375–393.
43. Weber Ch, Johnson M, Arceneaux K. Genetics, personality, and group identity. *Social Science Quarterly*. 2011;92:1314–1337.
44. Dawes CT, Cesarini D, Fowler JH, Johannesson M, Magnusson PKE, Oskarsson S. The relationship between genes, psychological traits, and political participation. *American Journal of Political Science*. 2014;58:888–903.
45. Weinschenk AC, Dawes CT. Genes, personality traits, and the sense of civic duty. *American Politics Research*. 2017;46:47–76.
46. Weinschenk AC, Dawes CT. The relationship between genes, personality traits, and political interest. *Political Research Quarterly*. 2017;70:467–479.
47. Littvay L, Weith PT, Dawes CT. Sense of control and voting: a genetically-driven relationship. *Social Science Quarterly*. 2011;92:1236–1252.
48. Hatemi PK, McDermott R, Eaves LJ, Kendler KS, MC Neale. Fear as a disposition and an emotional state: a genetic and environmental approach to out-group political preferences. *American Journal of Political Science*. 2013;57:279–293.
49. McDermott R, Hatemi PK. The relationship between physical aggression, foreign policy and moral choices: phenotypic and genetic findings. *Aggressive Behavior*. 2017;43:37–46.
50. Ksiazkiewicz A, Ludeke S, Krueger RF. The role of cognitive style in the link between genes and political ideology. *Political Psychology*. 2016;37:761–776.
51. Friesen A, Ksiazkiewicz A. Do Political Attitudes and Religiosity Share a Genetic Path?. *Political Behavior*. 2015;37:791–818.

52. Ksiazkiewicz A, Krueger RF. The role of genes and environments in linking the need to evaluate with political ideology and political extremity. *Social Justice Research*. 2017;30:381–407.
53. Hatemi PK, Verhulst B. Political attitudes develop independently of personality traits. *Plos One*. 2015;10:e0118106. https://doi.org/10.1371/journal.pone.0118106.
54. Verhulst B, Estabrook R. Using genetic information to test causal relationships in cross-sectional data. *Journal of Theoretical Politics*. 2012;24:328–344.
55. Boardman JD, Roettger ME, Domingue BW, McQueen MB, Haberstick BC, Harris KM. Gene–environment interactions related to body mass: school policies and social context as environmental moderators. *Journal of Theoretical Politics*. 2012;24:370–388.
56. Hatemi PK. The influence of major life events on economic attitudes in a world of gene–environment interplay. *American Journal of Political Science*. 2013;57:987–1007.
57. Segal NL. *Born Together, Reared Apart*. Cambridge, MA: Harvard University Press; 2012.
58. Bouchard Jr TJ, Segal NL, Tellegen A, McGue M, Keyes M, Krueger R. Evidence for the construct validity and heritability of the Wilson-Patterson conservatism scale: a reared-apart twins study of social attitudes. *Personality and Individual Differences*. 2003;34:959–969.
59. Bouchard Jr TJ, Segal NL, Tellegen A, McGue M, Keyes M, Krueger R. Genetic influence on social attitudes: another challenge to psychology from behavior genetics. In: DiLalla LF, ed. *Behavior Genetic Principles: Development, Personality and Psychopathology*. Washington, DC: American Psychological Association; 2004:89–104.
60. Falconer DS. *Introduction to Quantitative Genetics*. Edinburgh: Oliver and Boyd Ltd; 1960.
61. Jinks JL, Fulker DW. Comparison of the biometrical genetical, MAVA, and classical approaches to the analysis of human behavior. *Psychological Bulletin*. 1970;73:311–349.
62. Neale M, Cardon L. *Methodology for Genetic Studies of Twins and Families*. Dordrecht: Springer Science and Business Media; 1992.
63. Hatemi PK. The genetics of political attitudes. *ETD collection for University of Nebraska - Lincoln*. 2007:AAI3258734. https://digitalcommons.unl.edu/dissertations/AAI3258734 (Accessed on September 15, 2021).
64. Medland SE, Hatemi PK. Political science, biometric theory, and twin studies: a methodological introduction. *Political Analysis*. 2009;17:191–214.
65. Purcell S. Variance components models for gene–environment interaction in twin analysis. *Twin Research*. 2002;5:554–571.
66. Horwitz AV, Videon TM, Schmitz MF, Davis D. Rethinking twins and environments: possible social sources for assumed genetic influences in twin research. *Journal of Health and Social Behavior*. 2003;44:111–129.
67. Hettema JM, Neale MC, Kendler KS. Physical similarity and the equal-environment assumption in twin studies of psychiatric disorders. *Behavior Genetics*. 1995;25:327–335.
68. Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. A test of the equal-environment assumption in twin studies of psychiatric illness. *Behavior Genetics*. 1993;23:21–27.
69. Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. Parental treatment and the equal environment assumption in twin studies of psychiatric illness. *Psychological Medicine*. 1994;24:579–590.
70. Mazzeo SE, Mitchell KS, Bulik CM, Aggen SH, Kendler KS, MC Neale. A twin study of specific bulimia nervosa symptoms. *Psychological Medicine*. 2010;40:1203–1213.

71. Mitchell KS, Mazzeo SE, Bulik CM, Aggen SH, Kendler KS, MC Neale. An investigation of a measure of twins' equal environments. *Twin Research and Human Genetics*. 2007;10:840–847.
72. Derks EM, Dolan CV, Boomsma DI. A test of the equal environment assumption (EEA) in multivariate twin studies. *Twin Research and Human Genetics*. 2006;9:403–411.
73. Littvay L. Do heritability estimates of political phenotypes suffer from an equal environment assumption violation? Evidence from an empirical study. *Twin Research and Human Genetics*. 2012;15:6–14.
74. Eaves LJ, Hatemi PK. Transmission of attitudes toward abortion and gay rights: effects of genes, social learning and mate selection. *Behavior Genetics*. 2008;38:247–256.
75. Batrićević N, Littvay L. A genetic basis of economic Egalitarianism. *Social Justice Research*. 2017;30:408–437.
76. Hatemi PK, Hibbing JR, Medland SE, et al. Not by twins alone: using the extended family design to investigate genetic influence on political beliefs. *American Journal of Political Science*. 2010;54:798–814.
77. Hatemi PK, Medland SE, Eaves LJ. Do genes contribute to the 'gender gap'? *Journal of Politics*. 2009;71:262–276.
78. Funk CL, Smith KB, Alford JR, et al. Genetic and environmental transmission of political orientations. *Political Psychology*. 2013;34:805–819.
79. Loewen PJ, ChT Dawes. The heritability of duty and voter turnout. *Political Psychology*. 2012;33:363–373.
80. Bergner CL, Hatemi PK. Integrating genetics into the study of electoral behavior. In: Arzheimer K, Evans J, Lewis-Beck MS, eds. London: SAGE Publications; 2017. *The SAGE Handbook of Electoral Behaviour*. I:367–405.
81. Bartels L. Your genes influence your political views. So What? *Monkey Cage (blog)*. *Washington Post*. 2013. https://www.washingtonpost.com/news/monkey-cage/wp/2013/11/12/your-genes-influence-your-political-views-so-what/ (Accessed on September 15, 2021).
82. Hatemi PK. Genetic and neurocognitive approaches for comparative politics: a partnership between science and culture. *SSRN Electronic Journal*. 2010. http://doi.org/10.2139/ssrn.1539131.
83. Benjamin DJ, Cesarini D, Van der Loos MJHM, et al. The genetic architecture of economic and political preferences. *Proceedings of the National Academy of Sciences of the Unites States of America*. 2012;109:8026–8031.
84. Fowler JH, Dawes CT. Two genes predict voter turnout. *The Journal of Politics*. 2008;70:579–594.
85. Dawes CT, Fowler JH. Partisanship, Voting, and the Dopamine D2 Receptor Gene. *Journal of Politics*. 2009;71:1157–1171.
86. Settle JE, Dawes CT, Christakis NA, Fowler JH. Friendships moderate an association between a dopamine gene variant and political ideology. *Journal of Politics*. 2010;72:1189–1198.
87. Hewitt JK. Editorial policy on candidate gene association and candidate gene-by-environment interaction studies of complex traits. *Behavior Genetics*. 2012;42:1–2.
88. Notes from the Editors: American Political Science Review. 2013;107: iii–viii, https://doi.org/10.1017/S0003055413000105.
89. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. *Nature*. 2009;461:747–753.

90. Marouli E, Graff M, Medina-Gomez C, et al. Rare and low-frequency coding variants alter human adult height. *Nature*. 2017;542:186–190.
91. Yang J, Bakshi A, Zhu Z, et al. Genetic variance estimation with imputed variants finds negligible missing heritability for human height and body mass index. *Nature Genetics*. 2015;47:1114–1120.
92. Rietveld CA, Medland SE, Derringer J, et al. GWAS of 126,559 individuals identifies genetic variants associated with educational attainment. *Science*. 2013;340:1467–1471.
93. Okbay A, Beauchamp JP, Fontana MA, et al. Genome-wide association study identifies 74 loci associated with educational attainment. *Nature*. 2016;533:539–542.
94. Lee JJ, Wedow R, Okbay A, et al. Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. *Nature Genetics*. 2018;50:1112–1121.
95. Bell E, Kandler C, Rainer R. Genetic and environmental influences on socio-political attitudes: addressing some gaps in the new paradigm. *Politics and the Life Sciences*. 2018;37:236–249.
96. Hufer A, Kornadt AE, Kandler C, Riemann R. Genetic and environmental variation in political orientation in adolescence and early adulthood: A Nuclear Twin Family analysis. *Journal of Personality and Social Psychology*. 2020;118:762–776.
97. Keller MC, Medland SE, Duncan LE, et al. Modeling extended twin family data I: description of the cascade model. *Twin Research and Human Genetics*. 2009;12:8–18.
98. Eaves LJ, Hatemi PK, Heath AC, Martin NG. Modeling the cultural and biological inheritance of social and political behavior in twins and nuclear families. In: Hatemi PK, McDermott R, eds. *Man Is by Nature a Political Animal: Evolution, Biology, and Politics*. Chicago, IL: University of Chicago Press; 2011:101–184.
99. Hatemi PK, Smith KB, Alford JR, Martin NG, Hibbing JR. The genetic and environmental foundations of political, psychological, social, and economic behaviors: a panel study of twins and families. *Twin Research and Human Genetics*. 2015;18:243–255.

CHAPTER

Childhood development of psychiatric disorders and related traits

17

Isabell Brikell[a], Paul Lichtenstein[a], Henrik Larsson[a,b]

[a]*Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden*

[b]*School of Medical Sciences, Örebro University, Sweden*

17.1 Introduction

Genetic research in the area of child and adolescent psychiatry has increased greatly in the past two decades. Through the establishment of large-scale twin studies, which combine longitudinal data collection from early childhood through to adolescence with detailed phenotypic assessments, evidence from twin research has substantially changed our understanding of the etiology and development of childhood psychiatric disorders and related traits.[1] Based on more than 1000 published research articles, it is now clear that genetic factors play an important role in all psychiatric disorders.[2] Yet, the development of child psychiatric problems is the result of a complex interplay between genetic sensitivity and environmental risk-factors. Twin studies have not only established the relative influence of genetic and environmental factors in the etiology of child psychiatric disorders and traits, but also clarified developmental stability and change, causes of comorbidity, and begun to unravel complex gene–environment interplay. In this chapter, we provide an overview of what can be concluded about the etiology and development of childhood psychiatric disorders and traits from over two decades of twin research and discuss future direction for the field.

17.2 Heritability of childhood psychiatric disorders and traits

During the late 1980 and 1990s, a number of large twin registers and cohorts were established across the United States, UK, the Netherlands, Finland, and Sweden, with the aim of studying the early antecedents and development of psychiatric disorders and traits from childhood into early adulthood.[1] Findings from these samples have provided heritability estimates for most common childhood psychiatric disorders and traits, with result firmly establishing that genetic factors play a role in all childhood psychiatric problems, thereby changing both clinical and public perception of several childhood disorders. Autism spectrum disorder (ASD) provide perhaps the most striking example: ASD is a relatively rare, early onset, and often severe

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00006-4

neurodevelopmental disorder that was long considered to be caused by cold parenting, commonly referred as refrigerator mothers.[3] Early family studies of ASD showed that 2%–6% of siblings of children with ASD were themselves affected, which was nearly 100 times the population rate of diagnosed ASD at the time.[4,5] Twin-studies have subsequently confirmed that ASD can be largely attributed to genetic factors, with heritability estimated between 60% and 90%, and minimal impact of shared environmental factors.[2,3,6] High heritability has also been found for attention-deficit/hyperactivity disorder (ADHD), which is the most common neurodevelopmental disorder today, with a prevalence of 5%–10% in childhood.[7] Twin studies consistently estimate the heritability of ADHD to 70%–80% and find limited support for the influence of the shared family environment.[2,8,9] Whilst less research has focused on other neurodevelopmental disorders, a study of nearly 11,000 twins aged 9- and 12-year-old reported heritability of 70% for parent-rated developmental coordination disorder.[10] The same study found the heritability of tics disorder to be somewhat lower (56%), which in line with two other population-based twin studies of parent-rated tics in children.[10–12] Twin studies of learning difficulties generally find moderate heritability, ranging from 40% to 60%,[13,14] whilst studies of specific development disorders of langue, speech, scholastic skills, and motor functioning are largely lacking. A consistent pattern across twin studies of neurodevelopmental disorders in general, and ADHD and ASD in particular, is that there is limited evidence for the influence of the shared environment.[2,9] In contrast, twin studies of externalizing problems of childhood (i.e., antisocial behaviors, aggression, oppositional defiant disorder, and conduct disorder) often find a substantial contribution of the shared environment.[15] In a meta-analysis of twin and adoption studies of conduct problems, heritability was estimated to 45%–50%, whilst the shared environment accounted for 15%–20% of trait variance.[16] Twin studies of internalizing disorders and traits in childhood (e.g., depression, anxiety, phobias, compulsive behaviors) generally show somewhat lower heritability (<50%) as compared to neurodevelopmental and externalizing disorders.[2,17] Given that anxiety disorders often onset in childhood and are highly prevalent, with life-time prevalence at age 18 estimated to 32%,[18] numerous twin studies have focus on anxiety in childhood and adolescence. These studies have revealed low to moderate genetic influences on a wide range of anxiety measures including anxiety disorder, self-assessed and parent-reported disorder-related symptoms, and trait anxiety.[19–23] Twin research on depression prior to adulthood has primarily been conducted in adolescent population-based samples and focused on symptoms scores, with heritability estimated between 30% and 50% and significant variation observed by sex, age, and rater, and generally much lower heritability estimated in younger children.[24,25] Importantly, twin studies of internalizing disorder in childhood and adolescence have highlighted that more than half of the variance can be attributed to environmental factor, with many studies reporting a significant influence of the shared family environment.[17,26]

In summary, univariate twin and family based studies have unequivocally shown that all child psychiatric disorders and traits are heritable, yet the magnitude of genetic influence differ by disorder, as does the relative importance of the shared

and nonshared environment. In the remainder of this chapter, we move focus from univariate twin studies that estimate heritability of single disorders and traits, to multivariate and longitudinal research designs that utilize twin data to address important questions about etiology of childhood mental health across different levels of symptom severity, comorbidity, and development. Finally, we discuss consider gene–environment interplay and twin-methods used to study genetic confounding.

17.3 Childhood psychiatric disorder and population traits have shared genetic origins

It is increasingly recognized that many psychiatric disorders reflect an extreme expression of normal variation in the population, emphasizing continuity in the underlying causes, meaning that psychiatric disorders and the trait expression in the general population differ only in degree, but not nature. In contrast, a categorical perspective views psychiatric disorders as qualitatively different from variation across the normal range of the trait expression in the population, and as having their own pattern of rather distinct causes, meaning that psychiatric disorders differ from the trait expression in the general population in both degree and nature.

A core question in the discussion around a categorical versus dimensional perspective on psychiatric disorders is the question of whether the etiological underpinnings are the same across different levels of severity of psychiatric disorders and their corresponding population traits. This question has to some extent been addressed in twin studies, using the so-called DeFries–Fulker extremes analysis,[27] which explores genetic links between the extreme (clinical diagnosis) and the subthreshold levels of a trait by bringing together the dichotomized classification of psychiatric disorders (e.g., yes/no diagnosis) and the underlying quantitative dimension of the psychopathological construct (e.g., the total score of parent-rated symptom scale). The available research using the DeFries–Fulker analytic method has demonstrated statistically significant group heritability for some forms of child and adolescent psychiatric conditions. One study of 16,366 Swedish twins explored the genetic link between the extreme and the sub-threshold variation of DSM-IV ADHD symptoms. The estimated group heritability was around 0.60, depending on the level of severity for the imposed cutoff.[28] These findings are consistent with one early study of 583 same-sexed twin pairs using DSM–III–R ADHD symptoms.[29] Together, these studies suggest that ADHD is best viewed as the quantitative extreme of genetic and environmental factors operating dimensionally throughout the distribution of ADHD symptoms, indicating that the same etiologic factors are involved in the full range of symptoms of inattention, hyperactivity, and impulsivity. At least two twin studies using the DeFries–Fulker analytic method also support significant group heritability using several different definitions of autistic-like-traits.[30] Another study used a UK-based twin sample and applied a novel approach, referred to as the joint categorical-continuous twin model, to estimate the degree of etiological overlap between autistic-like-traits and categorical diagnoses of autism. This study reported a strong

genetic correlation of 0.70 between autistic-like-traits and categorical diagnoses of autism.[31] Few, if any, twin studies have estimated the group heritability for other forms of child and adolescent psychiatric disorders, such as depression, anxiety, and obsessive-compulsive disorder.[32]

17.4 Genetic contributions to comorbidity across childhood psychiatric disorders and traits

Comorbidity across psychiatric disorders is pervasive, representing the norm rather than the exception in child and adolescent psychiatry. An important question is therefore why psychiatric problems co-occur and multivariate quantitative genetic methods, which focus on investigating the covariance across conditions, are particularly suited to study how different psychiatric disorders cluster together and the extent to which comorbidity is explained by genetic and environmental factors. Multivariate twin studies have greatly advanced our understanding of the underlying causes of comorbidity in child and adolescent psychiatry. For example, ADHD and ASD could not be diagnosed together in the DSM-IV and the ICD-10, a demarcation that has been removed in DSM-5, in part due to cumulating evidence from bivariate twin studies showing a strong degree of genetic sharing across the two disorders.[32] A UK study of over 6000 twin pairs reported genetic correlations >0.50 between autistic-like-traits and ADHD-traits in the general population,[33] with even stronger genetic overlap found in a study of parent-rated ASD and ADHD symptoms in 16,858 Swedish twins, where the genetic correlation was 0.80.[10] Anxiety and depression provide another example where twin studies have found comorbidity is largely explained by common genetic factors.[34] In recent work,[19] a sample of 578 twins assessed at mean age 8 and 10 years, and a sample of 2619 twins/siblings assessed at mean age 15, 17, and 20 years, were used to study comorbidity between anxiety and depression at different developmental stages. In childhood, depression symptoms showed largely distinct genetic effects from anxiety. However, from adolescence onwards, genetic influences were significantly shared between depression and all anxiety subscales(19). These results are in agreement with several prior twin-studies suggesting a shared genetic basis for depression and anxiety that increases in magnitude with age.[17,34–37]

Genetic sharing is not only evident within the neurodevelopmental, internalizing, and externalizing spectrum but also across these dimensions. In a recent meta-analysis of 31 twin studies of comorbidity in ADHD, the genetic correlations (rg) across ADHD and externalizing (rg = 0.49 [0.37–0.61]), internalizing (rg = 0.50 [0.39–0.69]), and neurodevelopmental (rg = 0.56 [0.47–0.66]) traits were found to be of similar magnitude.[38] Based on the evident high degree of genetic sharing across psychiatric disorders, there has in recent years been an increase of multivariate twin studies focused on analyzing shared etiology across broad dimensions of child psychiatric disorders and traits. Two of the most common approaches to do so is the *independent pathways model* and the *common pathway model*,[39] which can model

genetic and environmental influences on covariance across multiple traits as well as estimate the extent to which etiological factors are trait-specific (see Rijsdijk and Sham[39] for an in-depth discussion of these models). Studies using variations of the *independent* and *common pathway model* have demonstrated that genetic influences tend to clusters within *internalizing* disorders and traits and within *externalizing* disorders and traits. For example, Cosgrove et al. (2011) analyzed symptoms of depression, anxieties, ADHD, oppositional-defiant and conduct problems in a sample of 1162 twin pairs and 426 siblings, and found that a latent internalizing factor and a latent externalizing factor provided the best fit to the data. Both factors where moderately heritable and correlated at 0.72, with 62% of the covariance between internalizing and externalizing problems explained by common genetic influences. Similarly, Lahey et al.[40] analyzed data on 11 internalizing and externalizing psychiatric traits from 1571 pairs of 9–17-year-old twins. Result revealed two separate, but highly correlated (0.89), sets of genetic factors influencing the internalizing traits and the externalizing traits. Importantly, an alternative model which in addition included a general genetic factor that influenced covariance across all traits, was found to provide a better fit to data. A single latent genetic factor was also found to explain as much as 31% of shared variance in parent-ratings of ASD, ADHD, tics, and learning difficulties in a twin study of 6595 twin pairs aged 9-or-12 years.[41] Together, this growing body of work suggests that psychiatric comorbidity is in part explained by a general genetic vulnerability that increases the risk for virtually all child and adolescent psychiatric disorders and traits. This latent factor is commonly referred to as the general psychopathology factor, analog to the general factor of intelligence, and the heritability of such a general psychopathology factor in childhood was estimated to 43% in one twin study.[42]

Beyond establishing broad genetic sharing across disorders, multivariate twin studies have also highlighted the degree of shared versus specific etiological influences for more specific symptom presentations within a child psychiatric disorder. Taking ADHD as an example, twin-analyses have found similar heritability for symptom dimensions of inattention and hyperactivity/impulsivity,[1,7] but only 50%–80% of genetic influences are shared across the dimensions.[21–24] It has also been demonstrated that dimension-specific genetic factors associates differently to comorbidities, with ODD/CD traits found to be more strongly genetically correlated with hyperactivity/impulsivity than inattention,[25] whilst learning difficulties are more closely genetically linked with inattention.[26–28]

Together, multivariate twin studies suggest a broad sharing of genetic risk across a general psychopathology factor,[24,25] as well as genetic sharing across three broad subdimensions of neurodevelopmental, internalizing, and externalizing problems. In contrast, environmental risk factors appear to be largely disorder/trait specific, suggesting that whilst genetic drive co-occurrence of childhood psychiatric disorder, environmental influences across developmental is what sets childhood-onset disorder apart. Findings from twin studies regarding the broad genetic sharing in psychiatry have already informed design and methods development in molecular genetics and neuroimaging psychiatric research.[43–45] Further, multivariate twin-studies have also

been used to illustrate a degree of genetic specificity for symptoms dimension of some child psychiatric disorders (e.g. ADHD, various types of phobias and anxiety), and their etiological associations with other childhood psychiatric traits and disorders. Future efforts in twin research have the potential to generate a more in-depth understanding of both the specific and broadly shared etiologic (genetic and environmental) structure underlying not just psychiatric disorders, but also a broader disease spectrum that includes comorbid somatic and neurologic conditions.

17.5 Stability and change in the development of childhood psychiatric disorders and traits

Given that childhood and adolescence are periods of rapid physical and mental growth, accompanied by profound changes in the social environment, a key issue in child psychiatry is how etiological factors influence the development of psychiatric disorders over time. In twin studies, two issues are relevant to do consider; first, does the degree to which genetic and environmental influences account for variance in a disorder change across time; and second, are the genes and environments that contribute to a disorder earlier in childhood the same as those that contribute later in adolescence? Longitudinal twin studies, where the same traits are measured in the same individuals on multiple occasions, are particularly suited to address such research questions.

Numerous twin studies have focused on the development of ADHD from childhood into early adulthood since it well established that ADHD symptoms, particularly hyperactivity/impulsivity, often decrease with age.[46] For example, in a study of 4000 young twin pairs with parent-ratings of ADHD symptoms collected at ages 2, 3, 4, 7, and 8 years, the *Cholesky* decomposition[47] (i.e., a longitudinal approach which provides age-specific estimates of A, C, D, and E, as well as estimates of the extent to which genetic and environmental factors that explain variance at one age continue to influence variance in the same trait at later ages) revealed that the heritability of ADHD was high at all ages (0.77–0.86), and a moderate phenotypic stability between ages that was mainly explained by stable genetic influences. In addition, there was evidence of new genetic influences, not shared with those acting earlier on, emerging at later ages.[48] A recent twin study applied *latent growth modeling*, that is, models which estimate genetic and environmental contribution to an *intercept,* representing the mean baseline level of a trait, and a *slope,* representing the mean change in that trait across measured time points, to study the developmental course of ADHD symptoms between ages 8 and 16 years in a sample of 8395 UK twin pairs.[49] Results showed that hyperactivity/impulsivity symptoms decreased sharply across time points, and more importantly, that although genetic factors were important for variation in both baseline symptoms and the change in symptoms over time (heritability was 81% and 90%, respectively), only around 40% of the genetic factors influencing baseline symptoms were shared with those underpinning the developmental course of hyperactivity/impulsivity symptoms. This pattern of results, with high heritability

at each age and evidence of both genetic stability, but also new genetic factors emerging at different developmental stages (referred to as genetic innovation), has been replicated in several developmental twin studies of ADHD.[50–52] In general, change in ADHD symptoms has been found to be explained by both genetic innovation, and age-specific nonshared environmental influences across mid-childhood and adolescence.[53] Much less twin research has investigated the etiology of ASD beyond childhood, however, studies addressing continuity in autistic-like-traits in the general population find strong evidence for genetic stability across ages, which is in line with the persistent nature of clinical ASD. A study of more than 6000 twin pairs followed from ages 8 to 12 years of age showed that autistic traits are relatively, with moderately to-highly heritable at each age. Moreover, phenotypic stability across ages was largely accounted for by genetic factors.[54] Similar results were found in a sample of 2500 twin pairs assessed at age 9 or 12 years and again at age 18. Although the stability of autistic-like-traits from childhood to early adulthood were weaker than that reported in younger ages, 85% of the phenotypic correlation across ages was explained by stability in additive genetic factors.[55]

Conduct problems, aggression, and antisocial behaviors from early childhood to mid-adolescence have been analyzed in at least 20 longitudinal twin-studies. Overall, a majority of studies have reported moderate-to-substantial phenotypic stability in externalizing problems, which is primarily explained by stable genetic factors, However, unlike for ADHD, stable shared environmental influences also appear to contribute to phenotypic stability.[53] In terms of age-related changes in aggression, conduct problem, and antisocial behaviors, both genetic, shared and nonshared environmental innovation have been found to be in play. In a UK sample of >3000 twins pairs assessed for conduct problems at age 4, 7, 9, 12, and 16, age-to-age correlation was around 0.5, with heritability estimated around 50%–60% at all ages. Both genetic (12%–22%) and shared environments contributed to stability (3%–17%) from age 4 to age 16, whereas symptom change over time was partly attributed to shared environments in childhood (3%–8%), and genetic (23%–40%) and nonshared environmental (19%–25%) innovation across all ages.[56] Notably, there is evidence of sex differences in the development of antisocial behaviors, reflected by a stronger influence of genetic factors on age-to-age stability in females, whilst shared-environmental factors also play a role in males.[57–59] For example, in a study of 1226 twin pairs, correlations between parent-rated antisocial behaviors at age 8–9 years and self-rated antisocial behaviors at age 13–14 years was entirely explained by stable genetic effects in females, whereas genetic (39%–40%) and shared-environmental factors (47%–51%) contributed equally in boys.[57]

Twin studies of anxiety and depression have often reported an increasing heritability from early childhood (with heritability estimates ranging from zero to low) into adolescence (where heritability estimates are around 30%–50%),[17,24,60] although not all studies find a pattern of increasing heritability.[61–63] However, it is important to note that changes in heritability across ages mat not only arise from true etiological changes but are sometimes better explained by methodical effects of changing assessment tools and rater over time. Specifically, parents- and teachers-report,

which are commonly used in childhood, and self-ratings, which are often used in adolescent, can tap into different, situation-specific behaviors. Further, moving from a single person rating both twins in a pair, to relying on each twin rating themselves tends to increase environmental variance, which can affect heritability estimates.[61,64] Such issues can partly be circumvented by using *multi-rater models*, where heritability is estimated for a latent factor, reflecting the shared variance between parents, teacher, and twin self-ratings, thereby accounting for rater- and time-specific effect. A *multi-rater Cholesky decomposition* was used to study development of anxiety/depression symptoms in a prospective, 4-wave longitudinal twin study with parent and self-reported assessment at ages at ages 8–9, 13–14, 16–17, and 19–20 years. When doing so, heritability estimates for anxiety/depression were high at all ages, ranging from 72% to 89% with no evidence for shared environmental influences.[65] In a comprehensive systematic review of longitudinal twin-studies,[53] evidence from 18 studies of various internalizing conditions, spanning the age range of 4–18 years, generally converged to highlight that genetic factors are predominant in driving stability, and new non-shared environmental factors in driving change in anxiety, fear, obsessive-compulsive, and depressive symptoms from childhood into adolescence. Genetic innovation appears to be of less importance for internalizing disorders and traits, and has in several studies been found to be limited to childhood, suggesting that the emergence of new genetic factors attenuates later in development.[53]

In summary, evidence from longitudinal twin studies have shown that stability in psychiatric disorder and traits across development is often largely explained by genetic stability, whereas environmental factors are more likely to be age-specific and responsible for change.[53] Age-dependent changes in heritability have been found for some internalizing and externalizing childhood psychiatric disorders and traits. Nevertheless, changes in raters (i.e., parent, teachers or self-report) and assessments instruments across time can also influence results in longitudinal studies and require careful considerations when interpreting evidence from longitudinal samples.[64] To date, there is still a lack of prospectively collected twin data with sufficient follow-up time for many important child and adolescent psychiatric disorders, most notably for ASD and other neurodevelopmental disorders.

17.6 Environmental influences on the developmental of childhood psychiatric disorders and traits

Twin studies have provided important insights on the role of the environment in the development of childhood-onset psychiatric disorders and traits. First, twin studies provide some of the strongest evidence that that environmental factors play a crucial role in childhood psychiatry, since no childhood psychiatric disorder shows 100% heritability. Second, twin studies allow researchers to study the different pathways through which environments influence the development childhood psychiatric disorders and traits.[66] One surprising, yet robust, finding from twin studies is that the shared family environment only accounts for a small amount of variance for many

childhood psychiatric disorders. In a meta-analyses of over 490 twin and adoption studies of childhood and adolescent psychopathology, shared familial factors (*C*) were found to account for 10%–19% of the variance in conduct disorder, oppositional defiant disorder, anxiety, depression, and broad dimensions of internalizing and externalizing problems. ADHD was the only childhood disorder to show no influence of C, suggesting that familial resemble for ADHD is solely driven by genetic factors.[15] Results also showed that the influence of the non-shared environment (*E*) was greater than *C* for all the included child psychiatric disorder. This does not mean that early family environment is unimportant, but rather it suggests that children from the same family are impacted by the shared environment in different ways. Differences in how children respond to their environment may in turn be partly driven by genetic differences, a phenomenon referred to gene–environment interplay.

A substantial number of twin studies have explored the relative contribution of genetic and environmental influences on many putative environmental measures, such as videotaped observations of parenting, as well as self-report measures of parenting styles and life events.[67] These studies generally reveal pervasive evidence for genetic influences across all types of environmental measures. A meta-analysis of twin studies reported an average heritability estimate of 27% across 35 different environmental measures. This included not only measures of the family environment, but also measures of peer groups, classroom environments, neighborhood characteristics, and life events.[68] Finding genetic influences on environmental measures provide evidence for gene–environment correlation (*rGE*), which refers *to a correlation between* a person's genetic disposition and the environments they inhabit. Three forms of *rGE* have been described within the twin- and family-research literature: passive, active, and evocative.[69] First, passive *rGE* describes the association between an individual's genotype and the environment in which the individual is raised, both of which are provided by the individual's biological parents. For example, a child may inherit genetic factors involved in conduct problems from his or her parent in whom the same genetic factors may be involved in harsh parental discipline. Second, active *rGE* involves the genetically influenced behavior of the individual seeking out an environment that "matches" the individual's genotype. For example, a child's genetically influenced conduct problems may lead the child to actively seek confrontation with their parents. Third, evocative *rGE* involves the genetically influenced behavior of the individual seeking or evoking a particular response from the environment. For example, a child's genetically influenced conduct problems may evoke harsh discipline from their parents.

One useful approach to further understand the processes that underlies *rGE* involves multivariate twin analysis, described in previous sections, using data from an environmental measure and a measure of a child psychiatric disorder or trait. In this approach, any type of *rGE* (passive, evocative, or active) is indicated if genetic effects on the environmental measure overlap with genetic effects on the measured psychiatric disorder or trait. In one twin study, bivariate genetic analysis was used to test the contribution of genetic and environmental factors to the association between parent–child hostility and ADHD symptoms in a twin sample of 886 twin pairs, aged 11–17 years. Results revealed that reported associations between parent–child

hostility and child ADHD symptoms could be largely attributed to genetic factors.[70] Another study using this approach indicated that a substantial part of the association between parental negativity and antisocial behavior and depressive symptoms in offspring could be attributed to genetic factors.[71] Burt et al[72] fitted a cross-lagged twin model to data on parent–child conflict and children's antisocial behavior assessed by maternal reports as well as child reports. Both parent–child conflict and antisocial behavior at age 11 were found to independently predict the other at age 14, providing support for a bidirectional effect model that allows for both parent-driven and child-driven processes. In addition, the result indicated that both the parent-driven and child-driven effects were a function of both genetic and environmental influences. Thus, this study suggests, on the one hand, that parent–child conflict contributes to childhood antisocial behavior via environmental mechanisms, but also that genetically influenced antisocial behavior evoke parent–child conflict. Similar findings of bidirectional effect for antisocial behavior has been observed in other samples and with other measures of parenting,[73] whereas genetically influenced child-effects has been found to have a more pronounced effect for internalizing problems.[74] Although several twin studies have explored the role of rGE for measures of ADHD, depression, and antisocial behaviors, little is known about such processes for the development of ASD.

One implication of *rGE* is that the correlation between a putative environmental measure and a child psychiatric disorder may not necessary reflect environmental causation. In other words, the association between an environmental risk factor and a child psychiatric outcome may be confounded by genetic factors. Disentangling whether an association between environmental risk factors and child psychiatric outcomes reflect environmental causation, genetic and/or environmental confounding, or are indicative of other complex reciprocal processes is crucial to further our understanding of the etiology and course of child psychiatric disorders, and has important implications for prevention strategies. One useful approach to address such questions is the *co-twin control design*. This is a special type of twin design that allows for a focused test of the potential influence of genetic and environmental confounds underlying the association between a specific risk factor and an outcome.[75] Specifically, twin siblings share genes (i.e., monozygotic, MZ, twin pairs have identical genomes, whereas dizygotic, DZ, twin pairs share on the average 50% of their segregating genes), intrauterine exposures, maternal factors, early environments and have identical gestational age. If an association between a specific risk factor and an outcome in a cohort of twins remains at the within-twin pair level, then factors specific to each individual is probably involved in the underlying causal pathway. In contrast, if the association disappears or substantially attenuates in the within-twin pair comparison, then familial factors (genetic and/or shared environments) are supported.[75] A number of co-twin control studies in childhood have explored the association between birth weight and ADHD. These studies have overall demonstrated that lighter-born twins have elevated ADHD symptoms compared to heavier-born twins,[76–80] which suggest that environmental effect involved in lower birth weight are in the causal pathway to elevated risk of ADHD symptoms. Another useful method to study genetic and

environmental confounds in associations between parent and child characteristics is the *children-of-twins (CoT) design.* Whilst there are several types of *CoT* designs, all involve using samples of twin parents and their children in order to distinguish genetic and environmental effects on the association between parents and children phenotypes. This is achieved by making comparisons of the relative magnitude of a series of intrafamilial correlations.[81,82] For example, *CoT* studies that include both MZ and DZ twin parents contrast the magnitude of the correlation between MZ and DZ twin parents, across their children and across parent–child, allowing for the estimation of the genetic and environmental contribution to the parent phenotype, the child phenotype, and the phenotypic association between the parent and child.[82] *CoT* studies suggests that associations between parent and child anxiety and depression appear almost entirely explained by environmental transmission rather than genetics, suggesting that growing up with parent with anxiety or depression increases the risk of offspring risk of emotional disorders even after accounting for shared genetic factors between parent and child.[82–84] In contrast, two *CoT* studies have found that intergenerational transmission of antisocial behaviors is explained by both genetic and environmental factor, with parental.[85,86]

In summary, twin studies have shown that there is a complex relationship between genes and environments in the development of child psychiatric disorders and traits. Clearly, environment is not an easily defined concept. Twin studies have shown that virtually all environmental factors suggested to be associated with child psychiatric disorders are, to some extent, influenced by genetic factors. However, twin studies have also been vital in identifying environmental factors that show persistent association, even after controlling for genetic confounding.

17.7 Implications & concluding remarks

In this chapter, we have described how twin research has advanced the understanding of how genetic and environmental factors contribute to developmental stability and change, causes of comorbidity, and complex gene–environment interplay for child psychiatric disorders. The key question is no longer "if" genetic factors are important, but rather "how" genetic factors influence the development of child psychiatric disorders and traits across time and in interplay with environmental factors. We conclude that multivariate and longitudinal quantitative genetic methods can be used to address fundamental questions about the etiology of child psychiatric disorders and traits across different levels of symptom severity, comorbidity, and development, as well as the influence of gene–environment interplay in child psychiatry. Several criticisms have (mostly historically) been raised against twins studies,[87–89] primarily questioning the so-called "equal environment assumption." However, numerous studies aiming to evaluate this (and other) assumption(s) converge to suggest that the twin method provide largely valid estimates.[90–93] Importantly, advances in molecular genetic techniques in recent years have replicated many findings from twin research,[32, 94–96] providing further support for the validity of the twin method. This

is essential, given that molecular genetic studies are very suitable for studying the genetics of constructs which are easily measured in very large samples, preferably hundreds of thousands of individuals (e.g., educational attainment or broad concepts of psychopathology), whereas many of the burning issues in the development of psychopathology requires more in-depth data collections. Thus, we believe that twin studies will continue to play a crucial role in research of child psychiatric disorders and traits also in the future. We have in this chapter highlighted several questions that need to be addressed in future quantitative genetic research. In particular, there is a lack of twin studies with long-term follow-up for many important forms of child and adolescent psychopathology, and more twin research is needed to clarify the role of gene–environment interplay and the impact of genetic confounding for associations between putative "environmental" risk factors and different forms of child psychiatric disorders and traits. A better understanding of genetic confounding is an important step toward identifying new, early intervention targets.

References

1. Verhulst FC, Tiemeier H. Epidemiology of child psychopathology: major milestones. *European Child & Adolescent Psychiatry*. 2015;24:607–617.
2. Polderman TJ, Benyamin B, de Leeuw CA, Sullivan PF, van Bochoven A, Visscher PM, Posthuma D. Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nature Genetics*. 2015;47(7):702–709.
3. Ronald A, Hoekstra RA. Autism spectrum disorders and autistic traits: a decade of new twin studies. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2011;156:255–274.
4. Bailey A, Phillips W, Rutter M. Autism: towards an integration of clinical, genetic, neuropsychological, and neurobiological perspectives. *Journal of child psychology and psychiatry, and allied disciplines*. 1996;37:89–126.
5. Smalley SL, Asarnow RF, Spence MA. Autism and genetics. A decade of research. *Archives of General Psychiatry*. 1988;45:953–961.
6. Tick B, Bolton P, Happe F, Rutter M, Rijsdijk F. Heritability of autism spectrum disorders: a meta-analysis of twin studies. *Journal of child psychology and psychiatry, and allied disciplines*. 2016;57:585–595.
7. Polanczyk GV, Willcutt EG, Salum GA, Kieling C, Rohde LA. ADHD prevalence estimates across three decades: an updated systematic review and meta-regression analysis. *International Journal of Epidemiology*. 2014;43(2):434–442.
8. Nikolas MA, Burt SA. Genetic and environmental influences on ADHD symptom dimensions of inattention and hyperactivity: a meta-analysis. *Journal of Abnormal Psychology*. 2010;119:1–17.
9. Posthuma D, Polderman TJ. What have we learned from recent twin studies about the etiology of neurodevelopmental disorders? *Current Opinion in Neurology*. 2013;26:111–121.
10. Lichtenstein P, Carlstrom E, Rastam M, Gillberg C, Anckarsater H. The genetics of autism spectrum disorders and related neuropsychiatric disorders in childhood. *The American Journal of Psychiatry*. 2010;167:1357–1363.
11. Ooki S. Genetic and environmental influences on stuttering and tics in Japanese twin children. *Twin Research and Human Genetics*. 2005;8:69–75.

12. Bolton D, Rijsdijk F, O'Connor TG, Perrin S, Eley TC. Obsessive–compulsive disorder, tics and anxiety in 6-year-old twins. *Psychological Medicine*. 2007;37:39–48.
13. Haworth CMA, Kovas Y, Harlaar N, et al. Generalist genes and learning disabilities: a multivariate genetic analysis of low performance in reading, mathematics, language and general cognitive ability in a sample of 8000 12-year-old twins. *Journal of Child Psychology and Psychiatry*. 2009;50:1318–1325.
14. Plomin R, Kovas Y. Generalist genes and learning disabilities. *Psychological Bulletin*. 2005;131:592–617.
15. Burt SA. Rethinking environmental contributions to child and adolescent psychopathology: a meta-analysis of shared environmental influences. *Psychological Bulletin*. 2009;135:608–637.
16. Rhee SH, Waldman ID. Genetic and environmental influences on antisocial behavior: a meta-analysis of twin and adoption studies. *Psychological Bulletin*. 2002;128:490–529.
17. Gregory AM, Eley TC. Genetic influences on anxiety in children: what we've learned and where we're heading. *Clinical Child and Family Psychology Review*. 2007;10:199–212.
18. Merikangas KR, He J-p, Burstein M, et al. Lifetime prevalence of mental disorders in US adolescents: results from the national comorbidity study-adolescent supplement (NCS-A). *Journal of the American Academy of Child and Adolescent Psychiatry*. 2010;49:980–989.
19. Waszczuk MA, Zavos HMS, Gregory AM, Eley TC. The phenotypic and genetic structure of depression and anxiety disorder symptoms in childhood, adolescence, and young adulthood. *JAMA Psychiatry*. 2014;71:905–916.
20. Thapar A, McGuffin P. Are anxiety symptoms in childhood heritable? *Journal of Child Psychology and Psychiatry*. 1995;36:439–447.
21. Lau JYF, Eley TC, Stevenson J. Examining the state-trait anxiety relationship: a behavioural genetic approach. *Journal of Abnormal Child Psychology*. 2006;34:18.
22. Bourdon JL, Savage JE, Verhulst B, et al. The genetic and environmental relationship between childhood behavioral inhibition and preadolescent anxiety. *Twin Res Hum Genet*. 2019;22:48–55.
23. Chen J, Yu J, Li X, Zhang J. Genetic and environmental contributions to anxiety among Chinese children and adolescents—a multi-informant twin study. *Journal of Child Psychology and Psychiatry*. 2015;56:586–594.
24. Thapar A, Rice F. Twin studies in pediatric depression. *Child and Adolescent Psychiatric Clinics*. 2006;15:869–881.
25. Rice F. Genetics of childhood and adolescent depression: insights into etiological heterogeneity and challenges for future genomic research. *Genome Medicine*. 2010;2:68.
26 Rice F, Harold G, Thapar A. The genetic aetiology of childhood depression: a review. *Journal of Child Psychology and Psychiatry*. 2002;43:65–79.
27. DeFries JC, Fulker DW. Multiple regression analysis of twin data: etiology of deviant scores versus individual differences. *Acta Geneticae Medicae Et Gemellologiae*. 1988;37:205–216.
28. Larsson H, Anckarsater H, Rastam M, Chang Z, Lichtenstein P. Childhood attention-deficit hyperactivity disorder as an extreme of a continuous trait: a quantitative genetic study of 8,500 twin pairs. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*. 2012;53:73–80.
29. Levy F, Hay D, McStephen M, Wood C, Waldman I. Attention-deficit hyperactivity disorder: a category or a continuum? Genetic analysis of a large-scale twin study. *Journal of the American Academy of Child and Adolescent Psychiatry*. 1997;36:737–744.

30. Lundström S, Chang Z, Kerekes N, Gumpert CH, Råstam M, Gillberg C. Autistic-like traits and their association with mental health problems in two nationwide twin cohorts of children and adults. *Psychological Medicine*. 2011;41:2423–2433.
31. Colvert E, Tick B, McEwen F. Heritability of autism spectrum disorder in a UK population-based twin sample. *JAMA Psychiatry*. 2015;72:415–423.
32. Martin J, Taylor MJ, Lichtenstein P. Assessing the evidence for shared genetic risks across psychiatric disorders and traits. *Psychological Medicine*. 2018;48(11):1759–1774.
33. Ronald A, Simonoff E, Kuntsi J, Asherson P, Plomin R. Evidence for overlapping genetic influences on autistic and ADHD behaviours in a community twin sample. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*. 2008;49:535–542.
34. Franić S, Middeldorp CM, Dolan CV, Ligthart L, Boomsma DI. Childhood and adolescent anxiety and depression: beyond heritability. *Journal of the American Academy of Child & Adolescent Psychiatry*. 2010;49:820–829.
35. Eley TC, Stevenson J. Exploring the covariation between anxiety and depression symptoms: a genetic analysis of the effects of age and sex. *Journal of Child Psychology and Psychiatry*. 1999;40:1273–1282.
36. Thapar A, McGuffin P. Anxiety and depressive symptoms in childhood–a genetic study of comorbidity. *Journal of Child Psychology and Psychiatry*. 1997;38:651–656.
37. Middeldorp CM, Cath DC, Van Dyck R, Boomsma DI. The co-morbidity of anxiety and depression in the perspective of genetic epidemiology. A review of twin and family studies. *Psychological Medicine*. 2005;35:611–624.
38. Andersson A, Tuvblad C, Chen Q, Du Rietz E, Cortese S, Kuja-Halkola R. Research review: the strength of the genetic overlap between ADHD and other psychiatric symptoms—a systematic review and meta-analysis. *Journal of Child Psychology and Psychiatry*. 2020;61(11):1173-1183.
39. Rijsdijk FV, Sham PC. Analytic approaches to twin data using structural equation models. *Briefings in Bioinformatics*. 2002;3:119–133.
40. Lahey BB, Van Hulle CA, Singh AL, Waldman ID, Rathouz PJ. Higher-order genetic and environmental structure of prevalent forms of child and adolescent psychopathology. *Archives of General Psychiatry*. 2011;68:181–189.
41. Pettersson E, Anckarsater H, Gillberg C, Lichtenstein P. Different neurodevelopmental symptoms have a common genetic etiology. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*. 2013;54:1356–1365.
42. Waldman ID, Poore HE, van Hulle C, Rathouz PJ, Lahey BB. External validity of a hierarchical dimensional model of child and adolescent psychopathology: tests using confirmatory factor analyses and multivariate behavior genetic analyses. *Journal of Abnormal Psychology*. 2016;125:1053–1066.
43. Lee PH, Anttila V, Won H, et al. Genomic relationships, novel loci, and pleiotropic mechanisms across eight psychiatric disorders. *Cell*. 2019;179:1469–1482 e11.
44. Grotzinger AD, Rhemtulla M, de Vlaming R, et al. Genomic structural equation modelling provides insights into the multivariate genetic architecture of complex traits. *Nature Human Behaviour*. 2019;3:513–525.
45. Boedhoe PSW, Rooij Dv, Hoogman M, et al. Subcortical brain volume, regional cortical thickness, and cortical surface area across disorders: findings from the ENIGMA ADHD, ASD, and OCD working groups. *American Journal of Psychiatry*. 2020;177:834–843.
46. Faraone SV, Biederman J, Mick E. The age-dependent decline of attention deficit hyperactivity disorder: a meta-analysis of follow-up studies. *Psychological Medicine*. 2006;36:159–165.

47. Neale MC, Cardon LR. *Methodology for Genetic Studies of Twins and Families*. Doordrecht, The Netherlands: Kluwer Academic Publishers; 1992.
48. Kuntsi J, Rijsdijk F, Ronald A, Asherson P, Plomin R. Genetic influences on the stability of attention-deficit/hyperactivity disorder symptoms from early to middle childhood. *Biological Psychiatry*. 2005;57:647–654.
49. Pingault J, Viding E, Galéra C. Genetic and environmental influences on the developmental course of attention-deficit/hyperactivity disorder symptoms from childhood to adolescence. *JAMA Psychiatry*. 2015;72:651–658.
50. Larsson H, Lichtenstein P, Larsson JO. Genetic contributions to the development of ADHD subtypes from childhood to adolescence. *Journal of the American Academy of Child and Adolescent Psychiatry*. 2006;45:973–981.
51. Chang Z, Lichtenstein P, Asherson PJ, Larsson H. Developmental twin study of attention problems: high heritabilities throughout development. *JAMA Psychiatry*. 2013;70:311–318.
52. Rietveld MJ, Hudziak JJ, Bartels M, van Beijsterveldt CE, Boomsma DI. Heritability of attention problems in children: longitudinal results from a study of twins, age 3 to 12. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*. 2004;45:577–588.
53. Hannigan LJ, Walaker N, Waszczuk MA, McAdams TA, Eley TC. Aetiological influences on stability and change in emotional and behavioural problems across development: a systematic review. *Psychopathology Review*. 2017;a4:52–108.
54. Holmboe K, Rijsdijk FV, Hallett V, Happe F, Plomin R, Ronald A. Strong genetic influences on the stability of autistic traits in childhood. *Journal of the American Academy of Child and Adolescent Psychiatry*. 2014;53:221–230.
55. Taylor MJ, Gillberg C, Lichtenstein P, Lundström S. Etiological influences on the stability of autistic traits from childhood to early adulthood: evidence from a twin study. *Molecular Autism*. 2017;8:5.
56. Lewis GJ, Plomin R. Heritable influences on behavioural problems from early childhood to mid-adolescence: evidence for genetic stability and innovation. *Psychological Medicine*. 2015;45:2171–2179.
57. Tuvblad C, Eley TC, Lichtenstein P. The development of antisocial behaviour from childhood to adolescence. *European Child & Adolescent Psychiatry*. 2005;14:216–225.
58. Meier MH, Slutske WS, Heath AC, Martin NG. Sex differences in the genetic and environmental influences on childhood conduct disorder and adult antisocial behavior. *Journal of Abnormal Psychology*. 2011;120:377–388.
59. Wesseldijk LW, Bartels M, Vink JM, van Beijsterveldt CEM, Ligthart L, Boomsma DI. Genetic and environmental influences on conduct and antisocial personality problems in childhood, adolescence, and adulthood. *European Child & Adolescent Psychiatry*. 2018;27:1123–1132.
60. Bergen SE, Gardner CO, Kendler KS. Age-related changes in heritability of behavioral phenotypes over adolescence and young adulthood: a meta-analysis. *Twin Res Hum Genet*. 2007;10:423–433.
61. Nivard MG, Dolan CV, Kendler KS, Kan KJ, Willemsen G, van Beijsterveldt CEM. Stability in symptoms of anxiety and depression as a function of genotype and environment: a longitudinal twin study from ages 3 to 63 years. *Psychological Medicine*. 2014;45:1039–1049.
62. Boomsma DI, van Beijsterveldt CE, Hudziak JJ. Genetic and environmental influences on anxious/depression during childhood: a study from the Netherlands Twin Register. *Genes, Brain, and Behavior*. 2005;4:466–481.

63. Patterson MW, Mann FD, Grotzinger AD, Tackett JL, Tucker-Drob EM, Harden KP. Genetic and environmental influences on internalizing psychopathology across age and pubertal development. *Developmental Psychology*. 2018;54:1928–1939.
64. Kan KJ, van Beijsterveldt CE, Bartels M, Boomsma DI. Assessing genetic influences on behavior: informant and context dependency as illustrated by the analysis of attention problems. *Behavior Genetics*. 2014;44:326–336.
65. Kendler KS, Gardner CO, Lichtenstein P. A developmental twin study of symptoms of anxiety and depression: evidence for genetic innovation and attenuation. *Psychological Medicine*. 2008;38:1567–1575.
66. Plomin R, DeFries JC, Knopik VS, Neiderhiser JM. Top 10 replicated findings from behavioral genetics. *Perspectives on Psychological Science : a Journal of the Association for Psychological Science*. 2016;11:3–23.
67. Plomin R, Bergeman CS. The nature of nurture: genetic influence on "environmental" measures. *Behavioral and Brain Sciences*. 2011;14:373–386.
68. Kendler KS, Baker JH. Genetic influences on measures of the environment: a systematic review. *Psychological Medicine*. 2007;37:615–626.
69. Plomin R, DeFries JC, Knopik VS, Neiderhiser J. *Behavioral Genetics*. New York: Worth Publishers; 2013.
70. Lifford KJ, Harold GT, Thapar A. Parent-child hostility and child ADHD symptoms: a genetically sensitive and longitudinal analysis. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*. 2009;50:1468–1476.
71. Pike A, McGuire S, Hetherington EM, Reiss D, Plomin R. *Family Environment and Adolescent Depressive Symptoms and Antisocial Behavior: A Multivariate Genetic Analysis: US*: American Psychological Association; 1996.
72. Burt SA, McGue M, Krueger RF, Iacono WG. Sources of covariation among the child-externalizing disorders: informant effects and the shared environment. *Psychological Medicine*. 2005;35:1133–1144.
73. Larsson H, Viding E, Rijsdijk FV, Plomin R. Relationships between parental negativity and childhood antisocial behavior over time: a bidirectional effects model in a longitudinal genetically informative design. *Journal of Abnormal Child Psychology*. 2008;36:633–645.
74. Moberg T, Lichtenstein P, Forsman M, Larsson H. Internalizing behavior in adolescent girls affects parental emotional overinvolvement: a cross-lagged twin study. *Behavior Genetics*. 2011;41:223–233.
75. McGue M, Osler M, Christensen K. Causal inference and observational research: the utility of twins. *Perspect Psychol Sci*. 2010;5:546–556.
76. Asbury K, Dunn JF, Plomin R. Birthweight-discordance and differences in early parenting relate to monozygotic twin differences in behaviour problems and academic achievement at age 7. *Developmental Science*. 2006;9:F22–F31.
77. Ficks CA, Lahey BB, Waldman ID. Does low birth weight share common genetic or environmental risk with childhood disruptive disorders? *Journal of Abnormal Psychology*. 2013;122:842–853.
78. Groen-Blokhuis MM, Middeldorp CM, van Beijsterveldt CE, Boomsma DI. Evidence for a causal association of low birth weight and attention problems. *Journal of the American Academy of Child and Adolescent Psychiatry*. 2011;50:1247–1254 e2.
79. Lehn H, Derks EM, Hudziak JJ, Heutink P, van Beijsterveldt T, Boomsma DI. Attention problems and attention-deficit/hyperactivity disorder in discordant and concordant monozygotic twins: evidence of environmental mediators. *Journal of the American Academy of Child and Adolescent Psychiatry*. 2007;46:83–91.

80. Pettersson E, Sjölander A, Almqvist C, Anckarsäter H, D'Onofrio BM, Lichtenstein P. Birth weight as an independent predictor of ADHD symptoms: a within-twin pair analysis. *Journal of Child Psychology and Psychiatry, and Allied Disciplines.*. 2015;56:453–459.
81. D'Onofrio BM, Turkheimer EN, Eaves LJ, Corey LA, Berg K, Solaas MH. The role of the children of twins design in elucidating causal relations between parent characteristics and child outcomes. *Journal of Child Psychology and Psychiatry*. 2003;44:1130–1144.
82. McAdams TA, Neiderhiser JM, Rijsdijk FV, Narusyte J, Lichtenstein P, Eley TC. Accounting for genetic and environmental confounds in associations between parent and child characteristics: a systematic review of children-of-twins studies. *Psychological Bulletin*. 2014;140:1138–1173.
83. McAdams TA, Rijsdijk FV, Neiderhiser JM, Narusyte J, Shaw DS, Natsuaki MN. The relationship between parental depressive symptoms and offspring psychopathology: evidence from a children-of-twins study and an adoption study. *Psychological Medicine*. 2015;45:2583–2594.
84. Eley TC, McAdams TA, Rijsdijk FV, Lichtenstein P, Narusyte J, Reiss D. The intergenerational transmission of anxiety: a children-of-twins study. *American Journal of Psychiatry*. 2015;172:630–637.
85. D'Onofrio BM, Slutske WS, Turkheimer E, Emery RE, Harden KP, Heath AC. Intergenerational transmission of childhood conduct problems: a children of twins study. *Archives of General Psychiatry*. 2007;64:820–829.
86. Silberg JL, Maes H, Eaves LJ. Unraveling the effect of genes and environment in the transmission of parental antisocial behavior to children's conduct disturbance, depression and hyperactivity. *Journal of Child Psychology and Psychiatry, and Allied Disciplines.*. 2012;53:668–677.
87. Burt CH, Simons RL. Pulling back the curtain on heritability studies: biosocial criminology in the postgenomic era. *Criminology: An Interdisciplinary Journal*. 2014;52:223–262.
88. Lewontin RC, Rose S, Kamin LJ. *Not in Our Genes*. New York, NY: Pantheon Books; 1984.
89. Richardson K, Norgate S. The equal environments assumption of classical twin studies may not hold. *British Journal of Educational Psychology*. 2005;75:339–350.
90. Conley D, Rauscher E, Dawes C, Magnusson PKE, Siegal ML. Heritability and the equal environments assumption: evidence from multiple samples of misclassified twins. *Behavior Genetics*. 2013;43:415–426.
91. Felson J. What can we learn from twin studies? A comprehensive evaluation of the equal environments assumption. *Social Science Research*. 2014;43:184–199.
92. Barnes JC, Wright JP, Boutwell BB, Schwartz JA, Connolly EJ, Nedelec JL. Demonstrating the validity of twin research in criminology. *Criminology: An Interdisciplinary Journal*. 2014;52:588–626.
93. Kendler KS, Neale MC, Kessler RC, Heath AC. Eaves LJ. A test of the equal-environment assumption in twin studies of psychiatric illness. *Behavior Genetics*. 1993;23:21–27.
94. Faraone SV, Larsson H. Genetics of attention deficit hyperactivity disorder. *Molecular Psychiatry*. 2018.
95. Plomin R, Simpson MA. The future of genomics for developmentalists. *Development and Psychopathology*. 2013;25(25th Anniversary Special Issue 4pt2):1263–1278.
96. Sullivan PF, Agrawal A, Bulik CM, Andreassen OA, Børglum AD, Breen G. Psychiatric genomics: an update and an agenda. *The American journal of psychiatry*. 2018;175:15–27.

CHAPTER

18

Happiness and well-being: The value and findings from genetic studies

Margot P. van de Weijer[a,b], Lianne P. de Vries[a,b], Meike Bartels[a,b]

[a]*Department of Biological Psychology, Faculty of Behavioural and Movement Sciences, Vrije Universiteit, Amsterdam, The Netherlands*

[b]*Amsterdam Public Health Research Institute, Amsterdam University Medical Centre, Amsterdam, The Netherlands*

18.1 What is well-being?

Within the past decade, there has been increasing interest in well-being (WB) as a research topic across different disciplines, including the field of behavior genetics. Moreover, there is a growing global recognition of WB as an important public policy goal, as shown through population-based surveys initiated by governments with the aim of systematic consideration of WB to inform decisions.[1–3] In this chapter, we discuss the relevance of behavioral genetic (twin-)studies to increase our understanding of individual differences in WB.

The term "well-being" embodies a multitude of concepts with varying meanings depending on context and discipline. Here, we focus on the meaning of WB as employed in psychology and social sciences. It is important, though, to first briefly mention its philosophical origin. Two ancient philosophical traditions are relevant in this context: hedonism and eudaimonism.[4] The hedonist tradition dates back several centuries before Christ, to philosophers such as the Cyrenaics who believed that pleasure was the highest good, and central to happiness or WB.[5] Thus, in the ancient hedonist definition, WB or happiness is equated to the sum of one's pains and pleasures. Eudaimonism, on the other hand, has a definition that is quite different from the hedonist perspective. Influenced by Aristotle's virtue ethics, the eudaimonic view on happiness centers around living a virtuous life.[6] From this point of view, the greatest fulfillment in life will come with the realization of one's potential and finding meaning in life. These descriptions provide only a brief overview of the two philosophies, but they do illustrate the appreciable distinction that exists between their definitions of WB.

In the current psychological literature, a distinction is often made between "subjective" well-being (SWB) and "psychological" well-being (PWB). This distinction can be traced back to the ancient distinction between hedonic and eudaimonic WB,

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00016-7

with SWB following from the hedonic tradition and PWB from the eudaimonic tradition. While different definitions exist, SWB is mostly characterized by high levels of positive affect, and low levels of negative affect, translating into a subjective evaluation of high satisfaction with life.[7] While life satisfaction reflects a more cognitive evaluation of WB that is not necessarily in line with hedonist ideas about happiness, the positive and negative affect dimensions of SWB are highly similar to the hedonist ideas about balancing pleasure and pains. Similar to how the eudaimonic definition was formulated as a response to the hedonic definition, the PWB definition was formulated as a response to the SWB definition. A critique of the SWB definition is that it does not capture important aspects of positive psychological functioning, such as self-fulfillment.[8] Therefore, PWB definitions of WB aim to include broader domains of positive functioning. For example, in Carol Ryff's definition of PWB, included domains are self-acceptance, positive relations with others, autonomy, environmental mastery, purpose in life, and personal growth.[8]

Theoretically, the distinction between SWB and PWB is clear. Empirically, however, the distinction is less clear-cut. While most research finds that WB is comprised of multiple related, yet conceptually distinct underlying dimensions,[9,10] discussion remains concerning the extent to which these underlying factors are correlated. Moreover, results indicate that there is a large overlap in the set of genes that influence SWB and PWB, with a higher genetic correlation than phenotypic correlation.[11,12] Additionally, many different measurement instruments are available to assess (different aspects of) WB.[13,14] This further complicates our interpretation of the interrelatedness of different WB constructs as these different measurement instruments might introduce additional variance.

Twin studies help us understand WB in multiple ways. First and foremost, by partitioning the variance of WB into genetic and environmental sources of variation, twin studies enable us to interpret the causes of individual differences in WB (Sections 18.2 and 18.3). Second, by examining the genetic and environmental sources of variation in phenotypes highly related to WB, we come one step closer to understanding the complexities of the WB construct (Section 18.4). The knowledge gained from existing twin studies of WB has fueled follow-up in-depth analyses in both genetic and environmental directions (Section 18.5), which again have led to the development of novel, more complex twin designs (Section 18.6). In what follows, we present these past, present, and future directions of research, demonstrating the transformational effect this research has had on our understanding of WB.

18.2 Earlier reviews on twin studies on well-being

In 2015, two comprehensive reviews on the causes of individual differences in WB were published.[15,16] Results of these twin-family studies into the genetic and environmental influences on WB revealed a range of heritability estimates, but when meta-analyses were used to estimate heritability across the studies the meta-analytic results converged on the heritability estimate. In the book chapter of Nes and

Roysamb, the weighted average heritability, across 13 independent studies including more than 30,000 twins (aged 12–88) from seven different countries, was estimated to be 40% (CI: 37%–42%).[16] Similarly, in the paper by Bartels, the weighted average heritability of WB, based on a sample size of 55,974 individuals, was 36% (34%–38%), while the weighted average heritability for satisfaction with life was 32% (29%–35%) (n = 47,750).[15]

These similar results, with an overlapping confidence interval, provide a more robust estimate of the genetic influence on WB. Both reviews and meta-analyses showed that both genetic and environmental influences are important for variation in WB. The meta-analyses indicate that genetic influences on WB are mainly additive and that the environmental influences appear to be nonshared.

18.3 New findings of twin studies on well-being

Since 2015, the twin design has been used in an additional 15 studies to investigate the heritability of WB using different measures of WB, and in combination with other variables, such as depression or social support, as described later. Fig. 18.1A and B summarizes the heritability estimates of all included twin studies in the earlier meta-analyses, and of the recent twin studies on WB. In addition, Table 18.1 summarizes the designs and findings of the recent twin studies of WB. The heritability estimates of the recent studies on WB vary somewhat (range: 0.27–0.67), but are mostly in line with the previous meta-analytic estimates. The effect of a shared environment is small but significant in a few studies in younger participants. In contrast to earlier studies, none of the recent studies reported evidence for nonadditive genetic effects.

Besides investigating the heritability of WB, many recent studies used the bivariate or multivariate approach to investigate the (genetic and environmental) covariance between WB and other variables. For example, Haworth and colleagues[17] reported moderate genetic correlations with depressive symptoms, Wang and colleagues[18] with social support, Wootton and colleagues[19] with positive and negative life events, and Luo et al.[20] reported a moderate genetic correlation with self-enhancement. Van t' Ent and colleagues[21] reported nonsignificant genetic correlations with subcortical brain volumes. In a small Polish twin sample, Milovanović et al.[22] and Sadiković et al.[23] investigated the covariance of life satisfaction with emotion regulation and personality traits. The genetic correlation with various forms of emotion regulation varied between 0.53–0.86. The genetic correlation between WB and personality traits varied from 0 (openness and agreeableness) to 0.61–0.71 (conscientiousness, extraversion, and neuroticism). The heritability of life satisfaction in relation to personality traits has also been investigated by Røysamb and colleagues in a larger sample.[24] The heritability of life satisfaction was estimated at 31% (22%–40%), of which 65% was explained by personality-related genetic influences (mainly neuroticism and extraversion). The remaining genetic variance was unique to life satisfaction.

Thege and colleagues[25] investigated genetic and environmental influences on happiness, life satisfaction, and general WB in a small Hungarian twin sample. The

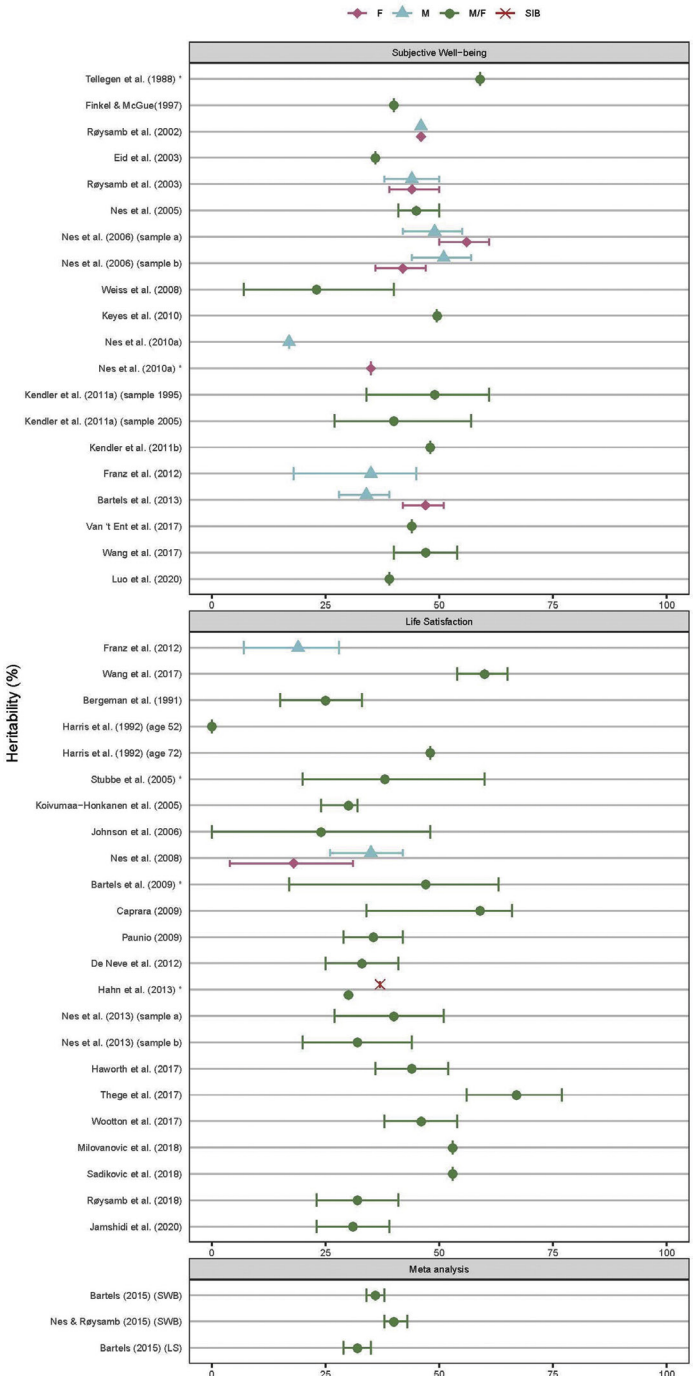


FIG. 18.1 (*Continued*)

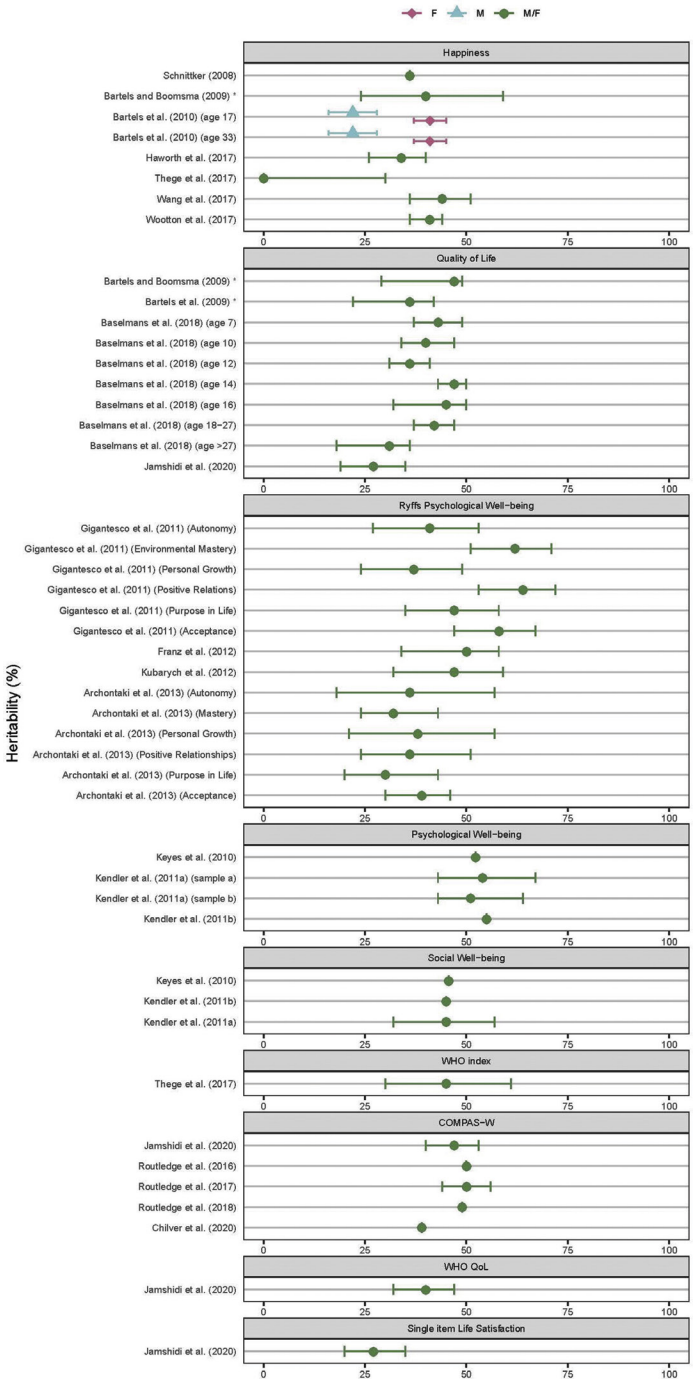


FIG. 18.1

(A) Heritability estimates for well-being. F = females, M = males, F/M = males and females, S = siblings. (B) Heritability estimates for well-being. F = females, M = males, F/M = males and females, S = siblings.

TABLE 18.1 Summary on twin studies for well-being since 2015.

References	Measure	Age	Sex	*N* twin pairs (MZ/DZ)	rMZ	rDZ	A (95 %CI)	C (95 %CI)	E (95 %CI)
Haworth et al.[17]	LS	16.32 (0.68)	M	1346 (693/653)	0.52	0.35	0.44 (0.36–0.52)	0.11 (0.05–0.18)	0.45 (0.42–0.48)
			F	1868 (989/879)	0.60	0.39			
			OS	1480		0.29			
	SHS		M	1347 (691/656)	0.43	0.19	0.34 (0.26–0.40)	0.06 (0.02–0.12)	0.60 (0.57–0.64)
			F	1867 (990/877)	0.41	0.30			
Thege et al. [25]	LS	43.27 (16.3)	M	37 (28/9)	0.67	0.46	0.67 (0.56–0.77)		0.33 (0.23–0.44)
			F	99 (72/27)					
	SHS				0.38	0.39	0.00 (0–0.30)	0.38 (0.10–0.61)	0.62 (0.45–0.82)
	WHO index				0.46	0.15	0.45 (0.30–0.61)		0.55 (0.39–0.70)
Van 't Ent et al.[21	Composite (SAT + HAP)	27.0 (4.1)	M/F	294 (171/123)	0.44	0.16	0.44		0.56
			Single	57 (32/25)					
			Siblings	87					
Wang et al.[18]	Positive Affect	17.85 (0.77)	M/F/OS	1133 (354/779)	0.49	0.19	0.47 (0.40–0.54)		0.53 (0.46–0.60)
	SHS				0.47	0.16	0.44 (0.36–0.51)		0.56 (0.49–0.64)

References	Measure	Age	Sex	*N* twin pairs (MZ/DZ)	rMZ	rDZ	A (95 %CI)	C (95 %CI)	E (95 %CI)
	LS				0.57	0.34	0.60 (0.54–0.65)		0.40 (0.35–0.46)
Wootton et al.[19]	LS	16.32 (0.68)	M	2200 (1142/1058)			0.46 (0.38–0.54)	0.10 (0.04–0.16)	0.44 (0.42–0.47)
			F	3058 (1640/1418)					
			OS	2370					
	SHS						0.41 (0.36–44)		0.59 (0.56–0.62)
Baselmans et al.[11]	Quality of Life		M/F/OS	42427 twins (16089/26338)					
		7			0.85	0.66	0.43 (0.37–0.49)	0.43 (0.37–0.49)	0.13 (0.12–0.16)
		10			0.79	0.62	0.40 (0.34–0.47)	0.41 (0.35–0.46)	0.20 (0.17–0.21)
		12			0.83	0.63	0.36 (0.31–0.41)	0.46 (0.41–0.50)	0.18 (0.17–0.20)
		14			0.46	0.25	0.47 (0.43–0.50)		0.53 (0.50–0.57)
		16			0.47	0.21	0.45 (0.32–0.50)		0.55 (0.51–0.59)
		18-27			0.42	0.16	0.42 (0.37–0.47)		0.58 (0.53–0.63)

(Continued)

TABLE 18.1 *Cont'd*

References	Measure	Age	Sex	*N* twin pairs (MZ/DZ)	rMZ	rDZ	A (95 %CI)	C (95 %CI)	E (95 %CI)
		>27			0.30	0.11	0.31 (0.18–0.36)		0.69 (0.64-0.75)
Reference	Measure	Age	Sex		rMZ	rDZ	A (95 %CI)	C (95 %CI)	E (95 %CI)
Milovanović et al.[22]/	LS	24.59 (7.11)	M	32 (23/9)	0.54	0.42	0.53		0.47
Sadiković et al.[23]			F	122 (98/24)					
			OS	28					
Røysamb et al.[24]	LS	57.11 (4.5)	M	537 (290/247)	0.35	0.05	0.32 (0.23–0.41)		0.68
			F	979 (456/523)	0.29	0.17			
Luo et al.[20]	Latent (LS + Affective WB)	18.29 (1.96)	M/F OS	492 (304/188) 116			0.39	0.12	0.49
Routledge et al.[27]	COMPAS-W	39.8 (12.7)	M	294 (202/92)			0.50		0.50
			F	449 (246/203)					
			OS	1483		0.16			
Routledge et al.[28]	COMPAS-W	39.77 (12.77)	M	598 twins (410/188)	0.51	0.22	0.50 (0.44–0.56)		0.50 (0.44–0.56)
			F	904 twins (496/408)					
Routledge et al.[29]	COMPAS-W	39.65 (12.73)	M	676 twins (472/204)			0.49		0.49
			F	979 twins (567/412)					

References	Measure	Age	Sex	*N* twin pairs (MZ/DZ)	rMZ	rDZ	A (95 %CI)	C (95 %CI)	E (95 %CI)
Chilver et al.[30]	COMPAS-W	40.0 (13.0)	M/F	422 (292/130)	0.43	0.08	0.39		0.61
Jamshidi et al.[31]	COMPAS-W LS WHO (psych QoL) Single item LS Single item QoL	39.64 (12.73)	M/F Single	1660 (968/334) 158			0.47 (0.40–0.53) 0.31 (0.23–0.39) 0.40 (0.32–0.47) 0.27 (0.20–0.35) 0.27 (0.19–0.35)		0.53 (0.47–0.60) 0.69 (0.61–0.77) 0.60 (0.53–0.68) 0.73 (0.65–0.80) 0.73 (0.65–0.81)
Well-being intervention	Composite (SHS + LS)	16.55(0.51)	M	158 (67/91)	0.55	0.32	Before: 0.48 (0.20–0.64)	0.07 (0.0–0.30)	0.44 (0.36–0.55)
Haworth et al.[32]			F	217 (100/117)	0.50	0.25	During: 0.45 (0.19–0.60)	0.06 (0.0–0.26)	0.49 (0.39–0.60)
					0.53	0.20	Follow-up: 0.48 (0.26–0.60)	0.03 (0.0–0.20)	0.49 (0.40–0.60)

SHS, subjective happiness scale; LS, life satisfaction; F, Females; M, Males; OS, opposite sex pairs.

results indicate a heritability of life satisfaction and general WB of 67% and 45% with no shared environmental effects. Happiness had a negligible heritability (0%), whereas 38% of the variance was explained by the shared environment. Due to the small sample, these results should be interpreted with caution.

A recent study in a Dutch twin sample[26] investigated the contribution of genetic and environmental factors on WB and depression across the lifespan. Genetic factors explained a substantial part of the phenotypic variance in WB during childhood, adolescence, and adulthood (range 31%–47%). In the younger samples, shared environmental influences explained a large part of the variation, but these disappeared with age. Regarding the association between WB and depression, the contribution of genetic factors increased from childhood to adolescence, meaning that environmental factors are important in explaining the relationship between WB and depressive symptoms in childhood, while in adolescence genetic factors play a larger role.

Whereas most recent studies used the most popular WB measures (e.g., the Satisfaction with Life Scale, Subjective Happiness Scale, or Cantril ladder), Routledge and colleagues[27–29] designed the COMPAS-W scale. The COMPAS-W scale is a composite index of subjective (hedonic) and psychological (eudaimonic) WB. The heritability of WB measured using this scale was estimated at 50%. Additionally, about half of the genetic influences on WB were shared with symptoms of depression and anxiety. Furthermore, Chilver et al. reported a small genetic association between WB and brain activation, as reflected by electroencephalography (EEG) power.[30] Recently, Jamshidi and colleagues compared the heritability estimates of the COMPWAS-W scale, Satisfaction with Life scale, and single-item measures of life satisfaction and quality of life.[31] Heritability estimates ranged from 23% to 47%, with the heritability of single-item questions being lower than multiple-item scales.

Lastly, Haworth and colleagues[32] investigated the effect of a WB intervention on the genetic and environmental variance components. The intervention lasted 10-weeks and consisted of online kindness and gratitude tasks. WB improved during the intervention and was significantly higher at follow-up. The contribution of genetic influences to the phenotypic variance remained consistent before, during, and after the intervention (respectively, 48%, 45%, and 48%). The contribution of nonshared environmental influences also remained constant, but new nonshared environmental influences emerged over time in response to the intervention. Thus, genetic influences stayed largely the same, whereas new environmental influences explained the changes in WB in response to the intervention.

To summarize, although the studies in the previous meta-analyses and the 15 newer studies use different types of contexts, WB measures, and sample sizes, the results seem to converge on a heritability estimate of about 40 to 50%.

18.4 Related phenotypes

As described in the previous sections, WB is not a unitary construct. Besides the multidimensionality of the construct itself in terms of its definition, there are also many phenotypes that are closely related to WB. We can identify different classes

of these related phenotypes: unfavorable outcomes that are negatively related to WB (e.g., depressive symptoms[26]), related but clearly distinct traits such as personality characteristics,[12] and highly related phenotypes that are sometimes difficult to conceptually separate from WB. In this section, we focus on the insights that twin studies have brought us for this last class of phenotypes. Specifically, we focus on optimism, meaning in life, self-esteem, and resilience.

18.4.1 Optimism

Optimism can be defined as the general expectation of positive versus negative outcomes in different domains of life and is often measured using the Life Orientation Test (LOT) or LOT-revised (LOT-R).[33] In the context of WB, optimism is related to lower negative emotions and higher, positive affect, and life satisfaction.[34–36] A large meta-analysis estimated phenotypic correlations of around 0.50 between optimism and the different aspects of WB.[37]

Fig. 18.2A provides an overview of the heritability estimates reported for optimism from the existing literature. All studies in this figure used the 6-item LOT-R to measure optimism,[19,38–43] with the exception of Plomin et al.[44] and Yuh et al.,[45] in which the 4-item LOT was used, and Mavioğlu et al.[38] and Whitfield et al.[46] whom used the 3-item LOT-R. Plomin et al.[44] were the first to study the causes of individual differences in optimism. Using a twin/adoption design, a heritability of 23% was reported for LOT-measured optimism, with the remaining 77% of the variance being accounted for by nonshared environmental factors. As depicted in Fig. 18.2A, the heritability estimates from later studies do not differ substantially across different studies, even though there is much variability in the confidence intervals.

18.4.2 Meaning in life

The meaning in life construct, like WB, knows many different operationalizations. One popular view on meaning in life is that it is a tripartite structure, consisting of three distinct subdomains: comprehension (one's life making sense), purpose (a sense of direction in life), and mattering (a sense of life having inherent value).[47,48] The relationship between WB and meaning in life is complex. While meaning in life can be viewed as an important *part of* eudaimonic/psychological WB,[8] it might also be interpreted as a route to or consequence of WB.[49,50] A correlation of around 0.50 has been reported between meaning in life and WB, i.e. life satisfaction or psychological WB.[51–53]

There have only been a few twin studies so far focusing on meaning in life (see Fig. 18.2A). As is evident from Fig. 18.2A, most studies find heritability estimates that are medium in effect, ranging from 33% to 52%.[18,19,54] Yet, Thege and colleagues report a heritability of 0% in their analysis of meaning life. However, given that the sample in this study was smaller than the previously described studies, these findings should be interpreted with caution.[41]

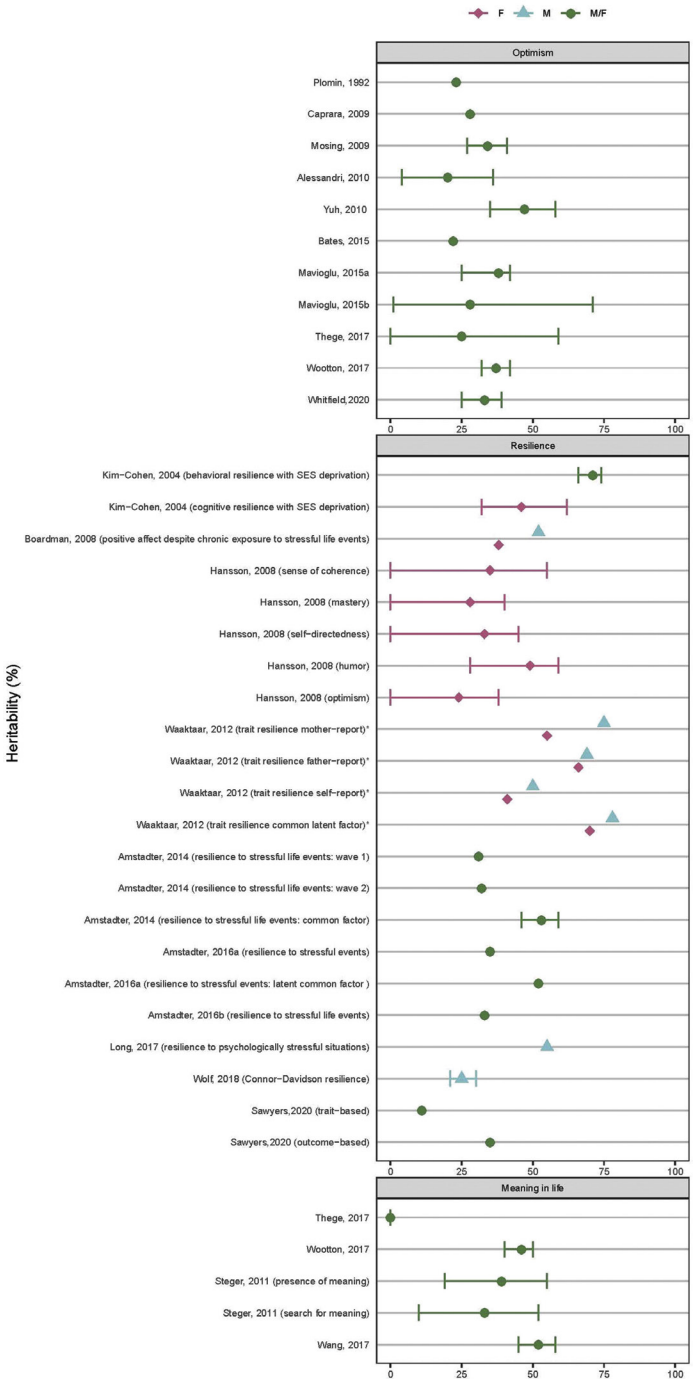


FIG. 18.2 *(Continued)*

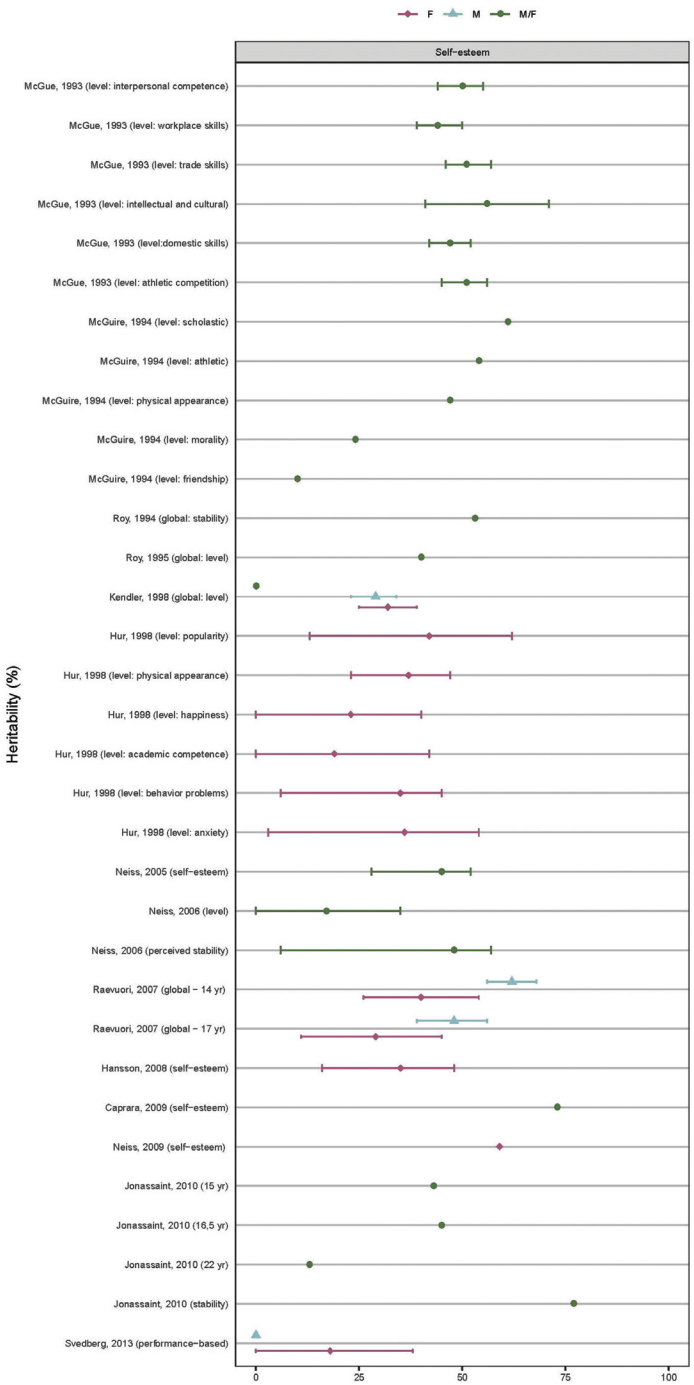


FIG. 18.2

(A) Heritability estimates reported for well-being related phenotypes. F = females, M = males, M/F = males and females. (B) Heritability estimates reported for well-being related phenotypes. F = females, M = males, M/F = males and females.

18.4.3 Self-esteem

The scientific study of self-esteem is one that has produced an abundance of literature. Often measured using the Rosenberg self-esteem scale,[55] self-esteem can be defined as one's affective or evaluative appraisal of the self, or the extent to which a person (dis)likes him- or herself.[56] Two components are central in the assessment of self-esteem: the level (i.e., the general appraisal of yourself), and the stability over time of this appraisal.[57] Moreover, we can interpret an individual's general self-esteem, but within a person self-esteem can also vary across different domains (e.g., intellectual, cultural). The correlation with WB is strong, as estimates of 0.50 and higher were reported.[58,59]

In a literature review performed in 2002, the results from behavioral genetic studies on self-esteem thus far were summarized.[57] For the review, results were split up for the level and stability of self-esteem, and within these categories, for general and domain-specific self-esteem. For the level of self-esteem, the results from different studies did not always converge. Nevertheless, overall it seems that about 30%–40% of individual differences in self-esteem level can be explained by genetic factors and that the remaining variation is accounted for by unique (but not shared) environmental factors. For domain-specific self-esteem levels similar results were reported, with heritability estimates around 50%, and a small or no role for shared environmental influences, both in childhood and adulthood. Yet, depending on which domain was studied, there is quite some variation in the estimates. For example, McGuire et al.[60] examined the level of self-esteem in the following five domains: scholastic competence, athletic competence, physical appearance, morality, and friendship. While the heritability of self-esteem in the scholastic domain was estimated at 61%, the heritability for self-esteem in friendship was substantially lower, 10% (also depicted in Fig. 18.2B). While there was less literature available for self-esteem stability, the heritability of stability in self-esteem seems to be similar to or even higher than the heritability at one time-point, with heritability estimates a little over 50%. This was true for both global self-esteem and domain-specific self-esteem.

Since this meta-analysis, many other twin studies on self-esteem have been published (see Fig. 18.2B). As can be seen in Fig. 18.2B, the heritability estimates varied considerably. However, this is likely due to the different definitions used for self-esteem, and the different age groups examined. For example, Jonassaint reported that in early adulthood, self-esteem is almost completely determined by the unique environment, with no role for genetic factors.[61] Raevuori and colleagues looked at genetic and environmental factors affecting self-esteem in boys and girls from age 14 to 17.[62] Their results show that the heritability of self-esteem is higher in boys than in girls in this age group.

18.4.4 Resilience

Psychological resilience can be defined as an individual's ability to recover after the experience of stress or trauma, returning to an optimal mental state, or as the

psychological outcome after adverse events.[63] Resilience and WB have been associated with many studies with a phenotypic correlation of around 0.50, and especially strong links between resilience and the cognitive and affective components of WB have been reported.[64] Resilience has been studied in different twin studies (Fig. 18.2A), but again with varying definitions of the construct. For example, Kim-Cohen and colleagues investigated individual differences in behavioral and cognitive resilience of children after economic deprivation (defined as lower antisocial behavior and higher IQ than predicted), and reported heritability estimates of 71% and 46%, respectively.[65] Hansson and colleagues performed analyses on specific resilience concepts (sense of coherence, mastery, self-directedness, self-worth, humor, and optimism), and estimated a moderate heritability of around 33%. Analyses of scales aimed at measuring psychological resilience reveal an even greater range of variation in heritability estimates. For example, using the Connor-Davidson Resilience Scale,[66] Wolf and colleagues estimated the explained variance of additive genetic effects and shared environment in military male twins at 25% and 15%, respectively.[67] However, in adolescents, the heritability of a latent resilience factor was estimated at 78% and 70% in boys and girls, using the Ego-Resilience scale.[68,69]

Alternatively, studies may use an outcome-based measure of resilience instead of questionnaires aimed at measuring resilience directly (trait-based).[70] For example, in a first study, resilience was defined as the residual of positive affect after controlling for stressors.[71] The heritability estimates were higher in men (52%) than in women (38%). In addition, Amstadter and colleagues defined resilience as the residual of internalizing symptoms after controlling for the number of stressful life events.[72] At two-time points, the heritability was stable, around 31%, with no sex differences. Sawyers et al. (2020) compared the etiology of trait-based and outcome-based resilience.[73] Only 15% of the heritability of outcome-based resilience was shared with trait-based resilience. In summary, variation in the definition of resilience (and in the sample) leads to a lot of variation in heritability estimates, demonstrating the need for a universal or commonly agreed-upon definition for resilience.

18.4.5 Multivariate models of positive psychological traits

Multivariate twin designs can answer the question how much of the phenotypic correlation between traits is accounted for by genetic and environmental factors. In addition, the overlap in genetic and environmental factors underlying multiple traits can be assessed. In other words, these designs help us understand why traits are related or tend to co-occur.

For example, a study by Caprara and colleagues[40] assessed the associations between self-esteem, optimism, and WB (in terms of life satisfaction). The analyses indicated a large overlap in genetic causes, with genetic correlations between 0.80 and 0.87. Likewise, Wootton and colleagues[19] investigated whether positive life events were genetically associated with SWB and related positive psychological traits including subjective happiness, life satisfaction, optimism, hopefulness, and gratitude measured at the age of 16. The WB traits were positively genetically correlated with

positive life events, and negatively with negative life events. However, these genetic correlations were moderate, ranging approximately from −0.5 to 0.5.

While the above studies are just two examples of studies applying multivariate models to WB and related traits, these types of investigations are becoming more frequent, and are fueling follow-up genetic molecular studies. In the next section, it will be shown how studies like these help with the design of so-called "multivariate genome-wide association meta-analysis,"[74] where the genetic overlap between related traits is used to increase power for genetic analyses.

To summarize, positive psychological traits, such as optimism, meaning in life, self-esteem, and resilience are related to WB with phenotypic correlations of around 0.50. Although the estimated heritability is tied to the specific construct, definition, sample, context, and methods used, around one-third of the variance in the related phenotypes can be explained by genetic factors, similar to what has been reported for WB. In addition, multivariate twin models show strong genetic correlations between WB and other positive psychological traits. These findings help us to further understand the complex nature of WB.

18.5 Specific molecular genetic and environmental influences

The introduction of this chapter already briefly mentioned that twin studies on WB fueled more in-depth analyses of genetic and environmental effects. To help frame the importance of findings from twin and family studies, it is useful to view them in conjunction with findings that probe the role genetic and environmental factors using other methods. Behavioral genetic studies have revealed that a substantial part (∼40%) of the variation in WB can be attributed to genetic influences and an obvious next step is to try to identify genomic regions associated with WB.

The first reliable molecular evidence for the genetic complexity of WB came from a method called GCTA (genome-wide complex trait analysis), where the proportion of phenotypic variance explained by all genome-wide SNPs (single nucleotide polymorphisms—DNA sequence variation of a single nucleotide) is estimated by comparing the phenotypic and genetic similarity across a group of unrelated individuals.[75] In a pooled sample of ∼11.500 unrelated genotyped Swedish and Dutch participants, WB was measured using the positive affect subscale of the Center for Epidemiology Studies Depression Scale (CES-D). Based on this approach, it was estimated that 12%–18% of the variance in WB was accounted for the additive effects of the SNPs measured on genotyping platforms.[76]

Next, the development of genome-wide association studies (GWASs), allowed for the first identification of specific genetic variants associated with WB. In a GWAS, millions of genetic variants are measured and regressed on a phenotype in a large group of individuals. In this way, the association between each genetic variant and an outcome of interest is tested with a strong correction for multiple testing, so that the chance of finding false positives is greatly reduced. The first successful GWAS for

WB ($N = 298{,}420$) was performed in 2016. This study led to the identification of 3 genetic variants associated with WB (defined as life satisfaction and positive affect).[77] The SNPs had estimated effects in the range of 0.015–0.018 s.d. per allele (each $R^2 \approx 0.01\%$). The high genetic correlations ($r_g > 0.75$) between life satisfaction, positive affect, neuroticism, and depressive symptoms suggest a common liability and this common liability was leveraged to increase the power to identify associated genetic variants. To this end, the latest GWAS for WB combined these three traits and coined them "the WB spectrum." In this study, 304 independent significant variant-phenotype associations were identified for the WB spectrum, with 148 and 191 associations specific for life satisfaction and positive affect, respectively. Biological annotation revealed evidence for enrichment of genes differentially expressed in the subiculum (part of the hippocampus) and enrichment for GABAergic interneurons. However, even with this progress, the identified variants account for only a small percentage of the variation, meaning that we still have a long road ahead. The first and only epigenome-wide association study approach, to identify differentially methylated sites associated with individual differences in WB, reports two sites (cg10845147, $P = 1.51 * 10–8$ and cg01940273, $P = 2.34 * 10–8$) that reached genome-wide significance following Bonferroni correction. Four more sites (cg03329539, $P = 2.76* 10\text{-}7$; cg09716613, $P = 3.23 * 10–7$; cg04387347, $P = 3.95 * 10–7$; and cg02290168, $P = 5.23 * 10–7$) were considered to be genome-wide significant when applying the widely used criterion of an FDR q value < 0.05. Gene ontology (GO) analysis highlighted enrichment of several central nervous system categories among higher-ranking methylation sites. However, replication of these results is warranted in larger samples.

Twin studies already taught us that about 40% of individual differences in WB can be explained by genetic factors. These follow-up analyses taught us about the genetic complexity of WB, with likely thousands of variants contributing to the trait. These studies also revealed that each genetic variant only contributes a tiny amount to the variation in WB, so that we cannot speak of a single "happiness gene" or a few "happiness genes" that assert substantial influence on WB.

While there is substantial genetic influence on variation in WB, the remaining majority of variance is caused by environmental influences. Again, while twin-and family-studies tell us something about the relative influence of the environment, they do not clarify which environmental influences are important. We can draw a few conclusions from the existing literature on the association between WB and environmental factors. On the socioenvironmental side, it seems that factors associated with social connectedness, such as the quality of social contacts[78] and social support[79] are important for WB. However, on the more contextual/physical environment side, there is not a lot of consensus on which environmental factors are important. Not only do studies produce contradicting results, there seems to be a lack of meta-analytic oversight. This lack of meta-analyses can mostly be explained by the fact that studies used varying designs, making it difficult to compare outcomes. There are some overview studies for specific environmental factors from the WB literature in general, but these studies also fail to present conclusive evidence. For example, Lovell and colleagues examined the association between exposure to biodiverse environments and WB and conclude that there is some evidence for a

small positive effect, but that much of the evidence is inconclusive.[80] Similarly, Vanaken, and Danckaerts[81] and Houlden and colleagues[82] examined the literature related to the relation between green space exposure and WB in children and adults, respectively. They both conclude there is limited evidence for a positive effect. Unfortunately, even though there is much literature examining the associations between different environmental variables and WB, it seems we are far from having a complete picture of these environmental influences.

For future research in this area, it is important that we continue with large-scale investigations into these environmental factors. For example, more homogeneity can be achieved by employing a design that is similar to that used in GWA studies, but includes WB and multiple environmental factors instead of multiple genetic variants. By performing such "environment-wide association studies," we can study the effect of environmental variables in different populations and geographical levels in a consistent manner. Ni and colleagues already applied such a design for WB, where they assessed the association between 194 psychosocial and behavioral factors and physical, mental, and social WB in a large Hong Kong sample.[83] They reported that only depressive symptoms, life satisfaction, and happiness were simultaneously associated with these three domains of WB. To develop a full picture of the WB *exposome* (i.e., the collective of exposures people experience, and how these exposures influence WB), it is important we continue this progress by studying other types of environmental factors in an environment-wide context, such as the physical and social environment. Moreover, as we have seen in this chapter, there is a considerable genetic influence on WB. Environmental factors are also partly under genetic control,[84] meaning that exposure to certain environments might be driven by genetic factors. Therefore, to fully understand the association between WB and environmental factors, this gene-environment interplay also needs to be considered. As mentioned earlier, there is a lot of inconsistent results from studies examining the environment in relation to WB. Part of this inconsistency might be explained by the fact that most studies do not use genetically sensitive designs. Twin research can help us elucidate the extent to which covariation between WB and environmental factors is genetic in nature, for instance using bivariate designs that partition covariance into genetic and environmental sources.

To conclude, while there are still hurdles to be overcome and many unanswered questions, considerable progress has been made over the past years in identifying genetic and environmental factors that influence WB. The above paragraphs already outlined some of the steps that have been/need to be taken to advance our understanding of WB. However, what was not mentioned yet is the way in which (extended) twin designs can help us further our understanding of WB. In Section 18.6, we elaborate on some interesting future directions for this type of research.

18.6 Future directions

In this final section, we present some interesting extensions of the classical twin design in terms of designs and outcome measures. More specifically, we discuss

the use of ecological momentary assessment, causality in terms of MZ difference models, and nuclear twin family designs (NTFDs).

18.6.1 Well-being fluctuations

Almost all existing twin studies examining the heritability of WB assess WB with questionnaires about general WB, happiness, or life satisfaction. However, like many other complex human traits, feelings of WB (e.g., mood) fluctuate over time and across different contexts.[85–87] The heritability of momentary WB (e.g., how happy do you feel in this moment?) has been assessed twice and resulted in low or even negligible estimates.[88,89] In a small twin sample, Riemann and colleagues measured moods across mood-inducing situations and estimated the heritability around 8%–16%.[88] More recently, Menne-Lothman et al. investigated momentary positive affect in female twins using the experience sampling method and reported a heritability of 0%.[89] The variance in momentary WB was completely explained by the environment, that is, the mood-inducing situation.

The heritability of fluctuations in WB has not been investigated, even though individual differences in WB and mood fluctuations have been reported. Some people show relatively stable levels of WB over the day and/or week, while others fluctuate a lot.[90–92] One way to capture the fluctuations and dynamic nature of WB is by applying an ecological momentary assessment (EMA) design. EMA involves the repeated assessment of the momentary experiences and moods of participants in real-time and in their natural environment.[93] Due to technological advances EMA studies can be conducted more easily with smartphones, a device ubiquitously present in our society. Future twin studies should make use of such designs to explore the contribution of genetic and environmental effects to the stability and fluctuations of momentary WB.

18.6.2 MZ difference/causality

Twin studies can also be used to investigate causal relationships between variables, as this design controls for genetic and shared environmental confounding. The co-twin control model makes use of discordant MZ (and DZ) twin pairs to determine whether an observed association is consistent with a causal effect of an exposure on an outcome.[94] For example, if MZ twins differ on an exposure variable, and also differ on the outcome (e.g., WB), we can conclude that the association between the variables is not due to confounding genetic or shared environmental factors affecting both variables as MZ twins share 100% of their genes.

In the field of WB, this causality analysis has only been applied to investigate the causal relationships between WB and exercise behavior[95] and mortality.[96,97] Stubbe and colleagues[95] reported that, even though exercisers were on average more satisfied with their lives and happier than nonexercisers, no evidence for a causal effect of exercise on WB was present using the co-twin method. Sadler and colleagues[96] and Saunders and colleagues[97] did find a causal association between higher WB and

lower mortality. Twin differences in WB predicted differential mortality within discordant pairs. Although the discordant twin method or (MZ) twin difference design is powerful to explore likely causal pathways between (environmental) factors and traits or outcomes, in the field of WB, the application of these methods is scarce. Future studies can use this powerful design to explore various causal influences on WB using twin samples.

18.6.3 Nuclear twin family design

In a classical twin study, the observed covariance between MZ and DZ twin pairs is used to make inferences about the relative influence of genes and environment. While the design has led to many important insights for WB (as was summarized in this chapter), it does have some limitations. In these models, we can only estimate as many parameters as there are pieces of information available. Since the unique environment parameter (e) can be estimated in all models (given that e is 1 minus the MZ correlation), and we observe the MZ and DZ covariance, we have three pieces of information available to us when using the classical twin design. This means we can only estimate three parameters: either additive genetic effects (a), shared environmental effects (c), and unique environmental effects (e), or (a), (e) and dominant genetic effects (d).

To increase the number of parameters that can be estimated, a simple solution is to include more family members between which we can estimate covariation. In the nuclear twin family design (NTFD), data on parents are included in addition to the twin data, meaning that we now also include the covariance between the parents, and the covariance between parents and children.[98] This allows for the simultaneous estimation of C and D, and also for the estimation of other interesting parameters: the potential effect of assortative mating, and potential vertical transmission. Assortative mating occurs when two spouses are more similar to each other than would be expected under a random mating pattern. While this can have many causes, the result is that these spouses are genetically more similar than two random individuals. Vertical transmission, in the context of the NTFD, describes the influence of the familial environment from nongenetic effects passed from parents to offspring. In the case of WB, this would mean that parental WB influences offspring WB through its effect on the familial environment. Importantly, the parental phenotype is influenced by the parental genotype. This vertical transmission from parental phenotype on offspring phenotype is thus not completely independent from genetic influences.

Thus, by including data on parents, the NTFD allows for the estimation of more parameters, and also provides more accurate estimates of the model parameters. Naturally, the design can be extended to include more family members (e.g., nontwin siblings) resulting in better-powered designs.[99] Importantly, this design is not new: it has been applied to many traits and has been improved by different people over the years. Yet, for WB, it seems that such an extended twin design has only been applied once. In a study in Norwegian twins and parents, Nes, Czajkowski, and Tambs[100] applied the NFTD to estimate nonrandom mating, cultural transmission, and shared environmental effects specific for regular siblings and twins. Their analyses revealed

the presence of nonrandom mating (spousal correlation of 0.26) and significant influence of the shared twin environment. The effect of vertical (cultural) transmission was estimated to be negligible. As this was the only extended NFTD study to date, it is not yet clear whether these results are consistent across different cultures/measures of WB. An interesting future direction would thus be to replicate these findings in different studies and with different measures.

18.7 Conclusion

The aim of this chapter was to summarize existing behavioral genetic research on WB, to show how this has directed WB research, and to provide a glance into directions for future research. While the last meta-analyses on twin studies for WB were published only five years ago, since then the field has developed rapidly: the first (300) genetic variants for WB were identified, the field is increasingly doubting existing definitions of WB and acknowledging the interrelatedness of different WB-related phenotypes, and are thinking about how to improve models for estimating sources of variation in WB. While there was first a focus on quantity, where the goal was to obtain the largest sample size possible at the cost of simple phenotyping, we are now transitioning to a focus on quality, with promising improvements in the measurement (e.g., EMA) and analyses (i.e., genetically informative designs) of WB ahead.

References

1 Zencey E. Measuring what matters. *Consumption and Well-Being in the Material World*: Springer; 2014:153–174. https://doi.org/10.1007/978-94-007-7368-4_8.
2 Stiglitz JE, Sen AK, Fitoussi J-P, Report by the commission on the measurement of economic performance and social progress, Paris, 2009.
3 Boelhouwer J. *Wellbeing in the Netherlands: the SCP life situation index since 1974. Doctoral dissertation*: Utrecht University; 2010.
4 Ryan RM, Deci EL. On happiness and human potentials: a review of research on hedonic and eudaimonic well-being. *Annu Rev Psychol*. 2001;52:141–166. https://doi.org/10.1146/annurev.psych.52.1.141.
5 O'Keefe T. The Cyrenaics on pleasure, happiness, and future-concern. *Phronesis*. 2002;47(4):395–416.
6 Waterman AS. The relevance of Aristotle's conception of eudaimonia for the psychological study of happiness. *Theor Philos Psychol*. 1990;10:39–44. https://doi.org/10.1037/h0091489.
7 Deci EL, Ryan RM. Hedonia, eudaimonia, and well-being: An introduction. *J Happiness Stud*. 2008;9:1–11. https://doi.org/10.1007/s10902-006-9018-1.
8 Ryff CD. Happiness is everything, or is it? Explorations on the meaning of psychological well-being. *J Pers Soc Psychol*. 1989;57:1069–1081. https://doi.org/10.1037/0022-3514.57.6.1069.

9 Gallagher MW, Lopez SJ, Preacher KJ. The hierarchical structure of well-being. *J Pers*. 2009;77:1025–1049. https://doi.org/10.1111/j.1467-6494.2009.00573.x.

10 Joshanloo M. Revisiting the empirical distinction between hedonic and eudaimonic aspects of well-being using exploratory structural equation modeling. *J Happiness Stud*. 2016;17:2023–2036. https://doi.org/10.1007/s10902-015-9683-z.

11 Baselmans BML, Bartels M. A genetic perspective on the relationship between eudaimonic –and hedonic well-being. *Sci Rep*. 2018;8. https://doi.org/10.1038/s41598-018-32638-1.

12 Baselmans BML, van de Weijer MP, Abdellaoui A, Vink JM, Hottenga JJ, Willemsen G. A Genetic Investigation of the well-being spectrum. *Behav Genet*. 2019;49:286–297. https://doi.org/10.1007/s10519-019-09951-0.

13 Linton MJ, Dieppe P, Medina-Lara A. Review of 99 self-report measures for assessing well-being in adults: exploring dimensions of well-being and developments over time. *BMJ Open*. 2016;6. http://doi.org/10.1136/bmjopen-2015-010641.

14 Cooke PJ, Melchert TP, Connor K. Measuring well-being: a review of instruments. *Couns Psychol*. 2016;44:730–757. https://doi.org/10.1177/0011000016633507.

15 Bartels M. Genetics of wellbeing and its components satisfaction with life, happiness, and quality of life: a review and meta-analysis of heritability studies. *Behav Genet*. 2015;45:137–156. https://doi.org/10.1007/s10519-015-9713-y.

16 Nes RB, Røysamb E. The heritability of subjective well-being: review and meta-analysis. *Genet Psychol Well-Being*. 2015:75–96. Oxford University Press. http://doi.org/10.1093/acprof:oso/9780199686674.003.0005.

17 Haworth CMA, Carter K, Eley TC, Plomin R. Understanding the genetic and environmental specificity and overlap between well-being and internalizing symptoms in adolescence. *Dev Sci*. 2017;20:e12376. https://doi.org/10.1111/desc.12376.

18 Wang RAH, Davis OSP, Wootton RE, Mottershaw A, Haworth CMA. Social support and mental health in late adolescence are correlated for genetic, as well as environmental, reasons. *Sci Rep*. 2017;7. https://doi.org/10.1038/s41598-017-13449-2.

19 Wootton RE, Davis OSP, Mottershaw AL, Wang RAH, Haworth CMA. Genetic and environmental correlations between subjective wellbeing and experience of life events in adolescence. *Eur Child Adolesc Psychiatry*. 2017;26:1119–1127. https://doi.org/10.1007/s00787-017-0997-8.

20 Luo YLL, Sedikides C, Cai H. On the etiology of self-enhancement and its association with psychological well-being. *Soc Psychol Personal Sci*. 2020. http://doi.org/10.1177/1948550619877410.

21 Van't Ent D, Den Braber A, Baselmans BML, Brouwer RM, Dolan CV, Hulshoff Pol HE. Associations between subjective well-being and subcortical brain volumes. *Sci Rep*. 2017;7(1):1–11. http://doi.org/10.1038/s41598-017-07120-z.

22 Milovanović I, Sadiković S, Kodžopeljić J. Genetic and environmental factors in emotion regulation and life satisfaction: A twin study. *Primenj Psihol*. 2018;11:399–417. https://doi.org/10.19090/pp.2018.4.399-417.

23 Sadiković S, Smederevac S, Mitrović D, Milovanović I. Behavioral genetics foundations of relations between personality traits and satisfaction with Life. *Primenj Psihol*. 2018. http://doi.org/10.19090/pp.2018.4.487-502.

24 Røysamb E, Nes RB, Czajkowski NO, Vassend O. Genetics, personality and wellbeing. A twin study of traits, facets and life satisfaction. *Sci Rep*. 2018. http://doi.org/10.1038/s41598-018-29881-x.

25 Thege BK, Littvay L, Tarnoki DL, Tarnoki AD. Genetic and environmental effects on eudaimonic and hedonic well-being: evidence from a post-communist culture. *Curr Psychol*. 2017;36:84–89. https://doi.org/10.1007/s12144-015-9387-x.

26 Baselmans BML, Willems YE, van Beijsterveldt CEM, Ligthart L, Willemsen G, Dolan CV. Unraveling the genetic and environmental relationship between well-being and depressive symptoms throughout the lifespan. *Front Psychiatry*. 2018;9. http://doi.org/10.3389/fpsyt.2018.00261.

27 Routledge KM, Burton KLO, Williams LM, Harris A, Schofield PR, Clark CR. Shared versus distinct genetic contributions of mental wellbeing with depression and anxiety symptoms in healthy twins. *Psychiatry Research*. 2016;244:65–70. https://doi.org/10.1016/j.psychres.2016.07.016.

28 Routledge KM, Burton KLO, Williams LM, Harris A, Schofield PR, Clark CR, Gatt JM. The shared and unique genetic relationship between mental well-being, depression and anxiety symptoms and cognitive function in healthy twins. *Cogn Emot*. 2017;31:1465–1479. https://doi.org/10.1080/02699931.2016.1232242.

29 Routledge KM, Williams LM, Harris AWF, Schofield PR, Clark CR, Gatt JM. Genetic correlations between wellbeing, depression and anxiety symptoms and behavioral responses to the emotional faces task in healthy twins. *Psychiatry Research*. 2018;264:385–393. https://doi.org/10.1016/j.psychres.2018.03.042.

30 Chilver MR, Keller AS, Park HRP, Jamshidi J, Montalto A, Schofield PR. Electroencephalography profiles as a biomarker of wellbeing: a twin study. *J Psychiatr Res*. 2020. http://doi.org/10.1016/j.jpsychires.2020.04.010.

31 Jamshidi J, Williams LM, Schofield PR, Park HRP, Montalto A, Chilver MR. Diverse phenotypic measurements of wellbeing: heritability, temporal stability, and the variance explained by polygenic scores. *Genes Brain, and Behavior*. 2020. http://doi.org/10.1111/gbb.12694.

32 Haworth CMA, Nelson SK, Layous K, et al. Stability and change in genetic and environmental influences on well-being in response to an intervention. *PLoS One*. 2016;11:e0155538. https://doi.org/10.1371/journal.pone.0155538.

33 Scheier MF, Carver CS. Optimism, coping, and health: assessment and implications of generalized outcome expectancies. *Health Psychology*. 1985;4:219–247. https://doi.org/10.1037/0278-6133.4.3.219.

34 Ben-Zur H, Adolescents H. The link between subjective well-being, internal resources, and parental factors. *J Youth Adolesc*. 2003;32:67–79. https://doi.org/10.1023/A:1021864432505.

35 Scheier MF, Carver CS. Effects of optimism on psychological and physical well-being: theoretical overview and empirical update. *Cognit Ther Res*. 1992;16:201–228. https://doi.org/10.1007/BF01173489.

36 Wrosch C, Scheier MF. Personality and quality of life: the importance of optimism and goal adjustment, in: Qual. *Life Res*. 2003:59–72. https://doi.org/10.1023/A:1023529606137.

37 Alarcon GM, Bowling NA, Khazon S. Great expectations: a meta-analytic examination of optimism and hope. *Pers Individ Dif*. 2013;54:821–827. https://doi.org/10.1016/j.paid.2012.12.004.

38 Mavioğlu RN, Boomsma DI, Bartels M. Causes of individual differences in adolescent optimism: a study in Dutch twins and their siblings. *Eur Child Adolesc Psychiatry*. 2015;24:1381–1388. https://doi.org/10.1007/s00787-015-0680-x.

39 Bates TC. The glass is half full and half empty: a population-representative twin study testing if optimism and pessimism are distinct systems. *J Posit Psychol*. 2015;10:533–542. https://doi.org/10.1080/17439760.2015.1015155.

40 Caprara GV, Fagnani C, Alessandri G, Steca P, Gigantesco A. Human optimal functioning: the genetics of positive orientation towards self, life, and the future. *Behav Genet*. 2009;39:277–284. https://doi.org/10.1007/s10519-009-9267-y.

41 Thege BK, Littvay L, Tarnoki DL, Tarnoki AD, Konkolÿ Thege B. Genetic and environmental effects on eudaimonic and hedonic well-being: evidence from a post-communist culture. *Curr Psychol*. 2017;36:84–89. https://doi.org/10.1007/s12144-015-9387-x.

42 Mosing MA, Pedersen NL, Martin NG, Wright MJ. Sex differences in the genetic architecture of optimism and health and their interrelation: a study of Australian and Swedish twins. *Twin Res Hum Genet*. 2010;13:322–329. https://doi.org/10.1375/twin.13.4.322.

43 Alessandri G, Vecchione M, Fagnani C, et al. Much more than model fitting? Evidence for the heritability of method effect associated with positively worded items of the life orientation test revised. *Struct Equ Model*. 2010;17:642–653. https://doi.org/10.1080/10705511.2010.510064.

44 Plomin R, Scheier MF, Bergeman CS, Pedersen NL, Nesselroade JR, McClearn GE. Optimism, pessimism and mental health: a twin/adoption analysis. *Pers Individ Dif*. 1992;13:921–930. https://doi.org/10.1016/0191-8869(92)90009-E.

45 Yuh J-I, Neiderhiser JM, Reiss D. Genetic and environmental influences on dispositional optimism and depressive symptoms. *Int J Hum Ecol*. 2010;11:15–23.

46 Whitfield JB, Zhu G, Landers JG, Martin NG. Pessimism is associated with greater all-cause and cardiovascular mortality, but optimism is not protective. *Sci Rep*. 2020. http://doi.org/10.1038/s41598-020-69388-y.

47 George LS, Park CL. Meaning in life as comprehension, purpose, and mattering: toward integration and new research questions. *Rev Gen Psychol*. 2016;20:205–220. https://doi.org/10.1037/gpr0000077.

48 Martela F, Steger MF. The three meanings of meaning in life: distinguishing coherence, purpose, and significance. *J Posit Psychol*. 2016;11:531–545. https://doi.org/10.1080/17439760.2015.1137623.

49 McMahan EA, Renken MDH. Eudaimonic conceptions of well-being, meaning in life, and self-reported well-being: Initial test of a mediational model. *Pers Individ Dif*. 2011;51:589–594. https://doi.org/10.1016/j.paid.2011.05.020.

50 Schueller SM, Seligman MEP. Pursuit of pleasure, engagement, and meaning: relationships to subjective and objective measures of well-being. *J Posit Psychol*. 2010;5:253–263. https://doi.org/10.1080/17439761003794130.

51 Steger MF, Kashdan TB. Stability and specificity of meaning in life and life satisfaction over one year. *J Happiness Stud*. 2007. http://doi.org/10.1007/s10902-006-9011-8.

52 Chamberlain K, Zika S. Religiosity, life meaning and wellbeing: some relationships in a sample of women. *J Sci Study Relig*. 1988. http://doi.org/10.2307/1387379.

53 Howell AJ, Passmore HA, Buro K. Meaning in nature: meaning in life as a mediator of the relationship between nature connectedness and well-being. *J Happiness Stud*. 2013. http://doi.org/10.1007/s10902-012-9403-x.

54 Steger MF, Hicks BM, Krueger RF, Bouchard TJ. Genetic and environmental influences and covariance among meaning in life, religiousness, and spirituality. *J Posit Psychol*. 2011;6:181–191. https://doi.org/10.1080/17439760.2011.569172.

55 Rosenberg M. *Society and the Adolescent Self-Image*. Princeton: Princeton University Press; 1965.

56 Baumeister RF, Tice DM, Hutton DG. Self-presentational motivations and personality differences in self-esteem. *J Pers*. 1989;57:547–579. https://doi.org/10.1111/j.1467-6494.1989.tb02384.x.

57 Neiss MB, Sedikides C, Stevenson J. Self-esteem: a behavioural genetic perspective. *Eur J Pers*. 2002;16:351–368. mbn@soton.ac.uk.

58 Schimmack U, Diener E. Predictive validity of explicit and implicit self-esteem for subjective well-being. *J Res Pers*. 2003. http://doi.org/10.1016/S0092-6566(02)00532-9.

59 Diener E, Diener M. Cross-cultural correlates of life satisfaction and self-esteem. *J Pers Soc Psychol*. 1995. http://doi.org/10.1037/0022-3514.68.4.653.

60 McGuire S, Neiderhiser JM, Reiss D, Hetherington EM, Plomin R. Genetic and environmental influences on perceptions of self-worth and competence in adolescence: a study of twins, full siblings, and step-siblings. *Child Development*. 1994;65:785–799. https://doi.org/10.1111/j.1467-8624.1994.tb00783.x.

61 Jonassaint CR. Heritability of self-esteem from adolescence to young adulthood. *New School Psychol Bull*. 2010;7(1):3–15.

62 Raevuori A, Dick DM, Keski-Rahkonen A, et al. Genetic and environmental factors affecting self-esteem from age 14 to 17: a longitudinal study of Finnish TWINS. *Psychol Med*. 2007;37:1625–1633. https://doi.org/10.1017/S0033291707000840.

63 Satici SA. Psychological vulnerability, resilience, and subjective well-being: the mediating role of hope. *Pers Individ Dif*. 2016;102:68–73. https://doi.org/10.1016/j.paid.2016.06.057.

64 Hu T, Zhang D, Wang J. A meta-analysis of the trait resilience and mental health. *Pers Individ Dif*. 2015;76:18–27. https://doi.org/10.1016/j.paid.2014.11.039.

65 Kim-Cohen J, Moffitt TE, Caspi A, Taylor A. Genetic and environmental processes in young children's resilience and vulnerability to socioeconomic deprivation. *Child Development*. 2004;75:651–668. https://doi.org/10.1111/j.1467-8624.2004.00699.x.

66 Connor KM, Davidson JRT. Development of a new resilience scale: the Connor-Davidson resilience scale (CD-RISC). *Depress Anxiety*. 2003;18:76–82. https://doi.org/10.1002/da.10113.

67 Wolf EJ, Miller MW, Sullivan DR, et al. A classical twin study of PTSD symptoms and resilience: evidence for a single spectrum of vulnerability to traumatic stress. *Depress Anxiety*. 2018;35:132–139. https://doi.org/10.1002/da.22712.

68 Block J, Kremen AM. IQ and ego-resiliency: conceptual and empirical connections and separateness. *J Pers Soc Psychol*. 1996;70:349–361. https://doi.org/10.1037/0022-3514.70.2.349.

69 Waaktaar T, Torgersen S. Genetic and environmental causes of variation in trait resilience in young people. *Behav Genet*. 2012;42:366–377. https://doi.org/10.1007/s10519-011-9519-5.

70 Kalisch R, Cramer AOJ, Binder H, Fritz J, Leertouwer I, Lunansky G. Deconstructing and reconstructing resilience: a dynamic network approach. *Perspect Psychol Sci*. 2019;14:765–777. https://doi.org/10.5281/zenodo.3228687.

71 Boardman JD, Blalock CL, Button TMM. Sex differences in the heritability of resilience. *Twin Res Hum Genet*. 2008;11:12–27. https://doi.org/10.1375/twin.11.1.12.

72 Amstadter AB, Myers JM, Kendler KS. Psychiatric resilience: longitudinal twin study. *Br J Psychiatry*. 2014;205:275–280. https://doi.org/10.1192/bjp.bp.113.130906.

73 Sawyers C, Kurtz ED, Sheerin C, Maes HH, Kendler KS, Amstadter AB. A behavioral genetic investigation of conceptualizations of resilience in a female twin sample. *Depress Anxiety*. 2020;37:532–539. https://doi.org/10.1002/da.23005.

74 Baselmans BML, Jansen R, Ip HF, van Dongen J, Abdellaoui A, van de Weijer MP. Multivariate genome-wide analyses of the well-being spectrum. *Nat Genet*. 2019;51:445–451. https://doi.org/10.1038/s41588-018-0320-8.

75 Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet*. 2011;88:76–82. https://doi.org/10.1016/j.ajhg.2010.11.011.

76 Rietveld CA, Cesarini D, Benjamin DJ, Koellinger PD, De Neve J-E, Tiemeier H. Molecular genetics and subjective well-being. *Proc Natl Acad Sci U S A*. 2013;110:9692–9697. https://doi.org/10.1073/pnas.1222171110.

77 Okbay A, Baselmans BML, De Neve J-E, Turley P, Nivard MG, Fontana MA. Genetic variants associated with subjective well-being, depressive symptoms and neuroticism identified through genome-wide analyses. *Nat Genet*. 2016;48:624–633. https://doi.org/10.1038/ng.3552.

78 Pinquart M, Sörensen S. Influences of socioeconomic status, social network, and competence on subjective well-being in later life: a meta-analysis. *Psychol Aging*. 2000;15(2):187–224. https://doi.org/10.1037/0882-7974.15.2.187.

79 Wang HH, Wu SZ, Liu YY. Association between social support and health outcomes: a meta-analysis. *Kaohsiung J Med Sci*. 2003;19:345–350. https://doi.org/10.1016/s1607-551x(09)70436-x.

80 Lovell R, Wheeler BW, Higgins SL, Irvine KN, Depledge MH. A systematic review of the health and well-being benefits of biodiverse environments. *J Toxicol Environ Heal B-CRITICAL Rev*. 2014;17:1–20. https://doi.org/10.1080/10937404.2013.856361.

81 Vanaken G-J, Danckaerts M. Impact of green space exposure on children's and adolescents' mental health: a systematic review. *Int J Environ Res Public Health*. 2018;15. https://doi.org/10.3390/ijerph15122668.

82 Houlden V, Weich S, Porto de Albuquerque J, Jarvis S, Rees K. The relationship between greenspace and the mental wellbeing of adults: a systematic review. *PLoS One*. 2018;13:e0203000. https://doi.org/10.1371/journal.pone.0203000.

83 Ni MY, Yao XI, Cheung F, et al. Determinants of physical, mental and social well-being: a longitudinal environment-wide association study. *Int J Epidemiol*. 2020;49(2):380–389. https://doi.org/10.1093/ije/dyz238.

84 Vinkhuyzen AAE, van der Sluis S, de Geus EJC, Boomsma DI, Posthuma D. Genetic influences on 'environmental' factors. *Genes, Brain, and Behavior*. 2010;9:276–287. https://doi.org/10.1111/j.1601-183X.2009.00554.x.

85 Eid M, Diener E. Global judgments of subjective well-being: Situational variability and long-term stability. *Soc Indic Res*. 2004;65:245–277. https://doi.org/10.1023/B:SOCI.0000003801.89195.bc.

86 Li Z, Yin X, Jiang S, Wang M, Cai T. Psychological mechanism of subjective well-being: a stable trait or situational variability. *Soc Indic Res*. 2014;118(2):523–534. http://doi.org/10.1007/s11205-013-0449-x.

87 Lyubomirsky S. Why are some people happier than others? The role of cognitive and motivational processes in well-being. *Am Psychol*. 2001;65(3):239. http://doi.org/10.1037/0003-066X.56.3.239.

88 Riemann R, Angleitner A, Borkenau P, Eid M. Genetic and environmental sources of consistency and variability in positive and negative mood. *Eur J Pers*. 1998;12(5):345–364. https://doi.org/10.1002/(SICI)1099-0984(1998090)12:5<345::AID-PER332>3.0.CO;2-Y.

89 Menne-Lothmann C, Jacobs N, Derom C, Thiery E, van Os J, Wichers M. Genetic and environmental causes of individual differences in daily life positive affect and reward experience and its overlap with stress-sensitivity. *Behav Genet*. 2012;42:778–786. https://doi.org/10.1007/s10519-012-9553-y.

90 Eid M, Diener E. Intraindividual variability in affect: Reliability, validity, and personality correlates. *J Pers Soc Psychol*. 1999;76(4):662. http://doi.org/10.1037/0022-3514.76.4.662.
91 Gadermann AM, Zumbo BD. Investigating the intra-individual variability and trajectories of subjective well-being. *Soc Indic Res*. 2007;81(1):1–33. http://doi.org/10.1007/s11205-006-0015-x.
92 Röcke C, Li SC, Smith J. Intraindividual variability in positive and negative affect over 45 days: do older adults fluctuate less than young adults? *Psychol Aging*. 2009;24(4):863. http://doi.org/10.1037/a0016276.
93 Shiffman S, Stone AA, Hufford MR. Ecolocial momentary assessment. *Annu Rev Clin Psychol*. 2008;4:1–32. https://doi.org/10.1146/annurev.clinpsy.3.022806.091415.
94 Vitaro F, Brendgen M, Arseneault L. The discordant MZ-twin method: one step closer to the holy grail of causality. *Int J Behav Dev*. 2009;33(4):376–382. http://doi.org/10.1177/0165025409340805.
95 Stubbe JH, de Moor MHM, Boomsma DI, de Geus EJC. The association between exercise participation and well-being: a co-twin study. *Prev Med (Baltim)*. 2007;44:148–152. https://doi.org/10.1016/j.ypmed.2006.09.002.
96 Sadler ME, Miller CJ, Christensen K, McGue M. Subjective wellbeing and longevity: a co-twin control study. *Twin Res Hum Genet*. 2011;14:249–256. https://doi.org/10.1375/twin.14.3.249.
97 Saunders GRB, Elkins IJ, Christensen K, McGue M. The relationship between subjective well-being and mortality within discordant twin pairs from two independent samples. *Psychol Aging*. 2018;33:439. https://doi.org/10.1037/pag0000248.
98 Keller MC, Medland SE, Duncan LE, Hatemi PK, Neale MC, Maes HHM. Modeling extended twin family data I: description of the cascade model. *Twin Res Hum Genet*. 2009;12:8–18. https://doi.org/10.1375/twin.12.1.8.
99 Posthuma D, Boomsma DI. A note on the statistical power in extended twin designs. *Behav Genet*. 2000;30:147–158. https://doi.org/10.1023/A:1001959306025.
100 Nes RB, Czajkowski N, Tambs K. Family matters: happiness in nuclear families and twins. *Behav Genet*. 2010;40:577–590. https://doi.org/10.1007/s10519-010-9365-x.

CHAPTER

Twin study of personality

19

Juko Ando
Keio Univerity, Tokyo, Japan

Personality is a cursory word when used normally: “Why did he act like that way?” “That’s because of his personality.” This statement provides no psychological explanation of his behavior and is merely a tautology. However, it sounds reasonable. The term *personality* is considered to be a concept that explains someone’s behavioral and psychological tendencies and predicts his/her behavior in a specific situation. G. Allport’s scientific definition of personality is the most popular and classic, stating "Personality is the dynamic organization *within* the individual of those psychophysical systems that determine his unique adjustment to his environment" (Allport, 1937, p. 48[1]). Thus, personality is usually considered an internal entity within an individual, determining the characteristics of his/her behavior and thoughts.

In contrast, a popular controversy known as the “person-situation debate” (Mischel, 1968[2]) challenged this perspective, stating that personality does not exist “within” a person but emerges in an interface “between” person and situation. Behaviorism is another opposing view, which argues that personality is an outcome of a history of stimulus-response contingencies in someone’s life.

Today, these extreme positions that deny “personality as an internal entity” (i.e. a social-cognitive theory of personality, or situationalism, radical behaviorism) have been eclipsed and views such as the more moderate “interactionism” or double-layer “trait/state” dichotomous theory of anxiety (Spielberger[3]) have gained popularity. Most psychologists acknowledge the existence of an internal entity, even partially, that underlies an individual’s personality.

Thus, the fundamental issue to be addressed is what this internal entity of personality is and how to describe and empirically verify it. One of the most reasonable explanations of this entity is genes. The study of twins can provide empirical evidence of the influence of genes.

If genes influence individual personality, an identical twin who shares the full range of genes will exhibit highly similar behavior in daily life. There is a pile of anecdotes reporting idiosyncratic similarity between identical twin siblings. For example, in one experimental case, which was attempted on a TV show, several pairs of identical twins who were placed into separate rooms with identical designs to have

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00034-9

a party with foods and drinks. Both twins enjoyed a free chat with each other and ate snacks on the same plates and drank from the same bottles placed on the same designed table in the same positions. Within 30 min, they exhibited interesting and astonishingly similar behaviors. One pair of twins played the same central role as the chairperson in the conversations between participants, of course being ignorant of what his cotwin was doing in the next room. Another pair of twins started singing the same song on karaoke. The same three pairs exhibited the same pattern of conversation "I live in/come from Gumma (a prefecture in Japan)" (Twin A siblings), "Wow, Gumma! I went/visited there last week." (Twin Bs), "I like Gumma, too." (Twin Cs) in exactly the same order. More impressive anecdotes that demonstrate the idiosyncratic similarity between identical twin siblings were reported in the Minnesota Study of Twins Reared Apart (Segal, 2012[4]).

Although these anecdotes are very impressive to provide evidence of some internal entity given by genes, they do not constitute scientifically firm evidences which are supported by some established theories of statistics and genetics. The next section introduces more scientifically elaborated studies of personality using the twin method.

19.1 Description of personality

Before introducing the outcomes of the twin studies of personality, we must first present a description and measure of personality. As mentioned earlier, the term *personality* is ambiguous. Broadly, personality contains almost all ranges of individual differences in psychological dimensions other than cognitive and information-processing abilities. This includes not only general personality traits but also emotional tendencies such as anxiety traits and well-being, social attitudes such as political preference, self-esteem, belief, value judgment, job satisfaction, religiousness. This is further complicated by the fact that the various names of so-called noncognitive (intellectual) ability aspects cannot be categorized simply as "noncognitive" because "noncognitive" refers to "cognitive" regulation of self (e.g., self-regulation), information processing (e.g., executive function), emotion (e.g., effortful control) recently. Thus, the term *personality* does not usually include psychopathology (e.g., schizophrenia and mood disorder) and developmental disorders (e.g., attention deficit hyperactivity disorder). However, personality disorders are considered to be a part of the personality.

Specifically, *personality,* in the mainstream of current personality psychology, refers to several psychological traits that are expressed in a certain taxonomic framework of specific theories. Many personality psychologists including Allport, adopted the "lexical approach," which involved quantitative judgment of words and sentences expressing an individual's personality (e.g., "warm," "nervous," or "I feel warm when I talk to my friend."). Allport and Odbert (1936)[5] scrutinized Webster's dictionary and identified 18,000 words that described human personality. This does not mean that there were 18,000 independent personality traits as some words (i.e., warm and tender) correlated with one another. Statistical techniques such as factor analysis categorized these lexicons into small numbers of clusters such as the two

systems (behavioral activation system [BIS], behavioral inhibition system [BAS] by Gray[6]), the giant three (neuroticism, extraversion, psychoticism by Eysenck[7]), the Big Five (neuroticism, extraversion, openness to experience, agreeableness, conscientiousness by Costa and McCrae[8]), six (honesty to humility, emotionality, extraversion, agreeableness (vs anger), conscientiousness, openness to experience [HEXACO] by Ashton and Lee[9]), seven (novelty seeking, harm avoidance, reward dependence, persistence, self-directedness, cooperativeness, self-transcendence; the temperament and character inventory by Cloninger[10]), even sixteen (16 personality factors by Cattell[11]). Some of these clusters were constructed based not only on the lexical approach but also on underlying neurobiological theories and evidence. Thus, to validate these trait theories and examine the relationships among them, it is necessary to describe these lexical theories from biological and genetic foundations through twin studies.

19.2 Twin studies of personality traits

Table 19.1 shows excerpts of twin correlation (i.e., index of resemblance between twin siblings, ranging from zero (no resemblance) to 1 (complete resemblance) or −1 (completely negative opposite-directing resemblance) and genetic/environmental proportions for the most popular personality traits of the Big Five.[12–16] Generally, twin studies on personality traits using any theoretical framework and their related characteristics produce robust results from a behavioral genetic point of view, that is, substantial genetic and nonshared environmental contributions with little or no shared environmental effects. They follow the "three laws of behavioral genetics" (Turkheimer, 2000[17]): (1) all human behavioral traits are heritable; (2) the effects of being raised in the same family are smaller than the effect of genes; and (3) a substantial portion of the variation in complex human behavioral traits is not accounted for by the effects of genes or families.

This is very different from the picture of cognitive abilities, which usually show substantial shared environment contribution because the DZ correlation is greater than half of the MZ correlation. A simple reason for this difference is that cognitive abilities grow with knowledge through information and learning opportunities given by the surrounding environment, especially the family environment during childhood and adolescence. On the contrary, personality traits are formed not by learned knowledge but by a complex combination of neurotransmitters such as dopamine and serotonin.

As shown in Table 19.1, twin correlations sometimes indicate nonadditive and additive genetic effects, as the MZ correlation is more than twice as large as the DZ correlation, but it is not very sensitive to detect its effect at a statistically significant level. It seems possible to detect the dominance effect with large combined samples[13,18] or extended twin family samples.[19–21] Loehlin et al. (1992) reported that 30% of N, 57% of E, 38% of O, 17% of A, 13% of C in the Big Five can be explained by nonadditive genetic effects. Kandler et al. (2019)[16] 30% of N (emotionality), 30% of A, 23% of C, 23% O in the HEXACO paradigm were explained

TABLE 19.1 Twin correlations and additive genetc (A)/nonshared environmental (E) contributions on the Big Five traits[a].

Samples	Trait	MZ	DZ	h^2	e^2
Jang et al. (1996) Canada	N	0.41	0.18	0.41	0.59
MZ=123p, 30.9yrs (11.8)	E	0.55	0.23	0.53	0.47
DZ=127p, 31.7yrs (11.7)	O	0.58	0.21	0.61	0.39
NEO-PI-R	A	0.41	0.26	0.41	0.59
	C	0.37	0.27	0.44	0.56
Loehlin et al. (1998) USA	N	0.43, 0.53, 0.44*	0.17, 0.25, 0.06	0.58	0.42
MZ=490	E	0.47, 0.60, 0.39*	0.01, 0.30, -0.06	0.57	0.43
DZ=317	O	0.39, 0.49, 0.36*	0.19, 0.27, 0.08	0.56	0.44
junior high	A	0.32, 0.46, 0.29*	0.06, 0.34, 0.18	0.51	0.49
*combination of 3 inventories	C	0.42, 0.53, 0.37*	0.21, 0.34, 0.14	0.52	0.48
Shikishima et al. (2006) Japan	N	0.45	0.18	0.46	0.54
MZ=470	E	0.48	0.12	0.46	0.54
DZ=210	O	0.52	0.25	0.52	0.48
14 - 30 yrs	A	0.37	0.12	0.36	0.64
NEO-PI-R	C	0.51	0.19	0.52	0.48
Riemann et al. (1997) Germany	N	0.53/0.40	0.13/0.01	0.68	0.32
MZ=84	E	0.56/0.38	0.28/0.22	0.68	0.32
DZ=34	O	0.54/0.49	0.34/0.21	0.79	0.31
32.98yrs (13.40)	A	0.42/0.32	0.19/0.17	0.66	0.34
NEO-FFI (self report / peer report)	C	0.54/0.40	0.18/0.18	0.75	0.25
Kandler et al. (2019) Germany	H	0.46	0.23	0.46	0.54
MZ=221	E(=N)[b]	0.58	0.16	0.58	0.42
DZ=352	X(=E)[c]	0.58	0.25	0.57	0.42
including twins' family	A	0.67	0.16	0.47	0.53
HEXACO	C	0.53	0.17	0.52	0.48
	O	0.66	0.27	0.63	0.34

[a] *N: neuroticidm, E: extraversion, O: opennes to experience, A: agreeableness, C: conscientiousness*
[b] *Emotionality in HEXACO is eqivalent to reverse of neuroticism in the Big Five.*
[c] *"X" is eqivalent of extraverion in the Big Five.*

by nonadditive genetic effects. Cloninger's temperament traits sometimes exhibited a dominance effect.[22,23] A recent meta-analysis of the genome-wide association study (GWAS) study for extraversion indicated that simple additive variance by common SNPs cannot explain a significant amount of variance but polygenic risk scores, weighted using linkage information, significantly predicted extraversion

scores in an independent cohort, showing that extraversion is a highly polygenic personality trait.[24]

Several studies have also reported shared environmental contributions to personality and related traits, especially deviated characters such as aggression[25–27] and attachment[28,29] in childhood. Cloninger's character dimensions also showed a substantial shared environmental effect in adolescence.[30]

19.3 Development trends of personality

Contrary to the increasing trend of heritability of cognitive abilities from childhood to adulthood,[31,32] the opposite trajectory was reported in studies on personality. One classic meta-analysis of developmental trends of twin resemblance[33] indicated that although both MZ and DZ twins become dissimilar in intelligence and personality traits, DZ becomes more dissimilar in intelligence, indicating an increase in heritability but not in personality. This tendency was clearly illustrated in the meta-analysis research by Tucker-Drob and Briley (Fig. 19.1, Fig. 19.2).[33,34]

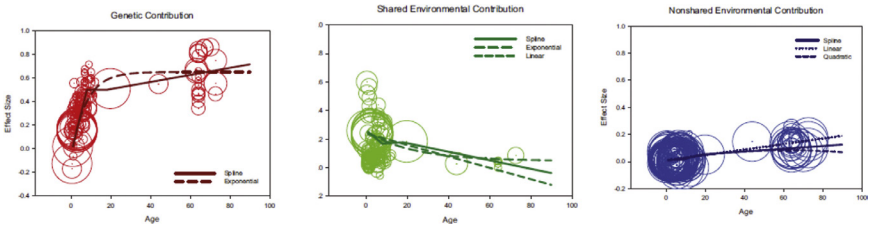


FIG. 19.1 Age-trends in heritability, nonshared environmentality of cognitive ability.
Circles surrounding data points are scaled by the weighting variable (described in analytic approach section) such that larger circles carried more weight in the analysis (Briley and Tucker-Drob, 2014).[32]

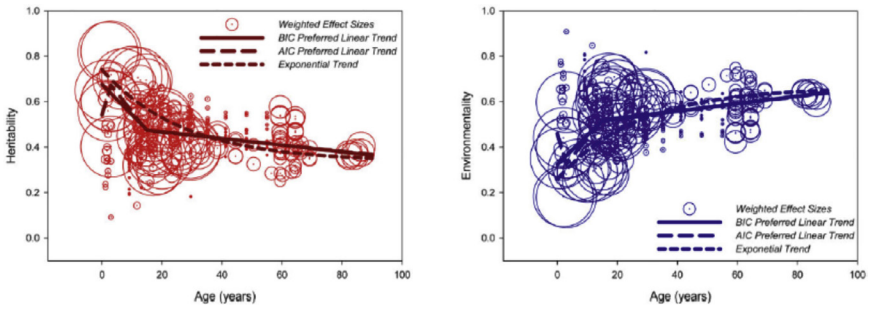


FIG. 19.2 Age-trends in heritability, nonshared environmentality of personality traits.
Circles surrounding data points are scaled by the weighting variable (described in analytic approach section) such that larger circles carried more weight in the analysis (Tucker-Drob and Briley, 2014).[34]

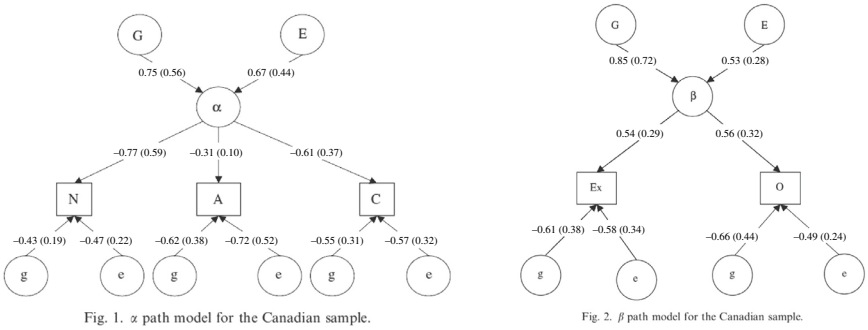


FIG. 19.3 Genetic structure of higher-order personality traits (Jang et al., 2006).[43]

Multivariate genetic analysis (i.e., bivariate and Cholesky decomposition analysis) of twin data indicated that phenotypic stability of personality and related traits are mainly explained by genetic factors and that their developmental changes are due to nonshared environmental changes[35–38] and genetic innovation.[39,40]

19.4 Genetic structure of personality

Personality traits are not completely orthogonal to each other. For example, five trait factors can be clustered into two higher-order factors phenotypically[41,42]; one consists of agreeableness, conscientiousness, emotional stability, called *alpha* or *stability,* the other consists of extraversion and intellect called *beta* or *plasticity* (Fig. 19.3). One twin study with a cross-national sample from three different countries (Canada, Germany, Japan) verified that this phenotypic two-factor structure reflects the underlying genetic structure of twins.[43] This study also revealed that genetic and nonshared environmental factor structures and phenotypic structures on 30 facets of NEO-PI-R (6 facets/domain × five domains) are highly congruent with one other and across nations. This suggested that the five-factor model has a strong biological basis and potentially indicates a common heritage of the human species.[44]

Twin studies are sensitive enough to detect genetic contributions not only at the level of domains and facets of personality traits but also at the top and bottom end of the personality hierarchy. Although personality structures are multidimensional, which contrasts with the single factor "g" of cognitive ability, personality traits can also be summarized to a single higher-order factor called "general factor of personality (GFP)."[45] This is positively loaded on Emotional Stability (an opposite direction of Neuroticism), extraversion, openness, agreeableness, conscientiousness and interpreted as a dimension of social adaptability and participation. It is hypothesized that GFP has an evolutionary emerging function that participates effectively in a social group and exhibits a genetic dominance effect.[46] The results of twin studies partially support this hypothesis.[47,48]

At the bottom of the personality, hierarchy is individual items of personality inventories. After eliminating facets and higher-order levels of personality structures, genetic contributions are still identified at each item level and called "nuances."[49]

Thus, the twin method in psychology can sensitively reveal genetic contributions within each level of the personality hierarchy.

19.5 Personality as a social behavior

Using the database of psychological literature (PsycINFO of American Psychological Association) more than 4000 articles are found when searching with the keywords "twin" and "personality" (about 2700 are found when "genetic" is added). This literature covers a range of psychological dimensions other than common personality traits such as social attitudes (e.g., political, religious, economic), undesired behavior (e.g., antisocial, aggressive, criminal, pathological, substance abusive). Using PubMed, 46,000 articles are found, which are more than 10 times as many as those from PsycINFO (approximately 23,000 are found when searching "twin," "personality," and "genetic"). This huge gap in the number of publications between psychological and medical fields can be because medical fields contain medical and biological (including molecular) characteristics that are not dealt with in the field of psychology. Thus, it is impossible to summarize all the literature completely, but this section introduces several interesting fields on social aspects of personality—political, vocational, economic, wellbeing, religious—using the twin method. Twin studies of personality have challenged and overcome the common prejudice that these social aspects tend to be considered as purely "social" and formed by environmental factors without any genetic influence.

The most impressive studies concern the genetic influence on political attitudes such as the liberal versus conservatism, individualism versus collectivism, authoritarianism versus authoritarian, which are considered to be outcomes of social learning. A cross-national meta-analysis of more than 12,000 pairs of twins from various countries demonstrated that approximately 40% of the variance in political attitudes can be explained by genes, 20% by shared environment, 40% by nonshared environment.[50]

This meta-analytic study also examined economic egalitarianism and showed substantial genetic influence. How about economic behaviors such as investment, saving, and earnings? These money-related behaviors also exhibited a genetic contribution of approximately 20%.[51] According to a study of more than 15,000 pairs of twins in Sweden,[52] genetic differences explained by approximately 33% of the variation in savings propensities across individuals and shared environmental contributed to a decrease in savings rates from young adulthood to middle age.

The economic situation is a significant factor of well-being. A systematic meta-analysis of 30 studies on twin-families with approximately 50,000 individuals on well-being and its related measures including satisfaction with life and happiness demonstrated that the weighted average heritability of well-being was 36 % and the weighted average heritability for life satisfaction was 32% with little or no shared environment.[53]

Religiousness or religiosity, that is, the beliefs or attitudes pertaining to religion, is associated with happiness and meaning in life,[54] and so many twin studies have examined this factor.[55–60] According to the literature, religiousness does not follow the second law of behavior genetics, indicating a substantial contribution of the shared environment. This is noteworthy as religiousness is correlated with personality,[61,62] which usually does not indicate any significant contribution of shared environment. This may be because scales of religiousness include religious customs such as church attendance, which tends to be shared by family members. Religiousness plays the role of the moderator of gene × environment interaction on drinking,[63] indicating that the heritability of alcohol consumption is lower in religious persons than in nonreligious people.

If religiousness is one end of human virtue, criminality may be the opposite end. Meta-analyses of twin and adoption studies concerning criminality and antisocial behavior[64] suggest that there are moderate additive genetic influences, nonadditive genetic influences, nonshared environmental influences on criminality, moderate additive genetic and nonshared environmental influences, modest shared environmental influences on aggression. This conclusion follows the three laws of behavioral genetics by Turkheimer (2000).[17] Several twin studies have suggested that juvenile delinquency is less heritable and shared-environmental factors are more influential than delinquency in adulthood,[65,66] but as discussed in this meta-analysis, this conclusion should be pending due to methodological problems such as measurement ambiguity.

19.6 Discordant identical twin method

The last part of this chapter discusses the discordant identical (monozygotic) twin method in which identical twin siblings with large phenotypic differences or different diagnoses are compared to identify the causal relationships between genetic/environmental factors and personality.

When examining the causal relationship between certain psychological or behavioral outcomes (X) and any specific environmental condition (Y), a mere correlation between X and Y or mean differences of X across groups categorized by Y are insufficient because information on Y contains not only individuals' specific environmental conditions but also underlying genetic and family (shared) environmental effects confound in Y. To eliminate these confounding factors from pure environmental factors specific to each individual, the discordant identical (monozygotic) twin method is effective because monozygotic twin pairs share the same genes and family (shared) environment and the difference between these identical twin siblings can sensitively reflect their individual (nonshared) environmental conditions. For example, if a within-pair difference of parenting style toward children (e.g., authoritative parenting) at a certain time point (e.g., 42 months of age) is correlated with within-pair differences in behavior problems (e.g., problems with peers) at 48 months of age but not vice versa (i.e., the within-pair difference at 42 months is less correlated with that of authoritative parenting), it can be concluded that a causal direction from parenting style to behavior problem is more plausible than in the opposite direction. The more

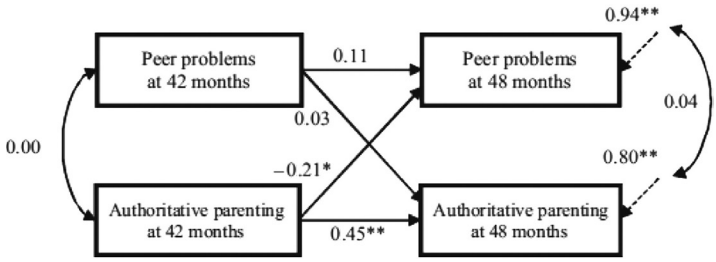


FIG. 19.4 Direction of causation between peer problems and authoritative parenting by the discordant twin method (Yamagata et al., 2013).[67]

* $p < .05$, ** $p < .01$.

sophisticated analysis such as cross-lag analysis applied to this kind of dataset can depict a clear causal picture (Fig. 19.4).[67]

Another interesting application of the discordant twin method is in economics, which aims to identify the causal relationship between education and earnings.[68–70] The causal direction of personality and earnings was challenged by this method. For example, "activity," a facet of extraversion (E) in the Big Five theory, is related to higher earnings, neuroticism (N) is related to lower permanent earnings in the labor market.[71]

The discordant identical twin method is also an important tool to detect epigenetic alteration in DNA (see Chapters 30 and 33 in the current book).

Recent behavior genetic studies have focused on personality and related traits at the molecular level. Several interesting findings have already been reported in large-scale GWAS consortia.[72,73] It is evident that molecular approaches, including epigenetic mechanisms, have become mainstream in this field. However, the classic twin method and its various applications remain powerful tools to identify and verify the complex causal mechanisms of genes and the broader environment of personality.

References

1. Allport GW. *Personality: A Psychological Interpretation*. New York, NY: H. Holt and Company; 1937.
2. Mischel W. *Personality and Assessment*. London: Wiley; 1968.
3. Spielberger CD. Theory and research on anxiety. In: Spielberger CD, ed. *Anxiety and behavior*. New York, NY: Academic Press; 1966.
4. Segal NL. *Born Together, Reared Apart: The Landmark Minnesota Twin Study*: Harvard University Press, USA; 2012.
5. Allport GW, Odbert HS. Trait-names: a psycho-lexical study. *Psychological Monographs*. 1936;47:i–171. https://doi.org/10.1037/h0093360.
6. Wilson GD, Barrett PT, Gray JA. Human reactions to reward and punishment: a questionnaire examination of Gray's personality theory. *British Journal of Psychology*. 1989;80:509–515.
7. Eysenck HJ. *Dimensions of personality*. London: Routledge & Kegan Paul; 1947.

8. Costa PTJr, McCrae RR. *NEO PI-R Professional Manual*. Odessa, FL: Psychological Assessment Resources, Inc; 1992.
9. Ashton MC, Lee K. Empirical, theoretical, practical advantages of the HEXACO model of personality structure. *Personality and Social Psychology Review*. 2007;11:150–166.
10. Cloninger CR. *The Temperament and Character Inventory (TCI): A Guide to its Development and Use*. St. Louis, MO: Center for Psychobiology of Personality, Washington University; 1994. ISBN 978-0-9642917-1-3.
11. Cattell RB, Stice GF. *The Sixteen Personality Factor Questionnaire*. 3rd ed. Champaign, IL: Institute for Personality and Ability Testing; 1962.
12. Jang KL, Livesley WJ, Vernon PA. Heritability of the big five personality dimensions and their facets: a twin study. *Journal of Personality*. 1996;64:577–591.
13. Loehlin JC, McCrae RR, Costa PT. Heritabilities of common and measure-specific components of the Big Five personality factors. *Journal of Research in Personality*. 1998;32:431–453.
14. Shikishima C, Ando J, Ono Y, Toda T, Yoshimura K. Registry of adolescent and young adult twins in the Tokyo area. *Twin Research and Human Genetics*. 2006;9:811–816.
15. Riemann R, Angleitner A, Strelau J. Genetic and environmental influences on personality: a study of twins reared together using the self- and peer report NEO-FFI scales. *Journal of Personality*. 2006;65(3):449–475.
16. Kandler C, Richter J, Zapko-Willmes A. The nature and nurture of HEXACO personality trait differences: an extended twin family study. *Zeitschrift für Psychologie*. 2019;227:195–206. https://doi.org/10.1027/2151-2604/a000378.
17. Turkheimer E. Three laws of behavioral genetics and what they mean. *Current Directions in Psychological Science*. 2000;9:160–165. https://doi.org/10.1111/1467-8721.00084.
18. Rebollo I, Boomsmsma DI. Genetic analysis of anger: genetic dominance or competitive sibling interaction. *Behavior Genetics*. 2006;36:216–228. http://doi.org/10.1007/s10519-005-9025-8.
19. Keller MC, William L, Coventry WL, Heath AC, Martin NG. Widespread evidence for non-additive genetic variation in Cloninger's and Eysenck's personality dimensions using a twin plus sibling design. *Behavior Genetics*. 2005;35(6):707–721.
20. Boomsma DI, Helmer Q, Nieuwboer HA, et al. An extended twin-pedigree study of neuroticism in the Netherlands twin register. *Behavior Genetics*. 2018;48:1–11. http://doi.org/10.1007/s10519-017-9872-0.
21. Bezdjian S, Baker LA, Tuvblad C. Genetic and environmental influences on impulsivity: a meta-analysis of twin, family and adoption studies. *Clinical Psychology Review*. 2011;31:1209–1223. http://doi.org/10.1016/j.cpr.2011.07.005.
22. Heiman N, Stallings MC, Young SE, Hewitt JK. Investigating the genetic and environmental structure of Cloninger's personality dimensions in adolescence. *Twin Research*. 2017;7:462–470. http://doi.org/10.1375/twin.7.5.462.
23. Keller MC, Coventry WL, Heath AC, Martin NG. Widespread evidence for non-additive genetic variation in Cloninger's and Eysenck's personality dimensions using a twin plus sibling design. *Behavior Genetics*. 2005;35:707–721. http://doi.org/10.1007/s10519-005-6041-7.
24. van den Berg SM, et al. Meta-analysis of genome-wide association studies for extraversion: findings from the genetics of personality consortium. *Behavior Genetics*. 2016;46:170–182. http://doi.org/10.1007/s10519-015-9735-5.
25. Borkenau P, Rainer R, Angleitner A, Spinath FM. Assessment issues in behavior-genetic research on personality. *Psychology: The Journal of the Hellenic Psychological Society*. 2002;9:212–225.

26. Tuvblad C, Fanti KA, Andershed H, Olivier F, Colins OF, Larsson H. Psychopathic personality traits in 5 year old twins: the importance of genetic and shared environmental influences. *European Child & Adolescent Psychiatry*. 2017;26:469–479.
27. Tarantino N, Tully EC, Garcia SE, South S, Iacono WG, McGue M. Genetic and environmental influences on affiliation with deviant peers during adolescence and early adulthood. *Developmental Psychology*. 2014;50:663–673.
28. Crawford TN, Livesley WJ, Jang KL, Phillip RS, Cohen PC, Ganiban J. Insecure attachment and personality disorder: a twin study of adults. *European Journal of Personality*. 2007;21:191–208.
29. Bokhorst CL, Bakermans-Kranenburg MJ, Fearon RMP, van IJzendoorn MH, Fonagy P, Schuengel C. The importance of shared environment in mother-infant attachment security: a behavioral genetic study. *Child Development*. 2003;74:1769–1782.
30. Lester N, Garcia D, Lundström S, et al. The genetic and environmental structure of the character sub-scales of the temperament and character inventory in adolescence. *Annals of General Psychiatry*. 2016;15:10. http://doi.org/10.1186/s12991-016-0094-2.
31. Haworth CMA, Wright MJ, Luciano M, et al. The heritability of general cognitive ability increases linearly from childhood to young adulthood. *Molecular Psychiatry*. 2010;15:1112–1120.
32. Tucker-Drob EM, Briley DA. Continuity of genetic and environmental influences on cognition across the life span: a meta-analysis of longitudinal twin and adoption studies. *Psychological Bulletin*. 2014;140:949–979.
33. McCartney K, Harris MJ, Bernieri F. Growing up and growing apart: a developmental meta-analysis of twin studies. *Psychological Bulletin*. 1990;107:226–237.
34. Briley DA, Tucker-Drob EM. Genetic and environmental continuity in personality development: a meta-analysis. *Psychological Bulletin*. 2014;140:1303–1331. http://doi.org/10.1037/a0037091.
35. Bratko D, Butkovic A. Stability of genetic and environmental effects from adolescence to young adulthood: results of croatian longitudinal twin study of personality. *Twin Research and Human Genetics*. 2007;10:151–157.
36. Takahashi Y, Yamagata S, Kijima N, Shigemasu K, Ono Y, Ando J. Continuity and change in behavioral inhibition and activation systems: A longitudinal behavioral genetic study. *Personality and Individual Differences*. 2007;43:1616–1625.
37. Kamakura T, Ando J. Genetic and environmental effects of stability and change in self-esteem during adolescence. *Personality and Individual Differences*. 2007;42:181–190.
38. Shikishima C, Hiraishi K, Takahashi Y, Yamagata S, Yamaguchi S, Ando J. Genetic and environmental etiology of stability and changes in self-esteem linked to personality: A Japanese twin study. *Personality and Individual Differences*. 2018;121:140–146.
39. Kawamoto T, Endo T. Genetic and environmental contributions to personality trait stability and change across adolescence: results from a Japanese twin sample. *Twin Research and Human Genetics*. 2015;18:545–556.
40. Lacourse E, Boivin, Brendgen M, Petitclerc A, et al. A longitudinal twin study of physical aggression during early childhood: evidence for a developmentally dynamic genome. *Psychological Medicine*. 2014;44:2617–2627. http://doi.org/10.1017/S0033291713003218.
41. Digman JM. Higher-order factors of the Big Five. *Journal of Personality and Social Psychology*. 1997;73:1246–1256.
42. DeYoung CG, Peterson JB, Higgins DM. Higher-order factors of the Big Five predict conformity: Are there neuroses of health? *Personality and Individual Differences*. 2002;33:533–552.

43. Jang KLW, Livesley J, Ando J, et al. Behavioral genetics of the higher-order factors of the Big Five. *Personality and Individual Differences*. 2006;41:261–272.
44. Yamagata S, Suzuki A, Ando J, et al. Is the genetic structure of human personality universal? A cross-cultural twin study from North America, Europe, Asia. *Journal of Personality and Social Psychology*. 2006;90:987–998. http://doi.org/10.1037/0022-3514.90.6.987.
45. Musek J. A general factor of personality: evidence for the Big One in the five-factor model. *Journal of Research in Personality*. 2007;41:1213–1233.
46. Rushton JP, Bons TA, Hur YM. The genetics and evolution of the general factor of personality. *Journal of Research in Personality*. 2008;42:1173–1185.
47. Rushton JP, Bons TA, Ando J, et al. A general factor of personality from multi-trait–multimethod data and cross–national twins. *Twin Research and Human Genetics*. 2009;12:356–365.
48. Loehlin JC, Martin NG. The general factor of personality: Questions and elaborations. *Journal of Research in Personality*. 2011;45:44–49.
49. Mottus R, Sinick J, Terracciano A, et al. Personality characteristics below facets: A replication and meta-analysis of cross-rater agreement, rank-order stability, heritability, utility of personality nuances. *Journal of Personality and Social Psychology, Journal of Personality and Social Psychology*. 2018;117:e35–e50. ttps://doi.org/10.1037/pspp0000202.
50. Hatemi PK, Medland SE, Klemmensen R, et al. Genetic influences on political ideologies: Twin analyses of 19 measures of political ideologies from five democracies and genome-wide findings from three populations. *Behavior Genetics*. 2014;44:282–294.
51. Cesarini DA. Essays on genetic variation and economic behavior. *Dissertation Abstracts International Section A: Humanities and Social Sciences*. 2011;71:2967.
52. Cronqvist H, Siegel S. The origins of savings behavior. *Journal of Political Economy*. 2015;123:123–169.
53. Bartels M. Genetics of wellbeing and its components satisfaction with life, happiness, quality of life: a review and meta-analysis of heritability studies. *Behavior Genetics*. 2015;45:37–156.
54. Steger MF, Hicks BM, Krueger RF, Bouchard Jr TJ. Genetic and environmental influences and covariance among meaning in life, religiousness, spirituality. *The Journal of Positive Psychology*. 2011;6:181–191.
55. Truett KR, Eaves LJ, Meyer JM, Heath AC, Martin NG. Religion and education as mediators of attitudes: a multivariate analysis. *Behavioral Genetics*. 1992;22:43–62.
56. Beer JM, Arnold RD, Loehlin JC. Genetic and environmental influences on MMPI factor scales: joint model fitting to twin and adoption data. *Journal of Personality and Social Psychology*. 1998;74:818–827.
57. Winter T, Kaprio J, Viken RJ, Karvonen S, Rose RJ. Individual differences in adolescent religiosity in Finland: familial effects are modified by sex and region of residence. *Twin Research*. 1999;2:108–114.
58. Koenig LB, McGue M, Krueger RF, Bouchard Jr TJ. Genetic and environmental influences on religiousness: findings for retrospective and current religiousness ratings. *Journal of Personality*. 2005;73:471–488.
59. Bradshaw M, Ellison C. Do genetic factors influence religious life? Findings from a behavior genetic analysis of twin siblings. *Journal for the Scientific Study of Religion*. 2008;47:529–544.

60. Button TMM, Stallings MC, Rhee SH, Corley RP, Hewitt JK. The etiology of stability and change in religious values and religious attendance. *Behavior Genetics*. 2011;41:201–210. http://doi.org/10.1007/s10519-010-9388-3.
61. Kandler C, Rieman R. Genetic and environmental sources of individual religiousness: the roles of individual personality traits and perceived environmental religiousness. *Behavior Genetics*. 2013;43:297–313. http://doi.org/10.1007/s10519-013-9596-8.
62. Ksiazkiewicz A, Friesen A. The higher power of religiosity over personality on political ideology. *Political Behavior*. 2019. http://doi.org/10.1007/s11109-019-09566-5.
63. Koopmans JR, Slutske WS, van Baal GCM, Boomsma DI. The influence of religion on alcohol use initiation: Evidence for genotype X environment interaction. *Behavior Genetics*. 1999;29:445–453.
64. Rhee SH, Waldman ID. Behavior-genetics of criminality and aggression. In Flannery DJ, Vazsonyi AT, Waldman ID, (Ed) "The Cambridge Handbook of Violent Behavior and Aggression", 77-90; New York, NY: Cambridge University Press; 2007.
65. DiLalla LF, Gottesman II. Heterogeneity of causes for delinquency and criminality: lifespan perspectives. *Development and Psychopathology*. 1989;1:339–349.
66. Cloninger CR, Gottesman II. Genetic and environmental factors in antisocial behavior disorders. In: Mednick SA, Moffitt TE, Stack SA, eds. *The Causes of Crime: New Biological Approaches*. New York, NY: Cambridge University Press; 1987:92–109.
67. Yamagata S, Takahashi Y, Ozaki K, Fujisawa KK, Nonaka K, Ando J. Bidirectional influences between maternal parenting and children's peer problems: A longitudinal monozygotic twin difference study. *Developmental Science*. 2013;16:249–259.
68. Ashenfelter O, Krueger A. Estimates of the economic return to schooling from a new sample of twins. *American Economic Review*. 1994;84:1157–1173.
69. Isacsson G. Estimating the economic return to educational levels using data on twins. *Journal of Applied Econometrics*. 2004;19:99–119.
70. Nakamuro M, Inui T. Estimating the returns to education using a sample of twins: the case of Japan. RIETI Discussion Paper Series 12-E-076 ;2012.
71. Maczulskij T, Viinikainen J. Is personality related to permanent earnings? Evidence using a twin design. *Journal of Economic Psychology*. 2018;64:116–129.
72. de Moor MHM, Costa PT, Terracciano A, et al. Meta-analysis of genome-wide association studies for personality. *Molecular Psychiatry*. 2012;17:337–349.
73. van den Berg SM, de Moor MHM, Verweij KJH, et al. Meta-analysis of genome-wide association studies for extraversion: findings from the genetics of personality consortium. *Behavior Genetics*. 2016;46:170–182. http://doi.org/10.1007/s10519-015-9735-5.

CHAPTER

Twin research in psychopathology

20

Lisabeth Fisher DiLalla[a], Matthew R. Jamnik[b], Riley L. Marshall[b], Emily Pali[b], David L. DiLalla[b]

[a]*Southern Illinois University School of Medicine*

[b]*Southern Illinois University Carbondale*

Psychological disorders have always been a major focus of behavior genetic twin research. Psychologists have attempted to examine the etiology of various psychological disorders not only to have a better understanding of the disorders themselves but also to attempt to devise effective preventions and interventions. The nature of the twin design has great allure for scholars of psychopathology. Unlike many psychological phenomena, it is either impossible or unethical to randomly assign study participants to a control versus experimental group when one of those groups involves a disorder. Luckily, the "natural experiment" of twins provides an opportunity to study people who share either half or all of their genes and much of their environment, especially during their formative years, but who may vary in terms of whether both twins develop a psychological disorder or only one does.

One particular twin-method example involves studying monozygotic (MZ) twin pairs where one member of the pair has a disorder and the other does not. Differences in genes cannot account for the different behavioral presentations. Accordingly, in this situation, it is possible to examine environmental influences that may differ between twins to determine whether those differences are related to one twin but not the cotwin being diagnosed with a disorder. Such an approach effectively controls for genetic influences on the disorder and allows examination of specific environmental influences that cause the twins to differ. However, there are a number of potential problems that make this approach difficult.[1] For example, do early environmental differences cause later mental illness, or are they manifestations of the illness that appear before the illness is fully realized? Could such early environmental differences be elicited by something that already differs between the twins that precedes the mental illness, such as more difficult childhood behaviors? Most often, these data are collected when the twins already are adults and diagnosed, so could the early differences be remembered inaccurately? It is not unusual for memories to change after a significant life event, such as a diagnosis of illness. Memories then become filtered through this new lens.[2] Finally, it is generally not possible, unless one conducts the

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00007-6

equivalent of a cradle-to-grave study, to know that the discordant twin might actually become concordant in the future.

The full twin design, with both MZ and dizygotic (DZ) twins, provides the opportunity to study the extent to which genetic as well as different types of environmental influences affect the occurrence of a psychological disorder (see Section 4 of this book for more details about the design of such studies). Again, the existence of this "natural experiment" allows researchers to study psychopathology as it naturally occurs in twins, often unevenly, allowing comparisons between twins. The expectation is that if the illness is at least partly a result of genotype, then MZ twins will be significantly more similar to each other than are DZ twins. Additionally, if the disorder is genetic and occurs along a spectrum (from a few symptoms to full-blown illness), then MZ cotwins of affected twins will be likely to have more symptoms than will DZ cotwins, and other biologically-related family members will also show some symptoms, but fewer than will cotwins.

A great challenge in studying psychopathology lies in the definition of the various illnesses. Accepted definitions of disorders change over time as we learn more about each disorder. Additionally, application of diagnostic standards may have a subjective component.[3] Accordingly, in twin studies of psychopathology it is critically important to employ reliable, well-validated diagnostic instruments.[3] Without such criteria, as might be the case in less well-controlled studies, rater bias may cloud diagnosis accuracy. In addition, there is scientific debate about the nature of psychopathology itself. For example, is it most accurate to think of psychopathology as a collection of discrete disease entities each with a clear and recognizable boundary? Or is it more accurate to view psychopathology in a dimensional fashion that highlights shades of gray? As is often the case, the truth likely lies somewhere in between, although recent work suggests that true, clearly defined categories of psychopathology may be relatively rare.[4] Researchers must make determinations about how to study these complex phenomena, and despite evidence that few disorders may truly be discrete, a categorical approach to diagnosis – you either have it or you don't – is commonly applied in research. Other studies approach the problem from the shades-of-gray perspective, focusing on what are sometimes called spectrum disorders. Such studies consider symptoms as part of a continuum, with people varying in terms of the number and degree of symptoms. Different twin design statistics must be used depending on how the psychopathology is measured.

There are a large number of psychological disorders that have been studied by behavior geneticists. In this chapter, we will focus on three that have been studied in great detail: schizophrenia, depression and bipolar disorders, and antisocial personality disorder (ASPD). A synopsis of twin studies that have been conducted on many different disorders can be found in the primer book on behavior genetics by Knopik, Neiderhiser, DeFries, and Plomin.[5]

Prior to discussing behavior genetic research related to specific psychological disorders, it is essential to define what is meant by heritability. This is covered in more detail in the chapters of this volume pertaining to methodology (section 4). Heritability reflects the degree to which genetic variation in a population contributes

to observed variation in an outcome of interest, in this case, a disorder or a constellation of symptoms. Heritability ranges from 0 to 1.0. If heritability is zero, this means that observed differences among individuals are completely the result of environmental variation. If heritability is 1.0, this means that all observed variation in the outcome of interest is the result of genetic variation among individuals. Heritability scores between 0 and 1.0 suggest that both genes and environment impact variation of the trait being studied.

There are several important ways that genetic factors might influence psychological outcomes. Single gene influences on complex behavioral phenomena are highly unlikely—so we do not expect that our search for answers will point to single genes that code for psychopathology. Influences on psychopathology are much more complicated than that, involving both multiple genes and multiple environmental influences. And the complexity increases: in terms of genetics, the genetic influences may be additive, whereby the effects of individual genes literally "add up" to determine overall genetic influence; alternately, genes may coact or interact with each other in nonlinear ways, resulting in so-called nonadditive genetic effects. Finally, it is understood that genes do not express themselves in isolation. Environmental influences are typically described as shared (for example, factors that siblings in the same family might experience) or unshared (for example, events that are unique to, or uniquely experienced by, an individual). Genes and environments coact and interact in a variety of ways. Ultimately, a complex interplay between genes and environments contributes to the disorders described in this chapter.

20.1 Schizophrenia

Schizophrenia is a psychological disorder that affects approximately 20 million people around the world,[6] with median lifetime prevalence of 4.0 per thousand individuals.[7] It is a chronic condition that includes symptoms such as hallucinations, delusions, grossly illogical thinking, poverty of speech, catatonia, and blunted/restricted affect.[8] Schizophrenia is a complex disorder that can cause much devastation and disability to a person experiencing it.[1] Due to its genetic nature, twin studies are valuable sources of information on this disorder. Due to its high proclivity to cause suffering and the problems experienced by those affected, studies examining its causes are of the utmost importance.

It is valuable to first acknowledge the contribution of adoption studies in our understanding of schizophrenia. Adoption studies also can help us tease apart environmental and genetic factors leading to the development of psychological disorders. This is because the adoptive parents contribute environmental influence but do not share genes with their adoptive children, whereas the biological parents share genes but not postnatal environments with their adopted-away children. For instance, in a study of children born to mothers with schizophrenia versus control mothers without schizophrenia, but all adopted into homes of parents without schizophrenia, the rate of schizophrenia in the children in the maternal schizophrenia group was 5.6%

versus 0.9% for children of mothers in the control group.[9] Because these adopted children were placed in homes with parents not diagnosed with schizophrenia, their environments were not affected by having a parent with schizophrenia. The higher rate of schizophrenia among adopted children born to biological mothers diagnosed with schizophrenia, as compared to the rate of schizophrenia among adopted children in the control group, suggests that schizophrenia has a significant genetic basis. Further, in this study, adopted children born to biological mothers with schizophrenia had a 14.8% prevalence of nonpsychotic latent schizophrenic symptoms, compared to 0.9% of the control group. This suggests that the propensity for schizophrenia-like symptoms also is influenced by genetic factors.

Similarly, twin studies help us to determine the heritability of disorders and the impact of the shared versus nonshared environment. Twin studies estimate heritability for schizophrenia to range from 41% to 83%, with an estimate, based on a national Danish cohort, of 79% for schizophrenia alone.[10] A similar heritability estimate of 73% was found for schizophrenia spectrum disorders,[10] which include schizoaffective disorder (a combination of symptoms of schizophrenia such as hallucinations and delusions as well as mood symptoms often seen in depression and bipolar disorder) and schizophreniform disorder (a briefer version of schizophrenia lasting one to six months). This may indicate that schizophrenia is influenced by a variety of genes, and that certain conditions must be met for these genes to be expressed in a way that produces symptoms of schizophrenia and schizophrenia spectrum disorders. A large number of studies now have shown that schizophrenia is highly genetic, with MZ twins expressing higher rates of concordance than DZ twins. At the same time, the concordance rates for schizophrenia among identical twins is roughly 50%.[11] This also highlights the clear influence of environmental factors that contribute to development of the disorder. Studies of identical twins who are discordant for schizophrenia provide a unique vantage point on environmental factors that may be protective or may serve as triggers for onset of disorder.

Other aspects of the etiology of schizophrenia can also be assessed using twin samples. In one study, when both twins were diagnosed with schizophrenia, the development of the disorder in one twin was shortly followed by the development of the disorder in the other twin (one to four years later for MZ twins and within six years for the sample's one concordant pair of DZ twins).[12] Heritability increases when schizophrenia symptoms are more broadly defined, such as including the psychosis that is present in bipolar disorder, versus when the definition is narrow (as in only diagnoses of schizophrenia), but only marginally so. This may indicate that the broad category of "psychosis-proneness" is highly genetic and occurs often between twin pairs when one is afflicted. This study also outlines the difficulties in studying schizophrenia in twins, as only approximately 0.02% of the entire population are twins diagnosed with schizophrenia, leading to small sample sizes for studies. Therefore, twin participation in research is critical, as is having large databases in twin research. Importantly, twin studies also have demonstrated that relatives of a twin diagnosed with schizophrenia show similar but less extreme behaviors, supporting the notion that this disorder exists on a spectrum from mild schizotypal behaviors to full-blown schizophrenia, with an underlying genetic cause to these behaviors.[13]

A schizophrenia and bipolar twin study in Sweden, the STAR study, has illuminated many findings on the genetic nature of psychiatric disorders.[14] They have identified endophenotypes related to schizophrenia. Endophenotypes are measurable traits that appear to coexist in many people with the disorder of interest but are not aspects of the disorder. Therefore, these traits may share genetic etiology with the disorder and they may be easier to study, possibly making it easier to find the genes that affect them. If so, then that will help us to identify genes related to the disorder. Some of the major findings of the STAR project are that: impulsivity is an endophenotype commonly found among participants with schizophrenia; genes linked to memory seem to also be linked to schizophrenia; and aspects of cerebrospinal fluid seem to be related to both schizophrenia and bipolar disorder, based on electron microscopy and microglia in the fluid. Future directions of the STAR study are to identify genetic markers linked to schizophrenia and bipolar disorder, as well as structural differences in the brains of affected persons. This is one of the largest studies on twins with schizophrenia and bipolar disorder and will likely be instrumental in helping us further understand these disorders.

It is likely that more extreme forms of schizophrenia have a stronger genetic influence. For example, one study found that early age of onset of schizophrenia was linked to a more heritable nature of the illness.[15] In this study, if one twin was diagnosed with schizophrenia before age 22, the second twin was five times as likely to also have schizophrenia. This effect was more pronounced in female twins. Because schizophrenia is more common in males, the incidence of the early age of onset in females specifically may indicate a greater genetic predisposition to develop the illness.

Much research is concerned with the spectrum nature of schizophrenia and related disorders. One study found that heritability rates were comparable for schizophrenia spectrum disorders compared to schizophrenia itself.[10] In another study, regional cerebral blood flow (rCBF) was examined in various brain regions in MZ and DZ twins in the Danish Twin Register.[16] rCBF was found to be heritable, and regions of the left thalamus and bilateral putamina were correlated with both schizophrenia and schizophrenia spectrum disorders. Higher rates of rCBF in the left putamen were found to be heritable; higher rates were present in MZ cotwins and less so in DZ cotwins who did not have schizophrenia when their twin did. Further, this study found that thalamic blood flow was greater in schizophrenia spectrum disorders, but not in schizophrenia. The researchers argued that this may be due to protective factors involved with taking antipsychotic medication. More of the participants with schizophrenia were taking antipsychotics than their peers with schizophrenia spectrum diagnoses. Given this evidence, these data provide compelling evidence for the efficacy of antipsychotic prescription for patients with schizophrenia and schizophrenia spectrum disorders alike.

Another study examined heritability of glutamate (a primary neurotransmitter essential for our nervous system) and its link to schizophrenia spectrum disorders.[17] This study found that glutamate rates in the anterior cingulate cortex and the left thalamus were heritable (29% and 16%, respectively). Further, glutamate levels in the left thalamus of MZ twins with schizophrenia spectrum disorders were similar to levels in their unaffected cotwins, and this effect was stronger than in DZ twins,

indicating genetic effects. This study indicates that the left thalamus, in particular, may be implicated in schizophrenia spectrum disorders, and that glutamate levels in this area are implicated in the illnesses as well. This may also indicate a phenotype that is present even in unaffected cotwins who do not have the disorder, further emphasizing a spectrum nature of the illness.

To conclude, twin studies have been instrumental in demonstrating that schizophrenia is heritable, as are disorders that fall on the schizophrenia spectrum. In fact, twin studies have demonstrated that similar genes are responsible for schizophrenia and other disorders that lie on that spectrum. Additionally, twin research has been helpful in identifying some endophenotypes of schizophrenia, which may lead to discovery of specific genes that contribute to this disorder. That information will be especially helpful in the creation of better pharmaceutical treatments for schizophrenia.

20.2 Depression and bipolar disorders

Depression is a disorder characterized by prolonged periods of sadness, hopelessness, numbness, and anhedonia.[8] Bipolar disorder is characterized by dramatic mood shifts from mania (extreme positive mood) to depression.[8] There are two types of bipolar disorder: bipolar 1, which is characterized by mania without depressive episodes; and bipolar 2, which includes both manic and depressive episodes. Depression and bipolar disorder have complex etiologies that involve both genetic and environmental factors. Many researchers have examined the etiologies of depression and bipolar disorder using the twin method. Research has more recently turned to examine the ways that depression and bipolar disorder relate to other phenotypes through genetic and environmental mechanisms.

20.2.1 Depressive disorders

Twin studies show that depression in adults is moderately heritable. Although there are sex differences in adult depression, with women substantially more likely than men to be diagnosed, there is little evidence of sex differences in the etiology of depression. Several studies have shown that the heritability of depression is about 30%–35% in both males and females.[18, 19] The remainder of the variance is due to nonshared / uniquely-experienced environmental factors in both sexes.

Depression in children is also caused by additive genetic and nonshared environmental factors. However, shared environmental factors also play a role in depression in children when adult informants are used rather than child self-report measures. For example, one study[20] found that there was a shared environmental component to childhood depression when parent and teacher reports were considered. Another study[21] found significant genetic, shared environmental, and nonshared environmental components for emotional problems in 7-year-old twins as reported by the strengths and difficulties questionnaire.[22] The fact that shared environment is significant when parents and teachers report about children suggests that parents and teachers may consider twins to be more similar to one another than they actually

are, or that observers such as parents and teachers have difficulty discerning the internalizing aspects of depressive symptoms. As such, it is important to be mindful of these comparisons between twins when attempting to understand their depression. A combination of self-report and other-report may be beneficial when diagnosing depression in children, particularly multiples (e.g., twins, triplets) who may be more readily compared to each other than other children and thus may provoke more biased reporting.

20.2.2 Bipolar disorders

Twin studies have shown that bipolar disorder is more heritable than depression, with some estimates approaching 80%,[23] with the remaining variance due to nonshared environmental factors. Bipolar disorders (e.g., bipolar 1 and bipolar 2) appear to be related to each other through genetic mechanisms. Specifically, twins who have bipolar 1 disorder often have cotwins who have bipolar 2 disorder.[24] In one study,[24] both bipolar 1 and bipolar 2 were heritable (73% and 58%, respectively), and the combined heritability of having either of these was 77%. Among MZ twins who had either bipolar diagnosis, 38% of their cotwins also had either bipolar 1 or bipolar 2. Among DZ twins who had either bipolar diagnosis, only 8% of their cotwins had one of the diagnoses. These findings suggest that the disorders may share an underlying genetic liability.

20.2.3 Depression and bipolar disorder

Depression and bipolar disorder may share a genetic liability that may cause cotwins or other family members of people diagnosed with one of these disorders to be more likely to develop either disorder. A broad definition of affective disorders that includes both depression and bipolar is slightly more heritable (89%) than a narrow definition that includes just bipolar disorder (85% in this study).[25] Although this difference is small, it suggests that there may be genes unique to depression that do not contribute to variance in bipolar disorder. Thus, twin research has shown that depression and bipolar disorder are substantially heritable. Nonshared environmental factors are also important. Twin research has been instrumental in advancing our understanding of how depression and bipolar disorder develop.

20.3 Antisocial personality disorder (ASPD)

Antisocial behavior (AB) and ASPD have many serious negative consequences (financial, social, and emotional) for society, perpetrators, victims, and their families.[26] Generally, twin studies investigating AB and ASPD have indicated significant heritability as well as both shared and nonshared environmental influences contributing to the emergence of these behaviors and diagnosis.[5]

A thorough review provides recent findings related to the genetic influences of ABs from a variety of genetic-based perspectives, including family-based designs.[26]

Raine[27] provides a detailed discussion of underlying mechanisms related to ASPD from a clinical perspective. A detailed discussion of the interplay between genetic and environmental influences (e.g., gene-environment interaction and gene-environment correlation) predicting antisociality also is available.[28] Here, we provide a general overview of findings on AB and ASPD from recent behavior genetic studies.

One theoretical framework for examining antisociality, the developmental propensity model,[29] proposes that the likelihood of learning AB across development is increased via the influence of interactions between children's genetically influenced characteristics and their environment.[30] This model suggests that individual differences in certain traits, like negative emotionality, daring, and callousness, contribute to differences in the social environment children select, evoke, and respond to, leading to a greater propensity for antisociality.[29] That is, these underlying individual differences in temperament facilitate social interactions that increase the likelihood for AB. For example, a child high in negative emotionality may respond to threats and frustrations with intense negative emotion, which may lead to antisocial interactions involving oppositional and aggressive behaviors. Using a twin sample, Lahey and colleagues[30] provide evidence in support of this model by demonstrating that the temperamental traits of high negative emotionality and daring and low prosociality in childhood (ages 10 to 17 years old) each independently predicted ASPD symptoms in adulthood (ages 22 to 31 years). Family based designs (twin and adoption studies) are useful for disentangling genetic and environmental influences and, thus, for further understanding the etiology of antisociality.[5]

20.3.1 Antisocial behavior (AB)

AB includes behaviors that are deviant, rule-breaking, aggressive, and/or involve other forms of misconduct.[31,32] In general, meta-analyses of twin studies on AB suggest high heritability (50%–60%), with shared and nonshared environmental influences around 15% and 25%–35%, respectively.[26,31] Research also indicates that genetic and shared environmental factors largely contribute to stability across development, whereas nonshared environmental influences contribute to developmental change.[33,34] This means that environmental influences that make siblings different from each other (nonshared or uniquely-experienced environment) are largely responsible for changes in these behaviors over time. Although the relative contribution of underlying genetic and environmental influences of these behaviors do not differ across sex,[35] findings do indicate higher overall scores for observed ABs in males compared to females.[34] Given these discrepant findings (i.e., indicating similar underlying etiologies but differing prevalence rates across males and females), more research investigating these nuances between sexes is needed.[35]

It is also worth mentioning two adoption studies that showed significant interaction between genetic and environmental factors. A seminal adoption study by Mednick, Gabrielli, and Hutchings[36] found that past criminal convictions of both biological parents (who shared genes with the adoptees) and adoptive parents (who shared environment with the adoptees) put children at greater risk for AB, but AB

was multiplicatively higher if both biological and adoptive parents had a history of criminal convictions (demonstrating gene-environment interaction). More recently, another study exploring the underlying etiology using an adoption design found the genetic and environmental influences of a child's birth father, but not birth mother, are significant contributors to AB.[37] Thus, findings from adoption studies are consistent with those from twin studies. Many important questions remain regarding genetic and environmental influences on AB and serve as reminders that genetically informed investigations are especially beneficial for uncovering important influences that shape children's perceptions, attitudes, and behaviors.[37]

20.3.2 Antisocial personality disorder

ASPD encompasses certain disruptive behaviors that surpass a threshold and meet clinical diagnostic criteria,[38] including aggressiveness, impulsivity, disregard for safety of self or others, and lack of remorse, in addition to chronic behaviors such as breaking the law, lying, and conning others for personal profit or pleasure.[8] Similar to AB, ASPD diagnoses have been shown to be influenced significantly by both genetic and environmental influences.[27] Genetic liability factors for ASPD remain mostly stable over time, and the main source of behavior change is related to environmental factors.[39] That is, the mean number of ASPD criteria has been shown to decrease over a 10-year period, despite results also indicating stable genetic influences (i.e., highlighting the salience of environmental influences).

Previous research suggested ASPD was multidimensional, including behaviors of aggressive-disregard and disinhibition,[40] meaning that these various forms of ASPD should be considered as different in terms of causes and symptoms. However, more recent twin work indicates otherwise. Common heritability (51%) has been shown across aggressive-disregard and disinhibition, suggesting a single dimension may underlie these behaviors.[38] A recent population-based twin study examining criteria for ASPD diagnoses provides further support for these recent findings.[41] This study also found that ASPD may commonly include aggression and/or disinhibition; however, dependent on unique idiosyncrasies for each individual, they may present somewhat differently.[41] This is in line with AB studies suggesting gene-environment interplay.[28]

It has been suggested that ASPD be considered a neurodevelopmental disorder, given that neural (e.g., prefrontal cortex, amygdala, striatum) and genetic markers (e.g., MAO-A), as well as early risk factors (e.g., birth complications, toxin exposure, traumatic brain injury), contribute to its developmental course.[27] This is in line with results from twin studies of ASPD, which indicate significant genetic and environmental effects contributing to diagnosis. Two recent twin studies have investigated some of these neurodevelopmental hypothesized markers for antisociality, including the personality traits of sensation seeking and impulsivity and resting heart rate (RHR).[42,43] Using two independent twin samples, Mann and colleagues[43] examined sensation seeking and impulsivity, which they hypothesized were potential AB endophenotypes (measurable components that exist between genotype and observable

phenotypes). Overall, this study found that large portions of genetic variance in AB were accounted for by these personality traits. They concluded that sensation seeking and impulsivity likely represent nonclinical or sub-threshold expressions of a polygenic risk (i.e., genetic liability) for AB. Similarly, Hammerton and colleagues[42] investigated the influence of sensation seeking and impulsivity on AB, alongside a hypothesized biological marker, RHR. Although results did not indicate a direct effect of RHR on AB, they did indicate an indirect effect of RHR through sensation seeking, which builds upon the results found by Mann et al.[43] Overall, the findings from these two studies suggest that sensation seeking may be one (potentially) influential marker of AB in need of further investigation.

In sum, the twin research literature suggests that both AB and ASPD have underlying genetic and environmental contributors.[38] For both, genetic variation plays an important role,[26] but so too do environmental influences.[39] As several reviews also conclude, more work is needed to further understand the underlying etiology of antisociality,[26,28,44] further underscoring the necessity (and utility) of family based designs (twin and adoption studies) to investigate the complex underlying factors contributing to ASPD and AB.

20.4 Implications and future directions

The work described in this chapter highlights the influence of genetic factors on certain major forms of psychopathology, as well as the complex interplay between genes and environments that contributes to the expression of psychological disorders. It is important to acknowledge that even in cases where genetic influence on a disorder is presumed to be strong, for example in the case of schizophrenia, the genetically identical cotwin of a diagnosed individual will be diagnosed with schizophrenia in only about 50% of cases. This means that half of the time the cotwin will *not* be diagnosed with schizophrenia. Clearly, possessing certain genes confers risk for the disorder, but how do we understand the ways in which environmental factors protect from or contribute to the ultimate expression of disorder?

Longitudinal studies of twins discordant for a disorder, though challenging and costly to conduct, could provide an important vantage point. Where and when, for example, do discordant identical twins begin to show signs of diverging? Some work in the area of schizophrenia suggests that the crucial environmental factors may even occur prenatally.[45] Continued work in this area may contribute to the identification of environmental interventions that could benefit individuals who are at risk for developing a given disorder. Other types of twin studies also are greatly advancing our understanding of the degree to which various psychopathologies share both genetic and environmental etiologies. For example, by studying twins, we have been able to demonstrate that some of the genes that are responsible for depression also are responsible for anxiety, but that different environmental experiences may lead someone down one path or the other.[46,47]

Also humbling is the recognition that although there appear to be clear genetic contributions to the expression of psychopathology, we have not identified major

genes of effect. Rather, multiple genes of modest individual effect contribute to psychological disorders, with the likelihood that there are likely multiple pathways through which an ultimate phenotype will be expressed. Still, advances in molecular genetics may contribute to the development of targeted pharmacological interventions for individuals diagnosed with various disorders. Twin studies provide essential upper limit estimates of heritability, thus augmenting molecular genetic research.[10] Such work might also spark ethical debates regarding whether genetically at-risk individuals who are asymptomatic or are experiencing prodromal symptoms, but are known to be at genetic risk, should be offered – or required to take – pharmacological treatment in a preventive fashion. The ethical implications of future research are enormous and require us to move ahead carefully, fully considering the possible ramifications of what we discover in terms of both genetic and environmental influences on various psychopathologies.[48]

In a similar fashion, twin research with at-risk individuals that focuses on environmental factors that trigger psychopathology, or protect against it, may contribute to advancement in the development of environmentally based prevention efforts and treatments. By studying twins, we can hold genetic influences constant and thereby better understand what environmental influences are significant triggers for psychopathology. Even more interesting, twin studies allow us to investigate some of the ways in which genes interact and correlate with different environmental influences, providing us with an even greater understanding of the building blocks of disorders.

Future research in this field will move beyond the twin design to investigate interactions among genes and between genes and environments. Epigenetics is the study of phenotypic changes in genetic expression as a function of environmental exposure. These epigenetic processes may lead to differences in MZ twin phenotypes,[49] and they appear to be significant for understanding variation in presentations of mental illness. Environmental experiences, especially during sensitive periods of development, may cause changes in genetic expression that may not be evident until later in development.[50] These "molecular scars" may be especially influential for mental illness,[49] as evidence for schizophrenia suggests.

In sum, the field of psychopathology owes a huge debt to twin studies for unraveling much of the puzzle of the story of psychopathology. Utilizing various types of naturalistic twin designs, researchers have been able to identify the degree to which genes influence many disorders and contribute to shared etiology across certain disorders. In so doing, they have further identified environmental conditions that may lead to one disorder or another, controlling for genetic influences rather than ignoring them. The logic and elegance of the twin design[46] has permitted the study of genetic and environmental factors that cannot be observed directly but whose effects can be measured. We believe that the picture is ultimately a hopeful one. Genes are not destiny – recall once again that identical twins who share all their genetic material are discordant for psychopathology in a substantial proportion of cases. We may not change genes, but we can continue to strive to understand the ways in which genes and environments intertwine to produce behavioral outcomes so that we might craft preventions and interventions that will have a positive personal and societal impact.

References

1. Gottesman II. *Schizophrenia genesis: The origins of madness*. New York, NY: W.H. Freeman and company; 1991.
2. Roediger HL, McDermott KB. Tricks of memory. *Current Dir Psychol Sci*. 2000;9:123–127.
3. Rogers R, Williams MM, Wupperman P. Diagnostic interviews for assessment of mental disorders in clinical practice. In: Llewellyn CD, Ayers S, McManus C, Newman S, Petrie KJ, Revenson TA, Weinman J, eds. *Cambridge Handbook of Psychology, Health and Medicine*. 3rd ed. King's College, London: Cambridge University Press; 2019:179–183.
4. Haslam N, Holland E, Kuppens P. Categories versus dimensions in personality and psychopathology: a quantitative review of taxometric research. *Psych Med*. 2012;42:903–920.
5. Knopik VS, Neiderhiser JM, DeFries JC, Plomin R. *Behavioral Genetics*. New York, NY: Macmillan Higher Education; 2016.
6. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet North Am Ed*. 2019;392:1789–1858.
7. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. *PLoS Med*. 2005;2:e141.
8. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Arlington, VA: American Psychiatric Pub; 2013.
9. Ingraham LJ, Kety SS. Adoption studies of schizophrenia. *Am J of Med Genet*. 2002;97:18–22.
10. Hilker R, Helenius D, Fagerlund B, et al. Heritability of schizophrenia and schizophrenia spectrum based on the nationwide Danish Twin Register. *Biol Psychiatry*. 2018;83:492–498.
11. Prescott C, Gottesman II. Genetically mediated vulnerability to schizophrenia. *Psychiat Clinics of North Amer*. 1993;16:245–267.
12. Klaning U, Trumbetta SL, Gottesman II, Skytthe A, Kyvik KO, Bertelsen A. A Danish twin study of schizophrenia liability: investigation from interviewed twins for genetic links to affective psychoses and for cross-cohort comparisons. *Behav Genet*. 2016;46:193–204.
13. Walter EE, Fernandez F, Snelling M, Barkus E. Genetic consideration of schizotypal traits: a review. *Front Psychol*. 2016;7:1–19.
14. Johansson V, Hultman CM, Kizling I, et al. The schizophrenia and bipolar twin study in Sweden (STAR). *Schizophr Res*. 2019;204:183–192.
15. Hilker R, Helenius D, Fagerlund B, et al. Is an early age at illness onset in schizophrenia associated with increased genetic susceptibility? Analysis of data from the Nationwide Danish Twin Register. *EBioMedicine*. 2017;18:320–326.
16. Legind CS, Broberg BV, Brouwer R, et al. Heritability of cerebral blood flow and the correlation to schizophrenia spectrum disorders: A pseudo-continuous arterial spin labeling twin study. *Schizophr Bull*. 2019;45:1231–1241.
17. Legind CS, Broberg BV, Mandl RCW, Brouwer R, Anhoj SJ, Hilker R. Heritability of cerebral glutamate levels and their association with schizophrenia spectrum disorders: A ^{1}[H]-spectroscopy twin study. *Neuropsychopharmacology*. 2019;44:581–589.
18. Sullivan PF Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. *Amer J Psych*. 2000;157:1552–1562.
19. Agrawal A, Jacobson KC, Gardner CO, Prescott CA, Kendler KS. A population based twin study of sex differences in depressive symptoms. *Twin Res Hum Gen*. 2004;7:176–181.

20. Happonen M, Pulkkinen L, Kaprio J, Van der Meere J, Viken RJ, Rose RJ. The heritability of depressive symptoms: multiple informants and multiple measures. *J Child Psych and Psychiat*. 2002;43:471–479.
21. Saudino KJ, Ronald A, Plomin R. The etiology of behavior problems in 7-year-old twins: substantial genetic influence and negligible shared environmental influence for parent ratings and ratings by same and different teachers. *J Ab Child Psych*. 2005;33:113–130.
22. Goodman R. Psychometric properties of the strengths and difficulties questionnaire. *J Amer Academy Child Adolesc Psych*. 2001;40:1337–1345.
23. Kendler KS, Pedersen NL, Neale MC, Mathé AA. A pilot Swedish twin study of affective illness including hospital-and population-ascertained subsamples: results of model fitting. *Behav Gen*. 1995;25:217–232.
24. Edvardsen J, Torgersen S, Røysamb E, Lygren S, Skre I, Onstad S, Øien PA. Heritability of bipolar spectrum disorders. Unity or heterogeneity? *J Affect Dis*. 2008;106:229–240.
25. McGuffin P, Rijsdijk F, Andrew M, Sham P, Katz R, Cardno A. The heritability of bipolar affective disorder and the genetic relationship to unipolar depression. *Arch Gen Psych*. 2003;60:497–502.
26. Gard AM, Dotterer HL, Hyde LW. Genetic influences on antisocial behavior: recent advances and future directions. *Curr Opin Psychol*. 2019;27:46–55.
27. Raine A. Antisocial personality as a neurodevelopmental disorder. *Annu Rev Clin Psychol*. 2018;14:259–289.
28. Beaver KM, Schwartz JA, Gajos JM. A review of the genetic and gene–environment interplay contributors to antisocial phenotypes. In: Morizot J, Kazemian L, eds. *The Development of Criminal and Antisocial Behavior*. New York, NY: Springer; 2015:109–122.
29. Lahey BB, Waldman ID. A developmental propensity model of the origins of conduct problems during childhood and adolescence. In: Lahey B, Moffitt T, Caspi A, eds. *Causes of Conduct Disorder and Juvenile Delinquency*. New York, NY: Guilford Press; 2003:76–117.
30. Lahey BB, Class QA, Zald DH, Rathouz PJ, Applegate B, Waldman ID. Prospective test of the developmental propensity model of antisocial behavior: from childhood and adolescence into early adulthood. *J Child Psychol Psychiatr*. 2018;59:676–683.
31. Ferguson CJ. Genetic contributions to antisocial personality and behavior: a meta-analytic review from an evolutionary perspective. *J Soc Psychol*. 2010;150(2):160–180.
32. Zheng Y, Cleveland HH. Differential genetic and environmental influences on developmental trajectories of antisocial behavior from adolescence to young adulthood. *J Adolesc*. 2015;45:204–213.
33. Burt SA, McGue M, Iacono WG. Environmental contributions to the stability of antisocial behavior over time: are they shared or non-shared? *J Abnorm Child Psychol*. 2010;38:327–337.
34. Wesseldijk LW, Bartels M, Vink JM, et al. Genetic and environmental influences on conduct and antisocial personality problems in childhood, adolescence, and adulthood. *Eur Child Adolesc Psychiatry*. 2018;27:1123–1132.
35. Burt SA, Slawinski BL, Carsten EE, Harden KP, Hyde LW, Klump KL. How should we understand the absence of sex differences in the genetic and environmental origins of antisocial behavior? *Psychol Med*. 2019;49:1600–1607.
36. Mednick SA, Gabrielli WF, Hutchings B. Genetic influences in criminal convictions: evidence from an adoption cohort. *Science*. 1984;224:891–894.
37. Knoblach RA, Schwartz JA, McBride M, Beaver KM. The association between genetic predisposition and parental socialization: An examination of gene–environment

correlations using an adoption-based design. *Int J Offender Ther Comp Crimino*. 2020;64:187–209.
38. Rosenström T, Ystrom E, Torvik FA, et al. Genetic and environmental structure of DSM-IV criteria for antisocial personality disorder: a twin study. *Behav Gen*. 2017;47:265–277.
39. Reichborn-Kjennerud T, Czajkowski N, Ystrøm E, et al. A longitudinal twin study of borderline and antisocial personality disorder traits in early to middle adulthood. *Psychol Med*. 2015;45:3121–3131.
40. Kendler KS, Aggen SH, Patrick CJ. A multivariate twin study of the DSM-IV criteria for antisocial personality disorder. *Biol Psychiatry*. 2012;71:247–253.
41. Whipp AM, Korhonen T, Raevuori A, et al. Early adolescent aggression predicts antisocial personality disorder in young adults: a population-based study. *Eur Child Adolesc Psychiatry*. 2019;28:341–350.
42. Hammerton G, Heron J, Mahedy L, Maughan B, Hickman M, Murray J. Low resting heart rate, sensation seeking and the course of antisocial behaviour across adolescence and young adulthood. *Psychol Med*. 2018;48:2194–2201.
43. Mann FD, Engelhardt L, Briley DA, et al. Sensation seeking and impulsive traits as personality endophenotypes for antisocial behavior: evidence from two independent samples. *Pers Individ Dif*. 2017;105:30–39.
44. DiLalla LF, Diaz E, Jamnik MR. Towards the dark side: temperament, personality, and genetics related to aggressive behaviors. In: Saudino K, Ganiban J, eds. *Behavior Genetics of Temperament and Personality*. New York, NY: Springer; 2020.
45. Torrey EF, Taylor EH, Bracha HS, et al. Prenatal origin of schizophrenia in a subgroup of discordant monozygotic twins. *Schiz Bull*. 1994;20:423–432.
46. Roysamb E, Tambs K. The beauty, logic and limitations of twin studies. *Norsk Epidem*. 2016;26:35–46.
47. Kendler KS, Eaves LJ, Loken EK, et al. The impact of environmental experiences on symptoms of anxiety and depression across the life span. *Psychol Sci*. 2011;22:1343–1352.
48. DiLalla LF, McCrary M, Diaz E. A review of endophenotypes in schizophrenia and autism: the next phase for understanding genetic etiologies. *Am J Med Gen Part C Seminars in Med Gen*. 2017;175:354–361.
49. Richetto J, Meyer U. Epigenetic modifications in schizophrenia and related disorders: molecular scars of environmental exposures and source of phenotypic variability. *Bio Psychiatr*; 2021;89(3):215–226.
50. van IJzendoorn MH, Bakermans-Kranenburg MJ, Ebstein RP. Methylation matters in child development: toward developmental behavioral epigenetics. *Ch Dev Perspect*. 2011;5:305–310.

CHAPTER

21 Cognitive aging: the role of genes and environments in patterns of change

Deborah Finkel[a,b], **Nancy L. Pedersen**[c,d], **Chandra A. Reynolds**[e]

[a]*Department of Psychology, Indiana University Southeast, United States*
[b]*Institute for Gerontology, School of Health and Welfare, Jönköping University, Sweden*
[c]*Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden*
[d]*Department of Psychology, University of Southern California, United States*
[e]*Department of Psychology, University of California – Riverside, United States*

21.1 General cognitive ability

In contrast to studies of normative cognitive aging, which focused on trajectories of performance on specific cognitive tasks,[1–3] much of the early focus of early twin and family studies of development was on IQ (or general cognitive ability, GCA). The majority of studies were on children, adolescents, and young adults with the explicit purpose of demonstrating that genetic influences were important for cognition and a variety of other developmental outcomes (e.g., temperament and personality).[4,5] Indeed, these early studies could demonstrate that cognitive ability was heritable, and the relative importance of genetic influences appeared to increase from infancy through early adulthood. This led some behavior geneticists to predict that with age, the heritability of cognitive ability will increase,[6] while others modeled a variety of different scenarios.[7]

Prior to the 1980s, there were no twin or family studies explicitly designed to study aging in general or cognitive aging in particular. The large twin registries in the Nordic countries did not have cognitive data on the adult twins. Other studies of adult twins that did have cognitive data were predominantly cross-sectional in design. In the 1980s and 1990s, several twin studies of aging were started in Sweden, Denmark, and the United States. The first results from the Swedish Adoption/Twin Study of Aging (SATSA), with twins on average 65 years of age, indicated that the heritability of GCA (80%) was substantially greater than estimates earlier in life (about 50%), whereas the heritability of general cognitive abilities in the OCTO-Twin study of octo- and nonagenarian twins was 62%. Longitudinal analyses of the Vietnam Era Twin Study of Aging (VETSA), representing the first half of the adult life span, indicate strong and mildly increasing heritable contributions from age 20 to 61 years (59% to 64%).[8] Longitudinal analyses of SATSA, representing the second half of the

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00005-2

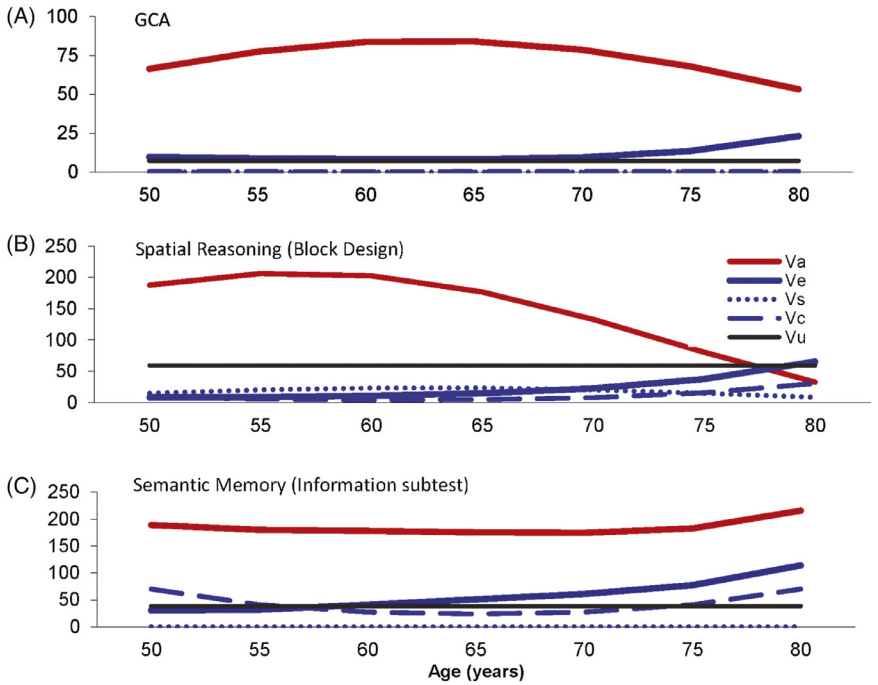


FIG. 21.1 Genetic and environmental contributions to cognitive aging: SATSA (adapted from ref.[9]

Notes. Va = additive genetic variation; Vs = shared rearing environmental variation; Vc = correlated environmental variation; Ve = nonshared environmental variation of growth model; Vu = variation unexplained by growth model.

lifespan, indicate that an apparent decrease in the heritability of GCA after 65 years reflected a decrease in genetic variance concomitant with an increase in environmental influences[9] (see Fig. 21.1A).

21.2 Specific cognitive abilities

Investigations of cognitive aging have identified different patterns of aging across domains of cognitive function, indicating faster rates of decline in age-sensitive domains (e.g., spatial abilities, processing speed, fluid abilities) and stability until late adulthood in aging-resilient domains (e.g., verbal abilities, crystallized abilities).[1–3]

In an attempt to understand these differences, twin studies of cognitive aging turned their focus from heritability of GCA to investigations of genetic and environmental contributions to age changes in specific cognitive abilities. Early work on cognitive aging, for example, demonstrated genetic influences on specific cognitive abilities independent of genetic influences on GCA[10] and diverse heritability estimates for different cognitive abilities,[11] indicating potential etiological differences in cognitive aging across domains. Subsequent research has leveraged both cross-sectional and longitudinal twin data to expand our understanding of the mechanisms that drive age changes in specific cognitive abilities. It is important to note that the methodological challenges associated with longitudinal studies also apply to longitudinal twin studies, particularly missing data due to selective drop-out and mortality. The effect of drop-out of older or less-healthy individuals typically biases the mean upward, suggesting higher performance levels than would be expected in the population. With regard to twin analyses, nonrandom drop-out may reduce the extent of variance, although the impact on genetic versus environmental components of variance is unclear.

21.2.1 Age changes in genetic variance

Differences in genetic and environmental influences across cognitive domains may arise from variable action of genetic factors and environmental influences, as well as their interplay over time. Increasing genetic variance could result from amplification of existing genetic factors, a form of genetic canalization,[12] or via mechanisms of the gene by environment interplay that continue to act and accumulate over the lifespan.[13] For example, positive feedback loops may result when we engage in behaviors that lead to experiences that in turn reinforce those behaviors, precipitating further experiences.[12] In contrast, aging is also associated with decreases in evolutionary pressures as individuals age beyond child-bearing years,[14] which could result in amplification of random or stochastic[15] and epigenetic processes[16] that may be reflected in increased environmental variance. Stability of environmental experiences in adulthood, paired with possible stability of gene action, may result in unchanging estimates of genetic and environmental variance for cognitive function.[12,17] Importantly, examining patterns of age changes in variance components across specific cognitive abilities enables testing theories that cognitive aging is driven by a single general phenomenon or results from a collection of aging processes unique to each specific ability.[18]

21.2.2 Traditional cognitive domains

Investigations of changes with age in genetic and environmental variance of specific cognitive abilities have generally focused on four major domains of functioning: verbal, spatial, memory, and processing speed. However, differences in measures, populations, and methods often result in inconsistent results across studies.[11] Two methods for resolving these differences are meta-analysis and analysis of pooled data (or pooled analysis). Meta-analysis involves a quantitative summary of published data, and the largest meta-analysis of twin data, incorporating millions of data points, suggested stable

heritability for verbal ability and memory over adulthood, but contained insufficient data about age differences in other cognitive domains to draw conclusions.[19] A recent meta-analysis focusing specifically on domains of cognitive aging integrated data from 19 twin and family studies from 12 countries including nearly 10,000 individuals from 3819 twin pairs ranging in age from 14 to 98 years.[20] Results indicated decreasing heritability for verbal ability after age 60, as well as modest decreases in heritability for processing speed. Estimates for general memory function and spatial ability were generally stable, with heritability peaking in midlife. These results suggest diverse aging processes in different cognitive domains, but meta-analyses of twin data are typically limited (by the published reports) to standardized components of variance. As a result, age changes in the underlying genetic and environmental variance components can be obscured by reliance on proportions of variance (heritability) instead of raw variance estimates. Moreover, sample age ranges may be reduced to mean age, further obfuscating the very age trend under investigation.

Pooled analyses, on the other hand, have the advantage of reanalysis of original raw data for both the cognitive measures and age, although harmonization of similar or identical measures from different studies can be challenging.[21] The Interplay of Genes and Environment across Multiple Studies (IGEMS) twin consortium recently reported a pooled analysis incorporating data from over 14,000 individuals from twin studies in Sweden, Denmark, and the United States.[22] Even though 47% of the sample overlapped with the meta-analysis reported by Reynolds and Finkel,[20] the difference in approach (pooled versus meta-analysis) marked a unique test of theories of aging. In fact, pooled analyses of twin data for vocabulary and synonyms tests indicated generally increasing genetic variance with age, in contrast to the decreasing heritability for verbal abilities reported by the meta-analysis.[20] Estimates of genetic variance for spatial ability and processing speed resulting from the pooled analyses were stable, similar to the results of the meta-analysis.

One limitation that both of these approaches shared was the reliance on cross-sectional data, and there is ample evidence that results from longitudinal studies may differ, both for examinations of mean performance and genetic and environmental influences on performance.[3,11] With longitudinal twin data, we can model the change trends over time and then examine genetic and environment influences on both the intercept (level at a given age) of that trend and the slope, or rate of change over time.[23] Applying growth models to SATSA data to evaluate change trends over time, we observed two patterns: (1) genetic variance decreased after age 65 years for spatial and speed abilities which are aging-vulnerable traits (see Fig. 21.1B), and (2) genetic variance increased for traits which are relatively age-resistant in terms of phenotypic change such as verbal abilities (see Fig. 21.1C). A review of longitudinal twin studies of cognitive aging reported strong heritabilities for the mean level in various cognitive domains, but typically much lower and more varied heritability estimates for rates of decline with age,[11] a pattern that was confirmed in the VETSA study.[8] To test theories that cognitive aging arises from a single general phenomenon or from a collection of processes unique to each cognitive ability, it is necessary to examine the extent to which genetic and environmental influences on intercepts

TABLE 21.1 Proportions of variance attributable to genetic (G) and environmental (E) factors common across cognitive domains and unique to each domain.

Cognitive domain	Common G factors	Unique G factors	Total G	Common E factors	Unique E factors	Total E
Verbal						
Intercept	49.99%	37.25%	87.24%	4.11%	8.65%	12.76%
Slope	30.21%	31.82%	62.03%	26.11%	11.87%	37.98%
Spatial						
Intercept	71.12%	19.98%	91.10%	5.85%	3.04%	8.89%
Slope	50.98%	4.86%	55.84%	44.05%	0.11%	44.16%
Memory						
Intercept	53.15%	37.00%	90.15%	4.37%	5.48%	9.85%
Slope	43.72%	1.70%	45.42%	37.78%	16.79%	54.57%
Speed						
Intercept	73.93%	12.99%	86.92%	6.08%	7.00%	13.08%
Slope	41.76%	14.50%	56.26%	36.08%	7.66%	43.74%

Adapted from Tucker-Drob et al.[24]

and rates of change are unique or overlapping across cognitive domains. Tucker-Drob and colleagues[24] applied a factor model that estimated general genetic and environmental influences common across cognitive domains and specific variance components unique to individual domains. As shown in Table 21.1, results indicate that genetic influences on cognitive aging are both unique to individual domains and general across domains. Both total genetic influences and common genetic influences are larger for intercepts (level at a given age) than for slopes (rates of change). In contrast, common environmental factors contributed a much larger portion to slopes than to intercepts. Across cognitive domains, values for shared and unique variance components varied considerably, indicating that multiple mechanisms likely play a role in aging in various cognitive domains.

21.2.3 Emerging cognitive domains

Recent advances in brain imaging have identified other domains of cognitive functioning fundamentally involved in aging processes, beyond conventional verbal, spatial, processing speed, and memory domains. For example, large age-related changes in brain structures are evident in areas involved in working memory, executive function, and language processing.[25] These abilities are important to successful daily functioning and indeed represent the more age-sensitive cognitive traits.[26] Working memory is identified by memory span tests requiring both processing and storage of information. Measures of working memory such as backward digit span and letter-number span are modestly heritable in mid to late adulthood, although it is unclear whether genetic influences are

stable across the lifespan or decrease somewhat with age.[20,22] Executive functions are separable traits from GCA and other traditional cognitive domains and include processes such as inhibitory control and set-shifting, working memory, planning, and verbal fluency among others.[27] Measures of executive functions may be particularly important for tapping subtle cognitive changes in midlife, but few twin studies of aging have incorporated the relevant tests. Results from those studies indicate modest heritability (29%–46%) for measures of executive function in midlife, with some evidence of decreasing heritability across adulthood.[28,29] Beyond simple vocabulary, measures of flexibility with language may play an important role in predicting cognitive decline, such as verbal and semantic fluency measures that require participants to start from a given semantic category or alphabetical letter and generate as many unique words as possible.[30] A pooled analysis of data from 21,856 adults from the IGEMS consortium found that heritability for verbal fluency decreased with age from 58% in midlife to 40% in late adulthood.[31]

21.2.4 Summary

In summary, examination of genetic and environmental influences on specific cognitive abilities indicates a variety of differences across domains: evidence for both increasing and decreasing genetic variance, and evidence for genetic and environmental factors common across domains and unique to individual tests. Thus, we find support for amplification of existing genetic factors across the lifespan in some cognitive domains and possible increases in random or stochastic processes in other domains. Although a portion of cognitive aging may be driven by a single general phenomenon, evidence also indicates aging processes unique to each specific ability. Ultimately, more longitudinal investigations of change from midlife through late adulthood incorporating measurements in multiple cognitive domains, possibly via meta-analyses and pooled analyses, are necessary to expand our understanding of the mechanisms of cognitive aging.

21.3 Molecular genetics

With the mapping of the human genome, researchers were able to investigate not just genetic and environmental variance, but the specific genes and genetic loci that underly the genetic variance in cognitive function. A recent systematic review highlights candidate gene and polygenic scores in studies of cognitive aging in mainly community-based or population-based samples of older adults.[32] Polygenic scores are based on the very small but cumulative contributions of hundreds or thousands of variants across of genes. They are formed by summing up each individual's alleles (SNPs) that contribute to a trait outcome in a genome-wide association study (GWAS) where each allele is weighted by effect sizes observed in the GWAS (see https://www.genome.gov/Health/Genomics-and-Medicine/Polygenic-risk-scores). The resulting score indicates an individual's composite genetic risk for developing the trait in

question. By and large, studies of dementia suggest a crucial role for apolipoprotein E that is coded by the *APOE* gene, the primary cholesterol transporter in the brain (https://ghr.nlm.nih.gov/gene/APOE). The *APOE* ε4 variant is a confirmed risk variant for Alzheimer's disease[33] and normative cognitive decline including GCA and episodic memory.[32,34] Specifically those who carry the *APOE* ε4 risk allele—associated with increase beta-amyloid deposition that is a key component of neuritic plaques, as well as tangles—show worse performance and longitudinal decline.[32,34] The size of the effect of the APOE ε4 allele on cognitive change, based on a meta-analysis of the Cognitive Ageing Genetics in England and Scotland (CAGES) samples,[34] suggests a decline in performance of about .2 standard deviations per 10 years, equivalent to a drop of 3 IQ points every 10 years. Polygenic risk scores have been examined for association with cognitive performance across the lifespan and/or cognitive aging based on GWAS of GCA[35] or IQ,[36] educational attainment,[37] and Alzheimer's disease adults.[32] Generally, findings support the contribution of many genes each of very small effect for cognitive performance across the adult lifespan[38] such that having a higher polygenic score for GCA and IQ predicts better performance on cognitive tests,[35,36] higher polygenic score for educational attainment is associated with genes that contribute to better cognitive performance[37,38] and a reduced risk for Alzheimer's disease.[37,38] With respect to cognitive decline, there may be small contributions of other AD-risk genes to cognitive performance and decline apart from *APOE*, for example, in related cholesterol or immune/inflammatory pathways, but the effects are fleeting across studies.[32] Likewise, studies of Alzheimer's disease risk show a smaller effect of measured polygenic factors apart from *APOE* as well.[33] Fig. 21.2 shows a word cloud that indicates the extent to which the top 25 identified

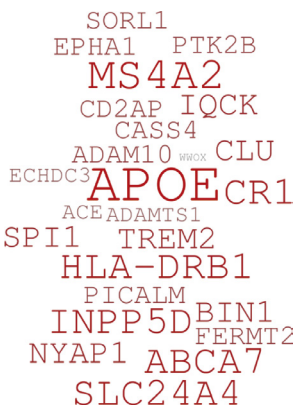


FIG. 21.2 Word cloud representing Top 25 gene loci in an Alzheimer's disease GWAS based on ref.[39]

Note. Non-*APOE* gene loci weighted by maximum priority ranking (c.f. Fig 21.2 and Supplementary Table 24 in ref.[39]); *APOE* assigned highest weight based on established evidence.

gene loci may be functionally involved in AD-relevant gene pathways, tissues and disease risk (i.e., AD/dementia risk above other disease traits) as identified in a 2019 AD GWAS meta-analysis;[39] we assigned *APOE* the highest priority score given its established role, as it had been excluded by design from the rankings.

Earlier work using twin methods sought to verify or disconfirm candidate gene associations with cognition and cognitive decline while controlling for family ancestry to counter the possibility of spurious effects, including *APOE* and other genes that participate in similar pathways.[40] However, in the wake of small effect sizes and failure to replicate many candidate associations in twin and other population-based samples,[41] the advent of GWASs provided more powerful ways to evaluate (and replicate) measured genetic contributions to complex traits such as cognitive aging. Indeed, data from twin studies are often included in large scale GWAS that examines single nucleotide polymorphisms (SNPs) associations with GCA and IQ,[35,36] and educational attainment.[37] Polygenic scores formed from multiple variants occurring within a particular gene known to affect dementia risk[42] or from variants within and across multiple genes based on GWAS of AD[43] are included in analyses of cognitive change and functioning in twins. As yet, it is uncommon to include polygenic scores within standard biometrical twin models though method development is emerging.[44,45]

21.3.1 Missing heritability?

In addition to polygenic scores, the contribution of all common SNP variants (SNP-heritability) contributing to GCA performance and change has been estimated on the basis of the tiny but varying amount allele-sharing among unrelated individuals using methods such as GCTA-GREML[46] which can be compared to heritability estimates from twin studies. Specifically, common variants explained 40% and 51% of crystalized and fluid performance in aging UK adults[47] and 21.5%–29% in consortia studies with samples from the United States, UK, Scandinavia, and Western Europe.[48,49] Moreover, common variants explained 24% of variation in change in GCA/IQ from childhood to late adulthood in Scottish samples, assuming invariance of the cognitive measurements.[50] In other studies of SNP heritability of mainly midlife adult performance levels in participants from the UK Biobank studies, heritability estimates run the gamut from 5% to 31% for memory, reaction time, executive functioning, and verbal-numerical reasoning.[51,52] The GTCA-GREML heritability estimates, while strong especially for GCA performance levels, are lower than comparable heritability estimates based on twin and family data.[22,53]

Reasons proposed for the gap between SNP- and twin-based heritabilities include that SNP arrays typically capture contributions of common not rare genetic variants, that only additive effects of genes are estimated (excluding nonadditivity such as dominance and epistasis), and that SNP-heritability methods do not capture gene-environment interactions.[46,54] However, it has also been proposed that twin studies

overestimate heritability perhaps because of possibly untenable assumptions such as the equal environments assumption whereby environmental factors contribute equally to both MZ and DZ twin similarity.[46,54] The missing heritability gap is not just observed for cognitive traits but indeed the gap is often even larger in other trait domains.[55] Approaches to testing the validity of twin-based heritabilities have included evaluating census data to avoid sampling biases of participating families, for example, in the case of educational achievement scores in primary school children,[56] and evaluating whole-genome sequencing data where the contributions of very rare variants can be accounted for in constructing SNP-based heritability estimates,[57] for example, adult BMI and height.[58] In these test-cases, estimated heritabilities were as high[58] or even higher[56] than twin studies, suggesting that twin studies are not consistently overestimating heritabilities per se and may more fully capture contributions of rare variants as well as nonadditivity. That said, additional influences often unaccounted for by both the classic twin models and SNP-heritability approaches are the influences of assortative mating and GE interplay,[57] such as gene by environment (GxE) interaction and gene-environment correlation (rGE).

21.3.2 Gene environment interplay

Twin and family based approaches may illuminate processes of direct versus indirect genetic influences, including rGE and GxE, unavailable to population-based GWAS studies of individuals.[57] rGE refers to environmental experiences that are associated with genetic factors, for example, via the pursuit of environmental experiences that fits with an individual's genetically influenced traits, such as an aspiring individual with design skills seeking training and occupations in architecture (active rGE or niche-picking).[59] GxE refers to the differential sensitivity to environmental experiences due to genetic factors.[59] Comparing differences in cognitive aging trends within pairs of MZ twins indexes the extent to which non-shared factors contribute to dissimilarity (E) and if the within-pair differences vary by genotype this may reflect one form of GxE.[60,61] Evidence for differential MZ pair differences has been observed for cognitive performance[62] and rate of change[63] as well as for traits associated with cognitive aging such as depressive symptoms and BMI in twin samples across multiple countries.[62] Heterogeneity in MZ within-pair differences as a general indicator of GxE has been observed to increase with age after age 40 years for spatial reasoning and short-term memory, but decrease with age for processing speed[62] whereas for BMI and depressive symptoms observed GxE effects were steady across age. Moreover, *APOE* may partly index the "G" in the GxE for cognitive performance with respect to spatial reasoning[62] and change in semantic memory over time[63] where those who do not carry the $\varepsilon 4$ risk variant show greater within-pair differences suggesting that those without the $\varepsilon 4$ risk variant may be sensitive to environmental factors that precipitate or buffer performance declines.[62,63] The "E" is harder to determine as many putative environments show genetic influences as well.[64] However, those who do not carry the $\varepsilon 4$ risk allele

may be sensitive to stress-related environmental aspects given that within-pair differences in depressive symptoms were associated with differences in semantic memory change.[63] Moreover, in the United States and Swedish MZ pairs, $\varepsilon 4$ noncarriers showed greater within-pair differences than $\varepsilon 4$ carriers in depressive symptoms, consistent with spatial reasoning and change in semantic memory, where in Danish MZ pairs, $\varepsilon 4$ carriers showed greater differences in depressive symptoms suggesting that GxE patterns may not be universal.[62]

A measurable index of GxE contributions to cognitive aging includes age-related epigenetic changes, such as age-related DNA methylation patterns observed in the brain, notably the hippocampus and prefrontal cortex showing reduced gene expression.[65] While DNA methylation typically results in reduced gene expression it is not invariably the case and reductions or activation of gene expression depends on the location of the methylation tag (typically residing between CG base repeats, called CpG's) within a gene as well as gene/genomic structures.[65] Altered DNA methylation in part may result from our behaviors and experiences and thus methylation patterns may be modifiable, although individual differences in methylation observed in whole blood tissues are partly heritable for many methylation sites.[66,67] Overall, in late life the heritability of CpG sites, including additive and nonadditive genetic influences, is about 24% at age 69 and 18% at age 79 years,[66] which is comparable to studies of younger twins.[67] Moreover, age-related CpG sites (i.e., where methylation patterns correlate with chronological age) are among the most heritable even in late life and across time (29%–39% broad heritability) with the most heritable-familial sites residing in genes in immune-inflammatory and neurotransmitter pathways.[66] A meta-analysis involving blood-based methylation data from nontwin and twin cohorts suggests some association of DNA methylation for GCA and verbal fluency performance levels but no sites predicted cognitive change in a smaller subsample.[68] However, an epigenome-wide (EWAS) study of cognitive change across midlife in 243 Danish MZ twin pairs based on blood DNA methylation suggests that altered methylation in genes involved in neuronal survival (e.g., *AGBL4*) may be relevant to differential GCA change using within-pair approach.[69] To our knowledge, no published study to date has looked jointly at the longitudinal change in DNA methylation and longitudinal change in cognition, instead relying on a single time-point of methylation, but such findings will be forthcoming in studies such as SATSA.

21.3.3 Summary

In summary, measured genetic influences have been uncovered for cognitive performance and change, albeit the magnitude of their contributions appears smaller than that found in twin and family studies. Genetic variants such as *APOE* index a salient measured influence on change in GCA as well as dementia risk. Moreover, GxE may be evident for *APOE* in combination with stress-provoking environmental experiences that contribute to accelerating change in specific genitive abilities. Polygenic contributions, beyond *APOE*, are evident for cognitive aging but the

magnitude of their contribution is less clear. Ultimately, large-scale longitudinal investigations of change from midlife through late adulthood, via GWAS and EWAS meta-analyses and pooled analyses, are necessary to expand our understanding of the measured polygenic and epigenetic contributions to cognitive aging.

21.4 Cognitive aging in context

Understanding the contexts in which cognitive aging occurs is fundamental to understanding cognitive aging processes, both within (physical) and outside (environmental) the individual. Cognition occurs within the brain, which is, of course, a physical entity that experiences physical aging. Cross-domain twin analyses can investigate whether unique and overlapping genetic factors that influence physical aging also play a role in cognitive aging. Moreover, cognitive aging occurs within an environmental context that may suppress or intensify the action of genetic factors via GxE interaction. To illustrate these internal and external contexts we will discuss one of the many physical correlates of cognitive aging (lung function) and one of the most impactful environmental factors (socio-economic status).

21.4.1 Lung function and cognitive aging

There are three possible mechanisms for cross-domain relationships between lung function and cognitive aging: physical health could impact subsequent cognitive function, cognitive function may underlie the maintenance of health and lifestyle habits that support lung function, or processes associated with aging could affect both cognition and lung function.[70] Pulmonary function predicts later cognitive function in older adults in population-based studies,[71,72] possibly as a result of processes such as hypoxia, reduced neurotransmitter function, increased systemic inflammatory processes, or a combination of factors. Cross-domain longitudinal twin studies of cognition and lung function report that genetic factors that contribute to lung function at baseline are associated with those contributing to cognitive function 6 years later,[73] suggesting a genetically influenced biological process common to both. Data from multiple waves of testing can be leveraged to investigate which variable changes first and contributes to subsequent changes in other variables.[74] An analysis of longitudinal lung and cognitive function across 19 years indicated that genetic variance for lung function preceded or was an early indicator of subsequent cognitive function changes with age.[75] For this cross-domain relationship, then, evidence suggests that genetic influences on physical health (lung function) impact subsequent cognitive function. In other domains, the relationship with cognitive aging may be more complex (e.g., cardiovascular health[76,77]).

21.4.2 Socioeconomic status and cognitive aging

An extensive literature documents that socioeconomic status (SES), including occupational status, income, and educational attainment, is associated with a

broad array of late-life outcomes including cognitive function.[78] GxE interaction occurs when the strength of genetic influence is moderated by environmental circumstances such as SES. Adverse environments raise the risk of poor outcomes for everyone, but the diathesis-stress model hypothesizes that high-risk environments (e.g., low SES) will have greater impact on high-risk genotypes.[79,80] Social compensation is an extension of the diathesis-stress model in which an enriched environment (high SES) prevents the expression of an underlying genetic vulnerability.[81] Social enhancement[80–82] and social distinction[79] models predict that the influences of high or low SES are not distributed equally, but rather, accrue preferentially to a subset of individuals with genotypes that are responsive to the social environment.

Extensive research has demonstrated that in childhood, genetic variance and heritability for intelligence tend to be diminished in lower SES rearing environments and maximized in higher SES rearing environments,[83] providing support for the social enhancement model of GxE interaction. A recent meta-analysis of the gene by SES interaction on cognitive functioning, however, indicated that results are not consistent across studies and countries.[84] In considering cognitive aging, it is possible that childhood SES has long-term direct or indirect effects on genetic variance in adult cognitive functioning or that the more proximal measure of attained adult SES plays a larger role in genetic influences on cognition. Twin studies have found little support for an association between childhood SES and genetic influences on adult cognitive functioning.[85,86] In fact, an analysis of within-pair differences in twins reared apart found that the association between childhood SES and adult cognition did not remain after adjusting for genetic factors, indicating that the association between SES and cognition could not be attributed to the causal effects of rearing environment on cognitive outcomes (Fig. 21.3).[87]

Because our own characteristics and behaviors contribute to our attained adult SES, it is possible that the association of adult cognition with adult SES differs completely from the relationship with childhood SES. Childhood experiences provide a foundation on which to build adult SES, contributing to passive gene-environment correlations (rGE). In contrast, we construct our adult SES through making choices and constructing our environments in a reflection of active gene-environment correlation.[13] A pooled analysis of data from the IGEMS consortium including over 12,000 individuals ranging in age from 27 to 98 years found mixed results for the nature of the relationship between adult SES and adult cognition, depending on the specific cognitive ability.[88] In some cognitive domains (verbal, spatial, memory), genetic variance was stable across levels of attained SES. In other domains (perceptual speed), genetic influences tended to be amplified in higher SES environments, providing modest support for the social enhancement model of GxE interaction. Perceptual speed is the cognitive component that declines most rapidly and universally with aging;[89] it is possible that speed is also uniquely susceptible to the benefits of enriching experiences that may accrue at higher levels of attained SES.

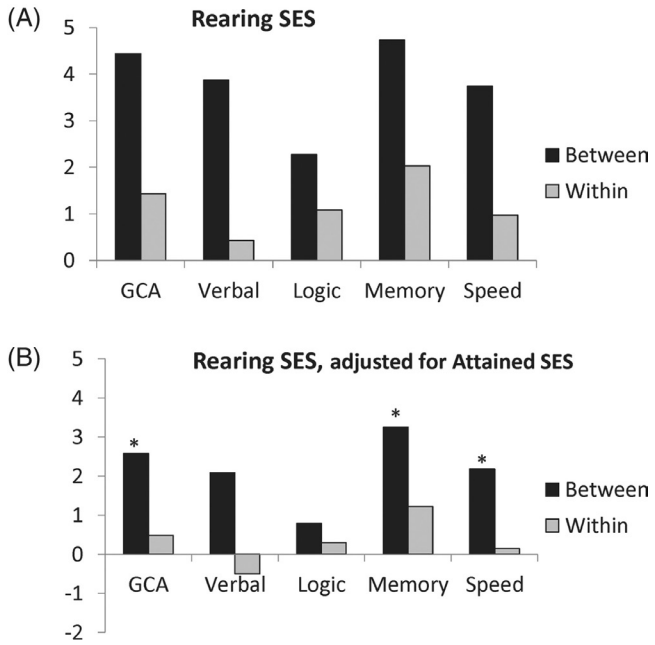


FIG.21.3 Results of between and within-pair analyses of the association between rearing SES and GCA and four specific cognitive domains in twins reared apart (adapted from ref.[87]: (A) unadjusted for attained SES, (B) adjusted for attain SES. *Notes*: **The between results (i.e. the pair averages) indicate the effect of rearing SES on the levels of cognitive abilities at age 65—equivalent to the population effects. The within-pair results (deviations from the pair mean, hence controlling for genetic and familial factors) are lower, indicating that the association between rearing SES and late life cognition cannot be considered causal.**

21.5 Future directions

Although considerable progress has been made since the growth of twin studies of older adults in the early 1980s, further work is necessary to achieve an understanding of the etiologies and dynamics underlying cognitive performance and change across adulthood. Future work in the field should include molecular genetics, cognitive ability phenotypes, focus on GE interplay with greater environmental specificity, and continued use of both quantitative and molecular approaches.[90] Molecular genetics has not yet helped resolve the distinction between normative cognitive aging and pathological aging. *APOE*, for example, is associated with both cognitive aging in the normal range and dementia risk.[32,34,91] Improved assessment and differentiation of normative cognitive aging, mild cognitive impairment, and various dementias will contribute to advances in our understanding of the etiology.[92] Moreover, understanding the complex interplay of specific environments and altered gene

expression, as indexed by DNA methylation and other epigenetic processes, may be helpful in understanding complex resilience and vulnerability factors that relate to differential patterns of cognitive aging and dysfunction.[93]

Developments in identifying specific genetic factors underlying cognitive aging are only beginning to be matched by innovations in specificity of environment measures. For example, interest in neighborhood-level effects on the experience of aging has been growing over the past 20 years.[94] Neighborhood-level factors encompass more than measures of individual socioeconomic position. Instead, measures can include relative socioeconomic position, experienced/subjective socioeconomic position, population density, food environment, characteristics of the built environment (e.g., distance to transportation, walkability), and social environment.[94,95] Growth in research on neighborhood-level factors reflects, in part, the influence of contextualized perspectives on functioning in late adulthood as an alternative to models that focus on individual-level variables.[96] One study of geography and cognitive aging used the co-twin control design to control for genetic and familial effects, thus highlighting the geographical variation in dementia rates in Sweden. Results indicated that dementia rates were significantly higher in sparsely populated areas.[97] Differences in quality and access to health care, environments, and educational and occupational opportunities during the 20th and now 21st centuries may play a role in qualitative differences in etiologies and GE interplay contributing to cognitive aging.

Our review of research demonstrates that both twin studies and molecular studies continue to serve as important resources for investigations of genetic and environmental factors contributing to cognitive aging.[98] Moreover, methods that combine estimates of variance components with identified polygenic factors show promise for increased understanding of genetic pathways[45,99] as will methods that reveal GE interplay of specific environmental and genetic factors, and resulting epigenetic consequences, that unfold across time.

References

1. Horn JL, Cattell RB. Age differences in fluid and crystallized intelligence. *Acta Psychologica*. 1967;26:107–129.
2. Lindenberger U. Lifespan theories of cognitive development*International Encyclopedia of the Social and Behavioral Sciences*: Elsevier Science. 2001;8848–8854.
3. Schaie KW. *Intellectual Development in Adulthood: The Seattle longitudinal study*: New York: Cambridge University Press; 1996.
4. Loehlin JC, Nichols RC. *Heredity, Environment, and Personality: A Study of 850 Sets of Twins*: Austin, TX: University of Texas Press; 2012.
5. Matheny AP, Jr. W, R. S, Nuss SM. Toddler temperament: stability across settings and over ages. *Child Development*. 1984;55:1200–1211.
6. Plomin R. *Development, Genetics, and Psychology*: Hillsdale, NJ: Lawrence Earlbaum Associates; 1986.
7. Hewitt JK, Eaves LJ, Neale MC, Meyer JM. Resolving causes of developmental continuity or "tracking." I. Longitudinal twin studies during growth. *Behavior Genetics*. 1988;18:133–151.

8. Lyons MJ, Panizzon MS, Liu W, et al. A longitudinal twin study of general cognitive ability over four decades. *Developmental Psychology*. 2017;53:1170–1177.
9. Reynolds CA, Finkel D, Mcardle JJ, Gatz M, Berg S, Pedersen NL. Quantitative genetic analysis of latent growth curve models of cognitive abilities in adulthood. *Developmental Psychology*. 2005;41:3–16.
10. Pedersen NL, Plomin R, Mcclearn GE. Is there G beyond g? (Is there genetic influence on specific cognitive abilities independent of genetic influence on general cognitive ability?). *Intelligence*. 1994;18:133–143.
11. Finkel D, Reynolds CA. Behavioral genetic investigations of cognitive aging. In: Kim Y-K, ed. *Handbook of Behavior Genetics*. New York, NY: Springer. 2009;101–112.
12. Tucker-Drob EM, Briley DA. Continuity of genetic and environmental influences on cognition across the life span: a meta-analysis of longitudinal twin and adoption studies. *Psychological Bulletin*. 2014;140:949.
13. Reynolds CA, Finkel D, Zavala C. Gene by environment interplay in cognitive aging. *Behavior Genetics of Cognition Across the Lifespan*: New York: Springer. 2014;169–200.
14. Kirkwood TB, Cordell HJ, Finch CE. Speed-bumps ahead for the genetics of later-life diseases. *Trends in genetics (Regular ed.)*. 2011;27:387–388.
15. Finch CE, Kirkwood TB, Kirkwood T. *Chance, Development, and Aging*: New York: Oxford University Press; 2000.
16. Lipman T, Tiedje LB. Epigenetic differences arise during the lifetime of monozygotic twins. *MCN: The American Journal of Maternal/Child Nursing*. 2006;31:204.
17. Sameroff AJ, Seifer R, Baldwin A, Baldwin C. Stability of intelligence from preschool to adolescence: the influence of social and family risk factors. *Child development*. 1993;64:80–97.
18. Rabbitt P. Does it all go together when it goes? The Nineteenth Bartlett Memorial Lecture. *The Quarterly Journal of Experimental Psychology*. 1993;46:385–434.
19. Polderman TJ, Benyamin B, De Leeuw CA, et al. Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nature genetics*. 2015;47:702.
20. Reynolds CA, Finkel D. A meta-analysis of heritability of cognitive aging: minding the "missing heritability" gap. *Neuropsychology Review*. 2015;25:97–112.
21. Blettner M, Sauerbrei W, Schlehofer B, Scheuchenpflug T, Friedenreich C. Traditional reviews, meta-analyses and pooled analyses in epidemiology. *International Journal of Epidemiology*. 1999;28:1–9.
22. Pahlen S, Hamdi NR, Aslan AKD, et al. Age-moderation of genetic and environmental contributions to cognitive functioning in mid-and late-life for specific cognitive abilities. *Intelligence*. 2018;68:70–81.
23. Mcardle JJ, Prescott CA, Hamagami F, Horn JL. A contemporary method for developmental-genetic analyses of age changes in intellectual abilities. *Developmental Neuropsychology*. 1998;14:69–114.
24. Tucker-Drob EM, Reynolds CA, Finkel D, Pedersen NL. Shared and unique genetic and environmental influences on aging-related changes in multiple cognitive abilities. *Developmental Psychology*. 2014;50:152.
25. Chavarría-Siles I, Fernández G, Posthuma D. Brain Imaging and Cognition. Behavior Genetics of Cognition Across the Lifespan. New York: Springer. 2014:235–256.
26. West RL. An application of prefrontal cortex function theory to cognitive aging. *Psychological Bulletin*. 1996;120:272.
27. Friedman NP, Miyake A. Unity and diversity of executive functions: individual differences as a window on cognitive structure. *Cortex; A Journal Devoted to the Study of the Nervous System and Behavior*. 2017;86:186–204.

28. Gustavson DE, Panizzon MS, Franz CE, et al. Genetic and environmental architecture of executive functions in midlife. *Neuropsychology*. 2018;32:18.
29. Kremen WS, Moore CS, Franz CE, Panizzon MS, Lyons MJ. Cognition in Middle Adulthood. Behavior Genetics of Cognition Across the Lifespan. New York: Springer. 2014.
30. Henry JD, Crawford JR, Phillips LH. Verbal fluency performance in dementia of the Alzheimer's type: a meta-analysis. *Neuropsychologia*. 2004;42:1212–1222.
31. Gustavson DE, Panizzon MS, Kremen WS, et al., IGEMS Consortium. Genetic and environmental influences on semantic verbal fluency across midlife and later life. *Behavior genetics*. 2021;51(2):99–109. doi:10.1007/s10519-021-10048-w.
32. Andrews SJ, Mcfall GP, Booth A, Dixon RA, Anstey KJ. Association of Alzheimer's disease genetic risk loci with cognitive performance and decline: a systematic review. *Journal of Alzheimer's Disease*. 2019;69:1109–1136.
33. Escott-Price V, Myers A, Huentelman M, Shoai M, Hardy J. Polygenic risk score analysis of Alzheimer's disease in cases without APOE4 or APOE2 alleles. *J Prev Alzheimers Dis*. 2019;6:16–19.
34. Davies G, Harris SE, Reynolds CA, et al. A genome-wide association study implicates the APOE locus in nonpathological cognitive ageing. *Molecular Psychiatry*. 2014;19:76–87.
35. Davies G, Lam M, Harris SE, et al. Study of 300,486 individuals identifies 148 independent genetic loci influencing general cognitive function. *Nature Communication*. 2018;9:2098.
36. Savage JE, Jansen PR, Stringer S, et al. Genome-wide association meta-analysis in 269,867 individuals identifies new genetic and functional links to intelligence. *Nature Genetics*. 2018;50:912–919.
37. Lee JJ, Wedow R, Okbay A, et al. Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. *Nature Genetics*. 2018;50:1112–1121.
38. Hill WD, Marioni RE, Maghzian O, et al. A combined analysis of genetically correlated traits identifies 187 loci and a role for neurogenesis and myelination in intelligence. *Molecular Psychiatry*. 2019;24:169–181.
39. Kunkle BW, Grenier-Boley B, Sims R, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. *Nature Genetics*. 2019;51:414–430.
40. Reynolds CA, Jansson M, Gatz M, Pedersen NL. Longitudinal change in memory performance associated with HTR2A polymorphism. *Neurobiology of Aging*. 2006;27:150–154.
41. Crouch DJM, Bodmer WF. Polygenic inheritance, GWAS, polygenic risk scores, and the search for functional variants. *Proc Natl Acad Sci*. 2020;117(32):18924–18933.
42. Reynolds CA, Zavala C, Gatz M, et al. Sortilin receptor 1 predicts longitudinal cognitive change. *Neurobiology of Aging*. 2013;34:1710 e11-8.
43. Logue MW, Panizzon MS, Elman JA, et al. Use of an Alzheimer's disease polygenic risk score to identify mild cognitive impairment in adults in their 50s. *Molecular Psychiatry*. 2019;24:421–430.
44. Dolan CV, Huijskens RCA, Minica CC, Neale MC, Boomsma DI. Incorporating polygenic risk scores in the ACE twin model to estimate A-C covariance. *Behavior Genetics*. 2021;51(3):237–249.
45. Minică CC, Dolan CV, Boomsma DI, De Geus E, Neale MC. Extending causality tests with genetic instruments: an integration of mendelian randomization with the classical twin design. *Behavior Genetics*. 2018;48:337–349.
46. Nolte IM, Tropf FC, Snieder H. Missing heritability of complex traits and diseases. *eLS*. 2019:1–9.

47. Davies G, Tenesa A, Payton A, et al. Genome-wide association studies establish that human intelligence is highly heritable and polygenic. *Molecular Psychiatry*. 2011;16:996–1005.
48. Davies G, Armstrong N, Bis JC, et al. Genetic contributions to variation in general cognitive function: a meta-analysis of genome-wide association studies in the CHARGE consortium (N=53949). *Molecular Psychiatry*. 2015;20:183–192.
49. Trampush JW, Yang ML, Yu J, et al. GWAS meta-analysis reveals novel loci and genetic correlates for general cognitive function: a report from the COGENT consortium. *Molecular Psychiatry*. 2017;22:336–345.
50. Deary IJ, Yang J, Davies G, et al. Genetic contributions to stability and change in intelligence from childhood to old age. *Nature*. 2012;482:212–215.
51. Davies G, Marioni RE, Liewald DC, et al. Genome-wide association study of cognitive functions and educational attainment in UK Biobank (N=112 151). *Molecular Psychiatry*. 2016;21:758–767.
52. Hagenaars SP, Cox SR, Hill WD, et al. Genetic contributions to trail making test performance in UK biobank. *Molecular Psychiatry*. 2018;23:1575–1583.
53. Reynolds CA, Phillips D. Genetics of brain aging – twin aging. *Reference Module in Biomedical Sciences*: Elsevier; 2015.
54. Young AI. Solving the missing heritability problem. *Plos Genetics*. 2019;15:e1008222.
55. Cheesman R, Selzam S, Ronald A, et al. Childhood behaviour problems show the greatest gap between DNA-based and twin heritability. *Translational Psychiatry*. 2017;7:1284.
56. Schwabe I, Janss L, Van Denb SM. Can we validate the results of twin studies? A census-based study on the heritability of educational achievement. *Front Genet*. 2017;8:160.
57. Young AI, Benonisdottir S, Przeworski M, Kong A. Deconstructing the sources of genotype-phenotype associations in humans. *Science*. 2019;365:1396–1400.
58. Wainschtein P, Jain DP, Yengo L, et al. Recovery of trait heritability from whole genome sequence data. *bioRxiv*. 2019:588020.
59. Knopik VS, Neiderhiser JM, Defries JC, Plomin R. *Behavioral Genetics*. New York, NY: Worth Publishers; 2017.
60. Fisher RA. The resemblance between twins, a statistical examination of Lauterbach's measurements. *Genetics*. 1925;10:569–579.
61. Martin NG, Rowell DM, Whitfield JB. Do the MN and Jk systems influence environmental variability in serum lipid levels? *Clinical Genetics*. 1983;24:1–14.
62. Reynolds CA, Gatz M, Christensen K, et al. Gene-environment interplay in physical, psychological, and cognitive domains in mid to late adulthood: is APOE a variability gene? *Behavior Genetics*. 2016;46:4–19.
63. Reynolds CA, Gatz M, Berg S, Pedersen NL. Genotype-environment interactions: cognitive aging and social factors. *Twin Res Hum Genet*. 2007;10:241–254.
64. Plomin R, Defries JC, Knopik VS, Neiderhiser JM. Top 10 replicated findings from behavioral genetics. *Perspect Psychol Sci*. 2016;11:3–23.
65. Harman MF, Martin MG. Epigenetic mechanisms related to cognitive decline during aging. *Journal of Neuroscience Research*. 2020;98:234–246.
66. Reynolds CA, Tan Q, Munoz E, et al. A decade of epigenetic change in aging twins: Genetic and environmental contributions to longitudinal DNA methylation. *Aging Cell*. 2020:e13197.
67. Van Dongen J, Nivard MG, Willemsen G, et al. Genetic and environmental influences interact with age and sex in shaping the human methylome. *Nature Communications*. 2016;7:11115.

68. Marioni RE, Mcrae AF, Bressler J, et al. Meta-analysis of epigenome-wide association studies of cognitive abilities. *Molecular Psychiatry*. 2018;23:2133–2144.
69. Starnawska A, Tan Q, Mcgue M, et al. Epigenome-wide association study of cognitive functioning in middle-aged monozygotic twins. *Front Aging Neurosci*. 2017;9:413.
70. Johnson W, Deary IJ, Mcgue M, Christensen K. Genetic and environmental transactions linking cognitive ability, physical fitness, and education in late life. *Psychology and Aging*. 2009;24:48.
71. Albert MS, Jones K, Savage CR, et al. Predictors of cognitive change in older persons: MacArthur studies of successful aging. *Psychology and Aging*. 1995;10:578.
72. Singh-Manoux A, Dugravot A, Kauffmann F, et al. Association of lung function with physical, mental and cognitive function in early old age. *Age*. 2011;33:385–392.
73. Emery CF, Pedersen NL, Svartengren M, Mcclearn GE. Longitudinal and genetic effects in the relationship between pulmonary function and cognitive performance. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*. 1998;53:P311–P317.
74. Mcardle JJ, Hamagami F. Structural equation models for evaluating dynamic concepts within longitudinal twin analyses. *Behavior Genetics*. 2003;33:137–159.
75. Finkel D, Reynolds CA, Emery CF, Pedersen NL. Genetic and environmental variation in lung function drives subsequent variation in aging of fluid intelligence. *Behavior genetics*. 2013;43:274–285.
76. S Laitala V, Kaprio J, Koskenvuo M, Raiha I, O Rinne J, Silventoinen K. Association and causal relationship of midlife obesity and related metabolic disorders with old age cognition. *Current Alzheimer Research*. 2011;8:699–706.
77. Vasilopoulos T, Kremen WS, Kim K, et al. Untreated hypertension decreases heritability of cognition in late middle age. *Behavior genetics*. 2012;42:107–120.
78. Lyu J, Burr JA. Socioeconomic status across the life course and cognitive function among older adults: an examination of the latency, pathways, and accumulation hypotheses. *Journal of Aging and Health*. 2016;28:40–67.
79. Boardman JD, Daw J, Freese J. Defining the environment in gene-environment research: lessons from social epidemiology. *American Journal of Public Health*. 2013;103(S1):S64–S72.
80. Reiss D, Leve LD, Neiderhiser J. How genes and the social environment moderate each other. *American Journal of Public Health*. 2013;103:S111–S121.
81. Shanahan MJ, Hofer SM. Social context in gene–environment interactions: retrospect and prospect. *Journals of Gerontology B*. 2005;60B:65–76.
82. Shanahan MJ, Boardman JD. Genetics and behavior in the life course: a promising frontier. In: Giele GHEAJZ, ed. *The Craft of Life Course Research*. New York, NY: Guilford. 2009:215–235.
83. Turkheimer E, Horn EE. Interactions between socioeconomic status and components of variation in cognitive ability. *Behavior Genetics of Cognition Across the Lifespan*. New York: Springer. 2014:41–68.
84. Tucker-Drob EM, Bates TC. Large cross-national differences in gene× socioeconomic status interaction on intelligence. *Psychological science*. 2016;27:138–149.
85. Grant MD, Kremen WS, Jacobson KC, et al. Does parental education have a moderating effect on the genetic and environmental influences of general cognitive ability in early adulthood? *Behavior genetics*. 2010;40:438–446.
86. Van Der Sluis S, Willemsen G, De Geus EJ, Boomsma DI, Posthuma D. Gene-environment interaction in adults' IQ scores: measures of past and present environment. *Behavior genetics*. 2008;38:348–360.

87. Ericsson M, Lundholm C, Fors S, et al. Childhood social class and cognitive aging in the Swedish adoption/twin study of aging. *Proceedings of the National Academy of Sciences*. 2017;114:7001–7006.
88. Zavala C, Beam CR, Finch BK, et al. Attained SES as a moderator of adult cognitive performance: testing gene–environment interaction in various cognitive domains. *Developmental Psychology*. 2018;54:2356.
89. Salthouse TA. The processing-speed theory of adult age differences in cognition. *Psychological Review*. 1996;103:403.
90. Finkel D, Reynolds CA. Future directions. In: Finkel D, Reynolds CA, eds. *Behavior Genetics of Cognition Across the Lifespan*. New York: Springer. 2014:281–294.
91. Belloy ME, Napolioni V, Greicius MD. A quarter century of APOE and Alzheimer's disease: progress to date and the path forward. *Neuron*. 2019;101:820–838.
92. Anderson ND. State of the science on mild cognitive impairment. *The Journals of Gerontology: Series B*. 2020;75:1359–1360.
93. Walker RM, Bermingham ML, Vaher K, et al. Epigenome-wide analyses identify DNA methylation signatures of dementia risk. *Alzheimers Dement (Amst)*. 2020;12:e12078.
94. Arcaya MC, Tucker-Seeley RD, Kim R, Schanke-Mahl A, So M, Subramanian SV. Research on neighborhood effects on health in the United States: a systematic review of study characteristics. *Social Science & Medicine*. 2016;168:16–29.
95. Besser LH, Mcdonald NC, Song Y, Kukull WA, Rodriguez DA. Neighborhood environment and cognition in older adults: a systematic review. *American Journal of Preventive Medicine*. 2017;53:241–251.
96. Krieger N. *Epidemiology and the People's Health: Theory and Context*. New York: Oxford University Press; 2011.
97. Russ TC, Gatz M, Pedersen NL, et al. Geographical variation in dementia: examining the role of environmental factors in Sweden and Scotland. *Epidemiology*. 2015;26:263.
98. Van Dongen J, Slagboom PE, Draisma HH, Martin NG, Boomsma DI. The continuing value of twin studies in the omics era. *Nature Reviews Genetics*. 2012;13:640–653.
99. Dolan CV, Huijskens RCA, Minică CC, Neale MC, Boomsma DI. Incorporating polygenic scores in the twin model to estimate genotype-environment covariance: exploration of statistical power. *bioRxiv*. 2019:702738.

CHAPTER

Twin studies of smoking and tobacco use

22

Jaakko Kaprio

Institute for Molecular Medicine FIMM, University of Helsinki, Helsinki, Finland

22.1 Introduction

Tobacco is derived from the plant *Nicotiana tabacum*, which originates from the Americas and was widely used there well before the arrival of Europeans over five centuries ago. The use of chewed or smoked tobacco spread subsequently in Europe. Only after the invention of the cigarette-making machine in the 1880s—over one hundred years ago—did cigarettes become the dominant form of tobacco use and a mass consumer product. Smoking was very common and wide-spread in many countries during the first half of the 20th century and mass-marketed by the tobacco industry.

After WWII, increasing evidence for detrimental effects of smoking on health accumulated, with major epidemiological studies showing the associations of smoking with lung cancer, chronic obstructive lung disease, and cardiovascular disease. Notable papers are the landmark studies of Doll and Hill[1] and Wynder and Graham.[2] These findings were summarized in several influential governmental reports, such as the 1964 Surgeon General's Report on Smoking and Health. Smoking was identified as a cause of these diseases based on the available scientific evidence. This conclusion of a causal relationship was not universally accepted, notably by the tobacco industry at that time. It was hypothesized that there was a third factor, namely genetic predisposition, that led to both smoking and later lung cancer. The genetic basis for smoking was suggested by the greater similarity of MZ than DZ pairs for smoking. Based on this, the well-known statistician, R.A. Fisher wrote in *Nature* in 1958[3,4] that the hypothesis of the causality of the association between smoking and disease, which was based on observational epidemiological evidence, could be tested using twins. By studying twin pairs in which one twin smoked and the other did not, it is possible to control for the unmeasurable (at that time) genetic predisposition. As a consequence of this debate, the Swedish Twin Register was established to test this hypothesis in the 1960s, and the Finnish Twin Cohort study was established in the 1970s for constructive replication. We return to the results of studies on twin pairs discordant for smoking later in the chapter.

In the decades following the Surgeon General's 1964 report, increasing evidence for the health effects of smoking on smokers emerged. Further, evidence accrued that

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00022-2

persons, who were not smokers but were exposed to cigarette smoke (i.e., second-hand smoke (SHS)) had an increased risk of disease. As a result, more people quit smoking and governments introduced multiple tobacco control measures during the last decades of the 20th century. By the end of the century, it was universally accepted that tobacco causes diseases and that nicotine in tobacco is addictive. Smoking and tobacco use decreased in many developed countries but has increased in many other parts of the world. Global tobacco control efforts have intensified since the Framework Convention on Tobacco Control was established in 2003. Overall, 25% of men and 5% of women smoked worldwide in 2015 (Lancet, 2017). A comprehensive introduction to the health, economic and environmental impact of tobacco is provided by the Tobacco Atlas (https://tobaccoatlas.org/). A comprehensive report on the diseases and disorders caused and/or associated with smoking is the U.S. Surgeon General 2014 landmark document "The Health Consequences of Smoking—50 Years of Progress."

22.2 Natural history of smoking behavior

Smoking is a complex behavior that results from the interplay of biological, psychological, and social interactions occurring in a given societal context. Smoking cigarettes has been and still is the most common form of tobacco use, and the early twin studies focused on cigarette smoking. The main addictive substance in tobacco is nicotine,[5] which can be delivered through other smoked tobacco products such as cigars and pipes. Smokeless tobacco also delivers nicotine, and there are many different forms of smokeless tobacco with varying degrees of harm (IARC, 2004). Over the past decade, e-cigarettes have proliferated as a nicotine-delivery device. This review will focus on studies of smoking, particularly of cigarettes as these are the most common form of use. In addition, smoked tobacco is the most harmful nicotine delivery method. Currently, twin studies are increasingly interested in nicotine, its metabolism and health effects. Many of the health effects of cigarettes have been attributed to components of tobacco smoke such as carbon monoxide, radioactivity from polonium, and the toxic mix of carcinogens found among the thousands of chemical ingredients that are inhaled by a smoker. These individual components have rarely been the subject of genetic studies to investigate interindividual differences in the effects of exposure.

To understand smoking and where genetic and environmental influences may exert effects, components of smoking behaviors have been identified and studied. Smoking begins with experimentation; about two-thirds of those persons who ever smoke an entire cigarette become regular smokers—which typically is defined as having smoked more than 100 cigarettes. Regular smoking may last decades, often a lifetime. After such *initiation* of smoking, tolerance develops and the amount smoked increases until the smoker reaches a fairly stable level of use. Thus, the smoker becomes exposed to ever-increasing amounts of smoke and the multiple compounds in smoke. Early studies, also in twins, distinguished only between having never smoked and being a regular smoker. A regular smoker, i.e. someone who smoked daily or almost daily might then continue to smoke for years or quit smoking. After successful

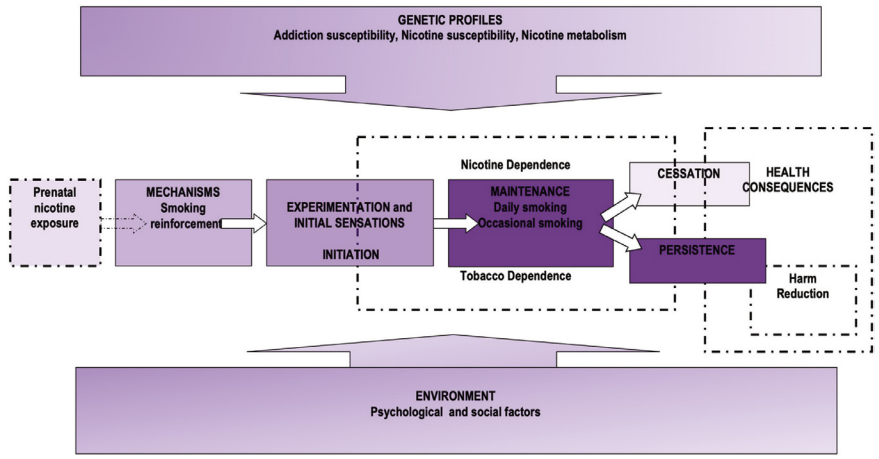


FIG. 22.1 Bio-behavioral model on development of smoking and nicotine/tobacco dependence.

English version translated from and based on Finnish original Figure 2 developed by Tellervo Korhonen, Ulla Broms, and Jaakko Kaprio and published in the Prevention and Treatment of Smoking and Nicotine Dependence Current Care Guidelines. Working group set up by the Finnish Medical Society Duodecim and the Finnish Cardiac Society. Helsinki: The Finnish Medical Society Duodecim, 2018 (referenced June 29, 2020). Available online at: www.kaypahoito.fi

smoking cessation, they would be classified as a former smoker. The *amount smoked daily* is one measure of tolerance; tolerance reflects the ability and needs to ingest increasingly larger amounts of a psychoactive agent to achieve the same desired psychological effect. This process of the brain adapting to the effect of the drug is known as neuroadaptation. Consequently, larger consumption leads to greater exposure to the toxic components of tobacco products. Nicotine dependence is reflected in difficulty quitting smoking successfully, craving, and withdrawal symptoms. Fig. 22.1 summarizes the development of smoking behavior and indicates the broad categories of factors affecting this development.

22.3 Twin studies past and present—the aim of the review

Almost all twin studies conducted in the last century use rather crude classifications of smoking status and analyzed quantity typically smoked. These studies have been reviewed extensively and I refer the reader to three reviews for details[6–8] of these older studies. Only at the end of the century, were there more detailed analyses of the amount smoked and degree of nicotine dependence. The twin studies on smoking quantity, nicotine dependence, and smoking cessation published in the first decade of the 21st century are summarized by Rose et al.[7]

The present review focuses on developments of study designs that extend beyond the classic twin design and estimation of heritability, including the introduction of novel measures of smoking behavior, and designs to test causal hypotheses linking smoking to health outcomes. The review does not encompass all published papers on twins and smoking, but I rather seek to highlight new approaches and some interesting findings over the past decade.

22.4 Genetic and environmental influences on smoking behavior

As reported by Rose et al.,[7] estimates of heritability of *smoking initiation* for studies published before 2008 come mainly from a fairly limited set of countries (Northern Europe, North America, and Australia), with study-specific estimates ranging from 0.22 to 0.72. Most of these studies also provided evidence for shared environmental effects. In the most definitive study so far based on data from 19313 pairs in 11 population-based twin samples from the United States, Europe, and Australia, adolescent smoking initiation was studied.[9] Altogether, 76,358 longitudinal assessments had been conducted of twins from same- and opposite-sex pairs between 1983 and 2007. Results showed that both additive genetic and shared environmental factors contribute to variance in initiation throughout adolescence. The estimate of additive genetic contributions increased from 15% to 45% from ages 13 to 19, while shared environmental factors were highest at age 13 (70%) and diminished by age 19 to about 40%. By young adulthood, shared environmental effects largely disappear.[10] Consistent with these findings, molecular genetic analyses from genome-wide association studies (GWAS) on almost one million adults[11] identified multiple genes related to smoking initiation. Common variants related to smoking initiation accounted overall for eight percent of the variation. Thus, a substantial fraction of the heritability from twin studies remains unaccounted for.

Some of that unaccounted variance can be assessed by analyzing better measures of smoking experimentation and of the process to becoming a *regular smoker*. Typically, an adolescent tries one cigarette and then progresses to smoke another. It may be pleasurable or there may be peer pressure to continue from smokers in the family and friend network. This leads to smoking becoming more regular but not necessarily daily. Eventually, most smokers become daily smokers, and the amount they smoke increases as they develop tolerance to nicotine. Many regular smokers but not all become *nicotine dependent*.

The process of developing dependence can be studied using multiple repeated measurements during adolescence. This was done in the Nicotine Dependence in Teens Study.[12] Interviews later in life provide another mode of data collection, such as in the Nicotine Addiction Genetics study, where detailed information was collected from twin pairs, where both twins were heavy smokers, and from their first-degree relatives.[13] Measures of initiation included the age at first puff on a cigarette, age at smoking a full cigarette and the time elapsed before the second

cigarette was smoked. The process of becoming a smoker can be rapid or slow, so the researchers also recorded the age when weekly smoking and daily smoking started. For all of these measures, similarity was greater among MZ than DZ pairs indicating the presence of genetic effects on the rate of uptake of smoking. Subsequent GWAS analyses sought to identify specific genes for the rate (or speed) at which a person becomes a regular smoker.[14] Unfortunately, the limited sample size did not permit definitive identification of associated genes. A large American twin study found modest heritability in the time (in years) from initiation of smoking to the age of first dependence symptoms ($a^2 = 0.24$) and age of onset of dependence ($a^2 = 0.18$),[15] but there was no evidence for shared environmental effects. In contrast, age at initiation showed genetic effects ($a^2 = 0.39$) and shared environmental effects ($c^2 = 0.15$).

Adolescents often experiment with a variety of drugs and may use for example alcohol when trying smoking. Thus, it was not surprising that age at initiation for smoking shares genetic influences with the age of initiation for alcohol use and cannabis.[16] In a subsequent study, the ordering of substance use was compared within-pairs, i.e. by looking at which of two siblings in a family used drugs first and in which order. This analysis conducted within families provided evidence that use of one drug does in fact lead to use of another.[17] A similar within-pair comparison of age of onset of regular smoking within MZ pairs suggested a direct causal effect of nicotine exposure on later degree of nicotine dependence.[18]

Also, *subjective effects* of various drugs, including nicotine, are heritable; indexed by traits such as dizziness on smoking one's first cigarette[19] or sensations felt during smoking of the first cigarette.[13] For subjective effects, there is also genetic covariation across different drugs.[20] Overall, there is substantial evidence from twin studies that genetic influences are shared across many aspects of smoking and other drug-use (including alcohol) behaviors[21,22] and more recently this has also been supported from molecular genetic studies.[11] Thus, when seeking to understand why some people are more liable to become users and addicted it is important to study use across different drugs.

In addition to studying persistence versus cessation of smoking as a binary trait,[7] there have also been studies of smoking expectancies, that is, beliefs about future outcomes such as quitting smoking. Smoking expectancies are dependent on the degree of nicotine dependence,[23] but strong genetic effects on most expectancies were not seen in that study. A longitudinal study from the Netherlands Twin Register,[24] asked participants whether they thought that they would be smoking in a year's time. This ability of a person to predict their future smoking status was called smoking expectancy, that is, the persons belief in what their smoking status would be in the future. The study showed that smoking expectancies corresponded to a realized smoking status better among never and former smokers than among current smokers. One could interpret this to mean that current smokers have an overly optimistic view of their ability to quit smoking. In addition, interindividual variation in smoking expectancy showed moderate heritability, 59% in adolescents versus 27% in adults. Molecular genetic work indicates that there are specific genetic variants underlying

the ability to quit successfully, and these only partially overlap with genes associated with the amount smoked and smoking initiation.[11] In that large meta-analysis of European populations, the genetic correlations were 0.33 between smoking initiation and amount smoked, 0.40 between smoking initiation and smoking cessation, and 0.42 between amount smoked and smoking cessation.[11]

Nicotine dependence has been assessed indirectly using a measure of heaviness of smoking, that is, cigarettes per day (CPD). This taps into one very central aspect of nicotine dependence, namely tolerance. The amount smoked is a major predictor of the ability to quit as heavier smokers find it harder to quit. More comprehensive unidimensional measures of nicotine dependence are based on psychiatric diagnostic criteria for substance use disorders, such as DSM-V. The Fagerström Test for Cigarette Dependence (FTCD), also known as the Fagerström test for Nicotine Dependence is a six-item brief assessment in wide-spread clinical use. Rose et al.[7] summarized seven studies conducted 1999-2007 that used one of these measures and reported a range of heritabilities from 0.40 to 0.75. In recent molecular genetic studies, the largest studies used CPD,[11] FTND,[25] and both FTND and one item on time to the first cigarette.[26] One of two major loci that stand out for CPD is the nicotinic receptor gene complex on chromosome 15, especially the functional variant D398N in *CHRNA5*. This variant accounts for about 1% of variance in CPD and 4%–5% of the variance in cotinine levels, cotinine being a biomarker of nicotine intake. The other major locus is found in the region of CYP2A6 on chromosome 19, which is of importance in nicotine metabolism as most nicotine is metabolized to cotinine by this enzyme. Overall measured genetic variants account for 8% of the variance in amount smoked.[11] Animal and imaging studies have confirmed the importance of D398N for nicotine dependence, while it is also used as a genetic instrumental variable in Mendelian randomization studies. Multiple other loci have been identified in these GWAs studies and together form the basis for constructing genetic risk scores. These aforementioned study designs and approaches are not within the scope of the present review, but without the prior twin studies, there would have been less interest in understanding the genetic architecture of nicotine dependence.

The speed at which nicotine is metabolized, mostly by activity of the CYP2A6 gene, is associated with smoking behavior. It has been observed that slower metabolizers smoke less and are able to quit smoking more readily. An index of metabolizing speed is the ratio of 3´hydroxycotinine to cotinine in plasma or urine[27] and is known as the nicotine metabolite ratio (NMR). In an observational study of Finnish twins who were current smokers, the heritability estimate of NMR was 81% (95% CI 70%–88%).[28] This is congruent with a smaller experimental twin study, which estimated the heritability of NMR to be 67%.[27] Known CYP2A6 alleles accounted for a small fraction of that heritability.[27] To identify more loci associated with NMR, GWAS have been conducted. These show that multiple single variants are associated with NMR, with nearly all in and around the CYP2A6 gene. A handful of genetic variants account for up to 40% of the variance in NMR, thus opening the possibility of assessing the speed of nicotine metabolism and of medications metabolized by CYP2A6 using genetic risk scores.[29] It should be noted that

most persons of European descent are fast metabolizers, while in other Ancestry groups there is a greater prevalence of slow metabolizers or they have a different genetic architecture of CYP2A6.[30] Thus, one can expect the heritability of nicotine metabolism speed to also vary by genetic ancestry in addition to varying environmental conditions.

Understanding where and when twin studies have been conducted is important when interpreting results from twin studies of smoking and tobacco use. Heritability estimates reflect the relative impact of genetic and environmental (i.e., all nongenetic effects). These environmental effects may act at many levels, from personal exposures and experiences to broad society-wide influences. Studies comparing results from different countries are particularly useful for understanding the influence of broad social effects. While most early twin studies of smoking were based on populations of European origin, there is much more diversity at present, with studies reported, for example from Sri Lanka,[31] and from Japan already in 1987.[32] There are multiple studies from China, the most recent being on the Chinese National Twin Registry,[33] which report a high heritability in male twins. A low estimate (28%) of heritability among adolescent Chinese has been reported.[34]

There are also temporal changes in the prevalence of smoking, and we do not understand fully all the reasons for such changes. While the gene pool does not change over a few decades, the way genes act may change as environmental influences change. Twin studies have examined changes in heritability at different time points within the same population.[35] In the Dutch population, there was no change in the heritability of smoking based on two surveys 10 years apart,[36] while in a Spanish study, year of birth influenced heritability among women but not men. The authors attributed this to a changing, more permissive environment, especially for women.[37] Thus, estimates of the relative role of genetic factors derived from twin studies need to be interpreted with respect to where and when studies have been conducted. In addition, the landscape of nicotine use is changing as smoking becomes less common, but other options for consuming nicotine such as e-cigarettes and smokeless tobacco appear to be on the rise. Future genetically informed studies will need to incorporate multiple smoking and nicotine-related behaviors. There is also a need to distinguish between effects due to nicotine and its use, and the effects due to the mode of administration of nicotine.

An example of a less studied smoking-related trait is exposure to SHS which has negative consequences for health. SHS also plays an important role in the likelihood to start smoking. SHS exposure may be work-place related but occurs most often at home. Exposure to SHS by one's spouse's smoking is the most studied. A Hungarian–American twin study reports evidence for substantial heritable effects on sensitivity to SHS (50%, 95% CI 19%–72%) and smoking-exposure-related opinions.[38] Peer influences are another kind of social-environmental influence, known to be of major importance in smoking initiation. As with spousal influences, the similarity of peers for smoking behavior can reflect direct peer influences (one's experiments with smoking among peers, some of whom already smoke) or can be due to homophily, that is, selection into groups with similar characteristics that increase the chance of

smoking initiation and progression to become a smoker. Analysis of data from the National Merit Twin Study suggested that homophily is a major explanation for peer similarity.[39]

22.5 Beyond twins

Traditional twin studies do not permit untangling several sources of variation such as effects specific to twins, assortative mating, and intergenerational transmission of social/cultural influences. However, expansion of the twin design to include information on relatives and spouses of twins permits testing more sophisticated models and estimation of additional sources of variation. In two very large samples from Virginia and Australia, with a total of 50,318 twins, spouses, parents, siblings, and children (55% women), assortative mating for smoking initiation was substantial and accounted for 10% of the variance.[40] When assortative mating was accounted for in the family analyses by Maes et al.,[40] additive genetic effects explained 53% (men) to 55% (women) of the variation in liability to smoking initiation, with smaller proportions of variance attributable to the effects of shared environment (18 in men and 11% in women) and unique environment (15% in both men and women). Cultural transmission, i.e. non-genetic influences from parents to children accounted for only 6% and 4%, respectively. A twin-specific variance component that represents the excess similarity of twins versus non-twin siblings was also estimated (9% and 15%). This may be attributed, in part, to the absence of age differences in twin siblings seen in ordinary sibling pairs,[41] but also the interactions of twins during adolescence that may be closer than for ordinary siblings.[42] One might speculate that this is the result of both twins experimenting with smoking when they are close to each other, but not if they have their own friends and peers. Further evidence for the role of shared environment comes from a Dutch Children of Twins study of smoking initiation and amount smoked.[43] Regression models of smoking initiation analyzing intergenerational data compared offspring of parents who were MZ or DZ twins and their uncles and aunts by the smoking status of the parental generation. The analysis also used polygenic risk score (PRS) analyses among those exposed and not exposed to smoking in childhood. The association of PRS with heavier smoking was found in the exposed group.

Epigenetics is one mechanisms by which both genetic variation and environmental factors affect health at the level of the cell, organ and whole organism. Epigenetic processes affect gene expression and are intimately involved in cell differentiation and maintenance of cell lines. Studies of one epigenetic process, namely DNA methylation, have provided insights into the action of environmental effects, such as smoking. It has been shown to have a major impact on methylation. Among studies of unrelated persons, the association of smoking with methylation may be confounded by various genetic and environmental sources of inter-individual differences in methylation. Indeed, twin studies reveal that the degree of methylation is, partly, heritable[44,45] and thus methylation profiles reflect both environmental and

genetic influences Twin studies have helped to establish a causal path from smoking to methylation, but not the reverse.[46] Despite the role of genetic variation in methylation, smoking status can be determined reliably using methylation data[47–49] because smoking has such a large impact of methylation As an example of another mechanism of action, twin pairs discordant for smoking have helped establish that smoking causally influences gene expression.[50]

22.6 Causes and consequences of tobacco use

In contrast to using the similarity of MZ and DZ twins to understand the genetic basis and architecture of smoking behavior, studies of twin pairs where one twin smokes and the other does not, have been a prototypical application of the cotwin control design. Due to ethical concerns and the widespread effects of smoking on the body, effects of smoking and nicotine in humans cannot be studied by interventions and clinical trials. Hence, scientists have sought to strengthen inference from observational data by using various quasi-experimental designs. By comparing family members who differ in their smoking behavior, some degree of control over familial and genetic effects can be gained. MZ pairs discordant for smoking are close to an ideal study design for providing evidence of the lack of genetic confounding between the association of smoking and later disease. By following up on twin pairs discordant for smoking for health outcomes of interest, additional evidence has been gleaned for the causal effects of smoking on the development of many somatic diseases. The evidence is further strengthened when smoking discordance, hence exposure, has been assessed well-before the onset of disease. A recent example is the sixfold incidence of lung cancer in the smoking twin compared to the nonsmoking cotwin in the large Nordic Twin Study of Cancer.[51] Smoking status was established at baseline among twins free of cancer and they were followed-up for several decades for cancer. A similar finding for smoking and snus use was seen with respect to type 2 diabetes mellitus from Sweden.[52] The causal nature of this association is further confirmed by an experimental demonstration of a habenula-pancreas axis linking the addictive and diabetes-promoting sides of nicotine.[53]

The role of smoking as a cause of mental disorders has been much more controversial, with some considering smoking to be secondary to the mental condition. The relationship of smoking to mental health is difficult to study as mental health develops in childhood and adolescence, and many mental disorders manifest early. Thus, it is plausible that smoking could be a consequence of poorer mental health. Alternatively, smoking can predispose to poor mental health. A further explanation is that common sets of environmental exposures or predisposing genes *t* affect both smoking and mental disorders. For any single disorder, all three explanations may apply. Twin studies have convincingly demonstrated the role of smoking in the development of schizophrenia[54] and the risk of suicides suicide.[55]

22.7 Conclusion

In future studies on smoking, its causes and consequences, I see that twin studies will continue to be of importance. In contrast to family studies, twin studies control for age differences, that can reduce the similarity of sib-pairs, and they provide good estimates of the relative contribution of genes and shared environment. Twin studies of smoking remind us that smoking is a quintessential example that illustrates how factors traditionally considered to be environmental exposures may reflect genetic sources of variation. In epidemiological studies even today, smoking is often considered to be a purely environmental exposure. Yet smoking behavior and the biological response to the multiple chemicals in tobacco are partially affected by our genes. Thus, epidemiological studies should take this into account in the set-up of study designs and interpretation of results.

While molecular genetic studies provide insights into the biological basis of smoking behavior and consequences of smoking, they require very large sample sizes to provide reasonably precise estimates of genetic contributions. Smaller twin studies can invest in more detailed (and hence more expensive) behavioral, physiological, or biochemical assessments to provide quantitative genetic estimates of reasonable accuracy. The results from the twin studies can then guide the design and focus of molecular genetic studies. Integrating molecular genetic approaches and knowledge of genetic variation, molecular biological characterization of functions (such as various omics) with innovative twin and twin-family designs should yield new findings that enhance our ability to understand the biology underlying nicotine dependence. Ultimately we seek insights into treatment modalities. Listening to and systematically studying life histories of smoking-dependent twins may be a tool for generating hypotheses on key social and psychological characteristics on the evolution of the relationship of smoking with the smoker. Finally, twin studies raise awareness of individual differences in the susceptibility to initiate and quit smoking, so that preventive actions can be made more effective. Treatment should be tailored to the needs of the patient who desperately wants to quit smoking but finds it very difficult.

References

1. Doll R, Ab Hill. A study of the aetiology of carcinoma of the lung. *Br Med J*. 1952;2:1271–1286 PubMed [citation] PMID: 12997741, PMCID: PMC2022425.
2. Wynder EL, Graham EA. Tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma; a study of 684 proved cases. *J Am Med Assoc*. 1950;143:329–336 PubMed [citation] PMID: 15415260.
3. Fisher RA. Cancer and smoking. *Nature*. 1958;182:596 PubMed [citation] PMID: 13577916.
4. Fisher RA. Lung cancer and cigarettes. *Nature*. 1958;182:108 PubMed [citation] PMID: 13566198.
5. Picciotto MR, Kenny PJ. Mechanisms of nicotine addiction. Cold Spring Harbor perspectives in medicine. 2020. pii: a039610. doi: http://doi.org/10.1101/cshperspect.a039610. [Epub ahead of print] PubMed [citation] PMID: 32341069

6. Li MD, Cheng R, Ma JZ, Swan GE. A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. *Addiction*. 2003;98:23–31 Review. PubMed [citation] PMID: 12492752.
7. Rose RJ, Broms U, Korhonen T, Dick DM, Kaprio J. Genetics of smoking behavior. Chapter 28. In: Kim YK, ed. *Handbook of Behavior Genetics*. New York, NY: Springer-Verlag; 2009:411–432 ISBN 978-0-387-76726-0.
8. Tyndale RF. Genetics of alcohol and tobacco use in humans. *Annals of Medicine*. 2003;35:94–121 Review. PubMed [citation] PMID: 12795339.
9. Maes HH, Prom-Wormley E, Eaves LJ, et al. A genetic epidemiological mega analysis of smoking initiation in adolescents. *Nicotine & tobacco research: official journal of the Society for Research on Nicotine and Tobacco*. 2017;19:401–409. http://doi.org/10.1093/ntr/ntw294 PubMed [citation] PMID: 27807125, PMCID: PMC5896552.
10. Do EK, Prom-Wormley EC, Eaves LJ, Silberg JL, Miles DR, Maes HH. Genetic and environmental influences on smoking behavior across adolescence and young adulthood in the virginia twin study of adolescent behavioral development and the transitions to substance abuse follow-up. *Twin Res Hum Genet*. 2015;18:43–51. http://doi.org/10.1017/thg.2014.78 PubMed [citation] PMID: 25662421, PMCID: PMC4720959.
11. Liu M, Jiang Y, Wedow R, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nature Genetics*. 2019;51:237–244. http://doi.org/10.1038/s41588-018-0307-5 Epub 2019 Jan 14. PubMed [citation] PMID: 30643251, PMCID: PMC6358542.
12. O'Loughlin J, Dugas EN, Brunet J, et al. Cohort profile: the nicotine dependence in teens (NDIT) study. *International Journal of Epidemiology*. 2015;44:1537–1546. http://doi.org/10.1093/ije/dyu135 Epub 2014 Jul 13. PubMed [citation] PMID: 25022274, PMCID: PMC4681104.
13. Loukola A, Wedenoja J, Keskitalo-Vuokko K, et al. Genome-wide association study on detailed profiles of smoking behavior and nicotine dependence in a twin sample. *Molecular Psychiatry*. 2014;19:615–624. http://doi.org/10.1038/mp.2013.72 Epub 2013 Jun 11. PubMed [citation] PMID: 23752247, PMCID: PMC3883996.
14. He L, Pitkäniemi J, Heikkilä K, et al. Genome-wide time-to-event analysis on smoking progression stages in a family-based study. *Brain Behav*. 2016;6:e00462. http://doi.org/10.1002/brb3.462 eCollection 2016 May. PubMed [citation] PMID: 27134767, PMCID: PMC4842934.
15. Huggett SB, Keyes M, Iacono WG, et al. Age of initiation and transition times to tobacco dependence: early onset and rapid escalated use increase risk for dependence severity. Drug and Alcohol Dependence. 2019;202:104-110. doi: http://doi.org/10.1016/j.drugalcdep.2019.04.027. Epub 2019 Jun 29. PubMed [citation] PMID: 31330330
16. Sartor CE, Agrawal A, Lynskey MT, Bucholz KK, Madden PA, Heath AC. Common genetic influences on the timing of first use for alcohol, cigarettes, and cannabis in young African-American women. Drug and Alcohol Dependence. 2009;102:49-55. doi: http://doi.org/10.1016/j.drugalcdep.2008.12.013. Epub 2009 Mar 3. PubMed [citation] PMID: 19261395, PMCID: PMC3032586
17. Richmond-Rakerd LS, Slutske WS, Deutsch AR, et al. Progression in substance use initiation: a multilevel discordant monozygotic twin design. *Journal of Abnormal Psychology*. 2015;124:596–605. http://doi.org/10.1037/abn0000068 PubMed [citation] PMID: 26098047, PMCID: PMC4573814.
18. Kendler KS, Myers J, Damaj MI, Chen X. Early smoking onset and risk for subsequent nicotine dependence: a monozygotic co-twin control study. *American Journal of*

Psychiatry. 2013;170:408–413. http://doi.org/10.1176/appi.ajp.2012.12030321 PubMed [citation] PMID: 23318372, PMCID: PMC3615117.

19. Haberstick BC, Ehringer MA, Lessem JM, Hopfer CJ, Hewitt JK. Dizziness and the genetic influences on subjective experiences to initial cigarette use. *Addiction*. 2011;106:391–399. http://doi.org/10.1111/j.1360-0443.2010.03133.x Epub 2010 Oct 19. PubMed [citation] PMID: 20955488, PMCID: PMC3017238.
20. Haberstick BC, Zeiger JS, Corley RP, et al. Common and drug-specific genetic influences on subjective effects to alcohol, tobacco and marijuana use. *Addiction*. 2011;106:215–224. http://doi.org/10.1111/j.1360-0443.2010.03129.x Epub 2010 Oct 19. PubMed [citation] PMID: 20955487, PMCID: PMC3006038.
21. Agrawal A, Madden PA, Bucholz KK, Heath AC, Lynskey MT. Initial reactions to tobacco and cannabis smoking: a twin study. *Addiction*. 2014;109:663–671. http://doi.org/10.1111/add.12449 Epub 2014 Jan 23. PubMed [citation] PMID: 24325652, PMCID: PMC3951663.
22. Korhonen T, Latvala A, Dick DM, et al. Genetic and environmental influences underlying externalizing behaviors, cigarette smoking and illicit drug use across adolescence. *Behavior Genetics*. 2012;42:614–625. http://doi.org/10.1007/s10519-012-9528-z Epub 2012 Feb 18. PubMed [citation] PMID: 22350186, PMCID: PMC3597348.
23. Kristjansson SD, Pergadia ML, Agrawal A, et al. Smoking outcome expectancies in young adult female smokers: individual differences and associations with nicotine dependence in a genetically informative sample. *Drug and Alcohol Dependence*. 2011;116:37–44. http://doi.org/10.1016/j.drugalcdep.2010.11.017 Epub 2010 Dec 30. PubMed [citation] PMID: 21194853, PMCID: PMC3105190.
24. Treur JL, Boomsma DI, Lubke GH, Bartels M, Vink JM. The predictive value of smoking expectancy and the heritability of its accuracy. *Nicotine & tobacco research: official journal of the Society for Research on Nicotine and Tobacco*. 2014;16:359–368. http://doi.org/10.1093/ntr/ntt155 Epub 2013 Oct 29. PubMed [citation] PMID: 24169813, PMCID: PMC3983427.
25. Quach BC, Bray MJ, Gaddis NC, et al. Expanding the genetic architecture of nicotine dependence and its shared genetics with multiple traits. *Nature Communication*. 2020;11:5562. http://doi.org/10.1038/s41467-020-19265-z PMID: 33144568; PMCID: PMC7642344.
26. Chen J, Loukola A, Gillespie NA, et al. Genome-wide meta-analyses of FTND and TTFC phenotypes. *Nicotine & tobacco research: official journal of the Society for Research on Nicotine and Tobacco*. 2020;22:900–909. http://doi.org/10.1093/ntr/ntz099 PMID: 31294817; PMCID: PMC7249921.
27. Swan GE, Lessov-Schlaggar CN, Bergen AW, He Y, Tyndale RF, Benowitz NL. Genetic and environmental influences on the ratio of 3′hydroxycotinine to cotinine in plasma and urine. *Pharmacogenet Genomics*. 2009;19:388–398. http://doi.org/10.1097/FPC.0b013e32832a404f PubMed [citation] PMID: 19300303, PMCID: PMC2849278.
28. Loukola A, Buchwald J, Gupta R, et al. A genome-wide association study of a biomarker of nicotine metabolism. *Plos Genetics*. 2015;11:e1005498. http://doi.org/10.1371/journal.pgen.1005498 eCollection 2015. PubMed [citation] PMID: 26407342, PMCID: PMC4583245.
29. Buchwald J, Chenoweth MJ, Palviainen T, et al. Genome-wide association meta-analysis of nicotine metabolism and cigarette consumption measures in smokers of European descent. *Molecular Psychiatry*. 2020. http://doi.org/10.1038/s41380-020-0702-z Epub ahead of print. PMID: 32157176.
30. El-Boraie A, Chenoweth MJ, Pouget JG, et al. Transferability of ancestry-specific and cross-ancestry CYP2A6 activity genetic risk scores in African and European populations.

Clinical Pharmacology & Therapeutics. 2020. http://doi.org/10.1002/cpt.2135 Epub ahead of print. PMID: 33300144.
31. Zavos HM, Kovas Y, Ball HA, et al. Genetic and environmental etiology of nicotine use in Sri Lankan male twins. *Behavior Genetics*. 2012;42:798–807. http://doi.org/10.1007/s10519-012-9544-z Epub 2012 May 19. PubMed [citation] PMID: 22610514.
32. Hayakawa K. Smoking and drinking discordance and health condition: Japanese identical twins reared apart and together. *Acta Geneticae Medicae Et Gemellologiae*. 1987;36:493–501 PubMed [citation] PMID: 3454511.
33. Zhang T, Gao W, Cao W, et al. The genetic correlation between cigarette smoking and alcohol drinking among Chinese adult male twins: an ordinal bivariate genetic analysis. *Twin Res Hum Genet*. 2012;15:483–490. http://doi.org/10.1017/thg.2012.31 PubMed [citation] PMID: 22853853.
34. Unger JB, Lessov-Schlaggar CN, Pang Z, et al. Heritability of smoking, alcohol use, and psychological characteristics among adolescent twins in Qingdao, China. *Asia-Pacific Academic Consortium for Public Health*. 2011;23:568–580. http://doi.org/10.1177/1010539509351052 Epub 2010 May 19. PubMed [citation] PMID: 20484245, PMCID: PMC4406051.
35. Boardman JD, Blalock CL, Pampel FC. Trends in the genetic influences on smoking. *Journal of Health and Social Behavior*. 2010;51:108–123 PubMed [citation] PMID: 20420298, PMCID: PMC3158572.
36. Vink JM, Boomsma DI. Interplay between heritability of smoking and environmental conditions? A comparison of two birth cohorts. *Bmc Public Health [Electronic Resource]*. 2011;11:316. http://doi.org/10.1186/1471-2458-11-316 PubMed [citation] PMID: 21569578, PMCID: PMC3112130.
37. Mezquita L, Sánchez-Romera JF, Ibáñez MI, et al. Effects of social attitude change on smoking heritability. *Behavior Genetics*. 2018;48:12–21. http://doi.org/10.1007/s10519-017-9871-1 Epub 2017 Sep 25. PubMed [citation] PMID: 28948422, PMCID: PMC5752747.
38. Tarnoki DL, Tarnoki AD, Littvay L, et al. Transmission of second-hand smoke sensitivity and smoking attitude in a family. *Annals of Agricultural and Environmental Medicine*. 2014;21:771–775. http://doi.org/10.5604/12321966.1129931 PubMed [citation] PMID: 25528918.
39. Wills AG, Carey G. Adolescent peer choice and cigarette smoking: evidence of active gene-environment correlation? *Twin Res Hum Genet*. 2013;16:970–976. http://doi.org/10.1017/thg.2013.51 Epub 2013 Aug 8. PubMed [citation] PMID: 23924806, PMCID: PMC3997114.
40. Maes HH, Morley K, Neale MC, et al. Cross-cultural comparison of genetic and cultural transmission of smoking initiation using an extended twin kinship model. *Twin Res Hum Genet*. 2018;21:179–190. http://doi.org/10.1017/thg.2018.22 Epub 2018 May 14. PubMed [citation] PMID: 29757125, PMCID: PMC6340705.
41. Verhulst B, Eaves LJ, Neale MC. Moderating the covariance between family member's substance use behavior. *Behavior Genetics*. 2014;44:337–346. http://doi.org/10.1007/s10519-014-9650-1 Epub 2014 Mar 20. PubMed [citation] PMID: 24647834, PMCID: PMC4058367.
42. Penninkilampi-Kerola V, Kaprio J, Moilanen I, Rose RJ. Co-twin dependence modifies heritability of abstinence and alcohol use: a population-based study of Finnish twins. *Twin Res Hum Genet*. 2005;8:232–244 PubMed [citation] PMID: 15989750.
43. Treur JL, Verweij KJH, Abdellaoui A, et al. Testing familial transmission of smoking with two different research designs. *Nicotine & tobacco research: official journal of the*

Society for Research on Nicotine and Tobacco. 2018;20:836–842. http://doi.org/10.1093/ntr/ntx121 PubMed [citation] PMID: 28575460, PMCID: PMC6685054.

44. Hannon E, Knox O, Sugden K, et al. Characterizing genetic and environmental influences on variable DNA methylation using monozygotic and dizygotic twins. *Plos Genetics*. 2018;14:e1007544. http://doi.org/10.1371/journal.pgen.1007544 eCollection 2018 Aug. PubMed [citation] PMID: 30091980, PMCID: PMC6084815.
45. van Dongen J, Nivard MG, Willemsen G, et al. Genetic and environmental influences interact with age and sex in shaping the human methylome. *Nature Communication*. 2016;7:11115. http://doi.org/10.1038/ncomms11115 PubMed [citation] PMID: 27051996, PMCID: PMC4820961.
46. Li S, Wong EM, Bui M, et al. Causal effect of smoking on DNA methylation in peripheral blood: a twin and family study. *Clin Epigenetics*. 2018;10:18. http://doi.org/10.1186/s13148-018-0452-9 eCollection 2018. PubMed [citation] PMID: 29456763, PMCID: PMC5810186.
47. Bollepalli S, Korhonen T, Kaprio J, Anders S, Ollikainen M. EpiSmokEr: a robust classifier to determine smoking status from DNA methylation data. *Epigenomics*. 2019;11:1469–1486. http://doi.org/10.2217/epi-2019-0206 Epub 2019 Aug 30. PubMed [citation] PMID: 31466478.
48. Philibert R, Dogan M, Noel A, et al. Dose response and prediction characteristics of a methylation sensitive digital PCR assay for cigarette consumption in adults. *Front Genet*. 2018;9:137. http://doi.org/10.3389/fgene.2018.00137 eCollection 2018. PubMed [citation] PMID: 29740475, PMCID: PMC5928210.
49. Sugden K, Hannon EJ, Arseneault L, et al. Establishing a generalized polyepigenetic biomarker for tobacco smoking. Transl Psychiatry. 2019;9:92. doi: http://doi.org/10.1038/s41398-019-0430-9. PubMed [citation] PMID: 30770782, PMCID: PMC6377665
50. Vink JM, Jansen R, Brooks A, et al. Differential gene expression patterns between smokers and non-smokers: cause or consequence? *Addiction Biology*. 2017;22:550–560. http://doi.org/10.1111/adb.12322 Epub 2015 Nov 22. PubMed [citation] PMID: 26594007, PMCID: PMC5347870.
51. Hjelmborg J, Korhonen T, Holst K, et al. Nordic twin study of cancer (NorTwinCan) collaboration. Lung cancer, genetic predisposition and smoking: the Nordic Twin Study of Cancer. *Thorax*. 2017;72:1021–1027. http://doi.org/10.1136/thoraxjnl-2015-207921 Epub 2016 Nov 14. PubMed [citation] PMID: 29054888.
52. Carlsson S, Kuja-Halkola R, Magnusson C, Lagerros YT, Andersson T. Tobacco and type 2 diabetes: is the association explained by genetic factors? *International Journal of Epidemiology*. 2019;48:926–933. http://doi.org/10.1093/ije/dyz002 PubMed [citation] PMID: 30726916.
53. Duncan A, Heyer MP, Ishikawa M, et al. Habenular TCF7L2 links nicotine addiction to diabetes. *Nature*. 2019;574:372–377. http://doi.org/10.1038/s41586-019-1653-x Epub 2019 Oct 16. PubMed [citation] PMID: 31619789.
54. Kendler KS, Lönn SL, Sundquist J, Sundquist K. Smoking and schizophrenia in population cohorts of Swedish women and men: a prospective co-relative control study. *American Journal of Psychiatry*. 2015;172:1092–1100. http://doi.org/10.1176/appi.ajp.2015.15010126 Epub 2015 Jun 5. PubMed [citation] PMID: 26046339, PMCID: PMC4651774.
56. Evins AE, Korhonen T, Kinnunen TH, Kaprio J. Prospective association between tobacco smoking and death by suicide: a competing risks hazard analysis in a large twin cohort with 35-year follow-up. *Psychological Medicine*. 2017;47:2143–2154. http://doi.org/10.1017/S0033291717000587 Epub 2017 Apr 12. PubMed [citation] PMID: 28399944, PMCID: PMC5551385.

SECTION

Health 6

CHAPTER

Anthropometric twin studies

23

Karri Silventoinen

Population Research Unit, Faculty of Social Sciences, University of Helsinki, Helsinki, Finland

23.1 Introduction

Human anthropometric has attracted a long time of interest going back to the time beyond modern scientific research. Already in the Hippocratic texts, overweight was mentioned as a risk factor of health,[1] and human height has routinely been measured in the conscription already in the 18th century since short stature was already at that time understood as a marker of inadequate nutrition which may have led to physical problems of the conscript.[2] Nowadays, the systematic measures of height, weight and in many countries also other anthropometric indicators, such as head circumference, are an important part of the health check-up of children since faltering in growth can reveal diseases, inadequate nutrition, or problems in the rearing environment of the child not possible to identify with other methods.[3] Also the globally increasing level of obesity during the last decades[4] and at the same time the persistent problem of malnutrition in many low and middle-income countries[5] have emphasized the need to understand the genetic and environmental factors affecting individual differences in body size and morphology.

Height and body mass index (BMI, kg/m^2), which is still the most widely used measure of obesity, have been the most intensively studied traits in human genetics including genetic twin and family studies. The roots of genetic family studies of height go back to the late 19th century when Galton found that the height of offspring can reliable be predicted from the parental height.[6] Pearson and Lee continued the research on the family resemblance of height in their paper published in 1903 where they presented the correlations of height between relatives in a large sample of British families. They correctly interpreted that the familial height correlations reflected the influence of genetic factors on height.[7] The insight of their research was shown by the fact that the heritability of height was later calculated based on the correlations they reported.[8] However, the real breakthrough in the analyses of the genetics of height and quantitative genetics, in general, happened in 1918 when Fisher published his seminal paper on the heritability of height, which first time presented the expected genetic correlations between different types of relatives thus creating the scientific foundation for quantitative genetics.[9]

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00024-6

The genetic family studies on BMI also have a long history of nearly 100 years. The first study reporting that overweight is clustered in families was published in 1923.[10] The authors correctly interpreted that this clustering indicates the role of genetic factors behind individual differences in BMI. The first twin study on any obesity indicator, skinfold thickness, was published in 1975,[11] and a few years later the first twin study on BMI was published.[12] After these seminal studies, the research progressed rapidly, and a meta-analysis published in 2012 already identified 27 heritability estimates based on family and 88 heritability estimates based on twin studies.[13]

Anthropometrical studies have thus been very important in the history of quantitative genetics and generally in public health. However, in spite of this long history, they still have important scientific and public health value. Obesity is one of the most important modifiable risk factors of many chronic diseases whereas height can be used at the population level as an indicator of inadequate childhood nutrition. Further, more detailed anthropometric measures can allow more detailed estimation of health risks.[14] Thus, it is not surprising that after more than 100 years of history, intensive research is continuing on different anthropometric indicators, and twin studies have solidly shown their continued value in this field.

This chapter will first present the estimates of how genetic and environmental factors contribute to individual differences in different anthropometric indicators. Then, the role of genetic and environmental factors on human development will be discussed. Finally, the chapter will deal with the issue how genes and environment do interplay when explaining the variation in anthropometric measures. The main emphasis is on twin studies, but these results will be positioned in the wider context of the rapidly developing area of molecular genetics. The main focus will be in simple anthropometric traits, especially height and BMI. This is partly because most of the previous studies have focused on height and BMI but also because these traits still have the highest value in public health since reliable information on them is widely available. However, also other traits typically measured in health check-ups and thus having importance in public health will be covered.

23.2 Genetic and environmental variation in anthropometric measures

In this section, the role of genetic and environmental factors on individual differences in various anthropometric traits will be discussed. Heritability estimates indicate how much genes explain on individual differences. For example, high heritability of height tells that in a certain cohort most of the differences between individuals are because of genetic differences. However, it does not indicate that the change of environmental factors could not have an effect on height. For example, the mean height of the global population has dramatically increased over the 20th century because of the change of environment.[15] This is not in contrast to the high heritability because the environmental change has happened at the level of population and so has not affected heritability if studying only one birth cohort or if adjusting the results for birth year.

23.3 Birth outcomes

Even when twins generally well represent the general population, birth-related outcomes are exceptions. Twin pregnancies are characterized by earlier gestational age, lower birth weight, and rapid catch-up growth, especially during the first year of life, when compared to singleton pregnancies.[16] Especially within monochorionic twins, who are always monozygotic (MZ), the vascularization of placenta can lead large discordance in birth size. This can inflate the estimates of shared environmental factors in birth-related outcomes because there is additional variation affecting MZ twins not taken into account in twin modeling.[17] Thus, the results on the role of genetic and environmental factors on birth-related outcomes need to be interpreted with caution.

The problem of the special features of twin pregnancies can be managed by using different designs to estimate the effects of genetic and environmental factors, each making different theoretical assumptions, and thus getting more reliable estimates. A Dutch study found that around equal shares of the variation of birthweight and length were caused by genetic factors, shared environmental factors, and unique environmental factors.[18] This study showed very similar results by using two different methods—parent-offspring trios of singletons and comparisons of MZ and dizygotic (DZ) twins. Both of these methods have possible sources of bias, but the uniform results give more evidence that both genetic and environmental factors are important for individual differences in birth size. Somewhat contrasting results on the role of the common environment were found in a Swedish study using information on maternal weight gain of MZ sisters, which did not find evidence on the role of shared environmental factors.[19] However, maternal weight gain only partly reflects the size of fetus. Further, the study was small and thus may not have enough statistical power to distinguish shared environmental variation from genetic variation.

Previous studies have thus given evidence that both genetic and environmental factors affect the birth size. The role of genetic factors can be because of genes affecting fetal growth directly but also indirectly through maternal genotype affecting the intrauterine environment. Environmental factors affecting fetal growth and birth size include maternal nutrition and smoking.[20] Also maternal socioeconomic position is known to affect birth size, probably indicating differences in behavioral factors between social classes. A factor having a large effect on birth size is gestational age. In the twin modeling, the effect of gestational age is modeled as a part of shared environment, and thus if the results are not correctly adjusted for gestational age, the role of shared environment in probably overestimated.

There is some evidence that the role of genetic and environmental factors on birth outcomes has changed over time. A large international twin study found that shared environmental factors explained a larger share of the variation of birth weight, length, and ponderal index (kg/m^3) than genetic factors.[21] Adjusting the results for gestational age explained a part of shared environmental variation, but it still remained substantial. However, there was more shared environmental variation in the

later cohorts born in 1990–1999 as compared to earlier cohorts born in 1970–1989, which led also to larger trait variation of these three outcomes. It is possible that this reflects the improved neonatal care in the later birth cohorts, which have led to the better survival of low birth weight newborns. The study also found that in East Asia there was less shared environmental variation in birth weight, length, and ponderal index than in Europe or in North America and Australia. This may suggest that Western social context can reinforce differences between families in the family level environmental factors affecting birth size.

23.4 Height

Height is one of the most highly heritable quantitative traits in humans. Height has also several properties making it an ideal trait in human genetic studies. It is approximately normally distributed, does not change in adult age excluding slight body shrinking in old age, and also the variation of height is largely similar in different populations in spite of differences in mean height.[22] Because of these advantages, height has been used in many genetic studies when new methods in human genetics have been developed. In a study using the whole genome, the genetic polymorphisms explained nearly the same proportion of height variation as found in twin studies.[23] This well shows that the theoretical assumptions of quantitative genetic studies proposed by Fisher already 100 years ago are valid.[9]

In spite of the high heritability of height, environmental factors have an effect on the differences in height. The influence of environmental factors on growth and height is well seen in the shorter stature of North Korean children as compared to their South Korean peers, which was more than 10 cm in mid-childhood, in spite of their common ethnic background.[24] Systematic socioeconomic height differences have also been found, which indicates that poor living conditions can affect growth in childhood and lead to shorter adult stature.[25] In twin studies, adult height shows a small but systematic shared environmental component: in most studies, shared environmental variation has been around 10% of the total variation, but in some cohorts it has been nearly 20%.[26,27] This systematic effect of shared environmental factors on the variation of height demonstrates that also the family environment has an effect on height. It is known that nutrition, especially protein intake during the period of fast growth in the first two years of life, has strong effect on growth, which can affect also adult stature.[28] However, also childhood diseases can affect growth, and in many populations they are interviewed with nutrition.[29]

The role of genetic and environmental factors on height can, however, change over the growth period. Fig. 23.1 presents the proportions of additive genetic, shared environmental, and unique environmental variances of height from infancy to adulthood based on a large international pooled twin study.[30,31] The role of shared environmental factors was substantial from 1 to 9 years of age when it explained from 20% to 40% of the height variation. However, after 9 years of age, the effect of shared environmental factors decreased, and at most of age groups until adulthood

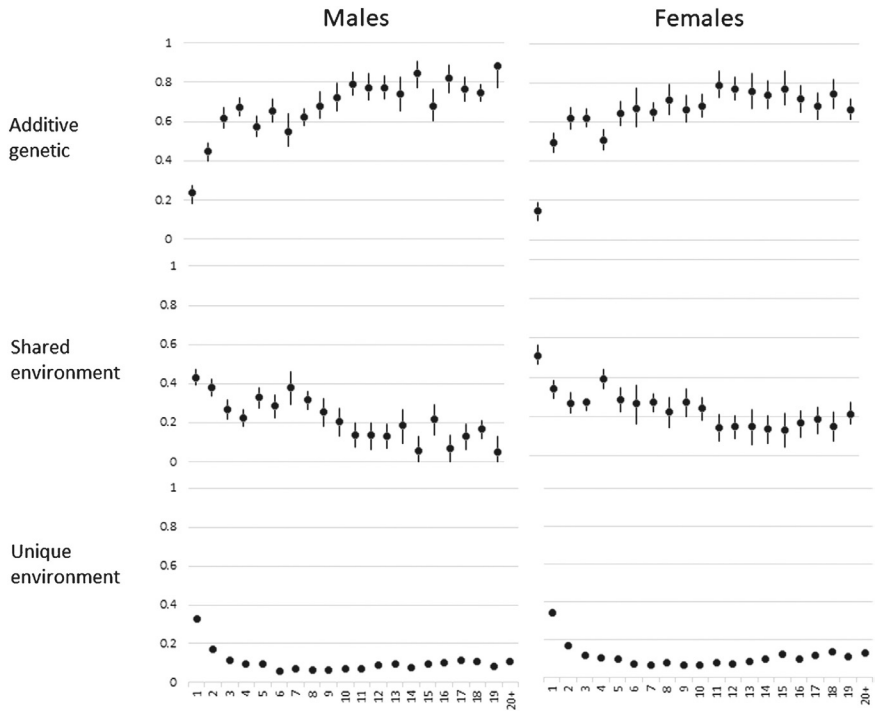


FIG. 23.1 The proportions of height variation explained by genetic, shared environmental, and unique environmental factors from infancy to adulthood.

explained from 10% to 20% of the height variation. Since the variation explained by unique environmental factors did not change, the differences in the effects of shared environmental factors over ages were mirrored in the heritability estimates, which were systematically lower in early and mid-childhood than at later ages. The proportions of genetic and environmental variations followed the same patterns in males and females.

The changing effect of shared environmental variation in height over childhood can well indicate the changing role of family environment in nutrition. After mid-childhood, the living conditions of children may become more equal when the nutrition of the child is not anymore totally depending on the family. Also, the effect of childhood diseases on growth can diminish at later ages thus decreasing environmental variation. The decrease of shared environmental variation can be linked with catch-up growth when the growth velocity will increase in children whose growth has previously been delayed thus compensating some of the height difference. However, more studies are needed to better understand this decreasing role of shared environment on growth, preferably also having information on nutrition intake and other factors affecting growth.

23.5 Body mass index

BMI is the anthropometric trait that probably received the most scientific interest in the recent decades, and human genetics is not an exception. The large interest in BMI is because obesity has become one of the most important modifiable risk factors of health and disability during the last decades.[32] Even as a simple indicator, BMI well indicates obesity at the populations level and is still the most widely used indicator of obesity in human genetics and public health.

Obesity is fundamentally caused by a long-term imbalance between eating (energy intake) and physical exercise (energy output). In the light of this principle, it may be surprising that previous twin studies on adult BMI have shown high heritability being at the level of 80% of the total BMI variation. In contrast to height, there is no evidence of the effect of shared environmental factors on BMI variation in adulthood.[13] When the genetics of BMI has been analyzed more detailed, it has been found that genes affect BMI largely through behavior. The genetic polymorphisms affecting BMI have been found to be expressed especially in the brain tissue, and twin studies have shown that genetic factors affect eating behavior, which can importantly contribute to the genetic variation of BMI.[33] Thus, the genetics of obesity interestingly shows how difficult it is to make distinction between genes and environment. When regarding obesity, eating behavior can be regarded as a part of environment. However, it is affected by genetic factors, and genes affect BMI largely through eating behavior.

The roles of genetic and environmental factors are, however, not constant but can change over the human life course. Fig. 23.2 presents the effects of genetic factors, shared environment, and unique environment on BMI variation from infancy to old age based on a large international twin study.[34,35] At 1–2 years of age, the heritability of BMI was over 60%, but it decreased to 50% at 4 years of age. This was because the variation of shared environment increased. However, after that age the heritability estimates increased again and were around 80% during adolescence and early adulthood. The increase in the heritability was because the effect of shared environmental factors decreased during late childhood and virtually disappeared in adolescence. The heritability estimates started to decline again during adulthood and in old age were around 50%, that is, at the level of heritability in infancy. However, in contrast to childhood, the decline of heritability during adulthood was because the increasing effect of unique environmental factors. Thus, the role of environment behind BMI variation is very different in childhood and adulthood. No differences in the proportions of genetic and environmental variance components were found between males and females.

The pattern of different influences of genetic and environmental factors on BMI from infancy to old age can indicate the changing dynamics of environment behind BMI variation over the human life course. In infancy, the baby can quite independently regulate eating since the caregiver mainly react to the crying and usually does not try to control eating. Thus, the genetic preferences affecting, for example, appetite can affect BMI. The situation changes in early childhood when parents have more control to the eating of their offspring. However, their control will gradually decrease

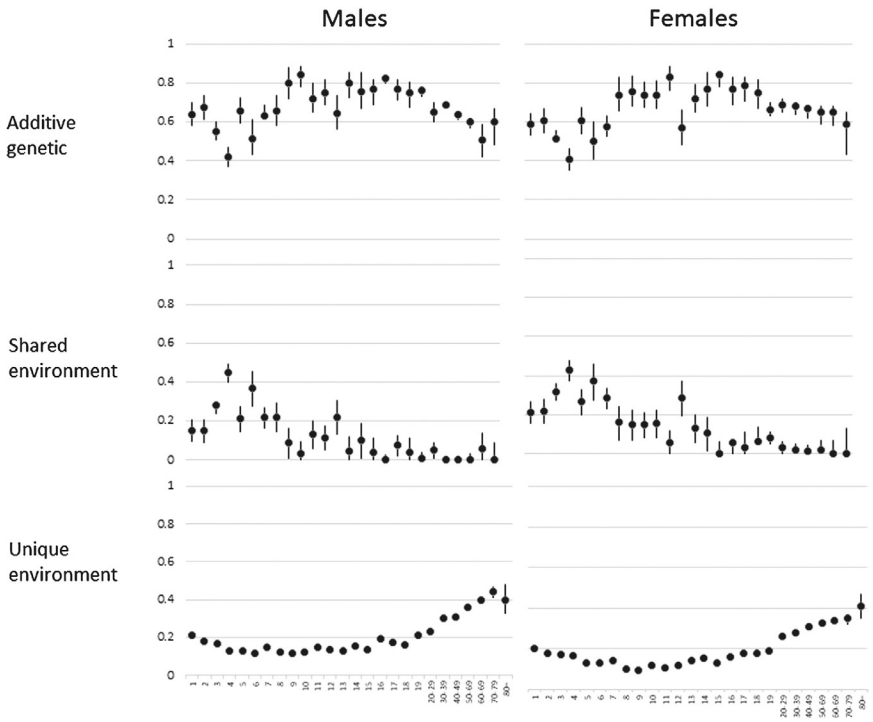


FIG. 23.2 The proportions of BMI variation explained by genetic, shared environmental, and unique environmental factors from infancy to old age.

in mid-childhood and largely disappear in adolescence when children get more independence. At that time, their own genetic preferences affecting eating styles and food preferences become more important seen as the increasing genetic variation of BMI. During adulthood, environmental influences start to increase again but, in contrast to childhood, these factors are not shared by cotwins. These environmental factors can be, for example, related to a spouse or a living environment affecting eating styles or the type of food available.

23.6 Other anthropometric measures

Even when height and BMI have received the most of scientific attention in twin studies on anthropometrics, there are also some information available on other anthropometric traits as well. Next, twin studies on the indicators having most value in public health will be discussed.

Head circumference is routinely measured in many countries in children health check-ups. Measuring head circumference is especially important in infancy since

abnormal development can reveal various neurological disorders. However, even the normal variation of head circumference is found to be associated with neurological development and thus offers information on risk factors of cognitive development.[36] A Japanese twin study found that both genetic and environmental factors affected the variation of head circumference during the first year of life, but the effect of a shared environment largely diminished after 7 months of age.[37] Similar results were found in a pooled study of Dutch and Australian twins, which found a shared environmental effect before four months of age but not anymore at later ages in childhood or in adulthood.[38] Somewhat different results on the role of shared environmental factors were found in a US twin study, which found that shared environmental factors affected head circumference and several other measures of head at 11 and 17 years of age.[39]

Chest circumference is a more rarely used indicator in regular health check-ups than head circumference. However, there is evidence that it is a good measure of nutritional status of newborn and may thus be an especially useful measure in developing countries.[40] A Japanese twin study found that shared environmental factors had a substantial effect on chest circumference until five months of age, but this effect started to decrease after that age and disappeared until 13 months of age.[41] Interestingly, a substantial part of shared environmental variation was common with birth weight; genetic factors also shared some variation with birth weight, but the effect was much smaller. This supports the hypothesis that chest circumference could be a surrogate measure for birth weight if, for example, a scale is not available or otherwise measuring birth weight is not feasible. However, chest circumference can also have additional value to birth weight as an indicator of fetal nutrition.

So far this chapter has focused only on single anthropometric measures. However, combining different measures can give more detailed information on body morphology. Somatotype is a method classifying body morphology into three components: endomorphy (relative fatness), mesomorphy (relative musculoskeletal development), and ectomorphy (relative linearity) based on detailed anthropometric measures.[42] Sometotype has been used especially in studies of elite athletes, but it has also value when studying the general population, especially because it is associated with physical fitness. Heritability estimates for somatotype are available in Belgian twin studies from mid-childhood to adulthood.[43,44] In a Portuguese study of children and adolescents, higher heritability estimates were found and genetic factors explained from 80% to 93% of variation of somatotype components.[45] This study did not find evidence on the role of shared environmental factors. Together, these studies showed that genetic factors are important for individual differences in somatotype. However, more studies are needed to confirm the role of shared environment behind the individual differences in somatotype traits.

23.7 Genetics of growth and development

As shown above, the relative effects of genetic and environmental factors on height and BMI change over aging. However, since the data were cross-sectional, these

studies cannot tell anything about the role of genetic and environmental factors behind human growth and development. This type of analysis needs longitudinal measures on the same children, preferably repeatedly during a long period of time. Next, the results on longitudinal twin studies on height and BMI will be discussed.

23.8 Growth in height

The characteristic pattern of human growth with the periods of rapid growth in infancy and during puberty and the period of slow growth in childhood has received a long-time research interest. The growth chart showing the normal growth pattern and allowing to identify abnormal growth is an important tool when following the physical development of the child. However, also growth within normal variation is found to be associated with health risks. Especially, catch-up growth has been found to be associated with health risks such as higher risk of cardiovascular diseases.[46] This probably indicates the fact that those children have higher environmental stress at earlier ages slowing growth and later compensated with catch-up growth affecting also further health.

Human growth is strictly genetically regulated even when living conditions, especially the lack of protein, can affect it. Twin studies allow to estimate genetic and environmental correlations between height at different ages. A Swedish twin study has a full series of longitudinal measures from 1 to 18 years of age in boys and thus allows studying this in detail.[47] This study showed that the genetic correlation was already as high as 0.73 between height at 2 and 18 years of age. This shows that largely the same set of genes affects human height during the whole growth period. However, also new genetic factors started to affect height at each age. Genetic factors explained nearly all of the correlations of height between different ages.

Puberty creates a special phase of growth, which has attracted a lot of scientific interest. Previous twin studies have shown that genetic factors have an important effect on the timing of puberty. A Swedish twin study showed that in boys more than 90% of the variation of the timing of pubertal growth spurt was explained by genetic differences.[48] A Finnish twin study showed that 30% of the genetic factors in girls and 50% in boys affecting the timing of the pubertal growth spurt affect also the timing of the development of secondary sexual characteristics largely used as a marker of puberty.[49] Thus, understanding the genetics of pubertal growth can also help to understand the genetics of sexual maturation, which is an important part of health check-ups in adolescence.

23.9 Development of body mass index

Such as height, also BMI shows a strong continuity from childhood to adulthood. Understanding the background of this tracking has important public health implications since overweight children have a high risk to become obese adults.

When studying the development of BMI, it is important to make distinction between childhood and adulthood. During childhood, body morphology and the proportions of different tissues change. This is especially notable during puberty when the proportion of muscle mass increases in boys and fat mass in girls.[42] Partly different sets of genes regulate the development of different tissues.[50] In adulthood, the change in BMI, which mainly means regular increase of weight, is because of the accumulation of fat mass. Thus, the genetics of change of BMI is a much simpler phenomena in adulthood than during the growth period of childhood.

Twin studies on the genetics of BMI development over childhood show that genetic factors are behind of the continuity. In a Swedish twin study having a full series of measurements from infancy to early adulthood, a genetic correlation with adult BMI was found already for BMI before 4 years of age. However, the genetic correlations increased after five years of age being 0.61 at 5 years of age and increasing after that. This suggests that after 4 years of age, a new genetic component starts to affect BMI.[51] This is also supported by molecular genetic studies showing that the effects of obesity candidate genes on BMI increase after early childhood.[33] As mentioned earlier, during this age period also the role of shared environment on BMI starts to decline and genetic variation to increase. Since many genetic variants associated with obesity are likely to be related to eating behavior, it is very possible that this new set of genes affecting BMI is related to eating and other behavioral factors. Eating preferences affecting adult BMI may thus be present already in mid-childhood, and this long-lasting continuity is underlined by genetic factors. However, more studies having longitudinal measures of eating styles and preferences in genetically informative data are needed to confirm this hypothesis.

Genetic factors have also effect on weight gain in adulthood. From low to moderate heritability estimates have been found in longitudinal twin studies from USA and Finland. Interestingly, the genetic correlation between weight gain and baseline BMI is modest or not existing at all.[52] This suggests that there is a specific set of genes affecting fat accumulation over adulthood, which is partly independent on genes affecting body size in early adulthood.

23.10 Gene–environment interactions

So far in this chapter, the effects of genes and environment have been treated separately. However, genes and environment can modify each other's effects, which is called as gene–environment interaction. This means that same genes can affect a trait differently in different environments or that a same environmental exposure has different effects depending on the genetic background of individual. In twin studies, the gene–environment interactions can be seen as differences in genetic variances depending on environment.[53] However, this needs a considerable statistical power, and thus the results based on small data sets should be treated with caution because they can easily lead to false positive results. Next, twin studies concerning height

and BMI will be discussed. Because strong statistical power is needed, all results are based on the large international database used also in Figs. 23.1 and 23.2.

23.11 Height

The idea that environmental factors can affect the heritability estimates of height is deeply involved in the idea how genes and environment affect growth and adult stature. According to a common hypothesis, humans have genetically determined maximum height, but inadequate nutrition and other environmental stress can affect growth leading to shorter adult stature. However, it is noteworthy that even when the mean height has increased globally during the 20th century, a similar increase has not been seen in the variation of height.[22] If the increase in mean stature would be caused by the fact that a larger proportion of population would reach the genetic maximum, it should lead to a systematic decrease in the variation of height since differences in environmental factors do not anymore affect differences in height between individuals. Next, we will see how large-scale twin studies can contribute to this issue.

When secular trends in the genetic and environmental influences on adult height over birth cohorts from the late 19th to the late 20th century were studied, no systematic differences in the genetic and environmental variation of height were found even when the mean stature expectedly increased.[31] Another design to analyze the effect of environment was used in a study on the heritability of height from infancy to adulthood according to parental education.[54] Also in this study, only weak evidence on the differences in the genetic and environmental variations of height between the categories of parental education was found even when the children of better-educated parents were taller than their peers whose parents had poorer education.

These previous twin studies thus challenge the long-lasting hypothesis that the heritability of height is lower in the presence of environmental stress slowing growth. It is noteworthy that in the late 19th and early 20th century, the standard of living was very low compared to the modern standards of high-income societies. Also, the mean height was dramatically lower compared to the recent birth cohorts also suggesting that environmental stress affecting growth has decreased. Thus, it is interesting that the heritability of height is largely constant in these very different environments.

23.12 Body mass index

The presence of gene–environment interactions in obesity has already been recognized for decades. A classic example of the interplay between genes and environment is the Pima Indians of Arizona. At the beginning of the 20th century, obesity and metabolic diseases were virtually unknown within the tribe, but the large changes in their living environment after the Second World War destroyed their traditional way of life leading to the westernized diet and lifestyle in general. Currently, the rate of obesity

and associated metabolic diseases within the tribe is the highest among the US ethnic groups.[55] This shows that the Pima Indians have high genetic susceptibility to develop obesity and related metabolic diseases, but this affects only in the presence of westernized lifestyle.

The mean BMI and the proportion of obesity have dramatically increased globally during the last few decades. This global change to a more obesogenic environment offers an interesting opportunity to study how the environmental change modifies the genetic and environmental variations of BMI. A study having measures of adult BMI from the 1940s to the 2010s found that at the same time when the mean BMI increased, also the genetic and environmental variances of BMI increased.[35] The similar differences were found between three cultural-geographic regions having different levels of mean BMI. The genetic and environmental variances were highest in North America and Australia representing a high level of obesogenic environment followed by Europe and being lowest in East Asia representing moderate and low levels of obesogenic environment, respectively. Similarly, when the genetic and environmental variances from infancy to adulthood were analyzed according to the parental education, the offspring of highly educated parents had lower BMI and less genetic and environmental variation than those whose parents had low education.[56]

Thus, twin studies using different measures of obesogenic environment—measurement year, cultural-geographic region, and parental education—have produced very similar results. In a more obesogenic environment, both genetic and environmental variances of BMI are higher than in a less obesogenic environment. This gives interesting information on the dynamics behind the obesity epidemic. A change in the environment seems to affect BMI largely through genetic factors. So those who have genetic susceptibility to get weight are especially vulnerable for the effects of obesogenic environment. Thus, they would also benefit most on the society level interventions to decrease obesity.

23.13 Conclusions

In this chapter, the results of previous twin studies on different anthropometric indicators were discussed. The overwhelming majority of previous studies have treated two indicators—height and BMI. They are both widely used indicators also in human genetics and public health in general. There are also other indicators widely used in public health, but which have received considerable less attention in twin studies. Thus, they would offer interesting new topics for further research.

Previous studies have shown interesting similarities but also differences in the genetic architecture of anthropometric indicators. Environmental factors shared by cotwins seem to have an effect on the individual differences in anthropometric traits only in childhood. The exception is height where this effect is still seen in adulthood. This is understandable since the effect of environmental factors affecting growth can have an effect on adult stature. This supports the hypothesis that at the population level adult height can be used as an indicator of childhood living conditions. On the

other hand, for BMI the role of shared environment is strongest in early childhood but it diminishes in mid-childhood and largely disappears during adolescence. This probably reflects the increasing independence of children from their parents leading to a higher influence of partly genetic preferences on eating behavior and through that to BMI.

On the other hand, the genetic and environmental variations of height and BMI react differently to environmental change. For height, the size of both genetic and environmental variations is largely independent on environment even when the mean height can differ. On the other hand, BMI shows both higher genetic and environmental variation in an obesogenic environment increasing mean BMI. This suggests that environmental factors affect BMI through genetic factors probably indicating the role of behavior behind individual BMI differences.

In conclusion, previous twin studies have shed new light to individual differences in body morphology. Genetic factors explain a major part of variation of anthropometric traits. Environmental factors shared by cotwins have effect in early childhood, and in height this effect is seen still in adulthood. Changes in environment have an effect on the genetic and environmental variations of BMI whereas for height they have only little effect. This reflects that even when ostensibly similar traits, the background of BMI is very different than height since it is sensible to eating behavior.

References

1. Haslam D. Obesity: a medical history. *Obesity Reviews*. 2007;8:S31–S36.
2. Kajava Y, Jalmari Y. Mittailungen über die Körpergrösse des finnischen Mannes Ende des 18. und Anfang des 19. *Jahrhundrets. Helsinki: Suomalainen tiedeakatemia*. 1926.
3. Himes JH. Why study child growth and maturation? In: Hauspie RC, Cameron N, Molinari L, eds. *Methods in Human Growth Research*. Cambridge: Cambridge University Press; 2004:3–26.
4. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128•9 million children, adolescents, and adults. *Lancet*. 2017;390:2627–2642.
5. Wells JC, Sawaya AL, Wibaek R, et al. The double burden of malnutrition: aetiological pathways and consequences for health. *Lancet*. 2020;395:75–88.
6. Galton F. Regression towards mediocrity in heriditary stature. *Journal of Antropological Institute*. 1886;15:246–262.
7. Pearson K, Lee A. On the laws on inheritance in man. *Biometrika*. 1903;2:356–462.
8. Crown JF, Kimura M. *Introduction to Population Genetics Theory*. New York, NY: Harper and Row; 1970.
9. Fisher RA. The correlation between relatives on the supposition of Mendelian inheritance. *Trans R Soc Edinburgh*. 1918;52:399–433.
10. Davenport CB. *Body-Build and its Inheritance*. Washington: Carnegie Institution of Washington; 1923.
11. Brook CG, Huntley RM, Slack J. Influence of heredity and environment in determination of skinfold thickness in children. *BMJ*. 1975;2:719–721.

12. Stunkard AJ, Foch TT, Hrubec Z. A twin study of human obesity. *JAMA*. 1986;256:51–54.
13. Elks CE, den Hoed M, Zhao JH, et al. Variability in the heritability of body mass index: a systematic review and meta-regression. *Front Endocrinol*. 2012;3.
14. Silventoinen K. Children's anthropometrics and later disease incidence. In: Komlos J, Kelly IR, eds. *The Oxford Handbook of Economics and Human Biology*. Oxford: Oxford University Press; 2015:1–20.
15. NCD Risk Factor Collaboration. A century of trends in adult human height. *Elife*. 2016;26(e13410).
16. Buckler JM, Green M. A comparison of the early growth of twins and singletons. *Annals of Human Biology*. 2004;31:311–332.
17. van Beijsterveldt CE, Overbeek LI, Rozendaal L, et al. Chorionicity and heritability estimates from twin studies: the prenatal environment of twins and their resemblance across a large number of traits. *Behavior Genetics*. 2016;46:304–314.
18. Mook-Kanamori DO, van Beijsterveldt CE, Steegers EA, et al. Heritability estimates of body size in fetal life and early childhood. *Plos One*. 2012;7:e39901.
19 Andersson ES, Silventoinen K, Tynelius P, Nohr EA, Sørensen TI, Rasmussen F. Heritability of gestational weight gain-a Swedish register-based twin study. *Twin Res Hum Genet*. 2015;18:410–418.
20. Valero De Bernabe J, Soriano T, Albaladejo R, et al. Risk factors for low birth weight: a review. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*. 2004;116:3–15.
21. Yokoyama Y, Jelenkovic A, Hur YM, et al. Genetic and environmental factors affecting birth size variation: a pooled individual-based analysis of secular trends and global geographical differences using 26 twin cohorts. *International Journal of Epidemiology*. 2018;47:1195–1206.
22. Eveleth PB, Tanner JM. *Worldwide Variation in Human Growth*. 2nd ed. Cambridge: Cambridge University Press; 2003.
23. Wainschtein P, Jain DO, Yengo L, Zheng ZTOPMed Anthropometry Working Group. Trans-omics for precision medicine consortium, Cupples, L.A. Recovery of trait heritability from whole genome sequence data. *bioRxiv*. 2019. https://doi.org/10.1101/588020.
24. Schwekendiek D. Height and weight differences between North and South Korea. *Journal of Biosocial Science*. 2009;41:51–55.
25. Steckel RH. Height and human welfare: recent developments and new directions. *Expl Econ Hist*. 2009;46:1–23.
26. Silventoinen K. Determinants of variation in adult body height. *Journal of Biosocial Science*. 2003;35:263–285.
27. Silventoinen K, Sammalisto S, Perola M, et al. Heritability of adult body height: a comparative study of twin cohorts in eight countries. *Twin Research*. 2003;6:399–408.
28. Perkins JM, Subramanian SV, Davey Smith G, Özaltin E. Adult height, nutrition, and population health. *Nutrition Reviews*. 2016;74:149–165.
29. Bozzoli C, Deaton A, Quintana-Domeque C. Adult height and childhood disease. *Demography*. 2009;46:647–669.
30. Jelenkovic A, Sund R, Hur YM, et al. Genetic and environmental influences on height from infancy to early adulthood: an individual-based pooled analysis of 45 twin cohorts. *Scientific Reports*. 2016;6(28496).
31. Jelenkovic A, Hur YM, Sund R, et al. Genetic and environmental influences on adult human height across birth cohorts from 1886 to 1994. *Elife*. 2016:e20320.

32. GBD 2015 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388:1659–1724.
33. Silventoinen K, Konttinen H. Obesity and eating behavior from the perspective of twin and genetic research. *Neuroscience and Biobehavioral Reviews*. 2020;109:150–165.
34. Silventoinen K, Jelenkovic A, Sund R, et al. Genetic and environmental effects on body mass index from infancy to the onset of adulthood: an individual-based pooled analysis of 45 twin cohorts participating in the COllaborative project of Development of Anthropometrical measures in Twins (CODATwins) study. *American Journal of Clinical Nutrition*. 2016;104:371–379.
35. Silventoinen K, Jelenkovic A, Sund R, et al. Differences in genetic and environmental variation in adult body mass index by sex, age, time period, and region: an individual-based pooled analysis of 40 twin cohorts. *American Journal of Clinical Nutrition*. 2017;106:457–466.
36. Bartholomeusz HH, Courchesne E, Karns CM. Relationship between head circumference and brain volume in healthy normal toddlers, children, and adults. *Neuropediatrics*. 2002;33:239–241.
37. Silventoinen K, Karvonen M, Sugimoto M, Kaprio J, Dunkel L, Yokoyama Y. Genetics of head circumference in infancy: a longitudinal study of Japanese twins. *American Journal of Human Biology*. 2011;23:630–634.
38. Smit DJA, Luciano M, Bartels M, et al. Heritability of head size in Dutch and Australian twin families at ages 0-50 years. *Twin Res Hum Genet*. 2010;13:370–380.
39. Silventoinen K, Iacono WG, Krueger R, McGue M. Genetic and environmental contributions to the association between anthropometric measures and IQ: a study of Minnesota twins at age 11 and 17. *Behavior Genetics*. 2012;42:393–401.
40. Goto E. Meta-analysis: identification of low birthweight by other anthropometric measurements at birth in developing countries. *Journal of Epidemiology*. 2011;21:354–362.
41. Silventoinen K, Kaprio J, Dunkel L, Yokoyama Y. Genetic and environmental influences on chest circumference during infancy: a longitudinal study of Japanese twins. *Paediatric and Perinatal Epidemiology*. 2012;26:553–560.
42. Malina RM, Bouchard C, Bar-Or O. *Growth, Maturation, and Physical Activity*. 2nd ed.: Champaign, IL: Human Kinetics; 2004.
43 Peeters MW, Thomis MA, Claessens AL, et al. Heritability of somatotype components from early adolescence into young adulthood: a multivariate analysis on a longitudinal twin study. *Annals of Human Biology*. 2003;30:402–418.
44. Peeters MW, Thomis MA, Loos RJ, et al. Heritability of somatotype components: a multivariate analysis. *International Journal of Obesity*. 2007;31:1295–1301.
45. Silventoinen K, Maia J, Jelenkovic A, et al. Genetics of somatotype and physical fitness in children and adolescents. *American Journal of Human Biology*. 2020(e23470).
46. Silventoinen K, Baker JL, Sorensen TI. Growth in height in childhood and risk of coronary heart disease in adult men and women. *Plos One*. 2012(1):e30476.
47. Silventoinen K, Pietiläinen KH, Tynelius P, Sørensen TIA, Kaprio J, Rasmussen F. Genetic regulation of growth from birth to 18 years of age: The Swedish Young Male Twins Study. *American Journal of Human Biology*. 2008;20:292–298.
48. Silventoinen K, Haukka J, Dunkel L, Tynelius P, Rasmussen F. Genetics of pubertal timing and its associations with relative weight in childhood and adult height: the Swedish Young Male Twins Study. *Pediatrics*. 2008;121:e885–e891.

49. Wehkalampi K, Silventoinen K, Kaprio J, et al. Genetic and environmental influences on pubertal timing assessed by height growth. *American Journal of Human Biology*. 2008;20:417–423.
50. Hasselbalch AL, Benyamin B, Visscher PM, Heitmann BL, Kyvik KO, Sørensen TIA. Common genetic components of obesity traits and serum leptin. *Obesity*. 2008;16:2723–2729.
51. Silventoinen K, Pietiläinen KH, Tynelius P, Sørensen TIA, Kaprio J, Rasmussen F. Genetic and environmental factors in relative weight from birth to age 18: The Swedish Young Male Twins Study. *International Journal of Obesity*. 2007;31:615–621.
52. Silventoinen K, Kaprio J. Genetics of tracking of body mass index from birth to late middle age: evidence from twin and family studies. *Obesity Facts*. 2009;2:196–202.
53. Boomsma DI, Martin NG. Gene-environment interactions. In: D'haenen H, den Boer JA, Wilner P, eds. *Biological Psychiatry*. New York, NY: John Wiley & Sons Ltd; 2002:181–187.
54. Jelenkovic A, Sund R, Yokoyama Y, et al. Genetic and environmental influences on human height from infancy through adulthood at different levels of parental education. *Science Reports*. 2020;10(7974).
55. Pratley RE. Gene-environment interactions in the pathogenesis of type 2 diabetes mellitus: lessons learned from the Pima Indians. *Proceedings of the Nutrition Society*. 1998;57:175–181.
56. Silventoinen K, Jelenkovic A, Latvala A, et al. Parental education and genetics of BMI from infancy to old age: a pooled analysis of 29 twin cohorts. *Obesity*. 2019;27:855–865.

CHAPTER

24

Twin studies of cardiorespiratory disease, daily cardiovascular activity and imaging

Adam D. Tarnoki[a], Gonneke Willemsen[b,c], Eco de Geus[b,c], David L. Tarnoki[a]

[a]*Medical Imaging Centre, Semmelweis University, Hungarian Twin Registry, Budapest, Hungary*
[b]*Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*
[c]*Amsterdam Public Health Research Institute, Amsterdam, The Netherlands*

24.1 Introduction

In 2019, the top three global causes of death consisted of noncommunicable cardiovascular and lung diseases, with ischemic heart disease being the number one cause of death, followed by stroke and chronic obstructive pulmonary disease[1]. Furthermore, cardiovascular diseases and chronic lung diseases, which also include asthma, are among the leading causes of morbidity worldwide.[1] Twin research provides important insights into the underlying causes of cardiorespiratory disease. Twin studies provide information on the degree of heritability for the various diseases and related traits, and may also add to our understanding of the interplay of genetic factors with various environmental risk factors (gene–environment interactions). A large body of twin studies on cardiorespiratory disease already exists. As it is impossible to review the full scope of the literature here, in part A of this chapter we provide examples of twin studies for a wide range of cardiovascular and respiratory diseases focusing on their results for the role of heritability and the interplay with the environment in cardiorespiratory disease. To fully understand disease development it is also important to understand related processes in a nonpatient population during everyday life. In part B of this chapter, an overview is provided of twin studies using ambulatory monitoring to determine the heritability of everyday variation in blood pressure and heart action. Ambulatory monitoring, however, is not the only way to capture disease processes. In the last few decades, imaging has undergone an extensive development and it now plays a crucial role in the detection and

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00042-8

characterization of most cardiorespiratory phenotypes. In part C of this chapter, we illustrate the opportunities which arise from combining twin research and imaging research.

24.2 Cardiorespiratory twin studies

Adam D Tarnoki, David L Tarnoki

24.2.1 Heritability of the most common cardiovascular diseases

24.2.1.1 Hypertension: blood pressure, blood pressure components, and vascular elasticity

Hypertension is a common complex polygenetic trait, traditionally considered to have a moderate genetic component that interacts with various environmental risk factors such as diet, physical activity, and alcohol consumption, which affects ≤1 billion adults globally.[2] Unraveling the multifactorial basis of essential hypertension has been a central question of numerous twin studies, which have demonstrated moderate heritability of 30%–65%, with the remaining variance explained by unique environmental factors. A meta-analysis of 17 studies found that the mean heritability of systolic (SBP) and diastolic (DBP) blood pressure was 54% and 49%, respectively. The effect of common environmental factors was negligible.[3] Gene-environment (GxE) interaction studies have found that several factors, such as education, eating habits, obesity, and the associated gut microbiota, may change the role of genetic factors.[4]

In the last decade, specific blood pressure components, such as the pulsatile component of blood pressure, pulse pressure (PP), and central blood pressure, have also received attention as they have been shown to predict later cardiovascular events.[5] Twin studies confirmed a moderate heritability for these blood pressure components, with a higher inheritance for the central blood pressure variables than the peripheral blood pressure values.[6]

Arterial stiffness is a dynamic property defined by vascular function and vascular wall structure, which is also a good predictor for future cardiovascular events.[5] Pulse wave velocity (PWV), characterizing vascular elasticity, and augmentation index (AIx), an indicator of wave reflection and peripheral vascular resistance have been also studied and a moderate genetic effect was observed for these traits.[7–10]

The genetic origin of the association of hypertension with BMI has also been the focus of a number of studies. These studies have shown that common genetic factors may for a large part explain the correlation of blood pressure components with BMI.[11–14] Blood pressure components were moderately correlated with BMI, largely because of shared genetic factors. However, for the association of BMI with brachial SBP and DBP, aortic SBP, and mean arterial pressure, acquired, modifiable factors were also found to be important.[11]

24.2.1.2 Carotid atherosclerosis

Carotid atherosclerosis, a risk factor of stroke, is one of the most commonly studied atherosclerotic phenotypes as the degree of carotid atherosclerosis can be easily obtained for the carotid artery using ultrasound. The timeline of atherosclerotic progression includes carotid stiffening, increased intima-media thickness (IMT), and plaque development.

Carotid IMT is a reliable marker of subclinical atherosclerosis. Twin studies attributed a moderate role to genetic factors (25%–60%).[15] The Healthy Twin Study provided evidence for segment-specific heritability of carotid IMT (48% for common, 38% for carotid bifurcation, and 45% for internal carotid artery (ICA), respectively) and a shared genetic variation was reported for the three carotid segments.[16]

Plaque formation in the carotid artery has been associated with a number of cardiovascular (e.g., myocardial infarction), retinal, and cerebral ischemia (stroke) complications. Based on the findings of a twin study involving Italian, Hungarian and American twins, heritability was 78% for the presence of carotid plaque, 74% for plaque-type based on its echogenicity, 69% for plaque size, 74% for plaque sidedness, 74% for plaque numerosity, 68% and 66% for the presence of plaque in carotid bulbs and proximal internal carotid arteries. Unique environmental factors were responsible for the remaining variance (22%–34%).[17] An Italian twin study reported additive genetics to be responsible for the variance of carotid plaques in 52%, with unique factors explaining the remaining variance in the trait.[18] Whole-genome association studies confirmed the role of 14 loci on chromosomes 14 loci with at least suggestive significance in the formation of carotid plaques.[19] Numerous single nucleotide polymorphisms (SNPs) have been linked to the increased risk for the development of subclinical or clinical carotid atherosclerosis, though only a minority of these genes seem to be potential future therapeutic targets.

Carotid flow velocities, determinate by ultrasound, showed a moderate and low (63% and 18%) heritability of the ICA peak systolic velocity and ICA/common carotid artery ratio. One of the few twin studies conducted for three traits showed little evidence for the role of genes as common (56%–63%) and individual environmental factors (37%–44%) explained the vast majority of the variance.[20] An Italian twin study showed carotid vascular wall elasticity to be moderately heritable (19%–46%).[21] These findings support the value of the prevention of modifiable environmental factors in case of altered carotid flow velocities.

24.2.1.3 Coronary atherosclerosis

The classic risk factors associated with coronary heart disease (CHD), which may lead to acute myocardial infarction, are well known. Genetic predisposition was shown to play a role in CHD in family studies, but limited information is available from twin studies. A Swedish large twin study involving 51065 same-sex twins showed that during the 40-year follow-up, the heritability of CHD decreased with increasing age, as well as with increasing levels of BMI, in both men and women. Thus, genetic factors may play a more prominent role for CHD development in the

absence of important environmental factors such as BMI.[22] Another Swedish twin study reported that, in general, probandwise concordances and intraclass correlations for angina pectoris (AP) and CHD death were greater in monozygotic (MZ) than dizygotic (DZ) twins among both sexes, indicating moderate heritability estimates for AP in both sexes (39% for males and 43% for females). The correlation between AP and CHD was almost exclusively explained by the influence of familial factors in both sexes, pointing to both shared genetic as shared environmental pathways.[23] Coronary calcification has been found to be moderately heritable in twins (67%, 95% CI: 37%–100%) when adjusted for age and sex, and overlapping genetic factors are largely responsible for the phenotypical resemblance of coronary and carotid or femoral atherosclerotic calcification.[24] These findings are supported by several published case studies where both twin brothers suffered acute myocardial infarction. In those cases, both twins usually have similar comorbidities as well as a similar course of the acute coronary syndrome.[25–26] This emphasizes the need for screening people with a higher risk of future myocardial infarction. GWAS studies of common SNPs already found some genomic regions explaining ~2.4% of coronary artery calcification's heritability.[27]

24.2.1.4 Aortic atherosclerosis and aneurysm

Calcified aortic plaque, assessed by computed tomography (CT), has shown a high heritability (61%), and the association between aortic wall calcification and increased arterial stiffness can be explained by a common genetic background.[28] Aortic atherosclerosis might lead to abdominal aortic aneurysm (AAA) formation, which is an abnormal dilation of the aorta and may progress to rupture and death. The Danish Twin Registry reported that the probandwise concordance rate for AAA was 2.5 times higher in MZ compared with DZ twins indicating a heritability of 77%.[29]

24.2.1.5 Peripheral arterial disease

Peripheral arterial disease (PAD) means the reduction of blood flow to the lower extremities due to slow and progressive narrowing, blockage, or spasms in a blood vessel.[30] The most common risk factors for PAD are well known, and also include genetic predisposition. A Swedish twin study confirmed the role of genetic (58%) and individual environmental factors (42%) in PAD development.[31] A study of 21 discordant twin pairs revealed that the twin with PAD was more likely to be sedentary and a persistent smoker.[31] An ultrasound twin study revealed a heritability of 44%–47% for common and superficial femoral IMT.[32] The variance in femoral plaques was due to genetic factors and the remaining 50% was explained by common (15%) and unique (35%) environmental factors. Sidedness and number of femoral plaques were mainly under genetic control. Femoral plaque composition was explained by genetics (64%) and unique environment (36%). Covariation between the liabilities to carotid and femoral plaques was mainly attributed to shared genes (77%).[18] Recent forays into GWAS and epigenetics studies have suggested an important role of environmental factors in DNA methylation, histone acetylation signatures, and miRNA regulation.[33]

24.2.2 Twin studies in frequent respiratory diseases

24.2.2.1 Lung function

A lung function test is a noninvasive method to investigate how well the lungs are working. Lung function variables (derived from spirometry) are moderately to highly genetically determined based on the results of multiple twin studies.[34–38] GWAS studies identified a number of genes related to respiratory function, such as TMEM132C, UNC93A, and TTLL2, and PPT2 on chromosome 21 in a Korean twin population.[39]

24.3 Twin studies of common chronic lung diseases

24.3.1 Chronic obstructive pulmonary disease (COPD)

COPD is a chronic inflammatory lung disease that causes obstructed airflow from the lungs, leading to airflow limitation by inflammation and destruction of the airways and lung parenchyma. The fast majority of COPD cases (80%–90%) are caused by smoking, which by itself is heritable, as discussed in Chapter 23. The susceptibility to develop severe COPD is strongly influenced by genetic factors (approximately 60%).[40] Heritability was also suggested for specific COPD components in a pilot study of one COPD-concordant and five COPD discordant twin pairs who underwent high resolution CT (HRCT).[41]Lung density and radiological markers of small airway disease (bronchial wall thickening, bronchiectasis, mucus plug formation, air trapping, and emphysema score) were very similar in identical twins, while other components were less similar among MZ twin pairs.[41] Behaviors, such as eating fruit, smoking, and alcohol use may also influence disease risk and those health behaviors are also in part heritable (see other chapters).

24.3.2 Chronic bronchitis

Chronic bronchitis, long-term inflammation of the bronchi, is characterized by chronic cough and sputum from the airways. A Danish twin study found a hereditary predisposition to chronic bronchitis with heritability estimates of 55% in women and 25% in men.[42] The heritability estimate for self-reported chronic bronchitis was a moderate 40% in Swedish samples, with common genetic factors explaining only 14% of the association with smoking.[43] Among twin pairs discordant for smoking, chronic bronchitis was significantly more common in the smoking twin compared with the nonsmoking cotwin.[44]

24.3.3 Asthma

Asthma is a chronic inflammatory disease of the airways. Its development was linked to genetic factors in around 35%–80%. A study in the Netherlands Twin Register confirmed high heritabilities for asthma (75%) and allergy (66%).[45] Childhood

asthma was also highly heritable (82%) in a Swedish twin study.[46] Nonadditive genetic influences may also be important, which may have consequences for gene hunting strategies. A population-based, cross-sectional Swedish twin study involving 612 MZ and same-sex DZ schoolchildren found that the association between asthma and exhaled nitric oxide level is to a large extent explained by genetics via allergen-specific IgE level and not blood eosinophils which might partly explain the clinical heterogeneity of asthma.[47] Environmental factors play a role in determining individual variations in the severity of asthma symptoms. A retrospective cohort study in twins aged 3–10 years showed that early life antibiotic use, particularly prescribed for respiratory infections, was associated with an increased risk of asthma.[48] In addition, epigenetic changes such as DNA methylation and histone acetylation can be modified by certain environmental factors, such as maternal nutrition, smoking, microbiome, xenobiotic exposure, and stress. Discordant twin studies found DNA methylation differences (HLX gene cg23603194) among asthma patients.[49]

24.3.4 Lung cancer

Smoking is the most important cause of lung cancer. However, genetic effects account for a significant amount of the variation in the liability to develop lung cancer. Heritability of lung cancer among current smokers was 41% and forever smoking pairs 37% according to the Nordic Twin Study of Cancer.[43] GWAS studies found an association with the *CHRNA5* functional D398N (rs16969968) variant, which was identified for smoking as well. This might explain the link with lung cancer.[50]

24.3.5 Exhaled biomarkers

Human-exhaled breath contains a mixture of over 3000 volatile organic compounds (VOCs), this exhaled breath pattern can be distinguished by pattern recognition using electronic noses (e-noses). Most human diseases such as lung cancer are affiliated with multiple chemical compounds. VOC pattern was determined by shared environmental rather than hereditary factors in twins.[51] The bronchodilator response to airway inflammation was studied in healthy twin pairs by measuring FEV1 before and after inhalation of 400 μg salbutamol. A heritability of 14.9% to 44% was found in twin studies.[38,52]

24.3.6 Obstructive sleep apnoea (OSA)

OSA is caused by the repetitive collapse of the upper airway during sleep and one of the major sources of excessive daily sleepiness and cognitive dysfunction. Hereditary factors explained the background of snoring.[53, 54] The heritability of OSA was studied using polysomnography. Heritability estimates for apnoea hypopnea index, respiratory disturbance index (RDI), and oxygen desaturation index ranged from 69% to 83%, while OSA was itself 73% heritable.[55] Genetic factors determinate the co-occurrence of OSA with hypertriglyceridaemia.[56]

24.3.7 In conclusion

Twin research has provided insight into the genetic and environmental factors and gene–environment interactions of cardiorespiratory diseases. Most studies confirmed the relevance of genetic factors and underline the role of screening high-risk individuals. Reducing cardiovascular risk factors is of paramount importance for individuals genetically susceptible to cardiovascular disease. Findings of twin studies may help guide personalized therapy for at-risk patients in the future, as well as prevention strategies, thereby reducing the incidence of chronic cardiovascular and lung diseases.

24.4 Gaining insight into the heritability of everyday cardiovascular function by twin studies

Gonneke Willemsen & Eco de Geus

24.4.1 Introduction

As outlined above, genetic variation plays a large role in the development of cardiovascular diseases. However, to fully understand the pathway from genes to disease, we also need to understand normal everyday functioning of the systems involved, not only in patients but also in the nonpatient population. For this purpose, it may not be sufficient to obtain momentary physiological measurements during a check-up, whether it be at a GP-office or during a large-scale population screening. Such momentary measurements are unlikely to capture all the individual variation in daily life, and the situation may also influence the measurement (think of the white coat effect on blood pressure). Studies have tried to mimic the response to daily challenges within the laboratory, measuring cardiovascular activity while exposing study participants to mental and/or physical challenges. Such laboratory stressors will often be of insufficient intensity and duration to trigger the full set of physiological responses that come into play when stress is "for real."[57] They will thus fail to reveal the slower counter-regulatory responses as well as allostatic adaptations that occur on a time scale of days or weeks. An example is the gradual build-up in resting blood pressure over the course of a stressful work week that subsides in the weekend.[58,59] Laboratory studies also preclude examination of the activities that may have the largest clinical relevance like job-related strain, marital conflict, child care or, at the other end of the spectrum, restful sleep. The solution to increase ecological validity of cardiovascular assessment has been to use ambulatory monitoring of cardiovascular signals in real-life settings, using the increasingly advanced technological solutions that enable this. Superior predictive validity for long term cardiovascular health has repeatedly been shown for ambulatory blood pressure, where full 24-h recordings proved better predictors for cardiovascular morbidity and mortality than laboratory or office measurements.[60–63]

24.4.2 Ambulatory studies of blood pressure and heart rate

Over the last three decades, several ambulatory studies have also been performed in twin families. These studies have focused mostly on the data obtained with an ambulatory blood pressure monitor with blood pressure as well as average heart rate (HR) obtained at intervals over a 24-h period.[64–71] The first twin study on 24-h blood pressure monitoring was published in 1994 and included only 28 MZ and 16 DZ male twin pairs. Participants wore a blood pressure monitor while freely moving around in a hospital, where they slept in the sleep laboratory. No exact heritability estimates were provided but based on the twin comparisons the authors concluded that genetic effects were present for the 24-h profile, in particular the daytime values, of DBP and HR.[64] While this study did not demonstrate genetic effects for SBP, two other small scale studies published in the 1990s,[65, 66] which allowed participants to engage in their normal day life, showed heritability for all three cardiovascular parameters, SBP, DBP, and HR. From 2003 onwards several larger twin studies on ambulatory blood pressure monitoring were conducted.[67–73] As can be seen in Table 24.1, the outcomes of these twin studies are, overall, very much in line with each other and show clear evidence for heritability of SBP (ranging from 32% to 71%) and DBP (ranging from 31% to 70%). Only one of the larger studies[67] included heritability estimates for HR, estimating this to be 70% in men and 51% in women, when including all participants.

Generally, the estimates for the different quantifications of the blood pressure or HR level (e.g., 24-h average, day-time average, night-time average) do not vary strongly across the larger studies. Differences between studies may be mostly due to choices in design and operationalization of the blood pressure measure. For instance, the lowest estimates for SBP (38% and 32%) were seen by Kupper et al.[68] for the morning and evening average, respectively, when excluding participants on antihypertensive medication (more on this later), while the outcomes for the 24-h average for SBP were closer together at 60%[67] and 70%.[69] Indeed, when Xu et al.[70] compared the heritability using data from two different twin registers (East Flanders Prospective Twin Survey and the Georgia Cardiovascular Twin Study) but with the same operationalization, they found that the estimates could be considered equal. Wang et al.[69] examined whether the same genes may influence daytime levels as night-time levels. The genetic correlation between daytime and nighttime levels was 0.77 for SBP and 0.66 for DBP indicating that common genes underlie the blood pressure levels during the day and night. However, additional unique genetic influences emerged for the nighttime levels. While most studies focused on the levels during the day and night, one study looked at particular aspect during the night, namely dipping.[71] In most individuals blood pressure decreases during the night, and not showing this drop may be related to an increased risk for mortality[74] and morbidity,[75] though being an extreme dipper at night may also be associated with an increased morbidity.[76] Wang et al[71] showed that this trait of having a nocturnal fall in blood pressure was highly heritable with an estimate of 59% for SBP and 81% for DBP.

So far, none of the studies conducting formal twin modeling of ambulatory measured blood pressure showed any evidence for the influence of common environmental factors. Although power may have been low to detect small effects of a shared twin environment, a fair conclusion is that the individual variation in blood pressure is predominantly

TABLE 24.1 Overview of the papers and heritability estimates referred to in the book chapter with regards to ambulatory blood pressure monitoring (ordered by publication date).

First author and year of publication	Sample	Age (years)	Cohort	Measurement	Variable	Heritability estimates[a]
Degaute et al. (1994)[64]	28 MZ & 16 DZ male pairs	Range: 16–36	Recruited via Belgian Twin Registers and university records	25-h measurement using Medilog blood pressure monitor	SBP	No evidence for genetic influences for any of the operationalizations
					DBP	24-hr: genetic effect
						Daytime: genetic effect
						Additional indicators: not clear
					HR	24-h: trend
						Daytime: trend
						Additional indicators: trend
Fagard et al. (1995)[65]	26 MZ & 27 DZ male pairs	Range: 18–38	East Flanders Prospective Twin Survey	Conventional measurement in the morning During the rest of the day and subsequent night measurements using Spacelab blood pressure monitor	SBP	Conventional: 64
						24-hr: 70
						Awake: 63
						Asleep: 72
						Awake crest: 61
						Awake 2pm-8pm: 4[d]
						Nighttime trough: 77
					DBP	Conventional: 73
						24-h: 73
						Awake: 55
						Asleep: 51
						Awake crest: 56
						Awake 2pm-8pm: 1[d]
						Nighttime trough: 57
					HR	24-h: 70
						Awake: 65
						Asleep: 52

(Continued)

TABLE 24.1 Cont'd

First author and year of publication	Sample	Age (years)	Cohort	Measurement	Variable	Heritability estimates[a]
Somes et al. (1995)[66]	38 MZ & 28 DZ pairs	Range: 15–17	The Medical College of Virginia Twin Study	24-h measurement using Accutracker blood pressure monitor	SBP DBP HR	24-h: 22 (incl all DZ pairs) 24-h: 38 (incl all DZ pairs) 24-h: 32 (incl all DZ pairs)
Vinck et al. (2001)[72]	150 MZ & 122 same sex DZ pairs	Range: 18–76 Analyses in all versus 3 age groups	East Flanders Prospective Twin Survey, registers from Leuven, Hasselt and Sint-Truiden	24-h measurement using Spacelab blood pressure monitor Conventional BP during separate visit	SBP DBP	Conventional: 62 (all ages) Daytime: 49 (all ages) Nighttime: 49 (all ages) Conventional 57 (all ages) Daytime 49 (all ages) Nighttime 50 (all ages)
Fagard et al. (2003)[67]	125 DZ, 97 MZ-DC & 128 MZ-MC	Range: 18–34	East Flanders Prospective Twin Survey	24-h measurement using Spacelab blood pressure monitor Conventional BP during separate visit	SBP DBP HR	Conventional 63 24-h average 60 Conventional 55 24-h average 62 Conventional 50 24-h average: 70 (men), 51 (women) Excluding MZ-MC: 66 (no gender difference)
Hottenga et al. (2005)[b,73]	165 MZ twins, 217 DZ twins, 184 singleton siblings	Range: 17–81	Netherlands Twin Register	Ambulatory measurements during the day using Spacelab blood pressure monitor	SBP DBP	Awake: 48 Awake: 44

First author and year of publication	Sample	Age (years)	Cohort	Measurement	Variable	Heritability estimates[a]
Kupper et al. (2005)[68]	230 MZ twins, 305 DZ twins and 257 singleton siblings	Mean = 31.3 (SD =11.2)	Netherlands Twin Register	Measurements during the day using Spacelab blood pressure monitor	SBP	Normotensive only/no antihypertensive medication/correction for antihypertensive medication Morning: 38/49/50 Afternoon: 50/57/57 Evening: 32/42/44
					DBP	Normotensive only/no antihypertensive medication/correction for antihypertensive medication Morning: 40/52/55 Afternoon: 55/61/63 Evening: 31/43/46
Wang et al. (2009)[71]	European Americans: 51 MZ and 54 DZ pairs, 30 singleton twins	Mean = 17.2 (SD = 3.4)	Georgia Cardiovascular Twin Study	24-h measurement using Spacelab blood pressure monitor	SBP	Day: 70 Night: 68 Day-night: 75 Night-specific: 30 Dipping: 59
	African-American: 36 MZ & 46 DZ pairs, 26 singleton twins				DBP	Day: 70 Night: 64 Day-night: 56 Night-specific: 43 Dipping: 81

(Continued)

TABLE 24.1 Cont'd

First author and year of publication	Sample	Age (years)	Cohort	Measurement	Variable	Heritability estimates[a]
Wang et al. (2011)[69]	European Americans: 104 pairs, 30 singleton twins African-American: 78 pairs, 30 singleton twins	Mean = 17.1 (SD = 3.4)	Georgia Cardiovascular Twin Study	24-h measurement using Spacelab blood pressure monitor Conventional Office BP[c]	SBP DBP	Office: 63 24-hr: 71 Office: 59 24-hr: 69
Xu et al. (2015)[70]	703 twins – 308 pairs and 87 singletons Meta-analysis including additional 242 white and 188 black twins	Range 18–34	East Flanders Prospective Twin Survey Meta-analysis including Georgia Cardiovascular Twin Study	24-h measurement using Spacelab blood pressure monitor Conventional BP (Office)	SBP DBP	Daytime: 60; meta: 63 Nighttime: 51; meta: 56 Nighttime-specific: 21; meta: 22 Daytime 54; meta: 59 Nighttime 46; meta: 53 Nighttime-specific 26; meta: 30

[a] *Model fitting results are presented when given.*
[b] *Heritability estimates for three separate laboratory studies were also included in the paper, but not presented here as they involved different participant groups.*
[c] *In addition, two 10-min stress tasks were performed in the laboratory. Data not presented as outside the scope of this chapter.*
[d] *Indicates occasions when common environment could not be excluded from the model.*
Note. Conventional or office BP is the average of several, generally three, sitting BP measurements conducted by or in the presence of the experimenter.

determined by genetic and unique environmental factors. This echoes similar findings for conventional blood pressure.[77] Several twin studies directly compared the heritability of ambulatory and conventional blood pressure measurements (e.g., the average of two or three measurements taken while sitting) obtained in the same individuals,[65,69,72] generally concluding that there were no differences in the extent of heritability of ambulatory and conventionally obtained blood pressure measurements. This was confirmed by Hottenga et al.[73] who compared blood pressure data from three laboratory twin studies and one ambulatory monitoring study. However, Wang et al.[69] suggested that there may be differences in the genes influencing conventional blood pressure measurements versus blood pressure measured over a prolonged period using ambulatory monitoring.

The outcomes of the twin studies on blood pressure heritability seem generalizable to other populations. Two studies[68,73] included in addition to twins also their singleton siblings. No differences in means, variances, and covariances emerged for blood pressure in twins and their singleton siblings, indicating that the results of twin studies may be generalized to nontwin populations. Also, studies exploring sex differences found no evidence for different heritability estimates for men and women in SBP or DBP.[67,69,71,73] Furthermore, Wang et al.[69,71] reported similar heritability estimates for European-American and African-American twins. The one study to examine age differences[72] pointed to a trend for a higher heritability in younger cohorts but this did not reach significance. Finally, Fagard et al.[67] examined whether the chorionicity of MZ twins (whether they were mono- or bichorionic) may influence the heritability of ambulatory blood pressure. Using data from the East Flanders Prospective Twin Study, the authors showed that chorionicity did not influence the heritability estimates for blood pressure. Only for HR gender differences emerged when monochorionic MZ twins were excluded.

Importantly, Kupper et al.[68] replicated previous findings from a family based design,[78] by showing that excluding participants on antihypertensive medication has substantial effects on heritability estimation. Heritability estimates were at its highest when, instead of excluding participants on antihypertensive medication, a correction was made for the average effects of the anti-hypertensive medication used. This correction should thus be applied in genetic investigations of ambulatory blood pressure as it provides the best reflection of the true population variance in blood pressure.

24.4.3 Ambulatory monitoring of other cardiovascular parameters

While the heritability of ambulatory monitored blood pressure and simultaneously measured HR has received much attention, very few heritability studies focused on the ambulatory monitoring of other parameters of cardiovascular function. These parameters are generally obtained by more continuous measurement of the heart function, extracting indicators of HR variability which may reflect parasympathetic or sympathetic influences on the heart. For an extensive explanation of these indicators and a general overview of the heritability of these indicators, the reader is referred to de Geus et al.[79]. The Netherlands Twin Register conducted several studies[80–82,84,85] on ambulatory cardiac parasympathetic nervous system activity using HR variability data obtained in twins and their singleton siblings with an ambulatory monitor of the electro- and impedance cardiogram (see box 24.1). As can be seen in Table 24.2, these studies demonstrated heritability for root mean square

Box 24.1 The monitor was developed for easy wear, allowing for normal everyday activities. To show the size of the monitor, it is here worn outside the clothing. The inset shows the monitor in more detail.

The Netherlands Twin Register has conducted a number of studies on daily life cardiovascular activity. The fast majority of these studies made use of the VU-AMS, which continuously monitors the electrocardiogram and impedance cardiogram. The monitor was developed at the Department of Biological Psychology, Vrije Universiteit to allow participants freedom of movement and enable the measurement of continuous heart action during several days to provide insight into the different factors influencing normal day cardiac action. Since this department also houses the Netherlands Twin Register (www.twinregister.org) this resulted in the largest twin study to date on ambulatory recorded indices of cardiac function. For an overview of the papers including the use of the VU-AMS, see www.vu-ams.nl/research.

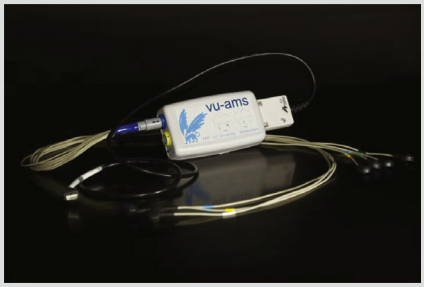


TABLE 24.2 Overview of the papers and heritability estimates with regards to ambulatory monitoring of heart action (as mentioned in the chapter and ordered by publication date).

First author and year of publication	Sample	Age	Cohort	Monitor	Variable	Heritability estimate (%)[a]
Kupper et al. (2004)[81]	218 MZ twins, 301 DZ twins and 253 singleton siblings	Men: mean = 1.3 (SD = 10.6) Women: mean = 30.8 (SD = 10.9)	Netherlands Twin Register	24-h ambulatory electro- and impedance cardiography using the VU-AMS	SDNN	Morning: 35 Afternoon: 36 Evening: 47 Night: 43
					RMSSD	Morning: 41 Afternoon: 48 Evening: 48 Night: 40
Kupper et al. (2005)[84]	222 MZ twins, 305 DZ twins and 253 singleton siblings	Mean = 31.0 (SD = 10.8)	Netherlands Twin Register	24-h ambulatory electro- and impedance cardiography using the VU-AMS	Repiration rate	Morning: 27 Afternoon: 44 Evening: 50 Night: 81
					lnRSA	Morning: 40 Afternoon: 49 Evening: 55 Night: 54
					Heart period	Morning: 37 Afternoon: 45 Evening: 40 Night: 48
Kupper et al. (2006)[80]	215 MZ twins, 296 DZ twins and 244 singleton siblings	Mean = 30.6 (SD = 10.4).	Netherlands Twin Register	24-h ambulatory electro- and impedance cardiography using the VU-AMS	PEP	Morning: 62 Afternoon: 62 Evening: 55 Night: 48
					PEP/LVET ratio	Morning: 58 Afternoon: 56 Evening: 48 Night: 35

(Continued)

TABLE 24.2 Cont'd

First author and year of publication	Sample	Age	Cohort	Monitor	Variable	Heritability estimate (%)[a]
Su et al. (2010)[83]	121 MZ twins and 77 DZ twins	MZ twins: mean = 54.3 (SD = 2.9) DZ twins; mean = 54.9 (SD = 2.7)	The Twins Heart Study, within the Vietnam Era Twin Registry	24-h ECG (Holter) monitor	lnTPow	Univariate: 63 Bivariate with BDI - unadjusted: 64 Bivariate with BDI - adjusted for covariates: 62
					lnULF	Univariate:59 Bivariate with BDI - unadjusted: 61 Bivariate with BDI - adjusted for covariates: 60
					lnVLF	Univariate: 57
					lnLF	Univariate: 43
					lnHF	Univariate: 56
Neijts et al. (2014)[82]	486 MZ twins, 517 DZ twins and 285 nontwin siblings	Mean = 33.5 (SD = 9.2)	Netherlands Twin Register	24-h ambulatory electro- and impedance cardiography using the VU-AMS	pvRSA	Including/excluding participants with a ceiling effect Sleep: 48/53 Sitting: 53/57 Active: 57/57 Max during night: 53 Max during night corrected for ceiling: 55 Daytime RSA-IBI slope: 35
					RMSSD	Including/excluding participants with a ceiling effect Sleep: 53/53 Sitting: 54/52 Active: 49/46
					SDNN	Including/excluding participants with a ceiling effect Sleep: 46/47 Sitting: 48/46 Active: 53/54
					IBImax	52% (corrected for ceiling effects)

First author and year of publication	Sample	Age	Cohort	Monitor	Variable	Heritability estimate (%)[a]
Neijts et al. (2015)[85]	486 MZ twins, 517 DZ twins and 285 nontwin siblings	Mean age 28.5 yrs (SD = 9.6) to 37.2 yrs (SD = 5.4) for the three different waves of data collection	Netherlands Twin Register	24-hr ambulatory electro- and impedance cardiography using the VU-AMS	IBI[a]	Sleep: 52 Leisure: 51 Wake: 52 Work (sitting): 69 Work peak level: 60 5 of 6 reactivity scores were significant: 29–40
					pvRSA[a]	Sleep: 46 Leisure: 43 Wake: 55 Work (sitting): 53 Work peak level: 33 4 of 6 reactivity scores were significant: 34 to 47
					PEP[a]	Sleep average: 38 Leisure average: 25 Wake average: 41 Work (sitting): 44 Work peak level: 39 3 of 6 reactivity scores were significant: 10–19

[a] *Outcomes of different bivariates models differed slightly for the mean level. In case of differences, the lowest value was taken.*

MZ, monozygotic; DZ, dizygotic; SD, standard deviation; SDNN, standard deviations of all normal-to-normal intervals; RMSSD, root mean square of successive differences between adjacent normal-to-normal intervals; ln, logarithm transformation; pvRSA, peak valley respiratory sinus arrhythmia; PEP, pre-ejection period; LVET, left ventricular ejection time; TP, total power; VLF, very low frequency; LF, low frequency; IBI, interbeat interval.

of successive differences between adjacent normal-to-normal intervals (RMSSD, 40%–54%), and for respiratory sinus arrhythmia (RSA, 33%–57%). The NTR also examined ambulatory pre-ejection period (PEP), an indicator of cardiac sympathetic nervous system activity.[80] The heritability for PEP ranged somewhat more than for the parasympathetic indicators, from 25% to 62%, depending on the sample and operationalization. When PEP was corrected for left ventricular ejection time heritability estimates ranged from 35% at night to 58% in the morning. A further indicator of autonomic activity, the standard deviations of all normal-to-normal intervals (SDNN) were also found to be heritable in these studies, with a narrow range from 35% to 48%.[81,82] Additional indicators of HR variability, obtained by spectral analysis were studied by Vaccarino et al.[83] who showed all to be highly heritable. Interestingly, they also found that common genes underlay the association of two indicators total power and ultra-low frequency with the score on the Beck Depression Inventory.

The NTR studies further showed that common genes influenced the parameters across the different periods of the day though at night new specific genes may also emerge.[80,81] Common genes also explain a large part of the association between closely related variables such as RSA with respiration rate and heart period[84] and SDNN, RSA, and RMSSD.[82] To gain more insight into the response to challenges during the day, Neijts et al.[85] expanded upon the findings by Kupper et al.[80] by operationalizing several definitions of reactivity (e.g., work levels while sitting versus average sleep or leisure levels). Significant heritability was seen for HR and parasympathetic reactivity (here indexed by RSA) and to a lesser extent for sympathetic reactivity (indexed by PEP). Further analyses showed that the response to the challenging periods of the day compared to resting levels was due to the emergence of additional genes influencing the response.

24.4.4 In conclusion

Overall, twin studies of ambulatory monitored cardiovascular activity show the importance of genetic factors for cardiovascular activity during the day and night. In addition, by studying cardiovascular function for prolonged periods of time valuable insights can be obtained in specific phenomena relevant to cardiovascular morbidity and mortality. Further studies are expected to provide more insight into the interplay of genetic and environmental factors on daily cardiovascular function and cardiovascular risk.

25.5 Imaging of twins

Adam D. Tarnoki, David L. Tarnoki

Imaging has developed rapidly in recent decades, with the emergence of new techniques. X-ray, mammography, and CT involve ionizing radiation. Therefore, most of these twin studies are mainly retrospective. Ultrasound propagates by sound waves, while magnetic resonance imaging (MRI) operates through a magnetic field.

Given their lack of harmful effect, these imaging modalities are frequently applied in twin studies. Here we briefly describe the techniques and present some examples to illustrate the enormous potential of imaging twin studies for gaining insight into disease processes.

25.5.1 X-ray

The use of X-ray has become the gold standard, among others, to analyze bone structure and abnormalities as well as certain thoracic abnormalities. There are some case studies with radiographs on twins, including a case study of a Scottish identical twin pair with recurrent right elbow dislocation[86] and a case of conjoined twins.[87]

Taking into account the effects of ionizing radiation, most twin studies analyze X-ray image data that were obtained in hospitals as part of the screening of potentially affected twins. A UK twin study examined the heritability of osteoarthritis of the hip joint which was between 58% and 64%.[88] Genetic determinants of hip joint morphometry and their relationship to hip cartilage thickness were also studied, and genetic factors accounted for most of the variation in minimal joint space and acetabular anatomy.[89]

Additional radiography studies analyzed the spine in the development of idiopathic scoliosis. Twin studies have shown that MZ twins are more often concordant for idiopathic adult scoliosis than DZ twin pairs. Phenotypic differences between MZ twins may also be the result of epigenetic differences. Genetic factors contributing to the spine curvature were also raised, as well as the severity of the curvature of scoliosis.[90, 91]

A population-based Korean twin study examined the origin of a common foot deformity, hallux valgus, in twins and their families with X-Ray. Heritability was estimated at 51% for hallux valgus and 47% for hallux valgus angle, and it was suggested that genetic vulnerability may be reinforced by lifestyle factors, such as shoe wearing habits or preference.[92]

Dual-energy X-ray absorptiometry (DEXA) was used for measuring bone mineral density and body composition in twins which indicated that 20% of adult hip axis length is associated with environmental factors. Accordingly, any environmental effects of physical activity or nutrition on hip geometry must occur before early teenage years.[93] Bone mineral density was strongly heritable in twins, especially in females at all locations using both DEXA and quantitative bone ultrasound (QUS), which may explain the importance of family history as a risk factor for bone fractures. Unshared environmental effects accounted for the rest of the variance with slight differences in magnitude across various bone regions, supporting the role of lifestyle in preventing osteoporotic fractures with various efficacy in different bone regions.[94]

25.5.2 Breast mammography

Compared to the traditional X-ray, breast mammography is performed with different physical parameters and photographic techniques. It is therefore suitable for detecting

subtle structural differences in the soft parts of the breast. For women of equivalent age, those whose breasts display greater white or bright areas on a mammogram—i.e. greater mammographic density—are at 1.8–6 times greater risk of developing breast cancer. Twin studies have reported that, under the assumptions of the classic twin model and after adjusting for age, BMI, and other determinants, the patterns of twin correlations for mammographic density measures are consistent with additive genetic factors explaining ~60% of their residual variances.[95,96] An Australian twin study also revealed that at least two common breast cancer susceptibility genetic variants were associated with mammographic density measures that predict breast cancer. These findings could help elucidate how those variants and mammographic density measures are associated with breast cancer susceptibility.[97] The heritability of the extent of dense and nondense areas within the dense breasts was also examined, and a negative genetic correlation was found between these two parameters. This may mean that the same genetic factors affect both parameters, but in different ways.[96] In a Korean twin study, the same high heritability of mammographic density was found as in Western women indicating that environmental factors are responsible for the differences in the risk of breast cancer across populations. An inverse additive genetic correlation was reported between dense and nondense mammographic area predicting that genes positively associated with dense area may have the opposite effect on nondense area.[98]

25.5.3 Ultrasound

Ultrasound is one of the most commonly involved imaging modality in twin research. The first twin studies using ultrasound began in the mid-1990s and have examined a wide variety of disorders. To name a few, in 1995, a study of twin pairs with polycystic ovary syndrome (PCOS, a disease where numerous small cysts are seen in the ovaries together with an abnormal amount of androgen production) showed that 5 of 19 pairs of MZ twins were discordant for PCOS. Accordingly, the authors concluded that PCOS may be a polygenic condition, an X-linked disorder, the result of an intrauterine or a postnatal event, or the result of an interaction between genetic and environmental factors.[99] In a Finnish twin study, transvaginal ultrasound was applied, and heritability of the number of uterine fibriods (myomas, benign tumors) was found to be 26%. The incidence of myomas was associated with a higher BMI, which is known to be a highly hereditary trait[100] (see Cancer and Twin Research chapter). A Hungarian twin study analyzed the background of the common liver lesion of nonalcoholic fatty liver disease and found this to have no genetic background, that is, common (74.2%) and individual (25.8%) environmental factors accounted for the variance of the disease.[101] Thyroid gland is easily examinable with ultrasound, therefore, various twin studies analyzed this endocrin organ. Based on a Danish twin study, genetic factors accounted for 71% of the individual differences in thyroid volume. This fits the observation that not all individuals develop goiter even in iodine-deficient areas.[102]

25.5.4 Computed tomography (CT)

CT is a diagnostic imaging procedure that involves rotating X-ray beams around the body to build cross-sectional images. In most cases, intravenous contrast material is administered in order to observe enhancement of certain organs or lesions for better characterization. Given the invasive nature and the ionizing radiation exposure, most twin studies are retrospective or case studies in this field or in the case of prospective study conducted with low or ultralow radiation.

A CT study on twin pairs found no significant differences between MZ and DZ twins in the development of paranasal sinuses which was mainly influenced by environmental factors, while the development of one common normal anatomical variant, the concha bullosa was partly genetically influenced.[103]

Since CT is the best choice for imaging the bone structure, several twin studies analyzed heritability of bone structures. To understand the genetic background of the microarchitecture of the distal tibia and distal radius and remodeling markers, female twin pairs aged 40 to 61 years underwent high-resolution peripheral quantitative CT. A substantial genetic component has been found, which indicates that middle-aged women differ in their bone microarchitecture and remodeling markers more because of differences in their genetic factors than differences in their environment.[104] The same group reported that a larger within twin pair difference in cortical porosity of the distal tibia was associated with a larger within twin pair differences in height. Accordingly, taller women assemble wider bones with relatively thinner and more porous cortices predisposing to fracture.[105]

25.5.5 Magnetic resonance imaging (MRI)

MRI is a noninvasive imaging technology that produces three-dimensional detailed anatomical images based on the detection of change in the direction of the rotational axis of hydrogene protons. Based on its noninvasive nature and lack of ionizing radiation, MRI is a popular multiparametric imaging modality in prospective study of twins.

Obesity is a common trait in the field of MRI twin research. Finnish twin researchers have shown by MR examination of long-term discordant twin pairs in terms of physical activity that regular physical activity is an important factor in preventing the deposition of high-risk adipose tissue, even though genetic determinants and childhood environmental factors play a role.[106,107] Australian researchers used MR to show a link between low birth weight and abdominal visceral and subcutaneous adipose tissue volume, which means that low birth weight is a high risk for abdominal obesity.[108] This is consistent with the results of epigenetic studies, as it reflects abnormal programming during pregnancy. An interesting example of high-risk adipose tissue accumulation was presented in 16 middle-aged (50–74 years) same-sex twin pairs with long-term discordance in physical activity habits. The inactive twins had 50% more visceral adipose tissue, 54% higher intramuscular adipose tissue, and 170% higher liver fat score compared to the physically active twins.[106]

The same Finnish research group also found a link between pancreatic fat content, insulin resistance and liver fat content.[109]

25.5.6 Neuroimaging

Neuroimaging is the other field where numerous twin studies have been conducted. To name a few, structural MRI data from the Human Connectome Project was used to investigate body mass index (BMI) associated differences in gray matter volume (GMV) within MZ twin pairs discordant for BMI. Heavier MZ twin siblings demonstrated less GMV within certain brain cortical areas. These results indicated that nongenetic influences and the mere presence of a higher BMI constitute relevant factors in the context of body weight-related structural brain alterations.[110]

A quantitative neuroimaging study in twins between 13 and 24 years of age using diffusion tensor imaging confirmed that genetic factors play a key role in the development of white matter microstructure.[111] MRI-visible dilated perivascular spaces (dPVS) in brain are common findings even in healthy young persons. A study on healthy young adult twins and nontwin siblings confirmed that dPVS volumes within basal ganglia and white matter were highly determined by genetic factors, especially in white matter.[112]

25.6 Future directions: radiogenomics and imaging epigenetics

The radiological and pathological sciences have developed closely together in recent decades, leading to the emergence of radiogenomics (or imaging genomics), a new branch of research that examines the relationships between radiological and histological features. Radiological tumor phenotypes can be used to provide noninvasive information on gene expression patterns, tumor subtypes, and even molecular biology data.[113] Radiogenomics has not yet been applied to discordant twins. However, valuable studies could be performed involving twins, investigating not only the radiomorphology but also the underlying epigenetic or environmental factors in the affected sibling compared to the healthy twin.[114]

The combination of epigenetics with imaging can answer questions whether various imaging phenotypes can predict epigenetic modification, that are related to organ (such as brain) structure, function, and metabolism, which impact disease risk and progression. The integration of genetic imaging methods with epigenetic markers in humans appears promising, especially in neuroimaging.[115] Imaging epigenetics will provide deeper insight into the causative pathogenetic and pathophysiological pathways through which genes and environment interrelate during life and impact physiology, pathophysiology, aging, and disease, especially in MZ twins discordant for a chronic disease.[114] This knowledge may open doors for the development of novel biomarkers and preventive and disease-modifying treatments.

References

1. World Health Organization (WHO): The top 10 causes of death. available at: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. (Accessed December 10, 2021).
2. Forouzanfar MH, Liu P, Roth GA, et al. Global burden of hypertension and systolic blood pressure of at least 110 to 115 mm Hg, 1990-2015. *JAMA*. 2017;317:165–182.
3. Wang B, Liao C, Zhou B, et al. Genetic contribution to the variance of blood pressure and heart rate: a systematic review and meta-regression of twin studies. *Twin Res Hum Genet*. 2015;18:158–170.
4. Marques FZ. Missing heritability of hypertension and our microbiome. *Circulation*. 2018;138:1381–1383.
5. Safar ME. Pulse pressure, arterial stiffness and wave reflections (augmentation index) as cardiovascular risk factors in hypertension. *Ther Adv Cardiovasc Dis*. 2008;2:13–24.
6. Tarnoki AD, Tarnoki DL, Stazi MA, et al. Heritability of central blood pressure and arterial stiffness: a twin study. *J Hypertens*. 2012;30:1564–1571.
7. Huang Y, Su S, Snieder H, Treiber F, Kapuku G, Wang X. Decreased heritability and emergence of novel genetic effects on pulse wave velocity from youth to young adulthood. *Sci Rep*. 2021;11:8911.
8. Ye C, Pan Y, Xu X, et al. Pulse wave velocity in elastic and muscular arteries: tracking stability and association with anthropometric and hemodynamic measurements. *Hypertens Res*. 2016;39:786–791.
9. Ge D, Young TW, Wang X, Kapuku GK, Treiber FA, Snieder H. Heritability of arterial stiffness in black and white american youth and young adults. *Am J Hypertens*. 2007;20:1065–1072.
10. Snieder H, Hayward CS, Perks U, Kelly RP, Kelly PJ, Spector TD. Heritability of central systolic pressure augmentation: a twin study. *Hypertension*. 2000;35:574–579.
11. Tarnoki AD, Tarnoki DL, Bogl LH, et al. Association of body mass index with arterial stiffness and blood pressure components: a twin study. *Atherosclerosis*. 2013;229:388–395.
12. Wu Y, Zhang D, Pang Z, et al. Multivariate modeling of body mass index, pulse pressure, systolic and diastolic blood pressure in chinese twins. *Twin Res Hum Genet*. 2015;18:73–78.
13. Wu T, Snieder H, Li L, et al. Genetic and environmental influences on blood pressure and body mass index in han chinese: a twin study. *Hypertens Res*. 2011;34:173–179.
14. Wang B, Wu T, Neale MC, et al. Genetic and environmental influences on blood pressure and body mass index in the national academy of sciences-national research council world war ii veteran twin registry. *Hypertension*. 2020;76:1428–1434.
15. Zhao J, Cheema FA, Bremner JD, et al. Heritability of carotid intima-media thickness: a twin study. *Atherosclerosis*. 2008;197:814–820.
16. Lee K, Sung J, Lee SC, et al. Segment-specific carotid intima-media thickness and cardiovascular risk factors in koreans: the healthy twin study. *Eur J Cardiovasc Prev Rehabil. Eur J Prev Cardiol.* 2012;19:1161–1172.
17. Tarnoki AD, Baracchini C, Tarnoki DL, et al. Evidence for a strong genetic influence on carotid plaque characteristics: an international twin study. *Stroke*. 2012;43:3168–3172.
18. Lucatelli P, Fagnani C, Tarnoki AD, et al. Genetic influence on femoral plaque and its relationship with carotid plaque: an international twin study. *Int J Cardiovasc Imaging*. 2018;34:531–541.

19. Franceschini N, Giambartolomei C, de Vries PS, et al. GWAS and colocalization analyses implicate carotid intima-media thickness and carotid plaque loci in cardiovascular outcomes. *Nat Commun*. 2018;9:5141.
20. Lucatelli P, Tarnoki AD, Tarnoki DL, et al. Genetic and environmental effects on carotid flow velocities: an international twin study. *Atherosclerosis*. 2013;231:205–210.
21. Fagnani C, Meneghetti G, Baracchini C, Tarnoki AD, Tarnoki DL, Schillaci G. Genetic and environmental components of carotid artery elasticity: an italian twin study. *Eur J Intern Med*. 2013;24:e53–e54.
22. Song C, Chang Z, Magnusson PK, Ingelsson E, Pedersen NL. Genetic factors may play a prominent role in the development of coronary heart disease dependent on important environmental factors. *J Intern Med*. 2014;275:631–639.
23. Zdravkovic S, Wienke A, Pedersen NL, de Faire U. Genetic influences on angina pectoris and its impact on coronary heart disease. *Eur J Hum Genet*. 2007;15:872–877.
24. Hernyes A, Piroska M, Fejer B, et al. Overlapping genetic background of coronary artery and carotid/femoral atherosclerotic calcification. *Medicina (Kaunas)*. 2021;57:252.
25. Kern A, Bojko K, Sienkiewicz E, Zarzecki A, Bil J. Non-st-elevation acute coronary syndrome due to a totally occluded coronary artery: a history of two twin brothers. *Wiad Lek*. 2020;73:201–202.
26. Murray SW, Cooper RM, Appleby C, et al. Double jeopardy: multi-modality imaging of mz "twin cap" atherosclerosis. *Atherosclerosis.* 2014;237:264–267.
27. Bielak LF, Peyser PA. Genetics of subclinical coronary atherosclerosis. *Curr Genet Med Rep*. 2018;6:116–123.
28. Cecelja M, Jiang B, Bevan L, Frost ML, Spector TD, Chowienczyk PJ. Arterial stiffening relates to arterial calcification but not to noncalcified atheroma in women. a twin study. *J Am Coll Cardiol*. 2011;57:1480–1486.
29. Joergensen TM, Christensen K, Lindholt JS, Larsen LA, Green A, Houlind K. Editor's choice—high heritability of liability to abdominal aortic aneurysms: a population based twin study. *Eur J Vasc Endovasc Surg*. 2016;52:41–46.
30. Fowkes FGR, Rudan D, Rudan I, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet*. 2013;382(9901):1329–1340.
31. Wahlgren CM, Magnusson PK. Genetic influences on peripheral arterial disease in a twin population. *Arterioscler Thromb Vasc Biol*. 2011;31:678–682.
32. Fejer B, Tarnoki AD, Tarnoki DL, et al. Heritability of the femoral intima media thickness. *Eur J Intern Med*. 2017;41:44–48.
33. Belkin N, Damrauer SM. Peripheral arterial disease genetics: progress to date and challenges ahead. *Curr Cardiol Rep*. 2017;19:131.
34. Ingebrigtsen TS, Thomsen SF, van der Sluis S, et al. Genetic influences on pulmonary function: a large sample twin study. *Lung*. 2011;189 323e30.
35. Hubert HB, Fabsitz RR, Feinleib M, Gwinn C. Genetic and environmental influences on pulmonary function in adult twins. *Am Rev Respir Dis*. 1982;125 409e15.
36. McClearn GE, Svartengren M, Pedersen NL, Heller DA, Plomin R. Genetic and environmental influences on pulmonary function in aging swedish twins. *J Gerontol*. 1994;49 264e8.
37. Hukkinen M, Kaprio J, Broms U, et al. Heritability of lung function: a twin study among neversmoking elderly women. *Twin Res Hum Genet*. 2011;14 401e7.
38. Wu T, Boezen HM, Postma DS, et al. Genetic and environmental influences on objective intermediate asthma phenotypes in dutch twins. *Eur Respir J*. 2010;36:261–268.

39. Kim WJ, Lee MK, Shin C, et al. Genome-wide association studies identify locus on 6p21 influencing lung function in the korean population. *respirol*. 2014;19:360–368.
40. Ingebrigtsen T, Thomsen SF, Vestbo J, et al. Genetic influences on chronic obstructive pulmonary disease - a twin study. *Respir Med*. 2010;104:1890–1895.
41. Tarnoki DL, Tarnoki AD, Lazar Z, et al. A possible genetic influence in parenchyma and small airway changes in COPD: a pilot study of twins using hrct. *Acta Physiol Hung*. 2014;101:167–175.
42. Hjelmborg J, Korhonen T, Holst K, et al. Lung cancer, genetic predisposition and smoking: the nordic twin study of cancer. *Thorax*. 2017;72:1021–1027.
43. Hallberg J, Dominicus A, Eriksson UK, et al. Interaction between smoking and genetic factors in the development of chronic bronchitis. *Am J Respir Crit Care Med*. 2008;177:486–490.
44. Meteran H, Thomsen SF, Harmsen L, KO K, Skytthe A, Backer V. Risk of chronic bronchitis in twin pairs discordant for smoking. *Lung*. 2012;190:557–561.
45. Willemsen G, van Beijsterveldt TC, van Baal CG, Postma D, Boomsma DI. Heritability of self-reported asthma and allergy: a study in adult dutch twins, siblings and parents. *Twin Res Hum Genet*. 2008;11:132–142.
46. Ullemar V, Magnusson PK, Lundholm C, et al. Heritability and confirmation of genetic association studies for childhood asthma in twins. Allergy. 2016;71:230–238.
47. Hedman AM, Kuja-Halkola R, Örtqvist AK, van Hage M, Almqvist C, Nordlund B. Genetic effects of allergen-specific ige levels on exhaled nitric oxide in schoolchildren with asthma: the stoppa twin study. *Pediatr Allergy Immunol*. 2021;32:709–719.
48. Slob EMA, Brew BK, Vijverberg SJH, et al. Early-life antibiotic use and risk of asthma and eczema: results of a discordant twin study. *Eur Respir J*. 2020;55:1902021.
49. Murphy TM, Wong CC, Arseneault L, et al. Methylomic markers of persistent childhood asthma: a longitudinal study of asthma-discordant monozygotic twins. *Clin Epigenetics*. 2015;7:130.
50. Gabrielsen ME, Romundstad P, Langhammer A, Krokan HE, Skorpen F. Association between a 15q25 gene variant, nicotine-related habits, lung cancer and copd among 56,307 individuals from the hunt study in norway. *Eur J Hum Genet*. 2013;21:1293–1299.
51. Tarnoki DL, Bikov A, Tarnoki AD, et al. Lack of heritability of exhaled volatile compound pattern: an electronic nose twin study. *J Breath Res*. 2014;8:016001.
52. Tarnoki DL, Medda E, Tarnoki AD, et al. Modest genetic influence on bronchodilator response: a study in healthy twins. *Croat Med J*. 2015;56:152–158.
53. Ferini-Strambi L, Calori G, Oldani A, et al. Snoring in twins. *Respir Med*. 1995;89:337–340.
54. Desai AV, Cherkas LF, Spector TD, Williams AJ. Genetic influences in self-reported symptoms of obstructive sleep apnoea and restless legs: a twin study. *Twin Res*. 2004;7:589–595.
55. Szily M, Tarnoki AD, Tarnoki DL, et al. Genetic influences on the onset of obstructive sleep apnoea and daytime sleepiness: a twin study. *Respir Res*. 2019;20:125.
56. Meszaros M, Tarnoki AD, Tarnoki DL, et al. Obstructive sleep apnea and hypertriglyceridaemia share common genetic background: results of a twin study. *J Sleep Res*. 2020;29(4):e12979.
57. Busscher B, Spinhoven P, de Geus EJ. Psychological distress and physiological reactivity during in vivo exposure in people with aviophobia. *Psychosom Med*. 2015;77:762–774.
58. Vrijkotte TG, van Doornen LJ, de Geus EJ. Effects of work stress on ambulatory blood pressure, heart rate, and heart rate variability. *Hypertension*. 2000;35:880–886.

59. Vrijkotte TG, van Doornen LJ, de Geus EJ. Overcommitment to work is associated with changes in cardiac sympathetic regulation. *Psychosom Med*. 2004;66:656–663.
60. Hansen TW, Jeppesen J, Rasmussen S, Ibsen H, Torp-Pedersen C. Ambulatory blood pressure monitoring and risk of cardiovascular disease: a population based study. *Am J Hypertens*. 2006;19:243–250.
61. Mallion JM, Baguet JP, Siche JP, Tremel F, de Gaudemaris R. Clinical value of ambulatory blood pressure monitoring. *J Hypertens*. 1999;17:585–595.
62. Verdecchia P. Using out of office blood pressure monitoring in the management of hypertension. *Curr Hypertens Rep*. 2001;3:400–405.
63. Ward AM, Takahashi O, Stevens R, Heneghan C. Home measurement of blood pressure and cardiovascular disease: systematic review and meta-analysis of prospective studies. *J Hypertens*. 2012;30:449–456.
64. Degaute JP, Vancauter E, Vandeborne P, Linkowski P. 24-Hour blood-pressure and heart-rate profiles in humans—a twin study. *Hypertension*. 1994;23:244–253.
65. Fagard R, Brguljan J, Staessen J, et al. Heritability of conventional and ambulatory blood pressures—a study in twins. *Hypertension*. 1995;26:919–924.
66. Somes GW, Harshfield GA, Alpert BS, Goble MM, Schieken RM. Genetic influences on ambulatory blood-pressure patterns - the medical-college-of-virginia twin study. *Am J Hypertens*. 1995;8:474–478.
67. Fagard RH, Loos RJF, Beunen G, Derom C, Vlietinck R. Influence of chorionicity on the heritability estimates of blood pressure: a study in twins. *J Hypertens*. 2003;21:1313–1318.
68. Kupper N, Willemsen G, Riese H, Posthuma D, Boomsma DI, de Geus EJ. Heritability of daytime ambulatory blood pressure in an extended twin design. *Hypertension*. 2005;45:80–85.
69. Wang XL, Ding XH, Su SY, Harshfield G, Treiber F, Snieder H. Genetic influence on blood pressure measured in the office, under laboratory stress and during real life. *Hypertens Res*. 2011;34:239–244.
70. Xu XJ, Su SY, Treiber FA, et al. Specific genetic influences on nighttime blood pressure. *Am J Hypertens*. 2015;28:440–443.
71. Wang XL, Ding XH, Su SY, et al. Genetic influences on daytime and night-time blood pressure: similarities and differences. *J Hypertens*. 2009;27:2358–2364.
72. Vinck WJ, Fagard RH, Loos R, Vlietinck R. The impact of genetic and environmental influences on blood pressure variance across age-groups. *J Hypertens*. 2001;19:1007–1013.
73. Hottenga JJ, Boomsma DI, Kupper N, et al. Heritability and stability of resting blood pressure. *Twin Res Human Genetics*. 2005;8:499–508.
74. Ohkubo T, Imai Y, Tsuji I, et al. Relation between nocturnal decline in blood pressure and mortality. the ohasama study. *Am J Hypertens*. 1997;10:1201–1207.
75. Hoshide S, Kario K, Hoshide Y, et al. Associations between nondipping of nocturnal blood pressure decrease and cardiovascular target organ damage in strictly selected community-dwelling normotensives. *Am J Hypertens*. 2003;16:434–438.
76. Kario K, Matsuo T, Kobayashi H, Imiya M, Matsuo M, Shimada K. Nocturnal fall of blood pressure and silent cerebrovascular damage in elderly hypertensive patients. advanced silent cerebrovascular damage in extreme dippers. *Hypertension*. 1996;27:130–135.
77. Evans A, van Baal GC, McCarron P, et al. The genetics of coronary heart disease: the contribution of twin studies. *Twin Res Human Genetics*. 2003;6:432–441.
78. Cui JSS, Hopper JL, Harrap SB. Antihypertensive treatments obscure familial contributions to blood pressure variation. *Hypertension*. 2003;41:207–210.

79. de Geus EJC, van Lien R, Neijts M, Willemsen G. Genetics of autonomic nervous system activity. In: Canli T, ed. *The Oxford Handbook of Molecular Psychology*. Oxford: Oxford University Press; 2015:357–390.
80. Kupper N, Willemsen G, Boomsma DI, de Geus EJ. Heritability of indices for cardiac contractility in ambulatory recordings. *J Cardiovasc Electrophysiol*. 2006;17:877–883.
81. Kupper NH, Willemsen G, van den Berg M, et al. Heritability of ambulatory heart rate variability. *Circulation*. 2004;110:2792–2796.
82. Neijts M, Van Lien R, Kupper N, Boomsma D, Willemsen G, de Geus EJ. Heritability of cardiac vagal control in 24-h heart rate variability recordings: influence of ceiling effects at low heart rates. *Psychophysiology*. 2014;51:1023–1036.
83. Kupper N, Willemsen G, Posthuma D, de Boer D, Boomsma DI, de Geus EJ. A genetic analysis of ambulatory cardiorespiratory coupling. *Psychophysiology*. 2005;42:202–212.
84. Neijts M, van Lien R, Kupper N, Boomsma D, Willemsen G, de Geus EJ. Heritability and temporal stability of ambulatory autonomic stress reactivity in unstructured 24-Hour recordings. *Psychosom Med*. 2015;77:870–881.
85. Su S, Lampert R, Lee F, et al. Common genes contribute to depressive symptoms and heart rate variability: the twins heart study. *Twin Res Hum Genet*. 2010;13:1–9.
86. Fazzi UG, Rymaszewski LA. Recurrent dislocation of the elbow in identical twins. *J Shoulder Elbow Surg*. 1996;5:401–403.
87. Muhammad S, Laraswati B, Violetta L. Radiology assessment of omphalopagus conjoined twins: a case report. *Radiol Case Rep*. 2022;17:1169–1174.
88. MacGregor AJ, Antoniades L, Matson M, Andrew T, Spector TD. The genetic contribution to radiographic hip osteoarthritis in women: results of a classic twin study. *Arthritis Rheum*. 2000;43:2410–2416.
89. Antoniades L, Spector TD, Macgregor AJ. The genetic contribution to hip joint morphometry and relationship to hip cartilage thickness. *Osteoarthritis Cartilage*. 2001;9:593–595.
90. Hermus JPS, van Rhijn LW, Ooij AV. Non-genetic expression of adolescent idiopathic scoliosis: a case report and review of the literature. *Eur Spine J*. 2007;16(Suppl 3):338–341.
91. Ponseti IV, Friedman B. Prognosis in idiopathic scoliosis. *J Bone Joint Surg Am*. 1950;32A:381–395.
92. Lee CH, Lee S, Kang H, et al. Genetic influences on hallux valgus in koreans: the healthy twin study. *Twin Res Hum Genet*. 2014;17:121–126.
93. Goulding A, Gold E, Cannan R, Williams S, Lewis-Barned NJ. Changing femoral geometry in growing girls: a cross-sectional dexa study. *Bone*. 1996;19:645–649.
94. Piroska M, Tarnoki DL, Szabo H, et al. Strong genetic effects on bone mineral density in multiple locations with two different techniques: results from a cross-sectional twin study. *Medicina (Kaunas)*. 2021;57:248.
95. Boyd NF, Dite GS, Stone J, et al. Heritability of mammographic density, a risk factor for breast cancer. *N Engl J Med*. 2002;347:886–894.
96. Stone J, Dite GS, Gunasekara A, et al. The heritability of mammographically dense and nondense breast tissue. *Cancer Epidemiol Biomarkers Prev*. 2006;15:612–617.
97. Odefrey F, Stone J, Gurrin LC, et al. Common genetic variants associated with breast cancer and mammographic density measures that predict disease. *Cancer Res*. 2010;70:1449–1458.
98. Sung J, Song YM, Stone J, Lee K, Jeong JI, Kim SS. Genetic influences on mammographic density in korean twin and family: the healthy twin study. *Breast Cancer Res Treat*. 2010;124:467–474.

99. Jahanfar S, Eden JA, Warren P, Seppälä M, Nguyen TV. A twin study of polycystic ovary syndrome. *Fertil Steril*. 1995;63:478–486.
100. Luoto R, Kaprio J, Rutanen EM, Taipale P, Perola M, Koskenvuo M. Heritability and risk factors of uterine fibroids—the finnish twin cohort study. *Maturitas*. 2000;37:15–26.
101. Tarnoki AD, Tarnoki DL, Bata P, et al. Heritability of non-alcoholic fatty liver disease and association with abnormal vascular parameters: a twin study. *Liver Int*. 2012;32:1287–1293.
102. Hansen PS, Brix TH, Bennedbaek FN, Bonnema SJ, Kyvik KO, Hegedüs L. Genetic and environmental causes of individual differences in thyroid size: a study of healthy danish twins. *J Clin Endocrinol Metab*. 2004;89:2071–2077.
103. Chaiyasate S, Baron I, Clement P. Analysis of paranasal sinus development and anatomical variations: a ct genetic study in twins. *Clin Otolaryngol*. 2007;32:93–97.
104. Bjørnerem Å, Bui M, Wang X, et al. Genetic and environmental variances of bone microarchitecture and bone remodeling markers: a twin study. *J Bone Miner Res*. 2015;30:519–527.
105. Bjørnerem Å, Bui QM, Ghasem-Zadeh A, Hopper JL, Zebaze R, Seeman E. Fracture risk and height: an association partly accounted for by cortical porosity of relatively thinner cortices. *J Bone Miner Res*. 2013;28:2017–2026.
106. Leskinen T, Sipilä S, Alen M, et al. Leisure-time physical activity and high-risk fat: a longitudinal population-based twin study. *Int J Obes (Lond)*. 2009;33:1211–1218.
107. Leskinen T, Rinnankoski-Tuikka R, Rintala M, et al. Differences in muscle and adipose tissue gene expression and cardio-metabolic risk factors in the members of physical activity discordant twin pairs. *PLoS One*. 2010;5:e12609.
108. Hng TM, McLean M, Cheung NW, Thompson CH. The interrelation of birth weight and regional lipid deposition: a twins study. *Metabolism*. 2006;55:561–562.
109. Hannukainen JC, Borra R, Linderborg K, et al. Liver and pancreatic fat content and metabolism in healthy mz twins with discordant physical activity. *J Hepatol*. 2011;54:545–552.
110. Weise CM, Bachmann T, Pleger B. Brain structural differences in mz twins discordant for body mass index. *Neuroimage*. 2019;201:116006.
111. Luo Z, Adluru N, Dean 3rd DC, Alexander AL, Goldsmith HH. Genetic and environmental influences of variation in diffusion mri measures of white matter microstructure. *Brain Struct Funct*. 2022;227:131–144.
112. Choi Y, Nam Y, Choi Y, et al. MRI-visible dilated perivascular spaces in healthy young adults: a twin heritability study. *Hum Brain Mapp*. 2020;41:5313–5324.
113. Mazurowski MA. Radiogenomics: what it is and why it is important. *J Am Coll Radiol*. 2015;12:862–866.
114. Tárnoki AD, Tárnoki DL. Imaging epigenetics and the radiogenomics. *Twin and Family Studies of Epigenetics*. United States: Elsevier; 2021:261–276. ISBN 978-0-12-820951-6.
115. Abdel Razek AAK, Alksas A, Shehata M, et al. Clinical applications of artificial intelligence and radiomics in neuro-oncology imaging. *Insights Imaging*. 2021;12:152.

CHAPTER

Pediatric twin studies

25

Catarina Almqvist[a,b], **Paul Lichtenstein**[a]

[a]*Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden*
[b]*Pediatric Allergy and Pulmonology Unit at Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden*

25.1 Introduction

Pediatric twin studies have been very useful to understand the most common somatic health disorders in childhood. Twin methodology has been used to report on heritability as well as risk factors, comorbidities, and consequences for pediatric respiratory, autoimmune, and hemato-oncological diseases. In a large meta-analysis of the heritability of human traits based on 50 years of twin studies, the majority of studies were however based on adult populations and very few with focus on childhood disorders.[1] Using advanced twin methodology in large twin populations, studies have been most useful to further the understanding of heritability and the role of early life risk factors, including those involved in the fetal origins or hygiene hypothesis, for childhood somatic health disorders. Pediatric research questions have however not yet utilized twin methodologies as much as for mental health disorders and there is a great potential to pursue future studies. In this chapter, we will review the causes, comorbidities, and consequences of pediatric somatic health disorders and point toward future studies.

25.2 Respiratory and allergic diseases

25.2.1 Causes

Asthma is one of the most common diseases in childhood with a prevalence of 6%–8% with both genetic and environmental causes. It is a complex disorder with a high heritability on average 70%[2–6] and major susceptibility loci at 17q21 (including *GSDMB/ORMDL3*), 6p21 (*HLA-DRB1*), 5q22 (*TSLP*), and 2q12 (*IL1RL1*).[7] The variance in disease liability explained by common variants seems to be higher for childhood-onset asthma than for adult-onset asthma.[8]

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00023-4

According to the *fetal origins hypothesis*, factors such as restricted growth in utero may result in insufficient energy supply for organ development and increased disease susceptibility in early childhood.[9] Several studies have reported that prematurity or low birth weight are risk factors for subsequent childhood asthma; however, twin studies can address the association between birth weight and asthma also taking genetics and shared environmental factors into account. As such, the association between fetal growth and asthma remains in twin comparisons, indicating that fetal growth per se influences the risk of asthma later in life.[10–12] Likewise, it also seems that the higher prevalence of asthma in twins compared to singletons can be explained by their lower gestational age and birth weight which suggests that twin studies are generalizable.[13] Some twin studies have also been able to study the association between fetal growth and lung function[14,15] or address genetic and environmental contributions of lung function and exhaled nitric oxide to childhood asthma phenotypes.[16,17]

Twin studies have also addressed the *hygiene hypothesis* which suggests that early childhood exposure to microorganisms (such as the gut flora and parasites) reduces the risk of asthma and allergic disease by contributing to the development of the immune system. The hygiene hypothesis was implied in conjunction with "westernisation" when the prevalence of asthma and allergic disease increased more rapidly than changes to the genome sequence would allow.[18] Timely, twin studies were able to study changes in prevalence (7.1% in 1994 to 10.8% in 2003) and heritability (79% in 1994 to 91% in 2003) of asthma over time.[19] Recently, a joint study from the Dutch and Swedish Twin Registries have addressed the association between exposure to antibiotics and asthma in childhood and found that children exposed to early life antibiotic were at higher risk of asthma which however may be confounded by other familial and genetic factors[20] and would speak against the hygiene hypothesis. There are several related research questions that could be addressed using twin methodology in the future.

Allergic diseases such as hay fever (or allergic rhinitis) and eczema (or atopic dermatitis) are also common in childhood and often comorbid with asthma. Twin studies have shown a high heritability for the diseases with 89% for eczema and 95% for rhinitis[21] and have contributed toward the fetal origins hypothesis to show a positive association between birth weight and childhood eczema, but not allergic rhinitis, independent of gestational age, shared environmental and genetic factors.[22]

25.2.2 Consequences

Twin studies have shown that there are no adverse effects of asthma on academic achievement[23] and twins have slightly better educational careers compared to those born as singletons.[24] There is a multitude of other research questions on consequences of asthma and allergic disease that could be attended to using twin methodology.

25.3 Autoimmune disorders

Type 1 diabetes is one of the most common chronic autoimmune diseases, with incidence rates in Finland and Sweden among the highest in the world. Studies from the Finnish Twin Registry have reported a pairwise concordance for Type 1 diabetes of 27.3% (95% CI 22.8–31.8) in MZ and 3.8% (2.7–4.9) in DZ twins and a probandwise concordance of 42.9% (26.7–59.2) in MZ and 7.4% (2.2–12.6) in DZ twins. The risk for Type 1 diabetes was highest in cotwins when the index twins were diagnosed at a very young age. In a model with additive genetic and nonshared environmental effects, 88% of phenotypic variance was explained by genetic factors[25] which is somewhat higher than the reported heritability estimate in Danish twins (71%) and a recent population-based Swedish twin study (81%) including adult twins.[26,27] Here, twin studies have also been helpful to elucidate how risk factors such as genetic disposition, age, and male sex are involved in the development of islet autoimmunity and positive autoantibodies followed by Type 1 diabetes.[28]

Celiac disease is another common childhood disorder with a prevalence of 3%. Twin studies have shown a high heritability of approximately 75% and the rest of the variation due to the non-shared environmental factors.[29] In the recent population-based Swedish twin study on heritability of organ-specific autoimmunity (although not specifically targeting the pediatric population), heritability was even higher for celiac disease 0.91 (95% CI 0.87–0.94).[27] In alignment with the fetal origins hypothesis, twin studies have also reported that higher birth weight is associated with an increased risk of celiac disease however with attenuated estimates when comparing discordant twin pairs in within-twin pair analyses.[30]

Although *inflammatory bowel disease* is not very common in childhood, higher concordance rates for monozygotic twins have been reported in Crohn's disease (50.0%) compared to ulcerative colitis (18.8%) which indicates a stronger genetic component in Crohn's disease.[31]

25.4 Hemato-oncological disorders

In childhood, the acute leukemias account for about 30% of all malignancies. A peak incidence of precursor B cell acute lymphatic leukemia, ALL, has emerged as socio-economic conditions have improved in countries worldwide. Identical infant twins with concordant leukemia were first described in the late 19th century, and neonatal blood spots in twins have been used to backtrack the first initiating genetic events within critical hemopoietic cells to fetal development in utero for most precursor B cell ALL and some cases of acute myeloid leukemia, AML.[32] These events may occur as part of normal fetal development. However, whether other factors (environmental or constitutional) are involved to increase the chance of these first genetic changes happening is unclear. For some leukemias, the first event appears sufficient to create a malignant clone but for the majority of ALL and AML further genetic changes are

required, probably postnatal.[33] Many environmental factors have been proposed as causative for leukemia but few preventative measures have emerged.

25.5 Comorbidity

In childhood, there is some comorbidity between common disorders, and pediatric twin studies have been useful to elucidate the mechanisms. Asthma, hay fever, and eczema often coexist in the same individuals,[34] partly because of a shared genetic origin (pleiotropy).[21] Recent genome-wide association studies, including both the Dutch and Swedish Twin Registries, have identified several loci that contain genetic risk variants shared between asthma, allergic rhinitis, and eczema, many of which dysregulate the expression of immune-related genes.[35,36] Moreover, comorbidities have been reported between asthma and attention deficit hyperactivity disorder, ADHD[37,38]; asthma, and Type 1 diabetes[39] as well as a familial aggregation of atopic diseases and depression or anxiety in children.[40] In the recent population-based twin study on coaggregation and heritability of organ-specific autoimmunity, coaggregation was more pronounced in MZ twins (median HR: 3.2, range: 2.2–9.2) than in DZ twins (median HR: 2.4, range 1.1–10.0), suggesting that disease overlap is largely attributable to genetic factors.[27] More recently, genetic informative twin studies including linkage-disequilibrium score regression and polygenic risk scores have addressed comorbidity between asthma and affective traits in an adult twin population and found that they may be partly due to shared genetic influences.[41] Similar studies are called for also in pediatric twin studies.

25.6 Conclusion

In summary, several pediatric twin studies have contributed to the understanding of causes of the most common somatic health disorders in childhood. There are yet many unanswered questions and future pediatric twin studies should expand on this to show for example long-term consequences of asthma, Type 1 diabetes, and gastrointestinal disorders, the role of familial confounding, and also the possibility to contribute toward multiomics studies.[42]

References

1. Polderman TJ, Benyamin B, de Leeuw CA, et al. Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nature Genetics*. 2015;47:702–709. http://doi.org/10.1038/ng.3285 Epub 2015 May 18.
2. Lichtenstein P, Svartengren M. Genes, environments, and sex: factors of importance in atopic diseases in 7-9-year-old Swedish twins. *Allergy*. 1997;52:1079–1086.
3. Skadhauge LR, Christensen K, Kyvik KO, Sigsgaard T. Genetic and environmental influence on asthma: a population-based study of 11,688 Danish twin pairs. *European Respiratory Journal*. 1999;13:8–14.

4. Koeppen-Schomerus G, Stevenson J, Plomin R. Genes and environment in asthma: a study of 4 year old twins. *Archives of Disease in Childhood*. 2001;85:398–400.
5. Thomsen SF, van der Sluis S, Kyvik KO, Skytthe A, Backer V. Estimates of asthma heritability in a large twin sample. *Clinical and Experimental Allergy*. 2010;40:1054–1061.
6. Ullemar V, Magnusson PK, Lundholm C, et al. Heritability and confirmation of genetic association studies for childhood asthma in twins. *Allergy*. 2016;71:230–238.
7. Demenais F, Margaritte-Jeannin P, Barnes KC, et al. Multiancestry association study identifies new asthma risk loci that colocalize with immune-cell enhancer marks. *Nature genetics*. 2018;50:42–53.
8. Ferreira MAR, Mathur R, Vonk JM, et al. Genetic architectures of childhood- and adult-onset asthma are partly distinct. *American Journal of Human Genetics*. 2019;104:665–684. http://doi.org/10.1016/j.ajhg.2019.02.022 Epub Mar 28.
9. Barker DJ. Fetal origins of coronary heart disease. *Bmj*. 1995;311:171–174.
10. Ortqvist AK, Lundholm C, Carlstrom E, Lichtenstein P, Cnattingius S, Almqvist C. Familial factors do not confound the association between birth weight and childhood asthma. *Pediatrics*. 2009;124:e737–e743.
11. Kindlund K, Thomsen SF, Stensballe LG, et al. Birth weight and risk of asthma in 3-9 year old twins: exploring the fetal origins hypothesis. *Thorax*. 2010;65:146–149.
12. Rasanen M, Kaprio J, Laitinen T, Winter T, Koskenvuo M, Laitinen LA. Perinatal risk factors for asthma in Finnish adolescent twins. *Thorax*. 2000;55:25–31.
13. Ullemar V, Lundholm C, Almqvist C. Twins' risk of childhood asthma mediated by gestational age and birthweight. *Clinical and Experimental Allergy*. 2015;45:1328–1336.
14. Ortqvist AK, Ullemar V, Lundholm C, et al. Fetal growth and childhood lung function in the Swedish twin study on prediction and prevention of asthma. *Annals of the American Thoracic Society*. 2017;14:1147–1153.
15. Nikolajev K, Korppi M, Remes K, Länsimies E, Jokela V, Heinonen K. Determinants of bronchial responsiveness to methacholine at school age in twin pairs. *Pediatric Pulmonology*. 2002;33:167–173. http://doi.org/10.1002/ppul.10059.
16. Nordlund B, Lundholm C, Ullemar V, van Hage M, Ortqvist AK, Almqvist C. The STOPPA twin study explains the exhaled nitric oxide and asthma link by genetics and sensitization. *Twin Res Hum Genet*. 2017;20:330–337.
17. Brew BK, Chiesa F, Lundholm C, Ortqvist A, Almqvist C. A modern approach to identifying and characterizing child asthma and wheeze phenotypes based on clinical data. *Plos One*. 2019;14:e0227091. http://doi.org/10.1371/journal.pone eCollection 2019.
18. Strachan DP. Hay fever, hygiene, and household size. *Bmj*. 1989;299:1259–1260.
19. Thomsen SF, van der Sluis S, Kyvik KO, Skytthe A, Skadhauge LR, Backer V. Increase in the heritability of asthma from 1994 to 2003 among adolescent twins. *Respir Med*. 2011;105(8):1147–1152.
20. Slob EMA, Brew BK, Vijverberg SJH, et al. Early-life antibiotic use and risk of asthma and eczema: results of a discordant twin study. *European Respiratory Journal*. 2020;55(4). (pii):13993003.02021-2019. http://doi.org/10.1183/.02021-2019 Print 2020 Apr.
21. van Beijsterveldt CE, Boomsma DI. Genetics of parentally reported asthma, eczema and rhinitis in 5-yr-old twins. *European Respiratory Journal*. 2007;29:516–521.
22. Lundholm C, Ortqvist AK, Lichtenstein P, Cnattingius S, Almqvist C. Impaired fetal growth decreases the risk of childhood atopic eczema: a Swedish twin study. *Clinical and Experimental Allergy*. 2010;40:1044–1053.

23. Brew BK, Soderberg J, Lundholm C, Afshar S, Holmberg K, Almqvist C. Academic achievement of adolescents with asthma or atopic disease. *Clinical and Experimental Allergy*. 2019;16:13371.
24. Hjern A, Ekeus C, Rasmussen F, Lindblad F. Educational achievement and vocational career in twins—a Swedish national cohort study. *Acta Paediatrica*. 2012;101:591–596.
25. Hyttinen V, Kaprio J, Kinnunen L, Koskenvuo M, Tuomilehto J. Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study. *Diabetes*. 2003;52:1052–1055. http://doi.org/10.2337/diabetes.52.4.1052.
26. Kyvik KO, Green A, Beck-Nielsen H. Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. *Bmj*. 1995;311:913–917. http://doi.org/10.1136/bmj.311.7010.913.
27. Skov J, Eriksson D, Kuja-Halkola R, Höijer J, Gudbjörnsdottir S, Svensson AM, et al. Co-aggregation and heritability of organ-specific autoimmunity: a population-based twin study. *European Journal of Endocrinology*. 2020;182:473–480. http://doi.org/10.1530/EJE-20-0049.
28. Triolo TM, Fouts A, Pyle L, Yu L, Gottlieb PA, Steck AK. Identical and nonidentical twins: risk and factors involved in development of islet autoimmunity and Type 1 diabetes. *Diabetes Care*. 2019;42:192–199. http://doi.org/10.2337/dc18-0288 Epub 2018 Jul 30.
29. Kuja-Halkola R, Lebwohl B, Halfvarson J, Emilsson L, Magnusson PK, Ludvigsson JF. Birth weight, sex, and celiac disease: a nationwide twin study. *Clin Epidemiol*. 2017;9:567–577. 10.2147/CLEP.S149181 eCollection 2017.
30. Kuja-Halkola R, Lebwohl B, Halfvarson J, Wijmenga C, Magnusson PK, Ludvigsson JF. Heritability of non-HLA genetics in coeliac disease: a population-based study in 107 000 twins. *Gut*. 2016;65:1793–1798. http://doi.org/10.136/gutjnl-2016-311713 Epub 2016 May 20.
31. Halfvarson J, Bodin L, Tysk C, Lindberg E, Järnerot G. Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. *Gastroenterology*. 2003;124:1767–1773. http://doi.org/10.016/s0016-5085(03)00385-8.
32. Greaves MF, Maia AT, Wiemels JL, Ford AM. Leukemia in twins: lessons in natural history. *Blood*. 2003;102:2321–2333. http://doi.org/10.1182/blood-2002-12-3817 Epub 2003 Jun 5.
33. Eden T. Aetiology of childhood leukaemia. *Cancer Treatment Reviews*. 2010;36:286–297. http://doi.org/10.1016/j.ctrv.2010.02.004 Epub Mar 12.
34. Pinart M, Benet M, Annesi-Maesano I, et al. Comorbidity of eczema, rhinitis, and asthma in IgE-sensitised and non-IgE-sensitised children in MeDALL: a population-based cohort study. *Lancet Respir Med*. 2014;2:131–140.
35. Ferreira MA, Vonk JM, Baurecht H, et al. Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. *Nature Genetics*. 2017;49:1752–1757. http://doi.org/10.038/ng.3985 Epub 2017 Oct 30.
36. Ferreira MAR, Vonk JM, Baurecht H, et al. Eleven loci with new reproducible genetic associations with allergic disease risk. *Journal of Allergy and Clinical Immunology*. 2018;19:012.
37. Mogensen N, Larsson H, Lundholm C, Almqvist C. Association between childhood asthma and ADHD symptoms in adolescence—a prospective population-based twin study. *Allergy*. 2011;66:1224–1230.
38. Holmberg K, Lundholm C, Anckarsater H, Larsson H, Almqvist C. Impact of asthma medication and familial factors on the association between childhood asthma and attention-

deficit/hyperactivity disorder: a combined twin- and register-based study: epidemiology of allergic disease. *Clinical and Experimental Allergy*. 2015;45:964–973.
39. Thomsen SF, Duffy DL, Kyvik KO, Skytthe A, Backer V. Relationship between type 1 diabetes and atopic diseases in a twin population. *Allergy*. 2011;66:645–647.
40. Brew BK, Lundholm C, Gong T, Larsson H, Almqvist C. The familial aggregation of atopic diseases and depression or anxiety in children. *Clinical and Experimental Allergy*. 2018;7:13127.
41. Lehto K, Pedersen NL, Almqvist C, Lu Y, Brew BK. Asthma and affective traits in adults: a genetically informative study. *European Respiratory Journal*. 2019;53. (pii):13993003.02142-2018. http://doi.org/10.1183/.02142-2018 Print 2019 May.
42. Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. *Genome biology*. 2017;18:83. http://doi.org/10.1186/s13059-017-1215-1.

CHAPTER

Twin-singleton differences

26

Kaare Christensen[a], **Matt McGue**[a,b]

[a]*The Danish Twin Registry, Department of Public Health, University of Southern Denmark, Odense, Denmark*

[b]*Department of Psychology, University of Minnesota, Minneapolis, MN, United States*

26.1 Why are twin-singleton differences of interest to twin research generally?

The majority of twin studies aim to shed light on the causes of individual differences in human health and behavior. An underlying assumption is that results from twin studies generalize to singletons despite twins starting out in life differently from singletons. Twins typically experience third-trimester intrauterine growth restriction and have a higher risk of both prematurity and being small-for-gestational-age. According to the hypothesis of "Fetal programming" or "Developmental Origins of Health and Disease" (DOHaD), impaired intrauterine growth increases the risk for a series of late-life diseases.[1,2] In infancy, childhood and adolescence twins usually have the unique experience of being reared with an age-matched sibling. Dizygotic twins have parents who are, on average, older than singleton parents and, since 1980 in high-income countries, artificial reproduction techniques have accounted for a large proportion of dizygotic twins. This potentially makes dizygotic twins characterized by being, on average, socially advantaged, but maybe biologically disadvantaged due to less fertile parents and potential side effects of artificial reproduction techniques.[3]

These known twin-singleton differences lead to questions important for twin research generally: (1) Is being a twin *per se* associated with certain health and behavior characteristics over the life course? (2) Could observed twin-singleton differences impact the generalizability of results from twin studies—generally or for certain specific phenotypes? This chapter will summarize twin-singleton differences and discuss their potential impact on the interpretation of twin studies.

26.2 Intrauterine and perinatal twin-singleton differences

Placenta anatomy is important for twin intrauterine growth. Dizygotic twin pregnancies arise from two different egg cells and two different sperm cells and will always have

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00018-0

two chorions (placentas) and two amnions. Monozygotic twin pregnancies derive from one fertilized egg from which two embryos later emerge. For about a third of the monozygotic twin pregnancies, the cleavage takes place within the first four days after conception and results in a placenta anatomy like dizygotic twin pregnancies: two chorions and two amnions. For about two-thirds of the monozygotic twins the split occurs four to seven days after conception and here the twins share the same chorion, but still have two amnions. For a small proportion of monozygotic twins (1%–2%), the cleavage occurs more than seven days after fertilization and results in a twin pregnancy in which the two twins share the same chorion and the same amnion. Twin pregnancies with only one chorion have an increased risk of the twin-to-twin transfusion syndrome, discordant fetal growth, perinatal morbidity, and mortality compared to the majority of twin pregnancies with two chorions.[4–7]

26.2.1 Intrauterine growth

Weight and length at birth have traditionally been used for assessing twin-singleton differences in intrauterine growth. The advantage of this approach is that very big population-based samples with reliable measures are available. Sankilampi et al. (2013)[8] used birth register data for infants born in 1996–2008 in Finland, which included more than half a million singletons and 15,000 twins. The median gestational age at birth was 37.1 and 40.0 weeks for twins and singletons, respectively. There were no twin-singleton differences in individual birth weight or length for births occurring before 30 gestational weeks, but thereafter twins diverged from singletons. For births at 37.0 gestational weeks, mean birth weight in twins was 400 grams lower than in singletons, and the birth length was 1.2 cm shorter. Alexander et al. (1998)[9] used the 1991–1995 US Natality Data Files to study birth characteristics of 3.6 million singletons and nearly a million twin individuals. Also, in this study, twins were on average delivered three weeks earlier than singletons and were nearly one kilogram lighter at birth (not taking into account differences in gestational age). In this bigger US sample, it was possible to detect twin-singleton differences in birth weight from 28 weeks of gestation. By 32 weeks of gestation, there was an approximate 300 gram difference in median birth weight between singletons and twins, and by 38 weeks of gestation, the number was around half a kilogram.

The disadvantage of using birth weight and length stratified for gestational age to assess intrauterine growth differences between twins and singleton is that twinning increases the risk of both intrauterine growth restriction and (iatrogenic) premature birth. This can lead to a so-called collider bias when stratifying for gestational age at birth and can create a spurious correlation between twinning and intrauterine growth—a bias that is reduced in studies using intrauterine ultrasound assessments.[10]

Grantz et al. (2016)[11] conducted a longitudinal, high-quality ultrasonography study of fetal growth in 171 pairs of dichorionic twins to avoid this bias. Using this approach, they found that twin fetuses start diverging significantly from the singleton growth standard around the beginning of 32 weeks of gestation. The twin-singleton fetal weight differences estimated in this ultrasonography study are substantially less

than the birth weight differences reported in the aforementioned large Finnish and US studies. For example, the ultrasound study estimated at 35 weeks of gestation a median fetal weight difference between twins and singletons of 200 grams—approximately half the estimate in the birth weight studies. The ultrasonography study also revealed that the deviation was asymmetrical with no twin-singleton differences in head circumference or femur length, but the abdominal circumference and estimated fetal weight trajectories of twin fetuses diverged significantly starting around 32 weeks of gestation. The asymmetrical intrauterine growth for twins in the third trimester is considered the result of maternal and placental constraints on fetal growth.

If the singleton growth standard is used, about one-third of twins would be classified as small for gestational age at week 32, and nearly 40% at week 35 of gestation. These NICHD Fetal Growth Study results are generally in agreement with previous larger cross-sectional ultrasonography studies.[12]

The potential long-term impact of the intrauterine growth disadvantage starting around the beginning of the third trimester will be discussed in the sections on twin-singleton differences in development, morbidity, and mortality.

26.2.2 Intrauterine risk factors occurring in twins but not in singletons

26.2.2.1 *Discordant fetal growth*

In a systematic review and meta-analyses of more than 10,000 twin pregnancies, it was shown that both dichorionic and monochorionic twin pregnancies discordant for fetal growth have substantially higher risk of intrauterine death than pregnancies concordant for fetal growth. The risk was most pronounced for the smaller fetus, and it increased with increasing differences in fetal growth. However, the risk of neonatal death was similar to that of fetal growth concordant twin pregnancies.[13]

26.2.2.2 *Twin-to-twin transfusion*

During intrauterine life, dichorionic twins (i.e., all dizygotic twins and about one-third of monozygotic twins) have separate circulatory systems. In contrast, nearly all monochorionic twins have vascular anastomoses that enable blood exchange between the two twins. This can lead to a number of adverse outcomes, most notably the twin-to-twin transfusion syndrome characterized by the "recipient twin" being volume-overloaded and the "donor twin" being volume-depleted. The syndrome is estimated to occur in 9%–15% of monochorionic twin pregnancies and, if untreated, it is associated with markedly increased perinatal morbidity and mortality, especially in the small group of monoamniotic twins. The introduction of endoscopic laser coagulation of placental anastomoses has improved the prognosis considerably for diamniotic twins with twin-to-twin transfusion syndrome.[14–16]

26.2.2.3 *Twin testosterone transfer*

Animal studies have indicated that intrauterine androgen hormone transfer from male fetuses to their female siblings can have a masculinizing effect on the female fetus.

This has prompted an interest in potential differences between females from opposite-sexed twins compared to females from same-sexed twins and female singletons. However, as pointed out by Ahrenfeldt et al.[17] in a recent review, the human studies of these differences in physiological and behavioral traits are conflicting. Cognition is the trait for which there is most support for this Twin Testosterone Transfer hypothesis, but even here the results are very divergent.

26.2.2.4 *Spontaneous single intrauterine fetal death*

Spontaneous single intrauterine fetal death in twin pregnancies or "Vanishing twin syndrome" is a spontaneous reduction of one of the fetuses in a twin pregnancy, either partially or completely. It is a common phenomenon and poses a risk to the surviving twin. Estimates vary depending on the study population and the methods used to assess the frequency.[18] In a study of more than 700 twin pregnancies conceived in a fertility clinic, more than one-third of pregnancies experienced the "Vanishing twin syndrome" before 12th week of gestation.[19]

In a systematic review and meta-analysis, death of one twin fetus was shown to be associated with increased risk of intrauterine death, prematurity, and brain damage in the co-twin.[20] This effect was most pronounced for monochorionic twins and for single intrauterine deaths occurring before 28 gestational weeks.

26.2.3 Congenital malformations

Twins have a higher prevalence of several major congenital malformations at birth. The increased risk is highest in monozygotic twins and, hence, also in same-sexed twins compared to opposite-sexed twins in studies with no information on zygosity.[21,22] Congenital heart diseases are among the most investigated as a major and relatively common birth defect. A very large register study from California found increased risk in twins compared to singletons in each of the 16 cardiovascular categories included, and for seven of them, the prevalence in twins was at least double that of singletons.[23] A Danish national register study that included zygosity information on the twins showed increased risk of congenital heart defects across all zygosity groups.[24] Acardia is an extremely rare congenital malformation occurring only in monochorionic twin pairs in which one twin has no heart and is receiving circulation from the other "donor" or "pump" twin.[25]

26.2.4 Perinatal mortality

Twin-singleton differences in perinatal mortality are complex. Overall, twins are at increased risk for perinatal morbidity and death primarily due to a high risk of preterm delivery. A direct comparison of the risk of perinatal death in twins and singletons will show substantially higher risk in twins. A nationwide study from the 21st century in the Netherlands showed an overall perinatal mortality rate for twin individuals of 6.6/1000 infants, while the corresponding number for singleton pregnancies was 4.1/1000 infants.[26] However, the study, in agreement with results

based on nationwide data from the US and Sweden, also found that the risk is highly dependent on gestational age at birth.[26–28] For the preterm period (28–36 weeks of gestation), the twin perinatal mortality rate was in fact markedly lower than in singleton pregnancies (10.4 vs 34.5/1000 infants). After the preterm period, twins have the highest perinatal mortality. One possible explanation for the lower perinatal mortality for twins delivered preterm could be that twins have "a good reason" for being born premature, namely the presence of another fetus in uterus, whereas a singleton premature birth could more often reflect an underlying and potentially undiagnosed pathology in the child, the placenta or the mother.

26.3 Twin-singleton differences in development

26.3.1 Neurodevelopment

Intrauterine growth restriction and prematurity are risk factors for adverse neurodevelopmental outcomes such as cerebral palsy and cognitive impairment. This applies to both twins and singletons; however, considering the higher risk of intrauterine growth restriction and prematurity in twins, a higher prevalence of adverse neurodevelopmental outcomes could be expected in twins compared to singletons.

26.3.1.1 Cerebral palsy

Twins do have higher prevalence of cerebral palsy than singletons and most pronounced for twins born at term, for growth restricted twins and for survivors after a "vanished twin." The increased risk is, however, found across the spectrum of gestational ages and birth weight, except maybe for very preterm or very low birth weight twins.[29,30] For infants with a birth weight of 2500+ g, the relative risk of cerebral palsy for twins compared to singletons has been estimated to be a factor of 3.3–5.5. Overall prevalence estimates have been in the intervals of 2.4–12.6 per 1000 twin survivors and of 1.2–2.3 per 1000 singleton survivors.[30]

26.3.1.2 Cognition

As reflected in several chapters in this book (Chapters 12, 23, and 30), one of the most studied phenotypes in twins is cognition and change in cognition over the life-course. Similarly, cognition or proxy measures for cognition such as school test scores are among the most well-studied in terms of twin-singleton differences. Two studies of twins born in the UK in the early mid-20th century show that twins at age 11 scored four to five IQ points lower than singletons, corresponding to about one-third of a standard deviation.[31,32] Another UK study of children born in the mid-20th century found a similar result. Moreover, when the study compared twins to their singleton siblings, hence controlling for many familial factors, it was found that the difference was six IQ points in favor of the siblings.[33] However, a Dutch study of more recent cohorts of twins was unable to detect differences of this size in a comparison with siblings.[34] A very powerful and reliable test of male twin-singleton differences in cognition was performed by Eriksen et al. (2012)[35] who linked two Norwegian national registers:

the birth register from which brothers could be identified and the military conscript register from which IQ scores at conscription at age 18–20 were available for males. The study comprised nearly half a million men born 1967–1984 and showed that the twin IQ was on average 0.1 standard deviation lower than the singleton IQ, both when unrelated singletons and when siblings acted as control groups.

The strong dependency between birth year and average twin-singleton differences in IQ test has been confirmed in meta-analyses showing differences in high-income countries of about one-third of a standard deviation in the early and mid-20th century birth cohorts, and trivial or no differences in more recent cohorts.[36,37] This pattern is likely to be the result of positive secular changes with better maternal health and living conditions as well as improvements in obstetrics and pediatrics. Interestingly in that context is a study by Hur and Lynn (2013)[38] of contemporary adolescent Nigerian twins and singletons, which showed a twin cognitive disadvantage similar in size to that found in mid-20th century birth cohorts in high-income countries.

26.3.1.3 School grades, academic achievements, and vocational career

Academic achievement tests are highly correlated with IQ and, furthermore, it is a "neurodevelopmental phenotype" likely to be of greater interest to parents of twins and to the twins themselves than is an IQ test score.[39] Due to nationwide health, social and educational registers, the Nordic twin registries have been able to provide very reliable and powerful designs to test twin-singleton differences in school grades, academic achievements, and vocational careers.

A study of school grades in the Danish 1986–88 birth cohorts showed that adolescent twins and singletons had nearly identical test scores in the ninth-grade test. The only difference observed was that twins, as expected, were overrepresented in the small group of very low birth weight newborns who were characterized by lower academic achievements. Otherwise, birth weight had a very small effect on performance, but in the large sample it was detectable, and it was found that for twins, this effect is best judged relative to what is normal for twins and not for singletons. For example, a birth weight of 2500 g, which is the 10^{th} percentile in singletons and about the median in twins in this sample, was associated with slighter lower mean test scores in singletons, but average mean test scores in twins.[39]

A nationwide Swedish study of the 1973–1981 birth cohorts[40] assessed ninth-grade scores, IQ test score at conscription (males only), educational achievement, and vocational career. The study included twins, siblings, and singletons and showed that twins did slightly better in ninth grade and more often completed a university education OR 1.16 (95% CI 1.02–1.21) compared to singleton siblings, despite male twins having slightly worse performance at the conscription test compared to male singletons. At age 27–35, employment rates, mean income and disability benefits were similar in twins and singletons.

26.3.1.4 Neurodevelopment in single twins

As previously mentioned, single twins (survivors after a vanished twin) have an increased risk of adverse pregnancy outcomes, e.g. growth restriction, prematurity,

and cerebral palsy. Therefore, it was surprising that Record et al. (1970)[31] showed that the four-five IQ point disadvantage found in twins from cohorts born in the 1950s did not exist among the subgroup of twins who lost their cotwin early in life, suggesting that social competition may be the cause of the twin disadvantage. However, newer nationwide register studies of much larger recent birth cohorts in Denmark and Sweden showed the opposite pattern, namely that twins with a deceased cotwin scored significantly lower on the academic tests than twins with a living cotwin.[39,40]

26.3.1.5 *The impact of assisted reproductive technology and socioeconomic position*

Dizygotic twins have parents who are, on average, older than singleton parents and, artificial reproduction techniques have since 1980 accounted for a large proportion of dizygotic twins in high-income countries.[41] There is also some evidence that assisted reproductive technology (ART) is associated with approximately a doubling of the monozygotic twinning rate[42,43] ART is associated with better than average parental education and socioeconomic position which again is associated with offspring IQ and educational achievements.[44–46] Therefore, it is key to control for parental socioeconomic position in twin studies of ART and later life neurodevelopment and generally in studies that are comparing twins to singletons in birth cohorts born after 1980 in high-income countries.

Using nationwide Danish register data, Spangmose et al. (2017)[47] investigated ninth-grade test scores for ART singletons and ART twins and compared them to spontaneously conceived singletons and twins. The crude mean test score was higher in both ART singletons and ART twins compared to spontaneously conceived singletons. However, when adjusting for parental socioeconomic characteristics and other confounders, the differences between the four groups were trivial. This is in line with a review by Briana and Malamitsi-Puchner (2019)[29] that concluded that currently there is no evidence that ART impacts on twins' neurodevelopment or general health but also that more long-term high-quality studies are needed.

26.3.2 Anthropometric development

26.3.2.1 *Height and body mass index*

Birth length and weight for twins are, as described previously in this chapter, smaller than singletons and mainly, but not completely, explained by shorter gestational age.[48] Smaller studies that compare growth in childhood for twins versus their siblings and the general population find a tendency for twins to be leaner, but also that the differences in height and body size disappear gradually before adulthood.[49,50] However, in nationwide population-based register studies with huge sample sizes, it is possible to detect small differences. Silventoinen et al. (2008)[51] found, in a study of more than 1 million Swedish males born 1951 to 1976, that male twins at military conscription were shorter, leaner, and had less muscle strength compared to singletons, but that the differences were a small fraction of a standard deviation. Eriksen et al. (2013)[52] estimated, in a similar register study of nearly half a million males in Norway born 1967–1984, that twins

were on average about half a centimeter shorter than singletons after adjustment of a series of background factors and about 1 cm shorter than their siblings. A 1 cm difference corresponds to about 0.15 standard deviation in the study sample. Eriksen and Tambs (2016)[53] used the same Norwegian dataset to demonstrate a slightly lower body mass index in twins compared to singletons and that the difference attenuated when birth weight was controlled for. This suggests that the body mass index difference could be attributed to lower intrauterine growth rate among twins. Preliminary Danish conscript data including twins also from the first half of the 20th century indicate that in the earlier cohorts anthropometric differences between twins and singletons were moderate but vanished in later-born cohorts. This secular change is very similar to what has been observed for cognition, and, again, the likely explanation for the progress is better maternal health, living conditions, and improvements in obstetrics and pediatrics.

Chapter 24 is dealing in more detail with anthropometric traits in twins.

26.4 Twin-singleton differences in behavior and personality

26.4.1 Lifestyle factors

A UK study of middle-aged females showed no twin-singleton difference in smoking prevalence or alcohol consumption.[54] For twin cohorts born after 1980, the high frequency of artificial reproduction technology use among the parents of dizygotic twins implies higher socioeconomic level in the twins' rearing environment and, hence, potentially a different lifestyle profile compared to the general population.

26.4.2 Behavior

Many smaller studies have found twin-singleton differences in the behavioral domain, and one potential factor may be that twins grow up with an age-matched sibling. According to the Adaptive hypothesis, twins could benefit from the socializing effects experienced by the cotwins while, on the other hand, the divided attention of the parents could lead to intrapair competition and dissociation. Rutter and Redshaw (1991)[55] concluded in a review that the general level of psychopathology in twins is broadly comparable to that in singletons and that socioemotional adjustment levels did not differ between twins and singletons. Similarly, van den Oord et al. (1995)[56] found that problem behaviors in twin infants were similar to those observed in singletons. A small Japanese study found that positivity toward one's sibling increased peer problems among monozygotic twins, whereas the opposite tendency was present among dizygotic twins and singleton siblings.[57]

Pulkkinen et al. (2003)[58] found no evidence in a large Finnish study of peer reports of adaptive behavior in 11–12-year-old twins and singletons for twin-singleton differences in externalizing problem behaviors (hyperactivity-impulsivity, inattention, aggression) or internalizing problem behaviors (depressive symptoms, social

anxiety). However, the same-sexed twins had a small advantage compared to singletons in adaptive behaviors (constructive, compliant, and active behavior), whereas the advantage was substantial in the opposite-sexed twins. More recently, Robbers et al. (2010)[59] studied Dutch twins age 6–12 and found that internalizing problems developed similarly for twins and singletons up to age 9 but at age 12, twins had less internalizing problems while the trajectories of externalizing behavioral problems were similar in twins and singletons.

Barnes and Boutwell (2013)[60] studied delinquency, polydrug use, victimization, and a long series of covariates in more than 1200 twins and 17,000 singletons in the US National Longitudinal Study of Adolescent Health, and they did not find evidence for systematic twin-singleton differences in many measures of behavior and development. Equally important, the study found similar effects of specific covariates on measures of antisocial behavior in twins and singletons.

26.4.3 Personality

Johnson et al., (2002)[61] studied personalities in nearly 13,000 individuals in the Minnesota Twin Family Study and compared the twins to singleton family members of the participating twins. The only consistent mean or variance difference in the Multidimensional Personality Questionnaire data was greater social closeness for twins than for singletons and the difference was very modest (0.1 standard deviation). Hence, the no substantial systematic twin-singleton differences were observed or, as the title of the paper stated it: *The personalities of twins: just ordinary folks*.

26.4.4 Divorce

Divorce is a phenotype that is influenced by behavior and personality. Jocklin et al. (1996)[62] found in the Minnesota study that a substantial proportion of the heritability of divorce risk consisted of genetic factors affecting personality. Petersen et al. (2011)[63] used the Danish civil registration system to study marriage and divorce rates among more than 35,000 twins and 80,000 matched singletons and found lower marriage rates among twins, especially among males and at younger ages. With age, the twin-singleton differences in marriage rate became smaller and fell to about 5%. For males, there were no twin-singleton differences in divorce rate, but female twins had a 13% lower divorce rate compared to female singletons. The interpretation may be that since twins have a partner from birth, they do not have the same need for marriage as singletons but have more experience in maintaining a relationship if they do marry.

26.5 Twin-singleton differences in morbidity and survival

26.5.1 Early life morbidity and survival

The high risk of intrauterine growth restriction and premature birth in twins results in excess infant morbidity and mortality in twins compared to singletons although

enormous progress in absolute terms has been made over the last 150 years. In the Danish 1870–1900 birth cohorts, which were the first to be included in the Danish Twin Registry, both twins survived to age 6 in only one-third of the pairs. Among singletons, 80% survived to age 6 in these cohorts and if twins had had the same mortality risk as singletons, two-thirds and not one-third of twin pairs should have made it to age 6.[64] In the 21st century in Denmark, 98% of twin individuals made it to age 6 whereas for singletons, it was more than 99%.[65]

A study based on large neonatal prospective databases concluded that when controlling for intrauterine growth restriction and prematurity there was no twin-singleton difference in contemporary neonatal mortality and morbidity rates.[66] Studies of very premature twins and singletons have provided mixed evidence may be partly due to small sample sizes.[67] Respiratory distress syndrome is the major cause of morbidity and mortality in preterm infants. A Finnish nationwide register study of more than 850,000 singletons and 23,000 twins showed no twin-singleton differences in respiratory distress syndrome if gestational age was taken into account.[68]

26.5.2 Adulthood morbidity and survival

26.5.2.1 The fetal origins hypothesis

According to the hypothesis of "Fetal programming" or "Developmental Origins of Health and Disease" (DOHaD), impaired intrauterine growth increases the risk for a series of late-life diseases. The hypothesis has been supported by animal studies and a large number of observational association studies in humans, although the "effect size" of the associations is generally very modest.[1,2] Philips (1993)[69] hypothesized that the impaired intrauterine growth, in particular for monozygotic twins, could impact the validity of classic twin studies. More generally, DOHaD research raised the important question whether the disadvantaged beginning of life for twins with a high frequency of prematurity, low birth weight, and an often challenging delivery would leave "scars" on the twins affecting their health and functioning later in life and make them biologically "different" from singletons. If that was the case, it could challenge the generalizability of scientific results obtained in twin studies. On the other hand, it could be hypothesized that the high intrauterine and perinatal twin mortality makes the surviving twin population more selected as only the strongest survive—especially in older cohorts. However, as documented below, large nationwide register studies of twin-singleton differences in birth cohorts spanning more than a century have shown no or small differences in morbidity and mortality between twins and singletons after the infant period.[70]

26.5.2.2 Morbidity

In a study of nearly 50,000 Swedish twins and their siblings and a population sample of singletons born 1932 to 1958 and followed through national registers through 2007, Öberg et al. (2012)[70] found the same cumulative risks of cancer, cardiovascular

diseases, and death in the three groups. Skytthe et al. (2019)[71] followed 260,000 Nordic twins with more than 30,000 incident cancers and found a marginally lower mortality and cancer incidence in twins compared to singletons.

The prevalence of type 2 diabetes was similar in a register study of nearly 80,000 Danish twins and a random sample of 220,000 age- and sex-matched individuals, which is in contrast to the results of Danish, Italian, and New Zealand small-scale clinical examinations of twins and singletons that demonstrated adverse effects of twin status on outcomes related to type 2 diabetes.[63] There is evidence from smaller studies that twins have less asthma than singletons in childhood and adolescence, whereas larger population-based studies tend to find similar estimates in twins and singletons.[72]

An exception to the pattern that large register-based studies with little room for selection bias find no or small twin-singleton differences in morbidity is a Danish study that found higher risk of schizophrenia but not bipolar disorders in dizygotic twins compared to the background population.[73,74] A smaller Israeli study was not able to confirm the excess risk of schizophrenia[75] and neither was a nationwide register study of Swedish twins that found a similar incidence of psychotic and affective disorders in twins and singletons and across zygosity groups.[76]

Monozygotic twins extremely discordant for birth weight provide a model for testing the impact of twin intrauterine growth restriction on later-life health and morbidity in a design where genetic and maternal factors can be controlled for. Frost et al. (2012)[77] studied more than 150 extremely birth weight-discordant young and middle-aged monozygotic twin pairs (median intrapair birth weight difference was 0.5 kilo) and found no indication for a lasting effect on glucose metabolism in the low birth weight twin compared to the high birth weight twin. Simmons et al. (1997)[78] studied older twins to assess the potential selection bias over a life course and found, through in-person interviews and testing, that octogenarian twins and singletons had similar health status and biobehavioral functioning.

26.5.2.3 Survival

A longitudinal study of Danish twins born 1870–1900 followed from age 6 through 1991 showed that mortality rates for twins were generally similar to those of the background population[64] and the Swedish study by Öberg et al. (2012)[70] mentioned in the previous section found similar mortality rates in twins, twin siblings, and singleton. The Danish study was later expanded to more than 100,000 twins born 1870–1990, and it was found that the mortality of monozygotic and dizygotic twin individuals differs slightly after taking into consideration the effects of birth- and age-cohorts, gender differences, and that twins are paired. However, no substantial or systematic differences remain when taking twins with unknown zygosity into account.[79] A study of cardiovascular mortality within the same population found similar rates in twins and singletons[80] but substantially decreased suicide risk among twins compared to singletons, potentially because twinships provide stronger family ties that may be protective.[81]

26.6 Twin-singleton differences in genetic studies

Many twin registries have collected data and biological material from a large number of twins for decades and have produced "omics" data on a substantial proportion of the twins. This makes the twin cohorts ideal for entering consortia that need very large sample sizes, e.g. GWAS-studies of complex traits. However, a key question is whether there are molecular differences between twins and singletons. Ganna et al. (2013)[82] sought to identify SNPs associated with being a twin *per se* and found no overall difference between twins and singletons. Only in stratified analyses did the authors find two SNPs that were genome-wide significant in dizygotic twins only. Mbarek et al. (2016)[83] identified two other robust genetic risk variants for dizygotic twinning.

Generally, twin samples can be used without bias in genetic studies if there are no genetic variants associated with either the trait under investigation or the fact of being twin. As monozygotic twinning is a random event, such associations are most likely to be found in dizygotic twins.

26.7 Conclusion

This chapter set out to answer the two questions: (1) Is being a twin *per se* associated with certain health and behavior characteristics over the life course? (2) Could observed twin-singleton differences impact the generalizability of results from twin studies - generally or for certain specific phenotypes? Twins do for sure differ from singletons in early life with a higher occurrence of prematurity, low birth weight, congenital malformations, neonatal morbidity, and perinatal death. Furthermore, dizygotic twins tend to have older parents, and, since 1980, also parents with above-average socioeconomic position due to the large proportion of dizygotic twins conceived with the help of ART in high-income countries. However, data from more than a century of birth cohorts show that these differences and their impact generally vanish with age and, for more recent birth cohorts, the twin-singleton differences are few and generally small after childhood. Even in traits for which there is a mean difference between twins and singletons, for example, IQ in older cohorts, it seems plausible that it is the same factors in twins and singletons that cause variation around their respective mean IQs. Therefore, twin-singleton differences will rarely be a challenge for using twin research together with other study designs and populations to understand the trait under study.

References

1. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet*. 1993;341:938–941.
2. Suzuki K. The developing world of DOHaD. *J Dev Orig Health Dis*. 2018;9:266–269.
3. Messerlian C, Maclagan L, Basso O. Infertility and the risk of adverse pregnancy outcomes: a systematic review and meta-analysis. *Human Reproduction*. 2013;28:125–137.

4. Hall JG. Twinning. *Lancet*. 2003;362:735–743.
5. Lewi L. Monochorionic diamniotic twin pregnancies pregnancy outcome, risk stratification and lessons learnt from placental examination. *Verhandelingen - Koninklijke Academie Voor Geneeskunde Van Belgie*. 2010;72:5–15.
6. Loos R, Derom C, Vlietinck R, Derom R. The east flanders prospective twin survey (Belgium): a population-based register. *Twin Research*. 1998;1:167–175.
7. Derom C, Thiery E, Rutten BPF, et al. the east flanders prospective twin survey (EFPTS): 55 years later. *Twin Res Hum Genet*. 2019;22:454–459.
8. Sankilampi U, Hannila ML, Saari A, Gissler M, Dunkel L. New population-based references for birth weight, length, and head circumference in singletons and twins from 23 to 43 gestation weeks. *Annals of Medicine*. 2013;45:446–454.
9. Alexander GR, Kogan M, Martin J, Papiernik E. What are the fetal growth patterns of singletons, twins, and triplets in the United States? *Clinical Obstetrics and Gynecology*. 1998;41:114–125.
10. Olsen J, Pedersen LH. Reproductive health indicators and fetal medicine - many things will change. *Scandinavian Journal of Work, Environment & Health*. 2016;42:561–562.
11. Grantz KL, Grewal J, Albert PS, et al. Dichorionic twin trajectories: the NICHD Fetal Growth Studies. *American Journal of Obstetrics and Gynecology*. 2016;215:e1–221.
12. Ong S, Lim MN, Fitzmaurice A, Campbell D, Smith AP, Smith N. The creation of twin centile curves for size. *Bjog*. 2002;109:753–758.
13. D'Antonio F, Odibo AO, Prefumo F, et al. Weight discordance and perinatal mortality in twin pregnancy: systematic review and meta-analysis. *Ultrasound in Obstetrics & Gynecology: The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2018;52:11–23.
14. Roberts D, Neilson JP, Kilby MD, Gates S. Interventions for the treatment of twin-twin transfusion syndrome. *Cochrane Database of Systematic Reviews (Online)*. 2014:CD002073.
15. Lewi L, Deprest J, Hecher K. The vascular anastomoses in monochorionic twin pregnancies and their clinical consequences. *American Journal of Obstetrics and Gynecology*. 2013;208:19–30.
16. Murgano D, Khalil A, Prefumo F, et al. Outcome of twin-to-twin transfusion syndrome in monochorionic monoamniotic twin pregnancy: systematic review and meta-analysis. *Ultrasound in Obstetrics & Gynecology: The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2020;55:310–317.
17. Ahrenfeldt LJ, Christensen K, Segal NL, Hur YM. Opposite-sex and same-sex twin studies of physiological, cognitive and behavioral traits. *Neuroscience and Biobehavioral Reviews*. 2020;108:322–340.
18. Evron E, Sheiner E, Friger M, Sergienko R, Harlev A. Vanishing twin syndrome: is it associated with adverse perinatal outcome? *Fertility and Sterility*. 2015;103:1209–1214.
19. Dickey RP, Taylor SN, Lu PY, et al. Spontaneous reduction of multiple pregnancy: incidence and effect on outcome. *American Journal of Obstetrics and Gynecology*. 2002;186:77–83.
20. Mackie FL, Rigby A, Morris RK, Kilby MD. Prognosis of the co-twin following spontaneous single intrauterine fetal death in twin pregnancies: a systematic review and meta-analysis. *Bjog*. 2019;126:569–578.
21. Rider RA, Stevenson DA, Rinsky JE, Feldkamp ML. Association of twinning and maternal age with major structural birth defects in Utah, 1999 to 2008. *Birth Defects Research. Part A, Clinical and Molecular Teratology*. 2013;97:554–563.

22. Doyle PE, Beral V, Botting B, Wale CJ. Congenital malformations in twins in England and Wales. *Journal of Epidemiology and Community Health*. 1991;45:43–48.
23. Hardin J, Carmichael SL, Selvin S, Lammer EJ, Shaw GM. Increased prevalence of cardiovascular defects among 56,709 California twin pairs. *American Journal of Medical Genetics. Part A*. 2009;149A:877–886.
24. Herskind AM, Almind Pedersen D, Christensen K. Increased prevalence of congenital heart defects in monozygotic and dizygotic twins. *Circulation*. 2013;128:1182–1188.
25. Botto LD, Feldkamp ML, Amar E, et al. Acardia: epidemiologic findings and literature review from the International Clearinghouse for Birth Defects Surveillance and Research. *Am J Med Genet C Semi Med Genet*. 2011;157C:262–273.
26. Vasak B, Verhagen JJ, Koenen SV, et al. Lower perinatal mortality in preterm born twins than in singletons: a nationwide study from The Netherlands. *American Journal of Obstetrics and Gynecology*. 2017;216 161.e1–161.e9.
27. Cheung YB, Yip P, Karlberg J. Mortality of twins and singletons by gestational age: a varying-coefficient approach. *American Journal of Epidemiology*. 2000;152:1107–1116.
28. Chen HY, Chauhan SP. Risk of neonatal and infant mortality in twins and singletons by gestational age. *American Journal of Perinatology*. 2019;36:798–805.
29. Briana DD, Malamitsi-Puchner A. Twins and neurodevelopmental outcomes: the effect of IVF, fetal growth restriction, and preterm birth. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2019;32:2256–2261.
30. Lorenz JM. Neurodevelopmental outcomes of twins. *Seminars in Perinatology*. 2012;36:201–212.
31. Record RG, McKeown T, Edwards JH. An investigation of the difference in measured intelligence between twins and single births. *Annals of Human Genetics*. 1970;34:11–20.
32. Deary I, Pattie A, Wilson V, Whalley L. The cognitive cost of being a twin: two whole-population surveys. *Twin Res Hum Genet*. 2005;8:376–383.
33. Ronalds GA, De Stavola BL, DA Leon. The cognitive cost of being a twin: evidence from comparisons within families in the Aberdeen children of the 1950s cohort study. *Bmj*. 2005;331:1306.
34. Posthuma D, De Geus EJ, Bleichrodt N, Boomsma DI. Twin-singleton differences in intelligence? *Twin Research*. 2000;3:83–87.
35. Eriksen W, Sundet JM, Tambs K. Twin-singleton differences in intelligence: a register-based birth cohort study of Norwegian males. *Twin Res Hum Genet*. 2012;15:649–655.
36. Voracek M, Haubner T. Twin-singleton differences in intelligence: a meta-analysis. *Psychological Reports*. 2008;102:951–962.
37. Babatunde OA, Adebamowo SN, Ajayi IO, Adebamowo CA. Neurodevelopmental outcomes of twins compared with singleton children: a systematic review. *Twin Res Hum Genet*. 2018;21:136–145.
38. Hur YM, Lynn R. Twin-singleton differences in cognitive abilities in a sample of Africans in Nigeria. *Twin Res Hum Genet*. 2013;16:808–815.
39. Christensen K, Petersen I, Skytthe A, Herskind AM, McGue M, Bingley P. Comparison of academic performance of twins and singletons in adolescence: a follow-up study. *Bmj*. 2006;333:1095–1097.
40. Hjern A, Ekeus C, Rasmussen F, Lindblad F. Educational achievement and vocational career in twins - a Swedish national cohort study. *Acta Paediatrica*. 2012;101:591–596.
41. Adashi EY. Seeing double: a nation of twins from sea to shining sea. *American Journal of Obstetrics and Gynecology*. 2016;214:311–313.

42. Aston KI, Peterson CM, Carrell DT. Monozygotic twinning associated with assisted reproductive technologies: a review. *Reproduction (Cambridge, England)*. 2008;136:377–386.
43. Vitthala S, Gelbaya TA, Brison DR, Fitzgerald CT, Nardo LG. The risk of monozygotic twins after assisted reproductive technology: a systematic review and meta-analysis. *Human Reproduction Update*. 2009;15:45–55.
44. Goody A, Rice F, Boivin J, Harold GT, Hay DF, Thapar A. Twins born following fertility treatment: implications for quantitative genetic studies. *Twin Res Hum Genet*. 2005;8:337–345.
45. Burt SA, Klump KL. How does the inclusion of twins conceived via fertility treatments influence the results of twin studies? *Twin Res Hum Genet*. 2012;15:746–752.
46. Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. *New England Journal of Medicine*. 2012;366:1803–1813.
47. Spangmose AL, Malchau SS, Schmidt L, et al. Academic performance in adolescents born after ART-a nationwide registry-based cohort study. *Human Reproduction*. 2017;32:447–456.
48. Buckler JM, Green M. A comparison of the early growth of twins and singletons. *Annals of Human Biology*. 2004;31:311–332.
49. Estourgie-van Burk GF, Bartels M, Boomsma DI, Delemarre-van de Waal HA. Body size of twins compared with siblings and the general population: from birth to late adolescence. *Journal of Pediatrics*. 2010;156:586–591.
50. Pietiläinen KH, Kaprio J, Rissanen A, et al. Distribution and heritability of BMI in Finnish adolescents aged 16y and 17y: a study of 4884 twins and 2509 singletons. *International Journal of Obesity and Related Metabolic Disorders*. 1999;23:107–115.
51. Silventoinen K, Magnusson PK, Tynelius P, Kaprio J, Rasmussen F. Heritability of body size and muscle strength in young adulthood: a study of one million Swedish men. *Genetic Epidemiology*. 2008;32:341–349.
52. Eriksen W, Sundet JM, Tambs K. Adult body height of twins compared with that of singletons: a register-based birth cohort study of Norwegian males. *American Journal of Epidemiology*. 2013;177:1015–1019.
53. Eriksen W, Tambs K. Is the twin-singleton difference in BMI related to the difference in birth weight? A register-based birth cohort study of Norwegian males. *American Journal of Human Biology*. 2016;28:566–573.
54. Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD, MacGregor AJ. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. *Twin Research*. 2001;4:464–477.
55. Rutter M, Redshaw J. Annotation: growing up as a twin: twin-singleton differences in psychological development. *Journal of Child Psychology and Psychiatry and Allied Disciplines*. 1991;32:885–895.
56. van den Oord EJ, Koot HM, Boomsma DI, Verhulst FC, Orlebeke JF. A twin-singleton comparison of problem behaviour in 2-3-year-olds. *Journal of Child Psychology and Psychiatry and Allied Disciplines*. 1995;36:449–458.
57. Nozaki M, Fujisawa KK, Ando J, Hasegawa T. The effects of sibling relationships on social adjustment among Japanese twins compared with singletons. *Twin Res Hum Genet*. 2012;15:727–736.
58. Pulkkinen L, Vaalamo I, Hietala R, Kaprio J, Rose RJ. Peer reports of adaptive behavior in twins and singletons: is twinship a risk or an advantage?. *Twin Research*. 2003;6:106–118.

59. Robbers SC, Bartels M, van Oort FV, et al. A twin-singleton comparison of developmental trajectories of externalizing and internalizing problems in 6- to 12-year-old children. *Twin Res Hum Genet*. 2010;13:79–87.
60. Barnes JC, Boutwell BB. A demonstration of the generalizability of twin-based research on antisocial behavior. *Behavior Genetics*. 2013;43:120–131.
61. Johnson W, Krueger RF, Bouchard Jr TJ, McGue M. The personalities of twins: just ordinary folks. *Twin Research*. 2002;5:125–131.
62. Jocklin V, McGue M, Lykken DT. Personality and divorce: a genetic analysis. *Journal of Personality and Social Psychology*. 1996;71:288–299.
63. Petersen I, Martinussen T, McGue M, Bingley P, Christensen K. Lower marriage and divorce rates among twins than among singletons in Danish birth cohorts 1940-1964. *Twin Res Hum Genet*. 2011;14:150–157.
64. Christensen K, Vaupel JW, Holm NV, Yashin AI. Mortality among twins after age 6: fetal origins hypothesis versus twin method. *Bmj*. 1995;310:432–436.
65. Ahrenfeldt LJ, Larsen LA, Lindahl-Jacobsen R, et al. Early-life mortality risks in opposite-sex and same-sex twins: a Danish cohort study of the twin testosterone transfer hypothesis. *Annals of Epidemiology*. 2017;27:115–120 e2.
66. Garite TJ, Clark R, Thorp JA. Intrauterine growth restriction increases morbidity and mortality among premature neonates. *American Journal of Obstetrics and Gynecology*. 2004;191:481–487.
67. Bodeau-Livinec F, Zeitlin J, Blondel B, et al. Do very preterm twins and singletons differ in their neurodevelopment at 5 years of age?. *Archives of Disease in Childhood. Fetal and Neonatal Edition*. 2013;98:F480–F487.
68. Marttila R, Kaprio J, Hallman M. Respiratory distress syndrome in twin infants compared with singletons. *American Journal of Obstetrics and Gynecology*. 2004;191:271–276.
69. Phillips DI. Twin studies in medical research: can they tell us whether diseases are genetically determined?. *Lancet*. 1993;341:1008–1009.
70. Öberg S, Cnattingius S, Sandin S, Lichtenstein P, Morley R, Iliadou AN. Twinship influence on morbidity and mortality across the lifespan. *International Journal of Epidemiology*. 2012;41:1002–1009.
71. Skytthe A, Harris JR, Czene K, et al. Cancer incidence and mortality in 260,000 Nordic twins with 30,000 prospective cancers. *Twin Res Hum Genet*. 2019;22:99–107.
72. Thomsen SF, Kyvik KO, Backer V. A note on twin-singleton differences in asthma. *Twin Res Hum Genet*. 2008;11:161–164.
73. Kläning U, Mortensen PB, Kyvik KO. Increased occurrence of schizophrenia and other psychiatric illnesses among twins. *British Journal of Psychiatry*. 1996;168:688–692.
74. Kläning U, Laursen TM, Licht RW, Kyvik KO, Skytthe A, Mortensen PB. Is the risk of bipolar disorder in twins equal to the risk in singletons? A nationwide register-based study. *Journal of Affective Disorders*. 2004;81:141–145.
75. Kleinhaus K, Harlap S, Perrin MC, et al. Twin pregnancy and the risk of schizophrenia. *Schizophrenia Research*. 2008;105:197–200.
76. Kendler KS, Pedersen NL, Farahmand BY, Persson PG. The treated incidence of psychotic and affective illness in twins compared with population expectation: a study in the Swedish Twin and Psychiatric Registries. *Psychological Medicine*. 1996;26:1135–1144.
77. Frost M, Petersen I, Brixen K, et al. Adult glucose metabolism in extremely birthweight-discordant monozygotic twins. *Diabetologia*. 2012;55:3204–3212.
78. Simmons SF, Johansson B, Zarit SH, Ljungquist B, Plomin R, McClearn GE. Selection bias in samples of older twins? A comparison between octogenarian twins and singletons in Sweden. *Journal of Aging and Health*. 1997;9:553–567.

79. Hjelmborg J, Larsen P, Kaprio J, et al. Lifespans of Twins: Does Zygosity Matter?. *Genes (Basel)*. 2019;10:166.
80. Christensen K, Wienke A, Skytthe A, Holm NV, Vaupel JW, Yashin AI. Cardiovascular mortality in twins and the fetal origins hypothesis. *Twin Research*. 2001;4:344–349.
81. Tomassini C, Juel K, Holm NV, Skytthe A, Christensen K. Risk of suicide in twins: 51 year follow up study. *Bmj*. 2003;327:373–374.
82 Ganna A, Ortega-Alonso A, Havulinna A, et al. Utilizing twins as controls for non-twin case-materials in genome wide association studies. *Plos One*. 2013;8:e83101.
83 Mbarek H, Steinberg S, Nyholt DR, et al. Identification of common genetic variants influencing spontaneous dizygotic twinning and female fertility. *American Journal of Human Genetics*. 2016;98:898–908.

CHAPTER

Twin studies of puberty and behavior

27

Holly T. Pham[a], Adriene M. Beltz[b], Robin P. Corley[c], Sheri A. Berenbaum[a,d]

[a]*Department of Psychology, The Pennsylvania State University, University Park, PA, United States*

[b]*Department of Psychology, University of Michigan, MI, United States*

[c]*Institute for Behavioral Genetics, University of Colorado Boulder, CO, United States*

[d]*Department of Pediatrics, The Pennsylvania State University, Hershey, PA, United States*

Puberty is important for psychological as well as physical development. Although puberty is strongly influenced by genes,[1] variations in pubertal development are also influenced by early life experiences[2] and can contribute to risk for behavior problems and poor health outcomes[3] or stress resiliency and positive outcomes.[4] In this chapter, we discuss how twin studies provide unique and rigorous information about the origins of pubertal variations and the links between puberty and behavior by accounting for genetic and common environmental influences in the associations. We specifically consider the nature and measurement of puberty, the ideal twin study on puberty, findings on gene/environment influences on pubertal development, and genetic covariations between puberty and behavior. We conclude with novel ways to best utilize twin puberty data, methodological concerns when interpreting findings, and suggestions for future research. In brief, many twin studies have revealed that pubertal timing is generally heritable in both girls and boys, and that genetic influences on behavior can change across puberty. These insights facilitate early detection of potentially maladaptive behaviors and identification of possible protective factors.

27.1 What is puberty?

Puberty is a set of linked processes under endocrine control (changes in adrenal and gonadal hormonal levels, and physical growth) that prepare adolescents for reproduction.[5–6] Adrenarche (maturation of the adrenal glands) generally starts at ages 6–9 in girls and ages 7–10 in boys and is responsible for body hair growth, body odor, and skin changes. Gonadarche (maturation of the ovaries and testes) generally starts at ages 8–14 in girls and ages 9–15 in boys and prompts increases in estrogens and androgens, and subsequent development of secondary sexual characteristics (e.g., growth of breasts in girls, growth of facial hair in boys). Physical growth during

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00030-1

puberty is seen in increases in height and weight, and changes in body size and composition in both sexes. Puberty ends when the individual reaches reproductive maturity and the pubertal processes of adrenarche, gonadarche, and physical growth are complete. This typically occurs at ages 15–17 in girls and ages 16–18 in boys. Although there are atypical variations of pubertal development (e.g., precocious puberty, or puberty that starts before age 8 in girls or before age 9 in boys), we focus on normative processes in this chapter.

It is important to differentiate aspects of puberty (i.e., status, timing, and tempo). Status refers to a certain point during the pubertal process; these points are often described by Tanner stages[7] that track the development of pubic hair (adrenarche) and breasts or genitalia (gonadarche) separately in girls and boys. Tanner stage 1 represents prepuberty, stages 2 through 4 represent increasing maturity, and stage 5 represents full adult development. Timing refers to the age at which an individual reaches a certain point in the developmental trajectory (e.g., menarche, peak height velocity, midpoint of puberty) relative to norms or same-age, same-sex peers. Tempo refers to the rate of pubertal development. Relatively few twin studies have examined tempo, and of those that do, there is a variety of ways in which tempo has been defined. This makes genetic (among other) data difficult to interpret, so we will not discuss tempo further in this chapter.

27.2 Measuring puberty

Table 27.1 shows major twin studies in which puberty has been examined, with a focus on puberty measurement. Study designs were prospective, retrospective, or cross-sectional. Measures differed in when they were administered and whether they assessed gonadarche (e.g., menarcheal age, breast growth, luteinizing hormones, gonadal hormones, voice breaking), growth (e.g., height), or a combination of processes (e.g., as assessed with common measures described later in this section). No studies included in Table 27.1 assessed only adrenarche. Status and timing were the two aspects of puberty that were most assessed. Most studies were cross-sectional and used child report. Cross-sectional studies varied in the ages of their samples, with some studies focusing on a specific year[12,21–22,24–25] while others recruited wider age ranges.[15–16,30–37] Prospective studies typically assessed across two or three measurement occasions,[8,12,20–23,27,38] with the exception of the Colorado Longitudinal Twin Study (LTS), which assessed puberty annually from ages 9–15.[9–10] For more information on how puberty is assessed in research, refer to papers outlining the importance of good puberty measurement.[6,39]

Pubertal status is best measured through physical examination by a health professional using standards for Tanner stages.[6] Because exams are invasive and must be performed by a trained clinician, it is more common to obtain information about pubertal development by having youth or their parents provide reports, using standard questionnaires, which contain descriptions to which the respondent compares him/herself.[26,40] For instance, the Pubertal Development Scale (PDS)[41] is a common measure that provides a total summary score based on the degree of development of secondary

TABLE 27.1 Puberty measurement in major twin studies.

Twin study	Paper(s)	Aspect	Study design	Report type	Measure	When assessed
Australian Twin Registry	8	Status	Prospective	Exam	Tanner	7–18 years (2 waves)
Colorado Longitudinal Twin Sample (Colorado LTS)	9–10	Timing	Prospective	Child	PDS[b] Menarcheal age	9–15 years (Annually)
Finnish Twin Cohort Study (FinnTwin)	11	Timing	Retrospective	Child	Menarcheal age	16 years
	12		Cross-sectional	Child	PDS	♀ 11.5 years ♂ 14 years
			Prospective	Biological	Height difference in standard deviations	♀ 11.5, 17.5 years ♂ 14, 17.5 years (2 waves)
	13	Development	Prospective	Child	PDS	12, 14 years (2 waves)
	14					11, 14 years (2-wave composite)
Michigan State University Twin Registry (MSUTR)	15[a]	Status	Cross-sectional	Child	PDS	10–28 years
	16					8–25 years
Minnesota Twin Family Study (MTFS)	17	Status	Cross-sectional	Child	PDS	11 or 17 years
	18					13–16 years
	15[a]					10–28 years
	19	Timing	Cross-sectional	Child	Menarcheal age	14–17 years
National Longitudinal Study of Adolescent Health (AddHealth)	20–23	Timing	Prospective	Child	Menarcheal age	11–21 years (3 waves)
	20–22		Retrospective	Child	Breast size during grade school	11–21 years

(Continued)

TABLE 27.1 Cont'd

Twin study	Paper(s)	Aspect	Study design	Report type	Measure	When assessed
					Body curviness during grade school	11–21 years
	21–22		Cross-sectional	Child	Physical development compared to peers	11–21 years
Netherlands Twin Registry (NTR)	24–25	Status	Cross-sectional	Child	Tanner	12 years
	26			Exam	Tanner	9 years
	27		Prospective	Exam	Tanner	9, 12 years (2 waves)
	28		Cross-sectional	Biological	Luteinizing hormone	9 years
Swedish Twin study of Child and Adolescent Development (TCHAD)	29	Status	Cross-sectional	Child	PDS	12–14 years
Texas Twin Project	30–33	Status	Cross-sectional	Child	PDS	7–20 years
	34–35					13–20 years
	30				Menarcheal age	7–20 years
				Biological	Gonadal hormones	13–20 years
Virginia Twin Study of Adolescent Behavioral Development	36	Onset	Cross-sectional	Mother	Voice breaking (CAPA)[c]	8–16 years
	37	Status	Cross-sectional	Child	Menarcheal age (CAPA)	8–17 years
	38	Timing	Prospective	Child	CAPA	8–17 years 3 waves

[a] *Indicates that the paper combined samples across twin studies.*
[b] *PDS: Pubertal Development Scale.*
[c] *CAPA: Child and Adolescent Psychiatric Assessment.*
Note: Number of waves in prospective study designs are provided in parentheses. Symbols for girls (♀) and boys (♂) are used when puberty assessment differed by sex.

sexual characteristics that reflect adrenarche, gonadarche, and physical growth. Both Tanner stages and the PDS are widely used and are able to measure status from early to late development. Despite some concerns about validity of the PDS in particular in measuring certain stages of puberty[42] and interrater reliability,[43] twin studies that use either Tanner stages or the PDS provide relatively good assessments of puberty.

Pubertal timing can be inferred from an individual's pubertal status (e.g., measured by the PDS) relative to age, peer group, or norm. Multiple assessments of status across adolescence[9–10] are necessary to achieve good estimates of the timing of different aspects of pubertal development, including onset, midpoint, and full attainment of adult characteristics.

Cross-sectional studies that attempt to use a single assessment of status to infer timing[12] risk conflating the two. Imagine, for example, a cross-sectional study of 11-year-old girls. If the pubertal status is indicated by age at menarche, then all girls who have not yet reached menarche will be considered "late." In the future, maybe half of those "late bloomers" will reach menarche before age 13, which is widely considered normative and on time, whereas the other half of the "late bloomers" will truly have late menarche (i.e., after age 13). In this study, a significant proportion of girls with on-time puberty would have been incorrectly labeled as late maturers.

Interpretation of pubertal timing findings from a single measure becomes even more difficult when a sample's age range is so wide[8,30–35] that it includes some participants who are just starting puberty and others who have already completed it. For instance, a cross-sectional study with 100 participants who range from 7 years old to 17 years old would include 100 data points spread across all five Tanner stages, whereas a prospective study with 100 participants who are all recruited at age 7 and are followed across puberty would eventually yield at least 100 data points for each Tanner stage. It would be difficult to make strong interpretations of puberty effects from the former study, especially compared to the latter study's much larger dataset.

The problem of conflating status with timing in cross-sectional studies and longitudinal studies that have only one puberty assessment is partly circumvented by asking people to report specific ages at which different aspects of puberty occurred. This is relatively easy to do in girls by asking for menarcheal age,[9–11,19–23,30,37] that is, when girls experience their first menstrual bleeding. But this is more difficult in boys who lack a distinct pubertal development marker. The Virginia Twin Study of Adolescent Behavioral Development assessed onset of voice breaking in male twins,[36] but this is not a clear equivalent to menarcheal age. Other puberty studies[44] have considered spermarche in boys, which is equivalent to menarche but is more difficult to measure (e.g., requires biological sampling). Additionally, reports of when a certain status has been reached are generally retrospective (often rated years after the event has occurred),[11,20–21] raising issues of recall bias such as validity based on how memorable the event was, how specific the event was to a particular age, and how far back the participant has to recall the event.

Biological measures of puberty include assessment of physical growth[12] and collection of hormonal assays (i.e., luteinizing hormones, testosterone, estradiol) as direct measurements of underlying hormonal changes during puberty.[28–30] Though

hormonal measures are considered more objective than physical exams and participant report, they can be influenced by other factors, such as diet, genes, and physiological rhythms. Interpretation of results from growth and hormone data should be taken with caution unless they are measured repeatedly over time (because in longitudinal studies individuals serve as their own baseline).

It is important to keep in mind that the timing of measurement is critical for the specific questions that researchers are trying to answer. For instance, assessments completed during age 12 are ideal for questions about the midpoint of puberty for girls but are limited for questions about pubertal onset. Additionally, differences across studies' findings might reflect differences in the ways that puberty was measured, so it is important to consider convergence across methods when interpreting results.

27.3 Variations in puberty: Gene and environment

Puberty is an essential biological process, and so is not surprisingly under strong genetic control. Much of the variation in both normative and abnormal pubertal development is attributable to genetic variation. Genome-wide association studies have revealed many genetic variations that can contribute to pubertal development.[45] For instance, over 380 different signals are associated with menarcheal age,[46] and genetic mutations are implicated in disorders of pubertal timing, such as Kallmann syndrome (delayed or absent puberty) and precocious puberty (early puberty).[47] Additionally, kisspeptin neurons in the hypothalamus are thought to regulate the release of gonadotropin-releasing hormone (GnRH), which serves as the catalyst for gonadarche. *KISS1*, *GNRHR*, *KAL1*, *FGFR1*, *FGF8*, *PROK2*, *CHD7*, and *WDR11*, just to name a few genes, are all implicated in the release of GnRH.[45] It is likely that multiple networks of genes in the central nervous system are all involved in the start, progression, and end of the different pubertal processes. Refer to Lee and Styne's review for more detail on genetic influences on puberty.[1]

Studies with singletons have found that genetic influences can explain about 46%–50% of variance in pubertal development in girls and 40%–45% in boys.[48–49] Thus, pubertal development is not fully determined by genes. The remaining variation in pubertal development in both girls (48%–49%) and boys (55%) can be explained by environmental influences.[48] Pubertal timing can be affected by many different aspects of the physical environment. For example, because puberty is heavily influenced by the endocrine system, endocrine-disrupting chemicals found in natural (e.g., tofu, soy) and synthetic (e.g., industrial solvents, fungicides, pesticides) products have been shown to either advance or delay puberty.[1] Childhood nutrition and diet have also been observed to have effects on pubertal timing, with intake of animal products related to earlier puberty and intake of vegetable protein related to later puberty.[50] Additionally, puberty can be changed by social experiences: accelerated by early family adversity, including father absence and parental harshness,[51] and delayed by extreme exercise.[52] Below we discuss evidence about the relative influences of genes and environment on puberty provided by twin studies.

27.4 Twin studies of puberty

Twin studies in puberty are uniquely useful (e.g., beyond large longitudinal studies of singletons) for providing insight into puberty and its links to behavior because they provide an opportunity to "control for," or hold constant, shared experiences and genetic background in order to examine effects on the behavior of other factors or experiences that differ between twins. Twin studies with large samples, strong puberty measures, and repeated data collection are ideal when studying contributors to pubertal variations. In reality, having all three components is difficult; it takes time and money to recruit and longitudinally assess many twin pairs, some of whom eventually drop out of the study. Most twin studies of puberty have examined pubertal timing, which is the focus of the following section. These studies reflect to what degree pubertal timing is heritable. Some studies have linked timing to psychological outcomes, which we discuss in the next section. These studies provide insight into the heritability of internalizing and externalizing behaviors across puberty.

27.4.1 Heritability of pubertal timing

Heritability of pubertal timing refers to how much variation in timing is due to genetic influence. The strongest evidence for heritability of pubertal timing comes from the Finnish Twin Cohort Study (FinnTwin) and the Colorado Longitudinal Twin Sample (LTS), due to the former's large sample size and the latter's use of annual PDS assessments across puberty. FinnTwin researchers used data from 20,000+ twin pairs across multiple birth cohorts to show that timing for secondary sexual characteristics is highly heritable. Same-sex monozygotic (MZ) twins were more similar in timing measured by the PDS (girls $r = .82$; boys $r = .72$) than were same-sex dizygotic (DZ) twins (girls $r = .51$; boys $r = .20$),[12] indicating that those who shared the same genes are more likely to be closer in age regarding the timing of pubertal. Colorado LTS provides important prospective data from ages 9–15. Growth curve estimates from pubertal development trajectories revealed timing to be highly heritable for both sexes (77% in girls, 62% in boys) in this study.[10]

The methodological strengths of these two studies are bolstered by other studies' findings on pubertal timing heritability. Timing was more correlated among MZ (girls $r > 0.99$; boys $r = 0.88$) than DZ (girls $r = 0.52$; boys $r = 0.44$) same-sex twins from the Virginia Twin Study.[38] Similarly, pubertal development was found to be moderately to strongly heritable in the Netherlands Twin Registry (NTR) when examined cross-sectionally in 12-year-olds, although there was notable overlap in the MZ and DZ estimates, likely owing to imprecision of the cross-sectional measurement (girls MZ *r*s 0.56–0.96 vs DZ *r*s 0.35–0.73; boys MZ $r = 0.97$ vs DZ $r = 0.75$).[24–25]

Estimates of environmental influences on pubertal timing are relatively smaller in twin studies than genetic influences, with effects ranging 0%–58% in girls and 0%–55% in boys.[10,14,25,30,38] The wide ranges for environmental influences across studies may be explained by differences in puberty measurement, with larger estimates from studies that either assessed puberty only once[25] or from a sample with

a wide age range.[30] Sample differences could also play a role in inconsistencies of these findings, and one way to address this issue is to dedicate more work to studying populations traditionally underrepresented in research. For instance, the Texas Twin Project has recruited a sample of twins that is almost 50% Hispanic or Latinx and about 20% Black.[53] Twin studies overall show that it is likely that variation in pubertal timing is much less influenced by environment than by genes, but more work with varied environments is needed.

27.4.2 Twin studies of links between puberty and behavior

Variations in pubertal timing are linked with many behavioral problems in singletons.[3] Girls with earlier pubertal timing are at higher risk for disordered eating and depressive problems starting in adolescence. Conversely, boys with off-time puberty are at higher risk for conduct and substance use problems starting in adolescence or young adulthood in some cases. Twin studies can help determine if there are common genetic factors that drive such connections between puberty and behavior as well as give insight into common environmental effects. As depicted in Fig. 27.1, we focus on studies linking puberty to internalizing (e.g., disordered eating, depression) and externalizing problems (e.g., rule-breaking, conduct disorder, risk-taking, substance use) because these associations have been most well-studied in twins.

Internalizing Behavior Problems. Data from the Minnesota Twin Family Study (MTFS) suggest that genetic influences on disordered eating (i.e., unhealthy eating

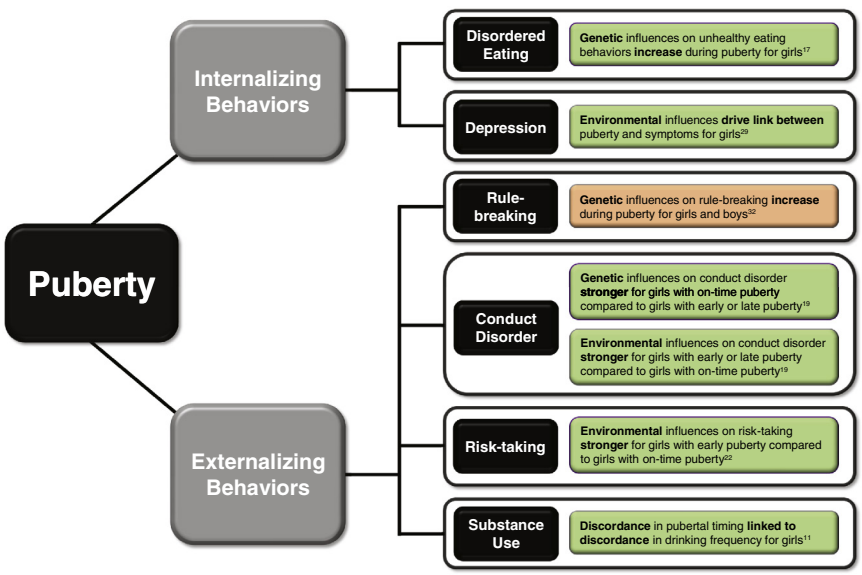


FIG. 27.1 Links found between puberty and behavior in twin studies.

Note. Findings for girls only are in green. Findings for girls and boys are in orange. There are no findings for boys only.

behaviors, such as restricting food, excessive exercise, and binge eating) increase at puberty. Genetic influences were found to be low (0% of variance) in prepubertal 11-year-old girls but moderate (54% of variance) in pubertal 11- and 17-year-old girls.[17] This suggests that increases in pubertal hormones influence gene networks important for disordered eating in girls. Nevertheless, puberty may not be important for genetic influences on other disordered eating risk factors and characteristics, including thin-ideal internalization and bulimia symptoms. Researchers working with data from the Michigan State University Twin Registry (MSUTR) examined whether pubertal development moderated associations between genetic and environmental influences and female twins' thin-ideal internalization (i.e., an individual's acceptance of the societal ideal that body thinness is attractive). The MSUTR data showed that thin-ideal internalization was more influenced by environment than genes in girls with no moderation effect of pubertal development;[16] this may be because body ideals can be socialized based on culture and context.[54] Additionally, menarcheal status was not found to moderate genetic or environmental influences on bulimia symptoms in a cross-sectional examination of 1502 participants aged 8–17 years from the Virginia Twin Study; heritability of bulimia symptoms did not differ in twins who were premenarcheal versus postmenarcheal.[37] Inconsistencies with MTFS and Virginia Twin Study findings may be due to differences in the measurement of puberty (i.e., midpoint of puberty in MTFS versus menarcheal status in Virginia Twin Study) or the age ranges of the samples themselves (i.e., same-age cohorts of 11- and 17-year-olds in MTFS versus a cohort ranging from 8 to 17 years old in Virginia Twin Study). It is also important to note that work on links between puberty and disordered eating in twin studies has been limited to girls, so it is not known whether genetic influences on disordered eating in boys follow the same pattern.

Puberty may moderate environmental rather than genetic influences on depression. Links between pubertal status and internalizing symptoms in girls were found to be largely driven by shared environmental influences in twins in the Swedish Twin study of Child and Adolescent Development (TCHAD).[29] In twin studies, shared environmental influences refer to nongenetic factors that twins have in common, such as parents and family (as opposed to nonshared environmental influences that twins do not have in common, such as friends and classrooms). Future research should examine whether these findings regarding shared environmental influences also hold true in boys. Genetic influences on internalizing problems did not change throughout puberty for Colorado LTS[10] and Texas Twin Project[33] girls and boys. Together, these findings suggest that, across development, the presence of depressive behaviors becomes more dependent on environment, such as stress exposure,[55] for girls specifically. Additionally, the findings from Colorado LTS and Texas Twin Project suggest that genetic influence stays consistent across puberty in both girls and boys. Though singleton data have shown that hormone levels at puberty can contribute to sex differences in depression that emerge during adolescence,[56] twin findings so far suggest that hormonal influences on sex differences do not change over time, particularly throughout puberty.

Externalizing Behavior Problems. Both pubertal status and timing have been found to influence the level of variation in externalizing behaviors. Heritability of rule-breaking increased with pubertal development for girls and boys; heritability was 19% for Texas Twin Project participants with a PDS score of 1 (pubertal development had not yet begun) and 71% for individuals with a PDS score of 4 (pubertal development was complete),[32] although findings are difficult to interpret as they conflate pubertal development with age-based trajectories of risk-taking (e.g., it is unclear whether puberty or age underlies the effects). MTFS work showed that genetic influences on conduct disorder were stronger for female twins who had on-time puberty whereas shared environmental influences were stronger for female twins who had early or late pubertal timing.[19] Girls from the National Longitudinal Study of Adolescent Health (AddHealth) with early menarche showed stronger links between their own risk-taking and the risk-taking of their peers than girls with on-time menarche.[22] The latter two findings suggest that genes may be more influential on behavior in a typical environment and less influential on behavior in an atypical environment for girls. Due to relative lack of data in opposite-sex twins allowing for examination of overlap in influences acting on externalizing behaviors, it is unknown at this point whether the same genetic and environmental influences on externalizing problems in girls are also present in boys.

Twin studies can be leveraged to examine variations in links between puberty and behavior within twin pairs, too. Discordant designs focus on differences between twins who have the same genetic influence and common experiences in order to isolate non-shared experiences that are likely to contribute to the differences.[57] FinnTwin girls who were discordant on menarcheal age (i.e., twins who did not start menarche at the same age) were also discordant on drinking frequency, a substance use measure; specifically, girls who started menarche earlier than their cotwins were more likely to report higher drinking frequency.[11] This is consistent with work in singletons showing that girls with earlier pubertal timing subsequently reported more substance use,[58] but this work is more compelling because of the nature of the discordant design available to twin studies, which is discussed further in a later section.

27.5 Other uses of twin data on puberty when studying behavior

Aside from twin study-specific modeling approaches, there are other useful ways to examine twin data when answering questions about puberty and behavior. These include attempts to replicate findings and examine sex differences.

27.5.1 Replication analyses

There is increasing emphasis on replication in many areas of research, including psychological science because several findings have not been reproduced.[59] This raises questions about sample size, measurement, and data analysis. Twins provide an opportunity for replication, especially because twin studies are often large: a research

question can be addressed in a sample of one twin from each pair and then repeated in another sample of the other twin from each pair. If findings are the same, then this increases confidence in the findings. If findings are not replicated, then this may indicate either that the original finding was a false positive or that there were issues in methodology when the study was conducted. So far, few studies looking at pubertal development in twins have taken advantage of replication analyses. One exception is a study from Colorado LTS. Results on estimated pubertal trajectories as well as correlations between pubertal timing and tempo, and internalizing and externalizing problems were generally consistent across replicates, suggesting that findings are robust.[9]

27.5.2 Examining sex differences

Twin data can help to understand if there are significant differences in the heritability of pubertal timing between girls and boys. Looking at opposite-sex twins who grow up in the same environment allows for examination of this question. Australian Twin Registry data suggest that pubertal status may be similarly heritable in girls and boys; 66 opposite-sex twins were concordant on pubertal status vs 24 opposite-sex twins who were discordant.[8] It is important to replicate this finding in a larger sample and to extend this finding by comparing it to heritability of pubertal status in same-sex twins. Additionally, work comparing opposite-sex twins on pubertal status should be expanded to investigations of whether heritability of pubertal timing differs by sex.

A more common way of studying sex differences with twin puberty data is to compare male and female same-sex pairs on puberty–behavior links in order to determine if there are different genetic variations influencing outcomes by sex. Colorado LTS researchers found no sex differences in whether puberty moderated heritability of behavior problems in male and female same-sex twins; puberty did not moderate genetic influences on behavior problems in either males or females.[10] Within the Texas Twin Project, sex did not influence how pubertal development is associated with reward sensitivity, but it was differentially associated with sensation seeking (positively in males and negatively in females).[35] These different effects found in the same study may be due to the different ways of operationalizing reward sensitivity (i.e., latent factor based on performance on multiple behavioral tasks) and sensation seeking (i.e., self-report on one questionnaire). Researchers often look at effects separately in each sex but do not compare them statistically, which will be important in moving forward with understanding sex differences in genetic influences on puberty–behavior links.

27.6 Methodological issues

There are sometimes significant concerns about puberty measurement in many studies, including twin studies. This is likely because puberty is not a focus of these studies, and pubertal timing assessment often is not consistently done with the best measures possible. Some studies look at puberty explicitly whereas others look at puberty incidentally. Improvements in measurement *are* occurring. For instance, the

Texas Twin Project is using multiple measures through multiple modes, including hormone assays. These strengths, however, pose some challenges. As previously mentioned, direct measurement of hormones can be influenced by other non-pubertal processes.

We also previously mentioned the need for research on populations traditionally underrepresented in research. Having a diverse sample is beneficial for increasing understanding of variations in pubertal timing that may be influenced by culture and context. Singleton studies have contributed to this literature. For instance, Black girls tend to start puberty earlier (e.g., earlier onset of breast and pubic hair development as well as earlier menarche) than White girls in the United States.[60] Additionally, pubertal timing's associations with behavioral outcomes can differ by racial/ethnic groups; early and later pubertal timing as measured by the PDS are more associated with depressive symptoms for Latina girls than for Black and White girls in the United States.[61] Such differences in puberty–behavior links may be driven by culture and context as well. For example, early pubertal timing was positively associated with externalizing problems for Caribbean Black girls whereas timing was not associated with externalizing problems for American Black girls.[62] These findings in singletons could be replicated in twin studies, which have the added benefit of a genetically-informative design. Though studies like the Texas Twin Project are helping contribute to puberty work by recruiting from different racial and ethnic populations, results from such studies of diverse samples are difficult to interpret if cross-cultural differences are not thoughtfully operationalized and if each race and ethnicity are not properly represented by large numbers.

Another issue some studies face when answering questions about puberty is accounting for wide age ranges common to cross-sectional twin studies. Having a wide age range causes confounds with general trends in adolescent development, making it difficult to know if something is occurring due to changing pubertal hormones specific to the individual or due to general adolescent processes like new friendships, parental conflict, or neural maturation.

27.7 Future directions

Opportunities are ripe for future work, and there are some knowledge gaps that twin studies can tackle. Work on the brain and neural development during puberty is emerging. Additionally, cultural influences on puberty links with behavior and brain can be explored. Designs unique to twin studies like discordant designs can also be used more in future puberty research.

27.7.1 Studying links between puberty and the brain

Twin studies are becoming recognized as an opportunity to study heritability of neural features and changes during puberty. Most work comes from the NTR and findings so far are about brain structure heritability at the start of puberty, though puberty was inferred from age and not explicitly linked to the brain.[26] Longitudinal examination

across puberty showed general brain and white matter volume to be heritable at both ages 9 and 12, and the negative association between increases in white matter growth and structural connectivity to be influenced by environmental factors.[27,63] This research is promising, but there are still knowledge gaps on function that can be filled. For instance, brain function underlying links in disordered eating identified in an earlier section could be explored, and brain function underlying reward sensitivity and sensation seeking may clarify conflicting results.

27.7.2 Potential impact of cultural differences

Thus far, it is unclear whether findings on puberty–behavior and puberty–brain links can be generalized across cultures. For example, American twin studies primarily focus on mental health outcomes whereas work on brain development during puberty comes from Dutch twin research. Future work could take an international, cross-cultural perspective by using existing data from twin studies around the world. The use of multiple data sets would also speak to questions about replicability.

27.7.3 Continued use of the discordant design

Researchers using twin data in future investigations of puberty can use a discordant design with which they can examine whether twins who have different pubertal development trajectories also have different behavioral outcomes or vice versa. Other than one study previously mentioned in this chapter,[11] the discordant design has not been applied widely to other work on twin puberty and behavior. As more researchers take advantage of this approach, more will be uncovered on how and through what mechanisms differences in behavior are impacted by differences in puberty.

27.7.4 Addressing limitations

We previously highlighted measurement, generalizability, and age specificity issues present in past studies of puberty. Future studies can overcome these limitations by making sure to use the best possible measures of puberty available, recruiting large enough numbers of different races and ethnicities, and following same-age individuals prospectively across adolescence. Researchers using data from existing studies can be transparent about study strengths and limitations, as findings from these data will still be informative as long as they are not overstated.

27.8 Conclusion

Twin studies have enhanced research on puberty by revealing that pubertal timing is highly heritable (about 70%) in both sexes and that puberty increases genetic influences on some behaviors at puberty. Twin studies have helped push puberty work forward because of their unique ability to parse genetic and environmental

influences. Not surprisingly, twin studies have just scratched the surface of what can be done, so there are many opportunities for future studies that take advantage of longitudinal assessments in functional neuroimaging data, studies of youth from wide ranges of cultures, and discordant designs to answer questions about puberty and behavior.

27.9 Takeaways

- Twin studies show that pubertal timing is highly heritable in both sexes.
- Pubertal development might increase genetic influences on behavior, especially disordered eating behaviors in girls. This suggests that pubertal processes activate genes important for those behaviors. There is less known about puberty–behavior links in boys due to relatively less available data.
- Future twin studies would benefit from greater attention to methodology, especially pubertal measurement and sample diversity.
- There is still much to discover about puberty and behavior links, such as sex differences, neural underpinnings, and cultural mechanisms. Twin studies are well-poised to continue filling knowledge gaps with their genetically informative design.

References

1. Lee Y, Styne D. Influences on the onset and tempo of puberty in human beings and implications for adolescent psychological development. *Hormones and Behavior*. 2013 1;64:250–261.
2. Belsky J. Early-life adversity accelerates child and adolescent development. *Current Directions in Psychological Science*. 2019;28:241–246.
3. Graber JA. Pubertal timing and the development of psychopathology in adolescence and beyond. *Hormones and Behavior*. 2013;64:262–269.
4. Romeo RD. Perspectives on stress resilience and adolescent neurobehavioral function. *Neurobiology of Stress*. 2015;1:128–133.
5. Blakemore SJ, Burnett S, Dahl RE. The role of puberty in the developing adolescent brain. Human Brain Mapping. 2010;31:926-33.
6. Dorn LD, Biro FM. Puberty and its measurement: a decade in review. *Journal of Research on Adolescence*. 2011;21:180–195.
7. Tanner JM. Growth at adolescence. Oxford (UK): Blackwell Scientific Publications; 1962.
8. Iuliano-Burns S, Hopper J, Seeman E. The age of puberty determines sexual dimorphism in bone structure: a male/female co-twin control study. *The Journal of Clinical Endocrinology & Metabolism*. 2009;94:1638–1643.
9. Beltz AM, Corley RP, Bricker JB, Wadsworth SJ, Berenbaum SA. Modeling pubertal timing and tempo and examining links to behavior problems. *Developmental Psychology*. 2014;50:2715–2726.
10. Corley RP, Beltz AM, Wadsworth SJ, Berenbaum SA. Genetic influences on pubertal development and links to behavior problems. *Behavior Genetics*. 2015;45:294–312.

11. Dick DM, Rose RJ, Viken RJ, Kaprio J. Pubertal timing and substance use: associations between and within families across late adolescence. *Developmental Psychology*. 2000;36:180–189.
12. Wehkalampi K, Silventoinen K, Kaprio J, et al. Genetic and environmental influences on pubertal timing assessed by height growth. *American Journal of Human Biology*. 2008;20:417–423.
13. Dick DM, Rose RJ, Pulkkinen L, Kaprio J. Measuring puberty and understanding its impact: a longitudinal study of adolescent twins. *Journal of Youth and Adolescence*. 2001;30:385–399.
14. Mustanski BS, Viken RJ, Kaprio J, Pulkkinen L, Rose RJ. Genetic and environmental influences on pubertal development: longitudinal data from Finnish twins at ages 11 and 14. *Developmental Psychology*. 2004;40:1188–1198.
15. Culbert KM, Burt SA, McGue M, Iacono WG, Klump KL. Puberty and the genetic diathesis of disordered eating attitudes and behaviors. *Journal of Abnormal Psychology*. 2009;118:788–796.
16. Suisman JL, Thompson JK, Keel PK, Burt SA, Neale M, Boker S, Sisk C, Klump KL. Genetic and environmental influences on thin-ideal internalization across puberty and preadolescent, adolescent, and young adult development. *International Journal of Eating Disorders*. 2014;47:773–783.
17. Klump KL, McGue M, Iacono WG. Differential heritability of eating attitudes and behaviors in prepubertal versus pubertal twins. *International Journal of Eating Disorders*. 2003;33:287–292.
18. Klump KL, Perkins PS, Burt SA, McGue MA, Iacono WG. Puberty moderates genetic influences on disordered eating. *Psychological Medicine*. 2007;37:627–634.
19. Burt SA, McGue M, DeMarte JA, Krueger RF, Iacono WG. Timing of menarche and the origins of conduct disorder. *Archives of General Psychiatry*. 2006;63:890–896.
20. Harden KP, Mendle J. Gene-environment interplay in the association between pubertal timing and delinquency in adolescent girls. *Journal of Abnormal Psychology*. 2012;121:73–87.
21. Harden KP, Mendle J, Kretsch N. Environmental and genetic pathways between early pubertal timing and dieting in adolescence: Distinguishing between objective and subjective timing. *Psychological Medicine*. 2012;42:183–193.
22. Kretsch N, Mendle J, Harden KP. A twin study of objective and subjective pubertal timing and peer influence on risk-taking. *Journal of Research on Adolescence*. 2016;26:45–59.
23. Mendle J, Moore SR, Briley DA, Harden KP. Puberty, socioeconomic status, and depression in girls: evidence for gene × environment interactions. *Clinical Psychological Science*. 2016;4:3–16.
24. Hoekstra RA, Bartels M, Boomsma DI. Heritability of testosterone levels in 12-year-old twins and its relation to pubertal development. *Twin Research and Human Genetics*. 2006;9:558–565.
25. van den Berg SM, Setiawan A, Bartels M, Polderman TJ, van der Vaart AW, Boomsma DI. Individual differences in puberty onset in girls: Bayesian estimation of heritabilities and genetic correlations. *Behavior Genetics*. 2006;36:261–270.
26. Peper JS, Schnack HG, Brouwer RM, et al. Heritability of regional and global brain structure at the onset of puberty: a magnetic resonance imaging study in 9-year-old twin pairs. *Human Brain Mapping*. 2009;30:2184–2196.
27. van Soelen IL, Brouwer RM, van Baal GC, et al. Heritability of volumetric brain changes and height in children entering puberty. *Human Brain Mapping*. 2013;34:713–725.

28. Peper JS, Brouwer RM, Schnack HG, et al. Cerebral white matter in early puberty is associated with luteinizing hormone concentrations. *Psychoneuroendocrinology*. 2008;33:909–915.
29. Marceau K, Neiderhiser JM, Lichtenstein P, Reiss D. Genetic and environmental influences on the association between pubertal maturation and internalizing symptoms. *Journal of Youth and Adolescence*. 2012;41:1111–1126.
30. Grotzinger AD, Briley DA, Engelhardt LE, et al. Genetic and environmental influences on pubertal hormones in human hair across development. *Psychoneuroendocrinology*. 2018;90:76–84.
31. Harden KP, Kretsch N, Tackett JL, Tucker-Drob EM. Genetic and environmental influences on testosterone in adolescents: Evidence for sex differences. *Developmental Psychobiology*. 2014;56:1278–1289.
32. Harden KP, Patterson MW, Briley DA, et al. Developmental changes in genetic and environmental influences on rule-breaking and aggression: age and pubertal development. *Journal of Child Psychology and Psychiatry*. 2015;56:1370–1379.
33. Patterson MW, Mann FD, Grotzinger AD, Tackett JL, Tucker-Drob EM, Harden KP. Genetic and environmental influences on internalizing psychopathology across age and pubertal development. *Developmental Psychology*. 2018;54:1928–1939.
34. Grotzinger AD, Mann FD, Patterson MW, et al. Twin models of environmental and genetic influences on pubertal development, salivary testosterone, and estradiol in adolescence. *Clinical Endocrinology*. 2018;88:243–250.
35. Harden KP, Mann FD, Grotzinger AD, et al. Developmental differences in reward sensitivity and sensation seeking in adolescence: Testing sex-specific associations with gonadal hormones and pubertal development. *Journal of Personality and Social Psychology*. 2018;115:161–178.
36. Pickles A, Crouchley R, Simonoff E, Eaves L, Meyer J, Rutter M, Hewitt J, Silberg J. Survival models for developmental genetic data: age of onset of puberty and antisocial behavior in twins. *Genetic Epidemiology*. 1994;11:155–170.
37. Rowe R, Pickles A, Simonoff E, Bulik CM, Silberg JL. Bulimic symptoms in the Virginia Twin Study of adolescent behavioral development: correlates, comorbidity, and genetics. *Biological Psychiatry*. 2002;51:172–182.
38. Eaves L, Silberg J, Foley D, et al. Genetic and environmental influences on the relative timing of pubertal change. *Twin Research and Human Genetics*. 2004;7:471–481.
39. Berenbaum SA, Beltz AM, Corley R. The importance of puberty for adolescent development: conceptualization and measurement. *Advances in Child Development and Behavior*. 2015;48:53–92.
40. Morris NM, Udry JR. Validation of a self-administered instrument to assess stage of adolescent development. *Journal of Youth and Adolescence*. 1980;9:271–280.
41. Petersen AC, Crockett L, Richards M, Boxer A. A self-report measure of pubertal status: reliability, validity, and initial norms. *Journal of Youth and Adolescence*. 1988;17:117–133.
42. Huang B, Hillman J, Biro FM, Ding L, Dorn LD, Susman EJ. Correspondence between gonadal steroid hormone concentrations and secondary sexual characteristics assessed by clinicians, adolescents, and parents. *Journal of Research on Adolescence*. 2012;22:381–391.
43. Dorn LD, Susman EJ, Nottelmann ED, Inoff-Germain G, Chrousos GP. Perceptions of puberty: adolescent, parent, and health care personnel. *Developmental Psychology*. 1990;26:322–329.
44. Chen TJ, Ji CY, Hu YH. Genetic and environmental influences on serum lipids and the effects of puberty: a Chinese twin study. *Acta Paediatrica*. 2009;98:1029–1036.

45. Aylwin CF, Toro CA, Shirtcliff E, Lomniczi A. Emerging genetic and epigenetic mechanisms underlying pubertal maturation in adolescence. *Journal of Research on Adolescence*. 2019;29:54–79.
46. Day FR, Thompson DJ, Helgason H, et al. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. *Nature Genetics*. 2017;49:834–841.
47. Herbison AE. Genetics of puberty. *Hormone Research in Paediatrics*. 2007;68(Suppl. 5): 75–79.
48. Ge X, Natsuaki MN, Neiderhiser JM, Reiss D. Genetic and environmental influences on pubertal timing: results from two national sibling studies. *Journal of Research on Adolescence*. 2007;17:767–788.
49. Towne B, Czerwinski SA, Demerath EW, Blangero J, Roche AF, Siervogel RM. Heritability of age at menarche in girls from the Fels Longitudinal Study. American Journal of Physical Anthropology. 2005;128:210-9.
50. Villamor E, Jansen EC. Nutritional determinants of the timing of puberty. *Annual Review of Public Health*. 2016;37:33–46.
51. Ellis BJ, McFadyen-Ketchum S, Dodge KA, Pettit GS, Bates JE. Quality of early family relationships and individual differences in the timing of pubertal maturation in girls: A longitudinal test of an evolutionary model. *Journal of Personality and Social Psychology*. 1999;77:387–401.
52. Warren MP. The effects of exercise on pubertal progression and reproductive function in girls. *The Journal of Clinical Endocrinology & Metabolism*. 1980;51:1150–1157.
53. Harden KP, Tucker-Drob EM, Tackett JL. The Texas twin project. Twin Research and Human Genetics. 2013;16:385-90.
54. Fallon A, Cash TF, Pruzinsky T. Culture in the mirror: sociocultural deerminants of body image*Body Images: Development, Deviance, and Change*. New York, NY: Guilford Press; 1990:80–109.
55. Hammen C. Stress and depression. *Annu. Rev. Clin. Psychol.* 2005;1:293–319.
56. Angold A, Costello EJ, Erkanli A, Worthman CM. Pubertal changes in hormone levels and depression in girls. Psychological Medicine. 1999;29:1043-53.
57. Huibregtse BM, Bornovalova MA, Hicks BM, McGue M, Iacono W. Testing the role of adolescent sexual initiation in later-life sexual risk behavior: a longitudinal twin design. *Psychological Science*. 2011;22:924–933.
58. Tschann JM, Adler NE, Irwin CE, Millstein SG, Turner RA, Kegeles SM. Initiation of substance use in early adolescence: the roles of pubertal timing and emotional distress. Health Psychology. 1994;13:326-33.
59. Open Science Collaboration. Estimating the reproducibility of psychological science. *Science.* 2015;349.
60. Herman-Giddens ME, Slora EJ, Wasserman RC, et al. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Pediatrics*. 1997;99:505–512.
61. Siegel JM, Yancey AK, Aneshensel CS, Schuler R. Body image, perceived pubertal timing, and adolescent mental health. *Journal of Adolescent Health*. 1999;25:155–165.
62. Carter R, Caldwell CH, Matusko N, Antonucci T, Jackson JS. Ethnicity, perceived pubertal timing, externalizing behaviors, and depressive symptoms among black adolescent girls. *Journal of Youth and Adolescence*. 2011;40:1394–1406.
63. Brouwer RM, Mandl RC, Schnack HG, et al. White matter development in early puberty: a longitudinal volumetric and diffusion tensor imaging twin study. *PLoS One*. 2012;7:1–10.

CHAPTER

Musculoskeletal twin studies

28

Munkh-Erdene Bayartai, Paulo H. Ferreira

The University of Sydney, Charles Perkins Centre, Sydney School of Health Sciences, Faculty of Medicine and Health

28.1 Introduction (MSK conditions)

Musculoskeletal disorders are one of the most pervasive conditions that contribute substantially to illness, pain, and disability, as well as global health and economic burden.[1] The burden of disease, causing people to have a health status less than an ideal health is usually assessed by the number of years lived with disability (YLD). The prevalence of musculoskeletal disorders, leading to YLD has risen by around 66% between 1990 and 2017.[1] During this period, YLD due to common musculoskeletal disorders, namely, osteoarthritis (degenerative joint disease characterized by breakdown and loss of cartilage in the joints) and low back pain have increased by 114% and 52%, respectively. Low back pain has also been found to be one of the top leading causes of YLD. In addition to the health burden of musculoskeletal disorders, these conditions have substantially contributed to the global economic burden. For example, in Australia between 2015 and 2016, the economic burden related to musculoskeletal disorders was the highest, $12.5 billion compared to other disease groups, around 7.8% of the total expenditure on health goods and services,[2] whilst in the United States, the economic impact of musculoskeletal disorders was $980 billion dollars, accounting for 5.7% of the gross domestic product in 2014.[3] Aside from the economic impact in Europe, where the overall costs of work-related musculoskeletal disorders alone could reach as high as 2% of the gross domestic product, musculoskeletal disorders, particularly work-related low back pain, have been reported to be the leading cause of people's absence from work.[4,5] Furthermore, the increasing economic impact of low back pain in Japan was estimated around 1.2 trillion yen in 2014 due to due to lost productivity.[6] Therefore, it is crucial for the scientific community to conduct sophisticated and robust research on musculoskeletal conditions to lessen the increasing global health and economic burden. Involving twins or incorporating twin designs in musculoskeletal studies can contribute to the advancement of musculoskeletal research.

In this chapter, we will review the applications, benefits, as well as potential caveats of incorporating twin approaches in musculoskeletal studies. First, we will

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00027-1

TABLE 28.1 Applications, advantages, and limitations of twin designs in musculoskeletal research.

Design	Examples	Advantages	Limitations
Classical twin design	Genetic factors explained 62% of the total variance of knee osteoarthritis[12]	Simple and no genotyping is required to partition the total variance of a trait into genetic and environmental factors[8]	Challenging to ensure the equal environment assumption between MZ and DZ twin pairs[8]
Cotwin control design in non-interventional musculoskeletal studies	Genetics and early shared environment potentially act as confounding factors for the association between physical activity and recent low back pain[35]	Almost perfect genetic control can be achieved by involving MZ twin pairs[30]	Including DZ twin pairs to investigate risk factors or exposures makes the design less efficient than involving MZ twin pairs[31]
RCTs involving twins	The beneficial effect of taking calcium supplementation on bone mineral density was observed in prepubescent children[39]	Involving concordant MZ twin pairs in RCTs provides an opportunity to reach the desired statistical power with fewer participants compared to involving unrelated singletons[30,38]	Study recruitment is challenging as both twins from each pair are required to be concordant phenotypically to participate in a RCT[38]

MZ, monozygotic*; DZ,* dizygotic*; RCT,* randomized controlled trial.

discuss the benefits and applications of using the classic twin design, which helps to partition the total variance of the trait of interest into genetic and environmental factors. This approach has been applied to better understand genetic and environmental contributions to various traits in relation to the musculoskeletal system. Second, we will discuss the cocontrol twin design, its applications, and possibilities of controlling confounding factors to investigate risk factors (exposures) of musculoskeletal conditions. In the final section, we will discuss the applications of the working with twins in randomized controlled trials. The benefits and applications of each twin design will be followed by a discussion of potential caveats associated with using these twin approaches in musculoskeletal research (Table 28.1).

28.2 How twins can help musculoskeletal research

28.2.1 The classical twin design in musculoskeletal research

The classic twin design, perhaps the most common in twin research, aims to partition the total variance of a trait into genetic and environmental contributions by estimating heritability (genetic influence on trait variance) based on twin resemblance.[7] The

influence of genetics on the trait of interest is demonstrated in the classic twin design when monozygotic twin pairs, who are genetically identical, are more similar than dizygotic twin pairs, who share half of their genes.[8] In other words, the genetic influence on the variance of a trait is expected when correlations within monozygotic twin pairs are higher than dizygotic twin pairs. Heritability estimates are found in almost all human traits,[9] meaning individual differences in almost all the traits are explained by a combination of genetic and environmental factors. Additionally, the heritability of most human traits ranges between 0.3 and 0.6, which is considered to be moderate heritability.[10] The classic twin design can be applied to both cross-sectional and longitudinal musculoskeletal studies.

The classic twin design has commonly been applied to musculoskeletal research to estimate genetic and environmental contributions to the heritability of various musculoskeletal conditions. Estimating the heritability of musculoskeletal conditions provides valuable information regarding the extent to which genetic and environmental factors influence musculoskeletal traits or conditions, in addition to important implications for genetic association studies designed to further understand the role of genetics. For instance, the heritability of low back pain, one of the most common musculoskeletal conditions, has been estimated to be between 27% and 67% depending on the severity of low back pain,[11] meaning environmental factors can explain between 33% and 73% of the variance of low back pain. Additionally, genetic factors explain approximately 62% of the total variance of knee osteoarthritis.[12] The classic twin design to estimate heritability has not only been applied in musculoskeletal conditions but also in musculoskeletal traits. For example, heritability estimates for lumbar lordosis and total lumbar range of motion in the sagittal plane, the reduction of which is often associated with low back pain,[13] have been found to be 77%[14] and 47%,[15] respectively. A recent systematic review investigating the heritability of musculoskeletal motion in healthy people found that the total variance of gait speed, lumbar range of motion, and motor coordination is partially influenced by genetics although very few studies to date have estimated the heritability of musculoskeletal traits in relation to human motion.[16] In that systematic review, a meta-analysis of within-pair correlations of monozygotic and dizygotic twins found that the influence of genetic factors explained one-third of the total variance in gait speed. As in the earlier studies, it is optimal to include both monozygotic and dizygotic twin pairs to determine the influence of genetic factors on the variance of a trait. Nevertheless, an interesting finding from a meta-analysis that pooled within-pair correlations only based on monozygotic twin data has shown that shared familial factors, including genetics, more strongly influence the response of body composition than cardiorespiratory fitness.[17] However, the heritability of musculoskeletal traits has still been less investigated than other traits, such as psychiatric, metabolic, and neurologic traits.[9] Therefore, there is a need for more research to investigate genetic and environmental contributions to various traits in relation to musculoskeletal system.

Applying advanced analytical approaches in the classic twin design may assist in precisely estimating heritability and identifying potential mechanisms through

which genetics influences a musculoskeletal trait or condition. The simplest way of estimating heritability based on differences in within-pair correlations between monozygotic and dizygotic twins in the classic twin design has been extended by sophisticated analytic approaches, such as structural equation modeling, which, although potentially useful in the field of musculoskeletal research, has a relatively small number of musculoskeletal studies to date. As an extension of the classic twin design, potential confounding factors for the trait of an interest, such as sex and age, can be adjusted in the analysis of twin data.[7] For example, a previous study that included 300 monozygotic and dizygotic twin pairs has used age-adjusted univariate models to estimate the heritability of lumbar flexibility.[15] Additionally, multivariate genetic analyses of twin data could particularly be useful to identify pleiotropic traits associated with musculoskeletal system, as well as musculoskeletal conditions. In pleiotropy, genes responsible for one trait play a role in other traits, meaning a change in a trait may influence other traits due to their genetic correlations.[18] One of very few studies that used the approach in musculoskeletal research has investigated a potential pathway through which genetics affect lumbar disc degeneration.[15] In that study, lumbar extension range of motion has genetically and inversely been correlated with intervertebral disc degeneration, suggesting increased lumbar disc degeneration was associated with reduced lumbar extension. Therefore, more musculoskeletal research is needed to use the classic twin design extended by advanced analytic approaches such as multivariate genetic analysis to better understand the role of genetics in musculoskeletal traits and conditions beyond the most commonly known heritability estimates.

There are potential limitations to consider when using the classic twin design in musculoskeletal research. Although heritability is commonly estimated by using the classic twin design, it is challenging to ensure the equal environment assumption where environments that monozygotic and dizygotic twin pairs shared are assumed to be equal. The violation of this assumption can potentially affect the accuracy of heritability estimates.[7,8] For example, heritability of a trait can be overestimated if environments that twins shared are more similar in monozygotic twins than dizygotic twins or underestimated if the environments are less similar in monozygotic twins than dizygotic twins. Therefore, ensuring the similarity of the trait of interest between monozygotic and dizygotic twins is important to minimize the impact of violating this assumption on the accuracy of heritability estimates. Additionally, heritability estimates should be interpreted with caution, as they can differ across different study methods, as well as different populations and change over time.[19] For example, a large twin study that included 37051 twin pairs from Australia, Denmark, Finland, Netherlands, Norway, Sweden, and the United Kingdom reported that the heritability estimate of exercise participation varied from 48% to 71% between these countries.[20] A longitudinal analysis of genetic and environmental influences on walking endurance in 130 older female twin pairs showed that the heritability estimated to be 40% at baseline increased to 60% at 3-year follow-up,[21] suggesting heritability estimates can change over time.

28.2.2 The cotwin control design in musculoskeletal research

The cotwin control design could potentially be one of the most useful study designs in musculoskeletal research. Interactions between environmental and genetic factors play a significant role on musculoskeletal conditions.[22] As a result of the interaction between these two, heterogeneity in the presentation and prognosis of musculoskeletal conditions are commonly observed across different individuals.[22] Some individuals can genetically be predisposed more than others to various musculoskeletal injuries and conditions, including low back pain and osteoarthritis.[23–25] For example, individuals carrying an allele G of IL6 SNPs rs1800795 and rs1800797 were found to have a higher risk of developing disc degeneration,[26] suggesting some individuals can be more susceptible to various musculoskeletal conditions due to their genetic backgrounds. A previous twin study has clearly shown how genetic factors tend to similarly influence the health status of individuals who share their genetic materials.[27] In that study, for example, monozygotic twins who share, on average, 100% of their genes, are a five times higher chance of developing low back pain than dizygotic twins who share half of their genetic materials when cotwin of each pair had previously experienced low back pain. Additionally, an increased risk of musculoskeletal pain has been found in people whose parents had previously experienced chronic musculoskeletal pain.[28] Therefore, both genetic and familial factors affecting the heterogeneity of musculoskeletal conditions are crucial to be controlled in musculoskeletal research, otherwise research outcomes could possibly be affected by heterogeneity across unrelated individuals. However, it is always challenging to control genetic and familial factors of research participants, especially it is even more complicated to account for diverse genetic backgrounds of different individuals in musculoskeletal research. Genetic and familial factors influencing heterogeneity across study participants can be more efficiently controlled in musculoskeletal research by involving twins than unrelated singletons.[29]

28.2.2.1 *The cotwin control design in noninterventional musculoskeletal studies*

Involving monozygotic twins as participants in twin research can primarily provide an opportunity to investigate the risk factors or exposures to musculoskeletal traits or conditions independent of genetic and familial confounding factors. Additionally, if an association between an exposure (risk factor) and a musculoskeletal condition is observed, it is also important to consider whether the association is confounded by genetic and familial factors using the cotwin control design. The cotwin control design can be applied to both observational and experimental studies to investigate associations between exposures and outcome variables based on within twin pair associations. One of the criteria for the cotwin control design in noninterventional studies is that monozygotic or dizygotic twin pairs are required to be discordant to investigate what exposures or risk factors (nonshared environmental differences) are associated with differences in outcome measures within twin pairs.[30] Involving monozygotic twins as participants make the design more powerful in investigating

exposures as monozygotic twins in a pair are almost perfectly matched for their genetic backgrounds, in addition to sex, age, and early shared environment.[31] There are some interesting studies that have been conducted using the cotwin control design to investigate exposures in relation to musculoskeletal system. Depression, a common mental condition is often associated with low back pain.[32] However, a previous cotwin control design study identified that the association between depression and low back found from a cross-sectional analysis of 2148 twins, including both monozygotic and dizygotic twins was reduced when only monozygotic twin pairs (controlling for genetic and familial factors) were included in the analysis.[33] The authors of that study then suggested that familial factors influencing both depression and low back pain appear to drive this association.[33] In both cohort and cross-sectional studies, engaging in moderate levels of physical activity is commonly associated with a reduced risk of low back pain.[34] However, a cross-sectional cotwin control study has found that an inverse association found between meeting the World Health Organization physical activity guidelines and recent low back pain was no longer statistically significant after controlling for genetics and early shared environment.[35] The authors of that study suggested that genetics and early shared environment potentially act as confounding factors for the association between physical activity and recent low back pain. These findings also show the benefits of incorporating cotwin control design in musculoskeletal research. However, very few studies to date have applied the cotwin control design to investigate the risk factors of musculoskeletal conditions.

Considerations of applying the cotwin control design in noninterventional studies can include the following: First, it can be challenging to find twin pairs who are discordant for risk factors or exposures. Second, it is crucially important to ensure having complete data of both twins in each pair to be able to conduct within-pair analyses. Third, although cotwin control studies can include dizygotic twin pairs to investigate risk factors or exposures, the use of such an approach makes the design less efficient than involving monozygotic twin pairs.[31]

28.2.2.2 Working with twins in randomized controlled trials

Involving twins as participants in randomized controlled trials (RCTs) could possibly be one of the most useful twin study designs contributing to the advancement of musculoskeletal research as RCTs are often referred as the cornerstone of clinical trials.[36] In RCTs, the effect of an intervention is determined by any differences observed over the course of a clinical trial between the different groups. Additionally, confounding factors such as age and sex that can affect participants' response are expected to be as similar as possible across treatment and control groups in RCTs.[37] The matching of these characteristics of participants, challenging for unrelated singletons, can almost perfectly be ensured by assigning monozygotic twin pairs into different groups in RCTs as they are identical for their genes, age, and sex. Therefore, the inclusion of monozygotic twin pairs who are phenotypically concordant makes the design efficient to determine the effect of an intervention. Additionally, involving the concordant monozygotic twin pairs in RCTs provides an opportunity to reach the desired statistical power with fewer participants compared to involving

unrelated singletons due to their high similarity.[30,38] Two interesting studies out of very few musculoskeletal studies that applied the cotwin control design to clinical trials have shown how calcium supplementation benefits bone density.[39,40] In the first double-blind trial that involved 70 pairs of monozygotic twins aged between six and fourteen showed that the randomly selected twins from each pair to take calcium supplementation (1000 mg) daily over three years had higher bone mineral density at almost all measured sites including in lumbar spine compared to the other siblings who were assigned into the placebo group.[39] However, the beneficial effect of taking the supplementation was only observed in prepubescent twins.[39] The second randomized cotwin control trial that included 42 twin pairs also used 1000 mg daily calcium supplementation to investigate its effect on bone mineral density over three consecutive 6-month periods but only in adolescent girls aged between 10 and 17.[40] In that trial, interestingly, a greater increase in bone mineral density in the spine and hip was observed in twins from each pair assigned to take the calcium supplementation compared to the other siblings in the placebo group at the end of the first six months. However, the within-pair difference was no longer significant after the first six months. The authors then suggested the importance of identifying the optimal timing for the effect of calcium supplement in long-term clinical trials.[40]

Although monozygotic twins are commonly included in RCTs for the reasons mentioned earlier,[38] it is also noteworthy that involving both monozygotic and dizygotic twin pairs in clinical trials could contribute to better understand the importance of genetic influence on participants' response to an intervention. For example, a recent clinical trial has investigated whether individualized participants' response to training is differed across different exercise modes along with the effect of genetic factors on their responses to endurance and resistant training. That study involved both monozygotic and dizygotic twin pairs who were randomized into the training modes.[41] Individualized participants' response was differed by different exercise modes and the authors suggested that individuals who fail to benefit from one type of exercise may respond to another type of exercise, showing the importance of an individualized exercise. Additionally, the influence of genetic factors on longitudinal changes in muscle strength and cardiovascular fitness to resistant and endurance training was not found to be substantial.[41] These studies show that incorporating the twin approach in RCTs could potentially contribute to the advancement of musculoskeletal research. However, the twin approach in RCTs to date has been underused, especially in musculoskeletal research despite its promising benefits.

The following potential caveats should be taken into account when applying the twin design in RCTs. First, study recruitment is challenging as both twins from each pair are required to be concordant phenotypically and meet eligibility criteria to participate in an RCT. Second, it is also crucially important to ensure both twins from each pair to participate during the whole study period in conducting within-pair comparisons. Third, twins in a pair may discuss about their treatments when they are assigned into different groups, which can lead to treatment contamination.[38] Therefore, blinding treatment allocation can be useful to minimize the risk of treatment contamination. For example, the earlier randomized cotwin controlled trial

successfully made calcium supplement exactly the same, in terms of appearance and taste, for both the treatment and placebo groups to investigate its effect on bone mineral density.[40]

Overall, we have discussed the applications, benefits, and potential considerations related to twin approaches in relation to musculoskeletal studies. The classical twin design is commonly used to estimate heritability of a trait, which helps to better understand genetic and environmental contributions to the trait of interest. The classical twin design has also been extended by advanced analytical approaches, such as structural equation modeling to precisely estimate heritability for musculoskeletal traits. However, there are critical assumptions of the classical twin design to be considered when estimating or interpreting heritability for musculoskeletal traits. Another important approach, perhaps the most useful in musculoskeletal twin research, is the cotwin control design to advance both interventional and noninterventional musculoskeletal studies. The cotwin control design is extremely helpful in clinical studies as this design can control genetic and familial factors affecting musculoskeletal condition or trait of interest by involving concordant monozygotic twin pairs. Therefore, incorporating these twin approaches in musculoskeletal studies can contribute to the advancement of musculoskeletal research.

References

1. Disease GBD, Injury I, Prevalence C. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet (London, England)*. 2018;392:1789–1858.
2. Australian Institute of Health and Welfare. *Disease Expenditure in Australia*: Australian Institute of Health and Welfare, Canberra; 2019.
3. United States Bone and Joint Initiative. BMUS: The Burden of Musculoskeletal Diseases in the United States 2014. http://www.boneandjointburden.org/.
4. Bevan S. Economic impact of musculoskeletal disorders (MSDs) on work in Europe. *Best Practice & Research Clinical Rheumatology*. 2015;29:356–373.
5. Cammarota A. Musculoskeletal conditions in Australia. October 11–12, Lisbon, 2007.
6. Montgomery W, Sato M, Nagasaka Y, Vietri J. The economic and humanistic costs of chronic lower back pain in Japan. *Clinicoecon Outcomes Res*. 2017;9:361–371.
7. Grasby KL, Verweij KJH, Mosing MA, Zietsch BP, Medland SE. Estimating heritability from twin studies. *Methods in molecular biology (Clifton, NJ)*. 2017;1666:171–194.
8. Mayhew AJ, Meyre D. Assessing the heritability of complex traits in humans: methodological challenges and opportunities. *Current Genomics*. 2017;18:332–340.
9. Polderman TJC, Benyamin B, de Leeuw CA, et al. Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nature Genetics*. 2015;47:702–709.
10. Symposium on Characterizing Human Psychological A. In: Bock G, Cardew G, eds. *Characterizing Human Psychological Adaptations*. Chichester: Wiley; 1997.
11. Ferreira PH, Beckenkamp P, Maher CG, Hopper JL, Ferreira ML. Nature or nurture in low back pain? Results of a systematic review of studies based on twin samples. *European Journal of Pain (London, England)*. 2013;17:957–971.

12. Neame RL, Muir K, Doherty S, Doherty M. Genetic risk of knee osteoarthritis: a sibling study. *Annals of the Rheumatic Diseases*. 2004;63:1022.
13. Laird RA, Keating JL, Ussing K, Li P, Kent P. Does movement matter in people with back pain? Investigating 'atypical' lumbo-pelvic kinematics in people with and without back pain using wireless movement sensors. *Bmc Musculoskeletal Disorders [Electronic Resource]*. 2019;20:28.
14. Bayartai M-E, Ferreira PH, Pappas E, et al. Genetic and environmental effects on lumbar posture, flexibility and motion control in healthy adults. *Musculoskeletal Science and Practice*. 2020;50:102253.
15. Battie MCL E, Videman T, Burton K, Kaprio J. Heritability of lumbar flexibility and the role of disc degeneration and body weight. *Journal of applied physiology (Bethesda, Md: 1985)*. 2008;104:379–385.
16. Bayartai ME, Kobayashi S, Ferreira PH, Pappas E, Pinheiro MB, Sullivan J. Heritability of motion in healthy people: a systematic review and multi-level meta-analysis. *Physical Therapy in Sport: Official Journal of the Association of Chartered Physiotherapists in Sports Medicine*. 2020;43:8–18.
17. Zadro JR, Shirley D, Andrade TB, Scurrah KJ, Bauman A, Ferreira PH. The beneficial effects of physical activity: is it down to your genes? A systematic review and meta-analysis of twin and family studies. *Sports medicine - open*. 2017;3:4.
18. Paaby AB, Rockman MV. The many faces of pleiotropy. *Trends in Genetics*. 2013;29:66–73.
19. Wray NV. Estimating trait heritability. *Nature Education*. 2008;1:1.
20. Stubbe JH, Boomsma DI, Vink JM, et al. Genetic influences on exercise participation in 37,051 twin pairs from seven countries. *PLoS One*. 2006;1:e22.
21. Ortega-Alonso AS S, Kujala UM, Kaprio J, Rantanen T. Longitudinal changes in genetic and environmental influences on older women's walking ability. *Scandinavian Journal of Medicine and Science in Sports*. 2009;19:669–677.
22. Diatchenko L, Fillingim RB, Smith SB, Maixner W. The phenotypic and genetic signatures of common musculoskeletal pain conditions. *Nat Rev Rheumatol*. 2013;9:340–350.
23. Goodlin GT, Roos TR, Roos AK, Kim SK. The dawning age of genetic testing for sports injuries. *Clinical Journal of Sport Medicine: Official Journal of the Canadian Academy of Sport Medicine*. 2015;25:1–5.
24. Loughlin J, Dowling B, Chapman K, et al. Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females. *PNAS*. 2004;101:9757–9762.
25. Khoschnau S, Melhus H, Jacobson A, et al. Type I collagen alpha1 Sp1 polymorphism and the risk of cruciate ligament ruptures or shoulder dislocations. *The American Journal of Sports Medicine*. 2008;36:2432–2436.
26. Kelempisioti A, Eskola PJ, Okuloff A, et al. Genetic susceptibility of intervertebral disc degeneration among young Finnish adults. *BMC Medical Genetics*. 2011;12:153.
27. Junqueira DR, Ferreira ML, Refshauge K, et al. Heritability and lifestyle factors in chronic low back pain: results of the Australian twin low back pain study (The AUTBACK study). *European Journal of Pain (London, England)*. 2014;18:1410–1418.
28. Lier R, Mork PJ, Holtermann A, Nilsen TIL. Familial risk of chronic musculoskeletal pain and the importance of physical activity and body mass index: prospective data from the HUNT study, Norway. *PLoS One*. 2016;11:e0153828.
29. Ferreira P, Craig J, Hopper JL. Research Note: Twin studies and their value for physiotherapy research. *Journal of Physiotherapy*. 2019;65:58–60.

30. Plomin R, Haworth CMA. Genetics and Intervention Research. *Perspectives on Psychological Science.* 2010;5:557–563.
31. Cherny SS. Advances in twin and sib-pair analysis. *American Journal of Human Genetics*. 2001;69:668.
32. Pinheiro MB, Ferreira ML, Refshauge K, et al. Symptoms of depression and risk of new episodes of low back pain: a systematic review and meta-analysis. *Arthritis Care & Research*. 2015;67:1591–1603.
33. Pinheiro MB, Ferreira ML, Refshauge K, et al. Genetics and the environment affect the relationship between depression and low back pain: a co-twin control study of Spanish twins. *Pain*. 2015;156:496–503.
34. Alzahrani H, Mackey M, Stamatakis E, Zadro JR, Shirley D. The association between physical activity and low back pain: a systematic review and meta-analysis of observational studies. *Scientific Reports*. 2019;9:8244.
35. Zadro JR, Shirley D, Amorim A, Perez-Riquelme F, Ordonana JR, Ferreira PH. Are people with chronic low back pain meeting the physical activity guidelines? A co-twin control study. *The spine Journal: Official Journal of the North American Spine Society*. 2017;17:845–854.
36. Hajebrahimi S, Mostafaie A, Sadeghi-Bazargani H. Evidence for the future - designing a clinical trial. *Indian J Urol*. 2011;27:494–497.
37. Kabisch M, Ruckes C, Seibert-Grafe M, Blettner M. Randomized controlled trials: part 17 of a series on evaluation of scientific publications. *Dtsch Arztebl Int*. 2011;108:663–668.
38. Sumathipala A, Yelland L, Green D, et al. Twins as participants in randomized controlled trials: a review of published literature. *Twin Research and Human Genetics: the Official Journal of the International Society for Twin Studies*. 2018;21:51–56.
39. Johnston CC, Miller JZ, Slemenda CW, et al. Calcium supplementation and increases in bone mineral density in children. *New England Journal of Medicine*. 1992;327:82–87.
40. Nowson CA, Green RM, Hopper JL, et al. A co-twin study of the effect of calcium supplementation on bone density during adolescence. *Osteoporosis International*. 1997;7:219–225.
41. Marsh CE, Thomas HJ, Naylor LH, Scurrah KJ, Green DJ. Fitness and strength responses to distinct exercise modes in twins: Studies of Twin Responses to Understand Exercise as a THerapy (STRUETH) study. *Journal of Physiology*. 2020;598(18):3845–3858.

CHAPTER

Contributions of twin studies to cancer epidemiology

29

Wendy Cozen[a,b,c], Esther Lam[a,c], Maryam Salehi[a,c], Victoria K. Cortessis[d,e], Tuong L. Nguyen[f], James G. Dowty[f], John L. Hopper[f], Shuai Li[f], Thomas M. Mack[d]

[a]*Division of Hematology-Oncology, Department of Medicine, School of Medicine, University of California at Irvine, Orange, CA, United States*

[b]*Department of Pathology, School of Medicine, University of California at Irvine, Orange, CA, United States*

[c]*Chao Family Comprehensive Cancer Center, University of California at Irvine, Orange, CA, United States*

[d]*Department of Population and Public Health Science, Keck School of Medicine, University of Southern California, Los Angeles, CA, United States*

[e]*Department of Obstetrics & Gynecology, Keck School of Medicine, University of Southern California, Los Angeles, CA, United States*

[f]*Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, University of Melbourne, Melbourne, VIC, Australia*

29.1 Introduction

Wendy Cozen and Victoria K. Cortessis

This chapter describes contributions that studies of twins have made to cancer epidemiology, and recent innovations in methodology for studying twins. Because there are over 3,000 published reports on the topic of cancer and twins, a comprehensive review is not feasible. Instead, we focus on unique contributions of twin studies to cancer epidemiology, explaining why comparable information cannot be provided by studies of singletons. Included topics are: 1) risk of cancer in twins compared to singletons; 2) patterns of occurrence of cancer in twins including concordance; 3) studies of acquired (i.e., non-genetic) risk factors for cancer in twins; 4) intraplacental (i.e., twin to twin) metastasis of infantile leukemia; 5) cancer screening, effects of treatment and survivorship; and 6) promising new approaches to modeling cancer risk based in studies of twins and families. We specifically excluded twin studies that simply confirm results initially reported in non-twin studies. Because readers may not be familiar with cancer research, epidemiology, modern technology used in genetic studies, or defined types of twins we include a brief description of these ideas and related terminology used in the chapter.

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00036-2

Cancer is defined as an uncontrolled growth originating from a single cell that clones itself and acquires mutations that permit continued growth and evasion of the host's immune response. Cancer is many diseases, which are distinguished by many factors including organ site, cell of origin, and the patient's age at the time of diagnosis (childhood cancer if diagnosed at 18 years of age or younger, adult cancer if diagnosed 18 years and older, and a special group that overlaps both age groups called adolescent/young adult (AYA) cancer diagnosed at 15–39 years of age). Diagnosis, treatment decisions, and survival depend on the cancer type, acquired mutations in the tumor, access to health care, host factors such as state of the immune response, and other considerations. Prevention and control of cancer and even treatment strategies depend on understanding the cause of the cancer, which is the realm of epidemiology that uses observational, as opposed to experimental, study designs. Since cancer is a complex chronic disease, the causes are often a combination of both genetic and non-genetic causes. Individual types of cancer, even within the same organ, often have at least some differences in sets of causes. Twin studies have made a unique contribution to understanding causes of cancer, and in addition, long-term problems experienced by cancer survivors as outlined in the sections below.

Epidemiology is a scholarly discipline that focuses on the study of disease in human populations to identify patterns of disease occurrence and outcomes and elucidate risk factors. Epidemiologists use specialized methodologies developed specifically to conduct valid research while protecting the privacy, rights and wellbeing of human subjects. Frequency of disease occurrence is called incidence, which in its simplest form is measured as the proportion of individuals in a defined population who develop the disease in a specified time. Relationships between suspected risk factors and incident (i.e., newly diagnosed) cancer are often studied by one of the three related approaches to epidemiologic research described this chapter.[1] Conceptually simplest are cohort studies, in which putative risk factors are measured for disease-free participants who are followed over time to assess incidence of the disease of interest among those with and without each factor of interest. Results are expressed as ratios of disease incidence among those with the risk factor and those without, termed risk ratio and abbreviated RR. Case-control studies are similar, but in this study design, a history of having each risk factor of interest is compared between those who develop disease (i.e., cases) and unaffected controls, who are ideally sampled from the same source population as the cases. The association between a putative risk factor and incident disease is estimated in case-control studies using the odds ratio, abbreviated OR, which compares the odds of developing the cancer with and without the risk factor. Cases enrolled in case-control studies already have the disease, and putative risk factors are assumed to have occurred prior to development of the disease; for inherited genotypes and early life exposures this is a valid assumption. However, it is important to consider whether exposure in some cases may have occurred after the onset of the cancer (even if not yet diagnosed), because of the cancer. For example, some cancer patients begin to lose weight prior to diagnosis before the disease is recognized. In such instances caution is required to

assure that exposures and risk factors caused by the disease are not mistaken for causes of the disease. Case-control studies are most commonly used to study rare diseases because cohort studies of rare diseases would require that prohibitively large groups of disease-free participants be enrolled and followed. In the third approach, investigators enroll only individuals with the putative risk factor, estimate disease incidence among them, and compare it to incidence of disease in the general population, accounting for demographic factors like age and sex. In studies of this type associations between putative risk factors and incident diseases are estimated as standardized incidence ratios (SIR).

Epidemiologic inferences about cancer causes are based partly on estimates of associations between putative risk factors and disease. Under ideal circumstances estimates of the RR, OR and SIR are expected to be greater than 1.0 if the factor is a cause, less than 1.0 if the factor protects from disease, and 1.0 if the factor has no influence at all on risk. However, limitations of individual studies can interfere with accuracy. For example, the role of chance (i.e., random error) must be considered. This is accomplished by calculating confidence intervals (CI), which depict the range of estimates expected if random error were the only limitation of a given study, and that study was conducted many times. A 95% CI denotes the range of values that would include the accurate (true) value in 95% of these hypothetical studies. If the 95% CI does not include 1.0, we consider the result to be "statistically significant", meaning unlikely to differ from 1.0 due to chance alone. If the study has many participants (i.e., a large sample size), the 95% CI will be narrower than if the study has few participants. A narrow confidence interval is more likely to exclude 1.0 and thus to be statistically significant. But because twins are uncommon, and most cancers are uncommon, sample sizes in twin studies of cancer are often small, making it difficult to identify statistically significant associations. On the other hand, because twins are matched on so many characteristics, twin studies are less vulnerable than studies of singletons (non-twins) to a second type of bias called confounding. Confounding occurs when the putative risk factor is correlated with other risk factors that may in fact be true causes. A classic example is the relationship between moles, childhood sun exposure and risk of melanoma. The size and number of moles are determinants of melanoma risk. But moles are also associated with childhood sun exposure, which is in turn, a risk factor for melanoma. Therefore, if childhood sun exposure is not accounted for, it could bias the true association between moles and melanoma risk, making moles seem like a more important cause than they really are. Confounding is far less likely in twin studies because twins have identical or very similar levels of exposures especially related to early life, parental exposures and genetics (since identical twins share the same genome and fraternal twins, on average, share half of their genomes). In this example, twins are likely to have similar levels of childhood sun exposure thus removing it as a potential source of bias so that the true exposure of interest, moles, can be isolated.

One set of risk factors that have been extensively explored with respect to cancer risk are inherited differences in DNA sequence, called genetic variants. These differences are usually single nucleotide polymorphisms (SNPs), referring to differences

in a single nucleotide. SNPs can now be measured efficiently and in great number using genome-wide association studies (GWAS)[2] usually conducted uing case-control methodology, comparing SNPs of cases to those of unrelated controls. The SNP-disease associations identified in GWAS studies are typically weak, but because the genetic variants are common, they can impact risk in a population. A measure called the polygenic risk score (PRS) is used to express the number of risk-associated genotypic variants an individual has inherited, together with strength of the association of each variant with the disease. Humans have additional forms of genetic variants. For example, at certain chromosomal locations some people have more nucleotides than others, These genetic variants are called insertion/deletion polymorphisms, or 'indels'. If an indel is known or postulated to influence function of a gene it might be studied as a risk factor, but such studies typically investigate only small numbers of indels. For SNPs, indels, and other forms genetic variants, the specific set of .DNA sequences that a person inherited is called that person's genotype.

Genetic variants found to be associated with risk can be categorized according to penetrance, defined as the probability that people who inherited the variant will develop the disease of interest. Examples of highly penetrant variantss are deleterious mutations causing loss of function of genes such as *BRCA1* and *BRCA2* that mediate repair of DNA damage. Risk of breast cancer and ovarian cancer is very high in the small number of women who inherited high penetrance variants of these genes, but those variants are so rare that they account for very small proportions of these cancers among all women. It is therefore also desirable to identify more common genetic variants that are associated with cancer risk although with lower penetrance, as might be identified in a GWAS.

Epidemiologists categorize twin pairs according to several criteria, which define groups of twins that provide particularly worthwhile information about certain kinds of questions. Zygosity refers to whether twins are identical (monozygotic, MZ) having arisen from a single egg and single sperm and thus a single zygote; or fraternal, like any other pair of full siblings (dizygotic, DZ), having arisen from two separate zygotes. Twins who are of the same sex can be either MZ or DZ, but unlike-sex twins are always DZ by definition. Finally, disease status of twins within a pair is called 'disease concordant' if both twins developed the disease of interest, or 'discordant' if only one twin of the pair developed the disease at the time of the study.

29.2 Risk of cancer in twins compared to singletons

Thomas Mack

Because twins differ biologically from singletons, it is of interest to identify unique differences in risk between the two groups. Some differences might derive from the nature of twinning, others from biologic interactions or acquired experience. There are only a few twin sources large enough to permit comparisons of cancer occurrence in twins compared to singletons. The largest and most recent comparison of cancer incidence rates between twins at all ages was based on the 260,000 same-sex twins

and the 30,000 cancer diagnoses registered in the NorTwinCan cohort, deriving from linkage between the twin registries of Denmark, Finland, Norway, and Sweden, and the respective national cancer registries.[3] The authors estimated that overall cancer incidence among all twins in comparison with the base population overall was almost identical among both males and females. Specifically, twins of both sexes combined had slightly lower SIRs for cancers of the kidney (SIR = 0.83; 95% CI = 0.76–0.89), lung (SIR = 0.89; 95% CI = 0.85–0.92), and colon (SIR = 0.90; 95% CI = 0.87–0.94). SIRs for malignant melanoma were also just under 1.0: 0.94 (95% CI = 0.86–1.03) among males and 0.98 (95% CI = 0.90–1.07) among females. The two most common cancers that occurred in twins occur almost exactly as frequently as in the (mostly singleton) population at large: breast cancer in women (SIR = 1.01; 95% CI = 1.00–1.07) and prostate cancer in men (SIR = 1.03; 95% CI = 0.98–1.04). Other sites, including the leukemias and lymphomas, showed slight differences between twins and the general population that could be explained by chance. The single substantial difference identified was for incidence of testicular germ cell cancer. This cancer occurs mostly in adolescent/young adult men and has become more common for reasons that are poorly understood. Risk of this cancer was greater in twins compared to the general population (SIR = 1.15; 95% CI = 1.02–1.30), and the difference was largely attributable to MZ twins, in whom the SIR reached 1.36 (95% CI = 1.12–1.65). Both males and females from like-sex DZ pairs have been compared to analogs from unlike-sex DZ pairs, showing no difference in cancer incidence, and thus failing to produce any evidence that in-utero hormonal influences act as major cancer determinants in adulthood.[4]

The only non-Nordic assessment of cancer incidence in adult twins was based on the Utah Population Database, linked to the Utah cancer registry.[5] This retrospective cohort study found no overall difference in relative risk (RR) for adult cancers among twins compared with singletons. With smaller numbers, most specific cancer deviations (as high as 1.3 for lymphoma and 1.7 for prostate cancer) were not significantly different from the null. However, incidence of malignant melanoma (a serious form of skin cancer) among like-sex twins was significantly lower at 0.53 (95% CI = 0.30–0.96).

A meta-analysis combining results of 7 studies of varying size, calendar year and study design examining the risk of testicular germ-cell cancer in twins compared with either the general population or a control group of singletons was conducted to improve the precision of the risk estimate.[6] The authors found a consistent increase in testis cancer among twins summarized by a pooled risk estimate of 1.31 (95% CI = 1.1–1.6), consistent according to source and zygosity/gender. Since more than 90% of testis cancers are germ cell malignancies, and since cryptorchidism (undescended testis) is a consistently strong risk factor for testis cancer,[7] the finding was interpreted as another form of the congenital malformations known to be more common among identical twins. Alternatively, some might hypothesize that this finding represented the influence of in utero maternal hormonal exposure.[8]

Evaluations of childhood cancer in twins (conventionally ages 0–14) compared to singletons are available from four sources; the Swedish Family-Cancer Database linked to the Swedish cancer registry,[9] the above-mentioned linkage implemented in the Utah

population,[5] an older comparison based on cases from the Connecticut Cancer Registry,[10] and a linkage between the cancer registries and birth records of five U.S. states (California, Minnesota, New York, Texas, Washington).[11] In the Swedish databases,[9] twins experienced lower overall risk of childhood cancer (SIR = 0.81; 95% CI = 0.69–0.94) compared to singletons, with an even larger deficit of nephroblastoma, a childhood cancer occurring in the kidney, (SIR = 0.34; 95% CI = 0.09–0.88). Both risk reductions were driven by same-sex twin pairs (overall cancer SIR = 0.77, 95% CI = 0.64–0.93); nephroblastoma SIR = 0.12, 95% CI = 0.00–0.71). Although the expected number was small, there was a deficit of bone and soft tissue sarcomas among same-sex twins. In the Utah databases,[5] as in adults, there was a slight deficit of all childhood cancers among twins compared to singletons (RR = 0.82; 95% CI = 0.55–1.24), although hematopoietic cancers (leukemia, lymphoma), accounting for half of the total, showed a non-significant increase among like-sex twins at RR = 1.28 (95% CI = 0.58–2,81). Although the sample sizes were very small, the non-significant deficits of solid tumors were in same-sex pairs at 0.58 (95% CI = 0.30–1.15). In Connecticut,[10] there was a 30% overall observed deficit of incident childhood cancers observed among twins (SIR = 0.7; 95% CI = 0.5–0.9) compared to the base population especially among young males. A non-significant 7% lower risk for all childhood cancers was again observed in the birth record linkage study of childhood cancers among twins and higher multiples compared to singletons in five U.S. states.[11] Risk of nephroblastoma was again lower, with an OR = 0.65 (95% CI = 0.39–1.09). Rates for ALL, AML, CNS cancers, embryonal cell cancers as a group, bone sarcomas and carcinomas were nearly identical to those for singletons. Among multiples, the incidence rate of Hodgkin lymphoma was non-significantly lower, and that of non-Hodgkin lymphoma non-significantly higher than incidence rates among singletons. Among those younger than 2 years, the risk of nephroblastoma was even lower, with an OR = 0.27 (95% CI = 0.09–0.86), as was that for neuroblastoma with an OR of 0.46 (95% CI = 0.25–0.84). This study found, not a deficit but a greater than 2-fold significant excess risk of soft tissue sarcomas (OR = 2.30; 95% CI = 1.12–4.69), mostly attributable to fibrosarcoma (OR = 5.81; 95% CI = 1.33–22.11). No clear explanation for these differences nor for the deficit of nephroblastoma was forthcoming although unlike germ cell testis cancers, nephroblastoma appears to originate in an anomalous embryologic cell rest (residual embryological cells). It is possible that the deficit in twins occurs because in some twin pairs one or both affected members with more serious anomalies died before birth.

29.3 Patterns of occurrence of cancer in twins

Thomas Mack and Wendy Cozen

Cancer concordance in twins can provide a crude estimate of heritability (e.g., contribution of inherited genetic factors to risk), especially when concordance in MZ and DZ twins are compared. As previous chapters have noted, MZ twins share almost 100% of their genome while DZ twins share, on average, 50%, like non-twin siblings. A caveat regarding the similarity of early childhood exposures and peer-mimicking behaviors (e.g., smoking), limits the interpretation of heritability.

Nevertheless, concordance evaluations simultaneously considering the separate roles of genetic inheritance, shared early environment, and separate individual adult environment among twins can be hypothesis-generating and can provide useful empirical information. Generally, assessment of twin cancer concordance rates requires large studies, because rates are lower than those for many other chronic diseases, such as asthma[12] and type I diabetes.[13]

Each twin registry offers an opportunity for the study of the contribution of heritability to disease risk, including cancers, if reliable distinctions can be made between MZ and DZ twin pairs, follow-up is feasible, and if it is possible to exclude biased ascertainment of disease-concordant pairs, especially MZ pairs. This is because twins from pairs in whom both are stricken with cancer may be more inclined to volunteer for studies than pairs in whom only one twin has cancer, and this bias will be even more pronounced for MZ compared to DZ twins. An example of a volunteer twin registry used for this purpose includes The International Twin Study,[14] an accumulation of roughly 11,000 twin volunteers ascertained through periodical advertising in the 1980's. Zygosity-specific concordance for breast cancer,[15] melanoma[16] and adolescent/young adult (AYA) Hodgkin lymphoma[17] was assessed excluding pairs who were concordant before the time of initial ascertainment to control for differential participation discussed above. Of note, this was the first study to demonstrate the exceptionally high SIR for unaffected twins of MZ probands for Hodgkin lymphoma in adolescents and young adults, with 0.1 cases expected and 10 observed in the unaffected MZ twins and is one of the largest existing collections of concordant Hodgkin lymphoma twin pairs (now at 15 MZ concordant pairs and 1 DZ concordant pair).

As mentioned in the previous section, the largest available body of population-based descriptive information on twins with cancer is that from the Nordic consortium of investigators, twin registries, cancer registries, and demographic resources of the Nordic countries: Denmark, Finland, Norway, and Sweden. In these countries, national databases of cancer, twins and health records exist that capture the entire population, circumventing potential biases inherent in volunteer registries. A publication describing the pattern of occurrence of cancer in 203,691 twins in the Nordic registries followed prospectively[18] included the frequencies of cancer concordant and discordant MZ and DZ twin pairs. When combined with the cumulative incidence in the base (mostly singleton) population, this provides an index of the familial risk among MZ twins. Three useful cancer site-specific indices of interest are provided in this paper.[18] One is the estimate of heritability based on concordance according to zygosity (Table 29.1), a second is an estimate of the shared environment component of the total variance of MZ twin concordance (after exclusion of the fractions of covariance attributable to inheritance and individual environment) and the third measure is the median interval between the diagnoses in cancer concordant MZ twin pairs. The first index is a crude measure of the role of genetic inheritance, the second crudely estimates the magnitude of acquired determinants occurring from conception until the individual twins separate and the third is a measure of non-genetic risk factors influencing timing of diagnosis. This index, along with the proportion of cancer discordance among MZ twins, crudely signifies the magnitude of non-genetic determinants.

TABLE 29.1 Biologic metrics from twins specific for each of 12 malignancies from the Nordic twin registries.[18]

Cancer site	DZ pairs	% DZ concordant	MZ pairs	% MZ concordant	% MZ familial risk	Rough Heritability (95%CI)	% Shared Environmental Variance (95%CI)	Median age difference in years, concordant MZ twins
Oropharynx	367	1.6	196	2.6	6.0	9 (0-60)	26 (0-65)	8.0
Stomach	663	2.3	352	4.0	6.8	22 (0-55)	6 (0-31)	10.3
Colon	1187	2.6	607	4.9	10.9	15 (0-45)	16 (0-38)	8.3
Rectum	784	1.7	454	3.1	6.6	14 (0-50)	10 (0-38)	10.3
Lung	1440	5.1	732	6.8	17.5	18 (0-42)	24 (7-40)	7.8
Melanoma	591	1.0	352	3.1	19.6	58 (43-73)	0	8.9
Breast	2364	6.0	1299	9.5	28.1	31 (11-51)	16 (0-31)	9.3
Endometrium	487	1.2	281	3.2	7.0	27 (11-43)	0	12.1
Ovary	431	0.9	230	2.6	8.7	39 (23-55)	0	(<5 pairs)
Prostate	1867	7.9	1004	19.6	38.0	57 (51-63)	0	3.7
Testis	126	2.4	95	5.2	13.8	37 (0-93)	24 (0-70)	(<5 pairs)
Kidney	376	0.5	201	2.5	6.7	38 (21-55)	0	(<5 pairs)
Bladder	883	1.5	489	3.7	9.9	30 (0-67)	0	7.1
Leukemia	975	0.1	636	0.9	2.0	57 (0-100	0	(<5 pairs)

The following table (Table 29.1) from Mucci et al.[18] itemizes these three indices for a selected group of common malignancies from the Nordic registries. Of note, to be included in the data generating the statistics, the twins would have to have survived to age 6, which excludes some cancers diagnosed at infant and toddler ages, such as infant leukemia.

Differences in zygosity-specific concordance has long been used to suggest heritability (problems with this assumption further discussed below in section 29.6) and here indicates a stronger heritable determination for melanoma, prostate cancer and leukemia compared to other cancers. This is supplemented by the estimated MZ familial risk level for prostate cancer but only modestly for melanoma, and not at all for leukemia. This suggests that concordance is not a very accurate measure of familial risk.

The cancers with a substantial shared environment contribution are oropharynx and lung, probably indicating a common peer-influenced teenage initiation of smoking,[19] and testis cancer, the only cancer on the list likely to have been initiated during gestation. Non-smokers show little evidence of heritability for lung cancer, whereas smokers do, suggesting heritability of smoking, the exposure, rather than lung cancer itself.[20] Of these, only testis cancer shows strong heritability. One might have expected to also see stomach cancer showing strong shared environmental determinacy, since infection with Helicobacter pylori is a cause and is acquired in childhood primarily from siblings.[21,22] Leukemia appears to have high heritability, but this is hard to interpret because leukemia (and the other broad categories of blood cancers examined in this paper) consists of multiple types with different risk factors and heritability.[23,24]

The third index shown in Table 29.1 is of differences in onset dates among cancer-concordant twins. The mean interval between cancer diagnoses is almost synchronous for infant acute leukemia[25] (see Section 29.4) and is an average 3.7 years for prostate cancer. Intervals for other concordant cancer diagnoses are much longer and range from 7 to 12 years. The short interval between both twins' prostate cancer diagnoses, together with the relatively high level of MZ concordance, indicates substantial genetic penetrance. Even so, more than 90% of the affected MZ twin pairs are disease-discordant, and thus either some individual adult exposure is playing a role (although no credible non-genetic determinants have been identified), or more likely, either competing causes of mortality have produced substantial twin discordance by chance, or (more unlikely), a large proportion of the cancers are initiated and progress by chance mutations independent of both inheritance and the environmentIn addition, chance mutations would not explain variation in incidence by time, geography or racial/ethnic group.

Despite the clinical importance of genetic variants causing different forms of colorectal cancer, neither the heritable nor the shared environmental influence upon these malignancies is very high, supporting the common view that individual lifestyle and diet are important.[26] From this table it appears that cancers of the ovary, endometrium, kidney and bladder are each moderately heritable and that there is little environmental contribution, however, pertinent adult risk factors are known

(reproductive factors,[27, 28] obesity,[29] and smoking,[30] respectively). While heritable determinants or modifiers may have obscured environmental differences between MZ twin pairs, limitations in power and unavoidable biases in information collection may have alternatively played a role.

That leaves female breast cancer, the most important cancer of women, and one with only moderate heritability with over 180 known genetic risk variants,[31, 32] moderate influence from the childhood environment, and clear adult determinants in the form of endogenous and exogenous hormones, alcohol, physical inactivity and sometimes radiation.[33] An enduring question is whether observed environmental exposures have any effect other than reflecting the effect of genetic modifiers.

29.4 Studies of acquired risk factors for cancer in twins

Wendy Cozen and Thomas Mack

Usual comparisons of cancer cases to similar but unrelated persons without cancer (case-control studies and similar studies) have provided much of what we know about cancer risk factors. According to the classic assumption for a valid case-control comparison, the control must come from the same source population as the control to avoid one form of confounding (alternative explanations based on differences in the comparison groups, see Introduction).[1] In practice, this assumption is difficult to achieve. The advantages of using cancer discordant twin pairs for these studies are several-fold. First, the unaffected twin control comes from the same source population as the case as much as is possible (literally the same womb), and thus bias from related factors is largely avoided. Second, comparative questions can be posed that can glean information not possible in unrelated case and controls, such as who had menarche first? Who weighed more at 20 years? Who sucked their thumb more? Who drank more milk growing up? These comparative questions can be easier to answer than questions requiring a definitive, specific answer. In addition, comparative questions can be asked of both the twins in a pair, providing a quality control check on agreement, again not possible in unrelated sets. Finally, when comparing biological exposures between affected and unaffected individuals such as infectious exposures (antibodies), metabolites, DNA methylation, etc. confounding by genetic factors is reduced, to the extent that genetics contribute to the biological construct being measured. Below we provide several examples of unique contributions to knowledge of cancer risk factors from twin studies that would not have been possible in singleton studies.

In an example, Swerdlow and colleagues identified, enrolled and received completed questionnaires from 60 twin pairs in England and Wales who were discordant for testis cancer.[34] Twins were asked comparative questions about growth and development, with the resulting observation that the twin with longer arms or legs at 18 was 2–3 times more likely to develop testis cancer than the twin with shorter arms or legs. More importantly, five twin pairs reported cryptorchidism in one twin and in

all five pairs, the cryptorchidism occurred in the case and not the unaffected control co-twin. Although the numbers were small, the tight matching on genetics and early childhood makes this observation more striking and provided early strong evidence that cryptorchidism was indeed a risk factor for testis cancer.

In another twin study, we[35] examined early childhood risk factors in Hodgkin lymphoma, a rare type of lymphoma that occurs mostly in adolescence and young adulthood, especially in economically developed countries. (In the previous section we described the inferred strong heritability based on the high SIR for unaffected twins of Hodgkin lymphoma cases).[17] We suspected that a deficit of exposures to microbes in early childhood was a risk factor ("too clean"), but evidence was difficult to obtain. In a study of 90 twin pairs in whom one twin had Hodgkin lymphoma and the other did not, twins were asked relative questions about which twin put more things in their mouth (including a pacifier, thumb or finger) as a toddler. Again, because these questions compared one twin's behavior to the other, they were easier to answer than questions requiring exact answers in absolute terms. In addition, both twins' answers could be compared to each other to see if they agreed. We found that the twin who put more items in their mouth as a toddler was 80% less likely to develop Hodgkin lymphoma as an adolescent or young adult, supporting the idea that early exposure to more microbes was protective. Moreover, the twins agreed on the comparative answer over 95% of the time, a bonus of a twin study. To follow up on the hypothesis about microbes, we conducted a small pilot study in 13 pairs of these Hodgkin lymphoma discordant twins to examine the number of bacterial species in their stool.[36] The hypothesis was that if the twin who developed Hodgkin lymphoma had less exposure to microbes as a young child, their gut would harbor fewer different species of bacteria (fecal microbiome). The fecal microbiome can be examined by extracting DNA from stool and sequencing a segment of the bacterial genome (16SrRNA) that permits bacterial classification. In this pilot study, the Hodgkin lymphoma survivor twin had significantly fewer bacterial genera in their stool compared to their unaffected twin, supporting the hypothesis that a lack of microbial diversity contributes to risk of adolescent/young adult Hodgkin lymphoma. The twin aspect here is important because the microbiome is formed early in life and has a genetic component; thus, by using matching twins discordant for the cancer, we controlled for these factors.

The final example concerns breast cancer. Breast cancer is a common malignancy as indicated from the pattern of occurrence among twins[18] and is a cancer of complex etiology, seemingly combining inherited, family-specific perinatal,[37] pubertal,[38] acquired behaviors involving reproduction (fewer children, late age at first pregnancy),[39] diet,[40] alcohol and tobacco,[41] and hormone usage.[42] These risk factors tend to cluster in two partially distinct etiologic pathways defined by an age at diagnosis before and after menopause, providing disparate causal patterns in the same cultural environment.[43] We[44] previously noted that risk to the MZ co-twin of a case does not gradually rise over elapsed age in parallel with other age-specific curves as expected but remains relatively constant from the fourth decade of life, consistent with the findings from the Nordic twin resource.[45]

From a case-control study[46] of 759 self-described MZ and 1052 DZ female twin pairs, we reached three tentative conclusions: 1) comparisons between a case and her unaffected DZ cotwin showed many of the risk factor differences seen between singleton cases and controls: taller, greater body mass index (a measure of obesity), longer reproductive period, later menopause, more medical problems in infancy (most associations were enhanced with evidence of familial breast cancer history); 2) When we compared a case to her unaffected MZ co-twin, to our surprise, none of these differences (including those related to puberty and endogenous hormones) were found, and this absence was unrelated to historical evidence of breast cancer in the family; and 3) Most dramatically, among MZ twins who were both affected with breast cancer, the twin who was diagnosed first had earlier markers of puberty (first menarche [onset of menstruation], first thelarche [noticeable breast appearance], and earliest age at first menstrual regularity in comparison to her later diagnosed twin sister. In fact, we found that the twin who had earlier menarche was nine times more likely to be the first twin in the concordant pair diagnosed with breast cancer. We interpreted this surprising evidence as suggesting that the subset of MZ twins who become concordant are those with an exceptional inherited risk that is manifested by extreme susceptibility to hormones secreted at puberty. These findings are now being tested in a new study conducted with a separate set of affected twin cases, this time with samples of DNA to see if the unusual findings depend on genetic risk variants.

29.5 Intraplacental metastasis of infantile leukemia

Wendy Cozen and Esther Lam

Prenatal origins of childhood malignancies have been proposed as a possible source of cancer development.[47] The hypothesis has focused mainly on acute lymphoblastic leukemia (ALL), the most common childhood cancer, and to a lesser extent, acute myeloblastic leukemia (AML), the second most common acute leukemia. The very large number of case reports of concordant twin pairs with childhood leukemia in the literature (well over 70) prompted Mel Greaves and his colleagues to examine clonal origins of this cancer in twins.[25] Infant and childhood leukemia are distinct etiological entities and are considered separately when evaluating concordance.[48] Concordant diagnoses for acute leukemia in twins are usually synchronous, occuring very close in time, and there is little evidence of similarly high synchronous concordance in DZ twins or non-twin siblings as would be expected with high genetic susceptibility or shared environmental risk factors.

Moreover, leukemia concordant MZ twins almost always shared a placenta, often with numerous anastomoses (blood vessel connections), and therefore, shared blood supply. The concept of intraplacental metastasis, in which the disease originated in one twin and spread to the co-twin in utero via a shared placenta, was initially suggested by Irving Wolman in 1962[49] but wasn't further explored until 1971 when Bayard Clarkson and Ed Boyse further developed the idea.[25] With technological

advances, it became clear that acute leukemia subtypes were defined by chromosomal translocations, where regions of one chromosome are transposed with regions of another, due to chromosomal instability.[23] For example, infant acute lymphoblastic leukemia is associated with a translocation of the short arm of chromosome 11 with any number of chromosomal fusion partners (11q23,3 (v;11q23.3);KMT2A-rearranged). In these types of chromosomal breaks and rearrangements, there are numerous possible breakpoint locations.[50]

Infants concordant for acute leukemia had identical breakpoints in both chromosomal partners in their diagnostic malignant blasts and, when available, in both twins' dried Guthrie card blood spots at birth, a highly unlikely event unless the malignant blasts were derived from the same clone.[51] Moreover, finding the identical translocations in the dried blood spots obtained from heel sticks at birth demonstrates that these neoplastic-transforming events occurred in utero, prior to diagnosis. This hypothesis is now an accepted mechanism explaining the high concordance of infant leukemia in twins and has led to further understanding the etiology of the acute leukemia in singletons. Some investigators propose that neuroblastoma, the most common extracranial childhood cancer in children, has a similar underlying mechanism,[52–54] but molecular evidence has proved elusive.

29.6 Cancer treatment, screening and survivorship in twins

Esther Lam and Maryam Salehi

Advances in cancer treatments have extended survival for many patients, emphasizing the importance of understanding late effects of cancer diagnosis and treatment. Utilizing within-twin pair comparisons in pairs discordant for cancer to study the effects of cancer and its treatments accounts for genetic and early environmental influences absent in a non-twin design. Unaffected twins of cancer patients offer an excellent control for studies of cancer screening and survivorship. The hypothesis that unaffected twins of cancer patients are more likely to seek screening for that cancer than the general population was tested using the International Twin Study based at USC in Los Angeles by Richardson and Mack and colleagues. In the first study[55] the investigators compared 591 of unaffected co-twins of breast cancer cases to 4000 women in a U.S. national general population sample and found that in the first year after their twin's breast cancer diagnosis, unaffected MZ co-twins sought mammograms and physician breast exams significantly more often than women in the general population, and significantly more than they did prior to their twin's diagnosis. However, this behavior did not last and dropped off to just slightly higher than pre-diagnosis baseline levels after several years, with only about 20% of the co-twins regularly obtaining a mammogram. Interestingly the durability was higher for twins whose sisters survived their breast cancer at least to the time of study participation. In a second study,[56] the investigators examined beliefs about risk in 672 unaffected co-twins of breast cancer cases from the same source (International Twin Study) and found that only half perceived that they were at higher risk

than the general population and most felt that they were not susceptible. Feeling susceptible was significantly correlated with screening behavior, consisting of mammogram and/or physician breast exam. Co-twins whose twin sisters with breast cancer were still alive were more likely to be screened. Using a subset of the same twins (369 co-twin sisters of breast cancer patients), the unaffected co-twins were randomized to an educational intervention about screening versus no intervention (control group).[57] The intervention consisted of a notebook and video mailed to the twins along with reminders and both groups completed a pre- and post-test questionnaire on mammograms and physician breast exams. Unaffected co-twins in the intervention group had 12.8% and 10.3% higher rates of physician breast exams and mammograms, respectively, than the control group. The same study design was also employed to evaluate screening behavior in 83 co-twin sisters of twins with colorectal cancer. Prior to the colorectal cancer diagnosis in their twin, the unaffected co-twins were being screened by occult blood test, digital exam or sigmoidoscopy at the same rate as the general population, but after the diagnosis, screening increased 15–20%, only to drop off again later. The authors concluded that for maintenance of regular screening, annual reminders addressing the motivating factors such as fear of cancer, perception of self-risk, guidelines updates, and family support should be sent to the co-twins.

The last paper[58] reported a two-fold higher likelihood of recent skin examination for moles among 50,044 members of the California Twin Program when the co-twin had history of melanoma; the likelihood increased to three-fold among MZ twins.

Another set of studies focused on cognitive function as an adverse effect of treatment. Cognitive function in cancer survivors was compared to that of their cancer-free co-twins using a retrospective, co-twin control design.[59] 702 twin pairs aged 65 and older discordant for cancer, excluding brain cancer, were identified from the linkage between the Swedish Twin Registry and the Swedish Cancer Registry. History of cancer diagnosis was statistically significantly associated with an almost three-fold increased risk for cognitive dysfunction in long-term (>5 years) cancer survivors compared to that of their unaffected co-twins. The interpretation was limited by the absence of cancer treatment information. In a later study using a subset of the same sample that had treatment information,[60] the researchers found that female cancer survivors were statistically significantly more likely to have cognitive impairment 3 or more years after cancer diagnosis and treatment as their unaffected co-twin (416 cancer-discordant twin pairs). When stratified by zygosity, results were significant only for same-sex female dizygotic pairs (OR = 3.50, 95% CI = 1.15–10.63). A 10-fold increased risk of cognitive impairment was found for survivors who were diagnosed with a gynecologic cancer compared to their unaffected co-twin, especially for those who had cancer treatment that disrupted ovarian functioning.

These studies show that twins have special value when examining effects of a cancer diagnosis and/or treatment on subsequent health or quality of life outcomes. The comparison with a twin, especially an identical twin, who did not experience these cancer and cancer treatment exposures, provides additional validity.

29.7 A novel epidemiological approach to quantify the familial and non-familial, genetic and non-genetic, measured and unmeasured causes of variation in risk

John L. Hopper, James G. Dowty, Shuai Li, Tuong L. Nguyen

We propose a novel approach to understanding genetic and non-genetic components of variation in risk. This model recognizes that there is a natural upper limit to the variation in risk due to genetic factors, but there is no upper limit to variation in risk due to non-genetic factors, and therefore due to all factors.

R.A. Fisher's seminal 1918 paper created the concept of variance components and applied it to estimate the variance in a measured continuously distributed outcome (trait) attributable to (unmeasured) genetic and non-genetic causes.[61] He did this by studying the trait associations (measured by the Pearson correlation coefficient) between relatives, including twin pairs, and thereby partitioned variance into components that had different and "statistically independent" mechanistic origins.

In the introduction to his paper, Fisher warned that: *"loose phrases about the "percentage of causation", which obscure the essential distinction between the individual and the population, should be carefully avoided*. That is, the major issue was the actual magnitude of the variance components, in particular the genetic variance, not a percentage or proportion. Fisher also abhorred the concept of "heritability" as a proportion and referred to it as having a "hotch-potch" of a denominator.[62] The breakthrough by Fisher was showing that the genetic variance would be transmitted to future generations and thereby maintained in the population, a fundamental step in reconciling Mendelian inheritance for binary traits with the genetic inheritance of continuous traits.[63, 64]

29.7.1 Variance of Age-specific Log Incidence Decomposition (VALID)

Variance of Age-specific Log Incidence Decomposition (VALID) is a model that can be used to decompose the familial, genetic and non-familial variance in risk. Risk can be defined as the age-specific log (incidence) if disease is seen as a dynamic process,[66, 67] but it could also be defined as the log (odds ratio) for studying risk to a given age,[68] or log (lifetime risk).[69, 70] Here we will focus on log (incidence) and allow it to be age-dependent, but the model can in essence apply to both situations.[67]

We assume that the relationship between a risk factor (which might also be a composite of risk factors) for a disease outcome can be viewed as a risk score which has a normal distribution in the population, and for which risk increases multiplicatively as the score increases. These characteristics have been observed for polygenic risk scores (PRS)[31] (see Introduction) and for other risk factors (at least once they have been suitably transformed). The risk score can be measurable or hypothetical. While this model might not represent reality for every risk factor, as a model for comparing combined risk factors (e.g., by familial versus non-familial or genetic versus

non-genetic), it appears to be a useful approximation to reality based on empirical evidence for measured genetic and non-genetic factors, at least for some diseases like breast cancer.

29.7.2 Measuring risk discrimination

The strength of the risk score, in terms of its ability to differentiate cases from controls on a population basis, can be assessed by the log odds ratio per standard deviation of the risk score. The risk score is the residual of the risk factor after it has been adjusted for age, sex, and perhaps other potentially confounding risk factors (OPERA)[71, 72] and should be standardized to have zero mean and unit variance for the population to which inference is being made. Note that one should not use the odds ratio per unadjusted standard deviation, as is unfortunately common practice when calculating the "odds per standard deviation".

There is a simple relationship between $\Delta = \log$ (OPERA) and the AUC given by

$$\mathrm{AUC} = \Phi(\Delta\sqrt{2}), \tag{29.1}$$

where Φ is the cumulative standard normal distribution; see Supplementary Material in.[70] Note that $\Delta = \log$ (OPERA) is the difference in the mean of the risk score between cases and controls, and is also referred to in different ways in different disciplines, such as by Cohen's D in psychology.[73]

The log (incidence) has variance

$$\sigma^2 = \Delta^2 = \left[\log\left(\mathrm{OPERA}\right)\right]^2 \tag{29.2}$$

and is equal to the difference in means between cases and controls on the log (incidence) scale.

29.7.3 The familial risk ratio caused by the familial aspects of a risk factor

For a relative of a particular type, rel, let the familial risk ratio $\mathrm{FRR}_{\mathrm{rel}}$ = the risk of disease for the relative of an affected person relative to that for the same type of relative of an unaffected person. Then a risk factor whose standardized risk score above has a risk gradient of $\Delta = \log$ (OPERA) will generate a

$$\mathrm{FRR}_{\mathrm{rel}} = \exp(r_{\mathrm{rel}}\Delta^2) \tag{29.3}$$

where r_{rel} is the correlation in the risk score for the given set of relatives.

This expression was in effect derived by Aalen[66] under the assumption of a multiplicative risk and a rare disease and shown to hold empirically for breast cancer under a more generalized logistic risk model by Hopper & Carlin.[68] Clayton[67] explicitly presented this equation and proved it for both the multiplicative and logistic risk models. As such, we consider this a Fundamental Equation in Genetic Epidemiology. It forms the basis of our model as it allows prediction about a familial risk factor, measured or unmeasured, to be interpreted in terms of disease association between pairs of relatives, such as twins pairs.

Note also that, from (29.1), (29.2) and (29.3),

$$\mathrm{AUC} = \Phi\left\{\left[\log\left(\mathrm{FRR}_{\mathrm{rel}}\right)/2r_{\mathrm{rel}}\right)\right]^{1/2}\right\} \tag{29.4}$$

If only genetic factors cause familial risk, then for first degree-relatives

$$\mathrm{AUC} = \Phi\left\{\left[\log\left(\mathrm{FRR}_{\mathrm{rel}}\right)\right]^{1/2}\right\} \tag{29.5}$$

Therefore, under this assumption, if the FRR for first degree relatives is 2, then AUC = 0.8 and $\Delta = 1.2$, whereas if it is 1.5, AUC = 0.74 and $\Delta = 0.9$.

29.7.4 Modeling the familial causes of variance in risk

Following the classic twin model including its assumption of equal shared environments, which maximizes the genetic component of variance, suppose that the variance in risk (either defined as log (incidence) or logit (cumulative risk)) can be decomposed into an additive genetic component (A) and a shared environment component (C). Therefore, the risk score represents the effects of one or more germline genetic factors so that r_{rel} can be modelled in terms of the kinship coefficients following Fisher[61] and subsequent expansions and modifications.[74]

For MZ twin pairs, for all intents and purposes $r_{rel} = 1$.

For DZ twin pairs and siblings, then

$$r_{\mathrm{rel}} = \left(0.5\mathrm{A} + \mathrm{C}\right)/\left(\mathrm{A} + \mathrm{C}\right) \tag{29.6}$$

If instead of C it is hypothesized that there are non-additive genetic effects at multiple loci which induce a dominance variance D, then

$$r_{\mathrm{rel}} = \left(0.5\mathrm{A} + 0.25\mathrm{D}\right)/\left(\mathrm{A}+\mathrm{D}\right), \tag{29.7}$$

This model can be extended to other relatives, and C could be modelled in terms of the extent to which the pairs of relatives cohabit, have cohabited, and have lived apart.[75–78]

29.7.5 Application of variance of age-specific incidence decomposition (VALID)

As in Hopper & Carlin,[68] we will consider breast cancer, except that this time we will model variance in age-specific log (incidence) rather than variance in logit (cumulative risk).

29.7.5.1 (i) Unmeasured familial factors

First, we consider unmeasured familial factors using twin pair disease associations estimated by the Nordic Twin Study[45] which takes into account the temporal nature of these large, combined, population-based family cohorts in a way that was lacking in an earlier publication.[79]

Column two of Table 29.2 shows that the FRR for MZ pairs ranges from 5.91 before age 50 to 2.04 after age 90, so column four shows that the maximum variance

TABLE 29.2 Familial Risk Ratio (FRR), twin pair covariance in log (incidence), additive genetic (A) and shared environmental (C) components of variance in log (incidence), and maximum area under the receiver operating characteristics curve (AUC_{max}) based on data from the Nordic Twin Study of Breast Cancer.[45]

	FRR		log (FRR)				
Age	*MZ*	*DZ*	*MZ*	*DZ*	*A*	*C*	AUC_{max}
<50	5.91	3.51	1.78	1.26	1.04	0.74	0.83
50-60	4.93	2.77	1.60	1.02	1.15	0.44	0.81
60-70	2.98	2.24	1.09	0.81	0.57	0.52	0.77
70-80	2.5	1.8	0.92	0.59	0.66	0.26	0.75
80-90	2.15	1.67	0.77	0.51	0.51	0.26	0.73
90+	2.04	1.68	0.71	0.52	0.39	0.33	0.72

Approximate confidence intervals that are symmetric on the FRR scale are presented in Moller et al.[45]

goes from 1.78 to 0.71, given that the MZ covariance and $r_{rel} = 1$. Column eight shows that the maximum AUCs that could be achieved from knowing all familial factors, and therefore all germline genetic factors, goes from 0.83 to 0.72 over these age ranges.

Under the classic twin model, column four shows that the variance of the additive genetic component (A) steadily decreases from 1.04 to 0.39, and column five shows that the variance of the shared environment component (C) also steadily decreases from 0.74 to 0.33. Therefore, the total familial variance goes from 1.78 to 0.72. Note that the ratio of these two components to one another, A:C, goes from 1.41 to 2.61 to 1.10 to 2.54 to 1.96 to 1.79 and 1.18, showing no evidence for a simple trend with age. Therefore, about two-thirds of the familial variance is attributed to genetic factors and one-third to environmental factors shared by twins.

29.7.5.2 *(ii) Measured familial factors*

The OPERA for the current best breast cancer polygenic risk score (PRS) is log (1.65) = 0.50 so the variance explained is 0.25.[31] Although the strength of this association appears to be similar across all ages, given the familial variance decreases with age (see Table 29.2) the proportion of familial risk explained by the PRS decreases with age. Given that for MZ pairs, the correlation in the PRS = 1, this PRS explains 0.25/1.78 = 14% of variance before age 50, increasing to 0.25/0.78 = 32% of variance after age 90.

Mammographic density adjusted for age and body mass index is a familial breast cancer risk factor that has a correlation of about 0.6 for MZ pairs and about 0.3 for DZ and sister pairs.[80,81] The risk gradient has an OPERA of about 1.5, so that it is associated with a variance in log (incidence) of about 0.16, of which 0.10 would be familial and 0.06 non-familial cf. 0.25 for the polygenic risk score above.

A large number of risk factors have been identified from questionnaire data, such as number of live births, age at menarche, age at menopause, weight, height, etc. The risk gradients for these are modest, with OPERAs in the range of 1.005 to 1.2.[71] There is, of course, error because easily obtained measurements and simple questions and recall are only surrogates for the true causes. These risk factors also are correlated in relatives, some only modestly due in part to theerror above. Therefore, these risk factors generate both familial, as well as mostly non-familial, components of variance. However, while the variance in log (incidence) from non-familial risk factors appears to be small compared with that due to known – let alone unknown – familial risk factors, there is no natural upper limit. Greater specificity of exposures will increase the variance due to known non-familial factors, as is being found for the mammogram-based risk factors discovered from digital mammography using artificial intelligence.[82]

29.8 Summary

For binary traits, such as having a cancer diagnosis, the natural scale on which to base genetic and environmental variance decomposition is the log (incidence), or the log (odds ratio) based on cumulative incidence e.g., to a specific age. There is an upper limit to the amount of genetic risk discrimination across a population and it is based on the disease association for MZ twin pairs. There is no such upper limit to non-genetic causes of variation,[83] so the concept of heritability as a percentage based on genetic variance divided by total variance in flawed. Moreover, familial risk ratio for cancer is typically highly age-dependent so the risk discrimination at a given age due to genetic and other familial factors differs substantially with age. This observation alone has profound implications for screening and risk reduction strategies, and for understanding the etiology of cancers which are typically multi-factorial diseases. We have demonstrated above how to apply the Variance of Age-specific Log Incidence Decomposition (VALID) model to understand the measured and unmeasured, familial and non-familial aspects of cancers, other diseases and conditions.

References

1. Rothman K. *Epidemiology, An Introduction*. 2nd Edition: Oxford University Press; 2012.
2. Uffelmann E, Huang Q, Munung N, de Vries J, Okada Y, Martin A, et al. Genome-wide association studies. Nat Rev Methods Primers 1:59.
3. Skytte A, Harris J, Czene K, Mucci L, Adami H, Christensen K, et al. Cancer incidence and mortality in260,000 Nordic twins with 30,000 prospective cancers. *Twi Res Hum Genet*. 2019(22):99–107.
4. Ahrenfeldt L, Skytthe A, Möller S, Czene K, Adami H, Mucci L, et al. Risk of Sex-Specific Cancers in Opposite-Sex and Same-Sex Twins in Denmark and Sweden. *Cancer Epidemiol Biomarkers Prev*. 2015;24(10):1622–1628.

5. Neale R, Mineau G, Whiteman D, Brownbill P, Murphy M. Childhood and adult cancer in twins: evidence from the Utah genealogy. *Cancer Epdemiology Biomarkers Prev*. 2005;14:1236–1240.
6. Neale R, Carriere P, Murphy M, Baade P. Testicular cancer in twins: a meta-analysis. *Br J Cancer*. 2008;98:171–173.
7. Banks K, Tuazon E, Berhane K, Koh C, De Filippo R, Chang A, et al. Cryptorchidisim and testicular germ cell tumors: comprehensive meta-analysis reveals that association between these conditions diminished over time and is modified by clinical characteristics. *Front Endocrinol*. 2013;3:1–11.
8. Horn M, Sriprasert I, Ihenacho U, Castelao J, Siegmund K, Bernstein L, et al. Systematic review and meta-analysis of testicular germ cell tumors following in utero exposure to diethysilbestrol. *JNCI Cancer Spectrum*. 2019;3(3):pkz045.
9. Murphy M, Bunch K, Chen B, Hemminki K. Reduced occurrence of cancer in twins compared to singletons: protection but by what mechanism?. *Pediatr Blood Cancer*. 2008;51:62–65.
10. Inskip P, Harvey E, Boice JJ, Stone B, Matanoski G, Flannery J, et al. Incidence of childhood cancer in twins. *Cancer Causes Control*. 1991;2:315–324.
11. Puumela S, Carozza S, Chow E, Fox E, Horel S, Johnson K, et al. Childhood cancer among twins and higher order multiples. *Cancer Epidemiology Biomarkers Prev*. 2009;18:162–168.
12. Strachan D, Wong J, Spector T. Concordance and interrelationship of atopic diseases and markers of allergic sensitization among adult female twins. *J All Clin Immunol*. 2001;108:901–907.
13. Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, Reunanen A, Eriksson J, et al. Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. *D. Diabetologia*. 1992;35(11):1060–1067.
14. Mack TM, Deapen D, Hamilton AS. Representativeness of a roster of volunteer North American twins with chronic disease. *Twin Res*. 2000;3(1):33–42.
15. Mack T, Hamilton A, Press M, Diedp A, Rappaport E. Heritable breast cancer in twins. *Br J Cancer*. 2002;87:294–300.
16. Cockburn M, Black W, McKelvey W, Mack T. Determinants of melanoma in a case-control study in twins. *Cancer Causes Control*. 2001;12:615–625.
17. Mack T, Cozen W, Shibata D, Weiss L, Nathwani B, Hernandez A, et al. Concordance for Hodgkin's disease in identical twins suggests genetic susceptibility to the young-adult form of the disease. *N Eng J Med*. 1995;332:413–418.
18. Mucci L, Hjelmborg J, Harris J, Czene K, Havelick D, Scheike T, et al. Familial Risk and Heritability of Cancer Among Twins in Nordic Countries. *JAMA*. 2016;315(1):68–76.
19. Hamilton A, Lessov Schlaggar C, Cockburn M, Unger J, Cozen W, Mack T. Gender Differences in Determinants of Smoking Initiation and Persistence in California Twins. *Cancer Epidemiol Biomarkers Prev*. 2006;15(6):1189–1197.
20. Hjelmborg J, Korhonen T, Holst K, Skytthe A, Pukkala E, Kutschke J, et al. Nordic Twin Study of Cancer (NorTwinCan) collaboration. Lung cancer, genetic predisposition and smoking: the Nordic Twin Study of Cancer. *Thorax*. 2017;72(11):1021–1027.
21. Blaser M, Chyou P, Nomura A. Age at establishment of Helicobacter pylori infection and gastric carcinoma, gastric ulcer, and duodenal ulcer risk. *Cancer Res*. 1995;55:562–565.
22. Gao L, Weck M, Raum E, Stegmaier C, Rothenbacher D, Brenner H. Sibship size, Helicobacter pylori infection and chronic atrophic gastritis: a population-based study among 9444 older adults from Germany. *Int J Epidemiol*. 2010;39(1):129–134.

23. Linet M, Morton L, Devesa S, Dores G. Leukemias. In: Thun MJ, Linet M, Cerhan J, Haiman C, Schottenfeld D, eds. *Cancer Epidemiology and Prevention*. Fourth Edition ed New York, New York, USA: Oxford University Press; 2018:715–745.
24. Clemmensen S, Harris J, Mengel-From J, Bonat W, Frederiksen H, Kaprio J, et al. Familial Risk and Heritability of Hematologic Malignancies in the Nordic Twin Study of Cancer. *Cancers*. 2021;13(12):3023.
25. Greaves MF, Maia AT, Wiemels JL, Ford AM. Leukemia in twins: lessons in natural history. *Blood*. 2003;102(7):2321–2333.
26. Giovannucci E, Nishihara R, Keum N, Wu K. Cancers of the colon and rectum. In: Thun MJ, Linet M, Cerhan J, Haiman C, Schottenfeld D, eds. *Cancer Epidemiology and Prevention*. Fourth Edition ed. New York, New York, USA: Oxford University Press; 2018:681–707.
27. Tworoger S, Shafrir A, Hankinson S. Ovarian Cancer. In: Thun MJ, Linet M, Cerhan J, Haiman C, Schottenfeld D, eds. *Cancer Epidemiology and Prevention*. Fourth Edition ed. New York, New York, USA: Oxford University Press; 2018:889–909.
28. Cook L, Meisner A, Weiss N. Endometrial Cancer. In: Thun MJ, Linet M, Cerhan J, Haiman C, Schottenfeld D, eds. *Cancer Epidemiology and Prevention*. Fourth Edition ed. New York, New York, USA: Oxford University Press; 2018:909–925.
29. Keum N, Song M, Giovannucci E, Eliassen A. Obesity and Body Composition. In: Thun MJ, Linet M, Cerhan J, Haiman C, Schottenfeld D, eds. *Cancer Epidemiology and Prevention*. Fourth Edition ed. New York, New York, USA: Oxford University Press; 2018:351–377.
30. Thun MJ, Freedman N. Tobacco. In: Thun MJ, Linet M, Cerhan J, Haiman C, Schottenfeld D, eds. *Cancer Epidemiology and Prevention*. Fourth Edition ed. New York, New York, USA: Oxford University Press; 2018.
31. Mavaddat N, Michailidou K, Dennis J, Lush M, Fachal L, Lee A, et al. Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. *Am J Hum Gene*. 2019;104:21–34.
32. Lilyquist J, Ruddy K, Vachon C, Couch F. Common Genetic Variation and Breast Cancer Risk—Past, Present, and Future. *Cancer Epidemiol Biomark Preven*. 2018;27(4): 380–394.
33. Brinton L, Gaudet M, Gierach G. Breast Cancer. In: Thun MJ, Linet M, Cerhan J, Haiman C, Schottenfeld D, eds. *Cancer Epidemiology and Prevention*. New York, New York, USA: Oxford University Press; 2018:861–889.
34. Swerdlow A, De Stavola B, Swanwick M, Mangtani P, Maconochie N. Risk factors for testicular cancer:a case-control study in twins. *Br J Cancer*. 1999;80(7):1098–1102.
35. Cozen W, Hamilton A, Zhao P, Salam M, Deapen D, Nathwani B, et al. A protective role for early childhood exposures and young adult Hodgkin lymphoma. *Blood*. 2009;114: 4014–4020.
36. Cozen W, Yu G, Gail M, Ridaura V, Nathwani B, Hwang A, et al. Fecal microbiota diversity in survivors of adolescent/young adult Hodgkin lymphoma: a study of twins. *Br J Cancer*. 2013;108:1163–1167.
37. Xue F, Michels K. Intrauterine factors and risk of breast cancer:a systematic review and meta-analysis of current evidence. *Lancet Oncol*. 2007;8(12):1088–1100.
38. Bodicoat D, Scoemaker M, Jones M, McFadden E, Griffin J, Ashworth A, et al. Timing of pubertal stages and breast cancer risk: the Breakthrough Generations study. *Breast Cancer Res*. 2014;16(1):R18.

39. Key T, Pike M. The role of oestrogens and progestagens in the epidemipology and prevention of breast cancer. *Eur J Cancer Clin Oncol*. 1988;24:29–43.
40. Linos E, Holmes M, Willit W. Diet and Breast Cancer. *Curr Oncol Rep*. 2007;9:31–41.
41. Cancer CGoHFiB. Alcohol, tobacco amd breast cancer – collaborative reanalysis of individual data from 53 epidemiolological studies, including 58515 women with breast cancer and 95057 women wthout the disease. *Br J Cancer*. 2002;87:1234–1245.
42. Lee S, Ross R, Pike M. An overview of menopausal oestrogen-progestin hormone therapy and breast cancer risk. *Br J Cancer*. 2005;92:2049–2058.
43. Liu L, Zhang J, Wu A, Pike M, Deapen D. Invasive breast cancer incidence trends by detailed race/ethnicity and age. *Int J Cancer*. 2011;130:395–404.
44. Peto JMT. High constant incidence in twins and other relatives of women with breast cancer. *Nat Genet*. 2000;26:411–414.
45. Möller S, Mucci L, Harris J, Scheike T, Holst K, Halekoh U, et al. The heritability of breast cancer among women in the Nordic twin study of cancer. *Cancer Epidemiology Biomark Prev*. 2016;25(1):145–150.
46. Hamilton A, Mack T. Puberty and genetic susceptibility to breast cancer in a case-control study in twins. *N Engl J Med*. 2003;348:2313–2322.
47. Marshall GM, Carter DR, Cheung BB, Liu T, Mateos MK, Meyerowitz JG, et al. The prenatal origins of cancer. *Nat Rev Cancer*. 2014;14(4):277–289.
48. Biondi A, Cimino G, Pieters R, Pui C. Biological and therapeutic aspects of infant leukemia. *Blood*. 2000;96(1):24–33.
49. Wolman I. Parallel responses to chemotherapy in identical twin infants with concordant leukemia. *J Pediatr*. 1962;60:91–96.
50. Wiemels J, Alexander F, Cazzaniga G, Biondi A, Mayer S, Greaves M. Microclustering of TELAML1 translocation breakpoints in childhood acute lymphoblastic leukemia. *Blood*. 2003;102(7):2321–2333.
51. Greaves M, Hughes W. Cancer cell transmission via the placenta. *Evol Med Public Health*. 2018;2018(1):106–115.
52. Boyd TK, Schofield DE. Monozygotic twins concordant for congenital neuroblastoma: case report and review of the literature. *Pediatr Pathol Lab Med*. 1995;15(6):931–940.
53. Tajiri T, Souzaki R, Kinoshita Y, Tanaka S, Koga Y, Suminoe A, et al. Concordance for neuroblastoma in monozygotic twins: case report and review of the literature. *J Pediatr Surg*. 2010;45(12):2312–2316.
54. Shatara M, Xavier AC, Dombkowski A, Cukovic D, Poulik JM, Altinok D, et al. Monozygotic twins with neuroblastoma MS have a similar molecular profile: a case of twin-to-twin metastasis. *Br J Cancer*. 2019;121(10):890–893.
55. Richardson J, Danley K, Mondrus G, Deapen D, Mack T. Mammography and physician breast exams after the diagnosis of breast cancer in a twin or non-twin sister. *Cancer Causes Control*. 1993;4(3):251–260.
56. Richardson J, Mondrus G, Deapen D, Mack T. Future challenges in secondary prevention of breast cancer for women at high risk. *Cancer*. 1994;74(Suppl 4):1474–1481.
57. Richardson J, Mondrus G, Danley K, Deapen D, Mack T. Impact of a mailed intervention on annual mammography and physician breast examinations among women at high risk of breast cancer. *Cancer Epidemiol Biomarkers Prev*. 1996;5(1):71–76.
58. Miller K, Langholz B, Zadnick J, Hamilton A, Cozen W, Mack T, et al. Prevalence and predictors of recent skin examination in a population-based twin cohort. *Cancer Epidemiol Biomarkers Prev*. 2015;24(8):1190–1198.

59. Heflin L, Meyerowitz B, Hall P, Lichtenstein P, Johansson B, Pedersen N, et al. Cancer as a risk factor for long-term cognitive deficits and dementia. *J Natl Cancer Inst*. 2005;97(11):854–856.
60. Kurita K, Meyerowitz B, Hall P, Gatz M. Long-term cognitive impairment in older adult twins discordant for gynecologic cancer treatment. *J Gerontol A Biol Sci Med Sci*. 2011;66A(12):1343–1349.
61. Fisher R. The correlation between relatives on the supposition of Mendelian inheritance. *Trans Roy Soc Edinburgh*. 1918;52:399–433.
62. Fisher R. Limits to intensive production in animals. *Br Agric Bull*. 1951;4:217–218.
63. Hopper J. Genetics for population and public health. *Int J Epidemiol*. 2017;46:8–11.
64. Hopper J. Debunking a myopic view of nature versus nurture. *Clin Experiment Optomet*. 2018;102(1):1–2.
65. Mackenzie D. *The social construction of scientific knowledge*. Edinburgh, Scotland: Edinburgh University Press; 1981:153–183.
66. Aalen O. Modelling the influence of risk factors on familial aggregation of disease. *Biometrics*. 1991;47(3):933–945.
67. Clayton D. Prediction and interaction in complex disease genetics: experience in type 1 diabetes. *PLoS Genet*. 2009;5(7):e1000540.
68. Hopper J, Carlin J. Familial aggregation of a disease consequent upon correlation between relatives in a risk factor measured on a continuous scale. *Am J Epidemiol*. 1992;136:1138–1147.
69. Pharoah P, Antoniou A, Bobrow M, Zimmern R, Easton D, Ponder B. Polygenic susceptibility to breast cancer and implications for prevention. *Nat Genet*. 2002;31(1):33–36.
70. Win A, Dowty J, Cleary S, Kim H, Buchanan D, Young J, et al. Risk of colorectal cancer for carriers of mutations in MUTYH, with and without a family history of cancer. *Gastroenterol*. 2014;146(5):1208–1211.
71. Hopper J. OPERA Odds per adjusted standard deviation: comparing strengths of associations for risk factors measured on different scales and across diseases and populations. *Am J Epidemiol*. 2015;182:863–867.
72. Nguyen T, Schmidt D, Makalic E, Maskarinec G, Li S, Dite G, et al. Novel mammogram-based measures improve breast cancer risk prediction beyond an established mammographic density measure. *Int J Cancer*. 2021;148(9):2193–2202.
73. Salgado J. Transforming the Area under the Normal Curve (AUC) into Cohen's d, Pearson's rpb, Odds-Ratio, and Natural Log Odds-Ratio: Two Conversion Tables. *Euro J Psych Applied Legal Context*. 2018;10(1):35–47.
74. Bulmer M. *The Mathematical Theory of Quantitative Genetics*: Clarendon Press; 1980.
75. Hopper J, Mathews J. Extensions to multivariate normal models for pedigree analysis. *Ann Hum Genet*. 1982;46:373–383.
76. Hannah M, Hopper J, Mathews J. Twin concordance for a binary trait. I. Statistical models illustrated with data on drinking status. *Acta Genet Med Gemellol (Roma)*. 1983;32:127–137.
77. Li S, Nguyen T, Wong E, Dugué P, Dite G, Armstrong N, et al. Genetic and environmental causes of variation in epigenetic aging across the lifespan. *Clin Epigenetics*. 2020;12:158.
78. Li S, Wong E, Dugué P-A, McRae A, Kim E, Joo J-HE, et al. Genome-wide average DNA methylation is determined in utero. *Int J Epidemiol*. 2018;47(3):908–916.
79. Lichtenstein P, Holm N, Verkasalo P, Iliadou A, Kaprio J, Koskenvuo M, et al. Environmental and heritable factors in the causation of cancer–analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med*. 2000;343(2):78–85.

80. Boyd N, Dite G, Stone J, Gunasekara A, English D, McCredie M, et al. Heritability of mammographic density, a risk factor for breast cancer. *N Engl J Med*. 2002;347(12):886–894.
81. Nguyen T, Schmidt D, Makalic E, Dite G, Stone J, Apicella C, et al. Explaining variance in the cumulus mammographic measures that predict breast cancer risk: a twins and sisters study. *Cancer Epidemiol Biomarkers Prev*. 2013;22(12):2395–4031.
82. Hopper J, Nguyen T, Schmidt D, Makalic E, Song Y, Sung J, et al. Going Beyond Conventional Mammographic Density to Discover Novel Mammogram-Based Predictors of Breast Cancer Risk. *J Clin Med*. 2020;9(3):627.
83. Stensrud M, Strohmaier S, Valberg M, Aalen O. Can chance cause cancer? A causal consideration. *Eur J Cancer*. 2017;75:83–85.

CHAPTER

Epigenetic studies of neurodevelopment in twins

30

Namitha Mohandas[a,b,c], Yuk Jing Loke[a], Yen Ting Wong[c], Garth Stephenson[c], Jeffrey M. Craig[a,c]

[a]*Environmental & Genetic Epidemiology Research, Molecular Immunity, Royal Children's Hospital, Parkville, VIC, Australia*

[b]*Department of Paediatrics, University of Melbourne, Parkville, VIC, Australia*

[c]*IMPACT Institute, School of Medicine, Deakin University, Geelong, VIC, Australia*

30.1 Introduction

Neurodevelopmental disorders (NDDs) describe a wide range of disabilities of motor function, cognition, behavior or communication impairments, and psychiatric disorders resulting from dysfunction in the growth or development of the brain. Common NDDs include communication disorders such as autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), intellectual disability; motor disorders such as developmental coordination disorder and cerebral palsy (CP); and other genetic disorders such as Rett syndrome or epilepsy. These conditions are likely to have an onset in early childhood and symptoms can range from developmental deficits affecting specific learning and control of executive functions, to global impairments of social skills or intelligence. Epilepsy can also be associated with increased morbidity and mortality with stigmatizing social and psychological repercussions. Early diagnosis is often difficult given the symptoms and behaviors of neurodevelopment often evolve, as the child grows older.

NDDs are complex and in the past decade, there has been substantial progress in their diagnosis and classification. In 2014, the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (American Psychiatric Association, 2013) classified NDDs based on adaptive functioning instead of intelligence quotient (IQ) scores.[1,2] Communication disorders were classified as a separate entity and motor disorders were reorganized. The classification system also reflects a developmental approach to the classification of each of the disorders. The global burden of disease study in 2016 (GBD 2016) reported that although the burden of mortality among children younger than 5 years decreased by half between 1990 and 2016, there was no corresponding improvement in nonfatal health outcomes among children with developmental disabilities globally.

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00032-5

Genetics has always played an important role in neuroscience research and great progress has been made to understand the inheritance patterns of NDDs. Such studies have advanced considerably, from detecting chromosomal abnormalities such as aneuploidy and microdeletions, to single gene defects and those with complex aetiology.[3] The advances in genetic technologies over the years demonstrate not just the range of genetic abnormalities associated with phenotypes, but also the complexity and variability of NDDs. With the advent of high throughput sequencing technologies, many causative genetic variants have been identified in individuals with NDDs. However, it is not clear whether only a relatively small number of common genetic variants are linked to the aetiology of NDDs, or if a large number of rare genetic variants are involved. Genetic risk scores for NDDs account for only 10% of phenotypic variance.[4] Teasing apart the complexity of genetic patterns of inheritance of NDDs largely involves twin and family studies that establish the magnitude of genetic and environmental components. Such studies provided considerable success in evaluating risk factors at disease-specific loci and genes.

30.2 The role of epigenetics in neurodevelopmental disorders

In 1942, Conrad Waddington coined the term "epigenetics" which literally means the layer (of regulation) above genes.[5] Waddington also hypothesized that epigenetics can respond to the environment. Studies attempting to understand the epigenetic mechanisms involved in the mediation of environment in development have been increasingly common in recent years. Epigenetics is also influenced by genetics and individual stochastic factors. The definition of epigenetics has evolved over time with some examples being "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail change in DNA sequence"[6] and "the structural adaptation of chromosomal regions so as to register, signal, or perpetuate altered activity states."[7] Each definition reflects a different aspect of epigenetic function.

30.2.1 The developmental origins of health and disease (DOHaD) hypothesis

Abnormalities that originate from environmental exposure in early life relate to maternal factors such as nutrition, stress, infections, alcohol, drugs, cigarette smoke, and some of these may affect embryonic development. David Barker in the 1980s found that the incidence of adverse outcomes, such as cardiovascular diseases, in individuals born with a low birth weight, was higher than those with a birth weight in the normal range. He hypothesized that low birth weight was due to a developing fetus adapting to its environment, which included low levels of fetal nutrients.[8] Barker also hypothesized that the adaptive response in the fetus would channel essential

nutrients to the development of the key organs such as the brain, while neglecting the development of other organs such as heart, causing in utero growth restriction and leading to higher chances of chronic illnesses of adulthood such as diabetes.[8–10] Low birth weight has been linked with neurocognitive outcomes in childhood, such as behavioral problems,[11] poor cognitive, language and behavioral skills,[12] as well as lower cortical surface area and volume in adolescence.[13] This was similar to the earlier "thrifty genotype" hypothesis by Neil, who proposed that in a food-scarce environment, the fetus would adapt to the low food environment by selecting "thrifty genes, enabling the child to survive in a food scarce situation in future."[14] However, food may be in abundance in later life stages, and the child's body may not be able to adapt to such an environment, leading to diabetes and related health complications. This adaptive mechanism was later termed the "Barker Hypothesis" then the "Fetal Origins Hypothesis" and is now known as the "Developmental Origins of Health and Disease (DOHaD)" hypothesis. Epigenetics is one of the main factors that may explain the DOHaD concept.

30.2.2 Epigenetic mechanisms

Understanding epigenetics, the "switch" that turns genes on and off, will help to identify how the environment brings changes to the genes that confer disease risk. There are many epigenetic modifications, the best-understood being DNA methylation. DNA methylation is the addition of the methyl molecule (CH_3) to the cytosine-guanine (CpG) dinucleotide of DNA by DNA methyltransferase (DNMT) enzymes.[15,16] DNA methylation state differs between cell types and tissues and its difference between individuals may account for variation in gene expression that may play a role in the expression of certain phenotypes.

Histone modification is another epigenetic mechanism that affects gene expression, by modifying the chromosome structure and function. Histones are proteins found in the eukaryotic cell that help in packaging and ordering DNA into structural units called nucleosomes. Histones undergo post-translational modifications such as acetylation, methylation, phosphorylation, ubiquitylation, and sumoylation. The changes to histone modifications lead to changes in chromatin function and therefore gene expression.[17,18] Noncoding RNA (ncRNA) molecules are also known to be involved in epigenetic regulation.

Epigenetic modifications in disease phenotypes can be due to genetic, developmental, or environmental factors but the exact contributions are unknown in most disorders. Importantly, many studies have shown that prenatal environment can influence epigenetic state, which is in turn related to risk for chronic disease.[19] Furthermore, such epigenetic states have been shown to be maintained for many years after an initial environmental event, examples of which are famine, maternal nutrient intake, and their links with obesity and heart disease.[20,21] Studies in animals have shown that disease-associated epigenetic state can be reversible after birth.[22] This information has huge implications for addressing human disease, namely that

epigenetic assays could be used to supplement other biomarkers to assist with (1) diagnosing chronic diseases; (2) assessing risk in very early childhood before onset of overt symptoms, and (3) designing therapeutic interventions.

Epigenetic state has been implicated as both a mediator and potential biomarker for neurodevelopmental diseases,[23] though these have been less well studied than other chronic diseases.[24,25] DNA methylation has been shown to be important for all aspects of brain development, homeostasis, plasticity, and response to injury.[26] Prenatal damage to the growth and development of the brain can result in serious neurological disorders. Understanding the underlying disease mechanisms and the development of potential biomarkers are key to better prediction and early diagnosis of such chronic conditions. Genetics play an important role in understanding the inheritance patterns of such disorders (discussed elsewhere in this book). However, there is increasing understanding that these disorders, like other chronic conditions, result from a combination of genes, environment, and developmental variation, the latter two being most prominent in early life.

30.3 The role of twins in studying epigenetics of NDDs

The study of twins offers a new opportunity to analyze the role of epigenetics in phenotypic variation because genetic variability within identical (monozygotic, MZ) twins is rare or absent.[27,28] MZ twins develop from one zygote that splits into two during the first few days of life, while dizygotic (DZ) twins develop from two different eggs fertilized by two separate sperm. The mechanisms of MZ twinning are not fully understood; however, it has been hypothesized that the time at which MZ embryos separate determines the development of each embryo and affects growth and development.[29]

30.3.1 Twin models

Twins can be valuable in understanding the importance of genetic and environmental influences on complex trait variation. Twin models such as the ACE model involved estimating proportion of variance due to additive genetic effects (A), common or shared factors (C), and nonshared or unique factors (E) for a given phenotype. Nonshared factors refer largely to the fetoplacental unit where there may be differences in placental size, morphology, location of cord insertion, blood flow, infection, etc., between the twin pairs. This nonshared environment can also include the normal developmental noise that would be different between twins. In chronic diseases, while the A (genetics) stays equal in MZ twins and the C (shared factors) is relatively small in size, the largest variance is from the nonshared factors.[30] Another twin model is the comparison of discordant MZ twins, called the cotwin control model. It is a powerful tool that can aid in the detection of biomarkers for various disorders, as it controls for shared genetic and shared environmental factors, enabling focus on nonshared factors as mechanisms to explain the disease discordance.

30.3.2 The use of twin models in epigenetic studies

An epigenome-wide association study (EWAS) is an analysis of genome-wide set of quantifiable epigenetic marks, such as DNA methylation, to identify associations between epigenetic variation and a phenotype of interest. EWASs have big implications for addressing human disease.

EWASs utilizing MZ twins offer greater power than studies of singletons, given the possibility of discordant MZ cotwin studies.[27,31] The advantage of using MZ twins is that they are perfectly matched for age, genetic background, sex, and family environment. The within-pair differences can be analyzed for an association to biological pathways that may contribute toward their discordance. Such studies also allow exploration of nonshared environmental influences on twins during early life. A wide variety of within-pair epigenetic and gene expression differences have been reported, indicating the role of stochastic and environmental factors in utero and in early life.[27,32–34]

In paediatric-onset NDDs such as ASD, ADHD, CP, and epilepsy, where the conditions are heterogeneous and disease mechanisms involved are complex,[35,36] the discordant cotwin control model is valuable to understand the epigenetic landscape of disease discordance. The next few sections will describe epigenetic studies using twins in common NDDs.

30.4 Epigenetic twin studies in autism spectrum disorder

ASD is a complex and heterogeneous disability that impacts social communication and behavior.[37] It was first described in the 1940s as a condition in which children lacked interest in other people.[38] Its definition has since been broadened and diagnosis of ASD is dependent on three major deficits: impaired communication skills, impaired social skills of interaction, and repetitive or restricted patterns of behavior.[39] As with all mental or psychiatric disorders, ASD often co-occurs with other NDDs such as intellectual disability, ADHD, anxiety disorder, etc.[40,41] The average age of diagnosis of ASD is four years, although in some cases it can be diagnosed at two years.[42] ASD has a higher prevalence in males than females with a ratio of approximately 4:1.[43,44] While an increasing prevalence can be attributed to improved characterization of ASD over the years,[45] an increase in environmental risk factors associated with the disorder cannot be ruled out.

The degree of variation in ASD phenotype caused by genetic variation has been estimated at between 40% and 90%.[46] However, like all other human complex diseases, it is likely caused by a combination of genes and environment. Regulation of DNA methylation is of growing interest in the pathophysiology of NDDs such as ASD, in order to capture trends that may help assist in creating methylation biomarkers. However, high levels of variability between individuals make it difficult to compare studies and replicate results. Few studies have investigated the role of DNA methylation on the landscape of ASD especially using twins as a study design. Wong and colleagues performed an EWAS using MZ twins discordant for ASD ($N = 50$ MZ twins,

six discordant for ASD, individuals from the Twins' Early Development Study, with a mean age of 15 years).[47] This study reported differentially methylated CpGs corresponding to the nuclear transcription factor Y gamma (*NFYC*) gene, with ASD cases having 8% higher DNA methylation compared to cotwin controls. Other genes identified as differentially methylated included dual-specificity phosphatase 2 (*DUSP2*) gene, with 5% lower methylation on average in ASD cotwins. This gene had previously been identified as a target of microRNAs misregulated in ASD.[47]

Wong et al. also evaluated probes associated with the scores of social, traits of communication, and repetitive behaviors and interests. DNA methylation at multiple CpG sites correlated with "Childhood Autism Spectrum Test" scores, including one in the putative promoter of the neurexin 1 (*NRXN1*) gene previously associated with ASD.[47]

Another recent EWAS was performed on samples from five pairs of ASD-discordant MZ twin pairs from the Children Development and Behavior Research Centre, Harbin Medical University, China, mean age 4 years). This study identified over 2000 differentially methylated genes, which were predominantly involved in the activation of the neurotrophin signaling pathway.[48] They also identified and validated significant methylation difference in the SH2B adaptor protein 1 (*SH2B1)* gene in ASD-discordant MZ twins than in ASD-concordant MZ twins as well as in a case-control cohort. This gene is located in one of the common chromosomal abnormal regions reported in autism where microdeletions were linked to developmental delay.[49]

Understanding DNA methylation is imperative to understanding the biological pathway underlying ASD, which may allow the development of early intervention biomarkers.

30.5 Epigenetic twin studies in attention-deficit hyperactivity disorder

ADHD is a NDD that relates to the delay in brain maturation and is characterized by attention deficit, impulsivity, and hyperactivity.[50] It is the most common psychiatric disorder in childhood and adolescence, affecting 5%–8% of school-age children.[51,52] The behavioral attributes of ADHD may continue into adulthood with a prevalence of 4%–6%,[53,54] associating with a range of long-term impairments such as risk of depression, substance use disorders to psychiatric comorbidities.[57–62] Two recent population-based studies revealed that 60%–90% of adults with ADHD had no history of ADHD from their childhood assessments.[55,56] Twins, family, and adoption studies have suggested ADHD has a high genetic component and estimated to be around 70%–80%.[57,58] However, the exact underlying mechanisms of ADHD are poorly understood, and this leads to a reconsideration of the developmental course and study design for ADHD.

To date, DNA methylation studies relevant to ADHD diagnoses or symptoms have been limited and with very little replication between analyses. Diagnoses of ADHD also differ between studies as there are varying symptoms found among children

with ADHD. There have been five studies on candidate genes approach primarily focused on genes related to dopamine function that suggests that their deficiencies impact ADHD development.[67–72]

The first EWAS meta-analysis of ADHD symptoms in twins was in three population-based adult cohorts: The Netherlands Twin Register (NTR, 2258 samples obtained from 2232 individuals from twin families, mean age 37 years), the Dunedin Multidisciplinary Health and Development Study ($N = 800$, unrelated individuals, age 38 years), and the Environmental Risk Longitudinal Twin Study (E-Risk, $N = 1631$ twin pairs, 56% MZ twins and 44% DZ twins, age 18 years).[59] In this study, DNA methylation was assessed in peripheral blood, and the authors identified six nonoverlapping differentially methylated regions (DMRs) in distinct subregions of the major histocompatibility complex (MHC), three in each of the NTR and Dunedin studies. The top DMR, in the MHC region, for the Dunedin study is part of the complement component (C4) gene family, *C4B*, *C4A*, which have been previously associated with schizophrenia.[60] In addition, CpGs associated with exposure to smoking were identified in the nonoverlapping DMR from the NTR and in five DMRs from the Dunedin study. In contrast, no significant ADHD associations were found in the E-Risk study.

A small-scale EWAS ($N = 14$ ADHD-discordant MZ twin pairs, peripheral blood DNA, median age 10.9 years) on children was performed by Chen and colleagues in 2018.[61] The authors focused their study on neuroanatomical, epigenetic, and genetic differences related to discordance for ADHD within MZ twin pairs.[61] Discordance for ADHD was correlated with the dimensions of deep brain structures (striatum) and the inferior or posterior cerebellum. Epigenetic differences were also identified in genes expressed in these "discordant" brain structures. Specifically, 68 of the 173 differentially methylated probes were enriched in shore and shelf regions (regions 0–2 kb and 2–4 kb from CpG islands, respectively) and 67 differentially methylated probes were enriched in enhancer regions (cis-regulatory regions of DNA). The affected twins had a significantly smaller right striatum and thalamus, and a trend toward a larger cerebellum in ADHD. The in vivo neuroanatomical imaging showed similar finding in studies of MZ twins discordant for autism,[62] Alzheimer's,[63] and schizophrenia.[64,65] Several genes previously associated with ADHD were also identified, such as myeloid ecotropic integration site homeobox gene *MEIS2*, which had increased methylation in ten of the affected twins[66] and the vasoactive intestinal peptide receptor 2 *VIPR2* gene, which had higher DNA methylation in three affected twins.[67] EWAS of ADHD has been promising but large and consistently phenotyped cohorts of MZ twins are needed to identify and increase the power for EWAS in ADHD.

30.6 Epigenetic twin studies of dimensions of cognitive development

Cognitive function is commonly assessed in individuals with NDDs and is conceptualized in domains of functioning. Examples of domains include sensation, perception, motor skills and construction, attention and concentration, memory, executive functioning, processing speed, and language or verbal skills.[68] Low performance in some or all

of these domains is associated with a range of cognitive impaired disorders, such as dementia, intellectual disability, and the NDDs already introduced in this chapter. Individual differences observed in cognitive development can be associated with a combination of genetic and environmental factors. Influences from these factors can vary across cognitive domains and age.

There are emerging EWAS analyses that have used discordant-twin model to investigate associations of DNA methylation and various domains in cognitive assessments. To date, published results of these within-pair analyses have been quite varied, ranging from no evidence of associations to suggestive identification of multiple candidate genes and biological pathways. Three papers[69,70] have analyzed associations between general cognitive scores and DNA methylation data from the Middle-Aged Danish Twins (MADT) ($N = 2298$, mean age = 56).[71] General cognitive composite scores in this cohort were computed based on six tests, which looked at verbal fluency, immediate word recall, delayed word recall, processing speed, attention, and working memory. The earliest paper ($N = 486$), by Starnawska and colleagues,[70] showed no evidence of the association between blood DNA methylation age and cognitive abilities in the MADT cohort. The second paper ($N = 486$), showed that a change in cognitive scores over 10 years was associated with *AGBL4* and *SORBS1* DNA methylation.[69] The former is important for neuronal survival,[72] and the latter is associated with Alzheimer's disease.[73] In the third and recent analysis integrating of EWAS and transcriptomic data (N=452), the study reported enriched gene sets related to "neuroactive ligand–receptor interaction," "neurotrophin signaling," "Alzheimer's disease," and "long-term depression."[70]

Several EWAS have focused on various memory domains of the cognitive test using the discordant-twin model. A DNA methylation dataset from the Older Australian Twins Study was analyzed in two papers, one looking at verbal memory ($N = 623$, mean age of 65)[74] and the other episodic memory ($N = 24$, mean age of 75).[75] The former reported no evidence of any associations with DNA methylation. However, the latter showed suggestive evidence of association with DNA methylation within the apolipoprotein A1 (*APOA1*) gene, which has been linked with mild cognitive impairment.[76] In an adult Spanish twin cohort ($N = 48$, mean age 37.8), DNA methylation of insulin-like growth factor 2 binding proteins 1-3 (*IGF2BP1-3*) was reported to be associated with working memory. The same research group have also shown that polymorphic variation in *DNMT3B*, a gene that plays a role in DNA methylation, was linked to different magnitudes of MZ within-pair IQ discordance.[77]

DNA methylation levels of within the dopamine receptor gene *DRD4* were reported to be associated with short-term memory in children twin cohort from Arizona ($N = 48$).[78] In addition, more dopaminergic genes such as *COMT*, *DBH*, *DAT1*, *DRD1*, and *DRD2* were reported to associate with observed MZ within-pair difference in response inhibitory control.[78] This is an important finding because dopamine has been implicated in various domains of cognition.[79]

Though there are no overlaps of candidate genes across these studies, a commonality is observed in the cognitive-related roles of these identified genes. Also, cohorts of different mean ages were investigated, mostly focused on adults and older

twins, with only one on children. As cognitive function is time- and domain-specific, further studies are warranted to better understand the role of epigenetics in cognitive functioning. Nevertheless, the current studies to date have set a strong platform to suggest the importance of epigenetics regulation in the development of cognition in every stage of life.

30.7 Epigenetic twin studies in cerebral palsy

CP is a clinical description of a group of heterogeneous motor impairment syndromes resulting from lesions or anomalies of the brain. They are usually nonprogressive but can still undergo changes in the severity through life. The condition generally arises in the early stages of development and is therefore considered to most likely have causes occurring in utero.[80] CP is the most common physical disability occurring in childhood[81] and preterm infants are at a higher risk of the disease.[82] CP risk is diagnosed between 1 and 2 years of age, on average, as the symptoms of this disorder are heterogeneous in nature thereby making it hard to define early. Primarily, CP affects movements and posture limiting physical capabilities. It can often also be accompanied by difficulties in sensation, perception, cognition, communication, and behavior as well as secondary musculoskeletal problems.[80]

It was once considered that all children with CP were either born premature or had a difficult labor associated with neonatal asphyxia. These events were considered to be directly linked to the sole cause of CP but are now considered to be risk factors in the development of CP.[83] Other demonstrated risk factors include intrauterine infection, changes in maternal blood pressure, and conditions associated with increased clotting.[84] Declining gestational age is another important risk factor for CP. Along with an early gestational age at birth; CP is 50% more likely to develop if associated with white matter injury or other brain injuries.[85] Perinatal factors such as chorioamnionitis (intra-amniotic infection) or other evidence of perinatal inflammation; transient hypothyroxinaemia (low maternal thyroid hormone levels) have been associated with the development of CP in premature infants. However, whether these factors act via brain damage or whether a direct link exists, is unclear. Like other NDDs, CP is also considered to have both a genetic and environmental influence although the extent of the influence of each is not clearly understood. Twin studies addressing the biological mechanisms of CP are not generally considered to represent the true cause of the disorder as factors associated with gestational age at birth influences CP[82,86]. Twins in general have a shorter mean gestational age at birth than singletons and have higher chances of preterm delivery. There have been a number of cases where the prevalence of CP is higher in twins compared to that of singletons, after adjusting for gestational age.[87]

While studies have indicated single nucleotide polymorphisms and copy number variations to be linked to CP, there is growing evidence that risk for CP is mediated by epigenetic mechanisms.[88] Dysregulation of methylation capacity and folate single- carbon metabolism has been reported in children affected with severe CP.[89]

Folate or single-carbon metabolism provides the carbon substrate (methyl group) required for DNA methylation. Several studies have looked at DNA methylation patterns in CP-affected and unaffected individuals to attempt to create a diagnostic biomarker that would allow for early intervention.[90] A major limitation, however, of such studies is the limited sample size and heterogeneous cohorts.

Twin studies provide more power to detect small differences compared to singletons when sample sizes are low. A recent EWAS ($N = 15$ CP-discordant twin pairs, Victorian CP Registry, Australia) identified DNA methylation differences around genes specific to immune function, cell adhesion, and inflammatory signaling.[91] In the study using DNA extracted from newborn blood spots (Guthrie cards), 33 genomic sites were differentially methylated within CP-discordant twin pairs associated with 25 genes, enriched in immune response signaling. A DMR within the lymphotoxin alpha (*LTA*) gene, which has been reported to play a role in inflammation and brain development, mediating preterm birth and white matter brain injury, was also found to be consistently differentially methylated in CP cases across all twin pairs. Likewise, a 10kb gene region within the Lck interacting transmembrane Adaptor 1 (*LIME1*) gene was differentially methylated in CP-affected twins across all pairs. Subsequent EWAS studies[92,90] have also implicated relevant immune genes to be associated with the pathophysiology of CP. Immune cells from inflammation can be caused by neonatal hypoxia-ischaemia caused by brain injury. This association of inflammation and perinatal brain injury may suggest one of the epigenetic mechanisms behind CP. Studies of phenotypically discordant MZ twins have allowed for within-twin pair comparisons allowing the genetic and environmental components of complex human disorders to be better identified.

30.8 Epigenetic twin studies in epilepsy

Epilepsy is a group of brain disorders marked by sudden, recurrent episodes of motor or nonmotor disturbance, loss of consciousness or convulsions, and is associated with excessive electrical activity in the brain. The aetiology of epilepsy includes a wide variety of phenomena, including traumatic brain or head injury, developmental brain malformations, and genetic causes. The absence of an underlying structural brain abnormality is termed as "idiopathic epilepsy" and can be classified into generalized and focal epilepsies. The role of genetics in epilepsy has long been described with various studies characterizing the patterns of inheritance of epilepsy, and the molecular explanations for these patterns.[108–111]

The genetic component of epilepsies has been defined by familial and twin studies prior to the genomic era, and further strengthened by the discovery of genes mutated in epilepsy. One of the first twin studies in epilepsy was by Sillanpää in 1998,[93] with a cohort consisting of over 27,000 twin pairs in total from the Finnish Twin Cohort Study and including 316 cases of epileptic seizures occurring in 310 twin pairs, both MZ and DZ. The results suggested that there was a higher concordance rate in (observed to expected ratio = 5.48) in MZ twins as opposed to DZ

pairs (observed to expected ratio = 2.12). Genome-wide expression was also investigated in five discordant and four concordant MZ twins with idiopathic absence epilepsy and healthy controls.[94] Using microarrays, the authors identified genes that were differentially expressed, and 16 of those genes were validated using real-time quantitative reverse transcription PCR (qRT-PCR). However, genetic variants currently associated with epilepsy account for only a minor portion of the variation in all epilepsies. The complicated genotype-phenotype correlations in epilepsy indicate that other modifying factors are responsible for determining the specific subtype. Moreover, twin studies have shown differing case-wise concordance estimates (proportion of epilepsy–concordant pairs as a proportion of concordant and discordant pairs combined) for idiopathic generalized epilepsies (MZ= 0.77; DZ= 0.35) and focal epilepsies (MZ= 0.40; DZ = 0.03).[95] The lack of complete case-wise concordance estimates in MZ twin pairs who are genetically identical indicates that presence of other factors, such as nonshared factors, may contribute to discordance.[96] There is growing evidence that epigenetic change plays a major role in neurodevelopment, brain maturation, and brain function as well as epileptogenesis.[97] Previous studies of human brain tissues from temporal lobe epilepsy have demonstrated specific DNA methylation patterns associated with epilepsy.[98–100] However, as with other NDDs, it is evident from twin studies that the high level of discordance of epilepsy in MZ twins suggests the role of nongenetic factors in the aetiology of the disease. Mohandas and colleagues used a discordant MZ twin model to assess variation of DNA methylation in idiopathic epilepsy ($N = 15$ discordant twin pairs, Twins Research Australia, Epilepsy Research Centre Database, Queensland Institute of Medical Research and Epilepsy Queensland, mean age of 47).[101] The study identified genes such as calcium and potassium voltage-gated channel genes, *KCNH5* and *CACNB2* that was significantly different between the focal and generalized subtypes of epilepsy.[101] Analysis of DMRs identified genes such as *PM20D1* and *GFPT2*, which have a role in neuronal pathways. Separate DNA methylation analysis of discordant focal and generalized epilepsy twin pairs demonstrated top DMR-associated genes *OTX1*, *GDNF*, and *DLX5*.[101] Biological functions of these genes support these regions as plausible candidate biomarkers for epilepsy.

30.9 Current issues for study of NDDs in twins

30.9.1 Can twin studies tease out cause versus effect?

Two major types of biomarkers are diagnostic biomarkers that provide discrete and objective indication of diagnostic status, and screening biomarkers, which would allow determination of risk status of a condition. Biomarkers can be of significant translational value as they allow the determination of diagnostic risk prior to the appearance of behavior symptoms, thereby making early detection and intervention possible in the case of disorders such as CP. Minimizing the functional and social impacts of NDDs such as epilepsy would represent a significant advancement for large numbers of children with these life-long conditions which not only impact

on the individual child but on their families too. Twins can be used in longitudinal studies where DNA methylation patterns at various time points can be measured, which allows to understand the onset of disease if not already present at birth and go beyond association studies to shed light on causation.[27]

30.9.2 Incidence of neurodevelopmental disorders in twins versus singletons

Epigenetic analysis utilizing twins from longitudinal studies could contribute toward the development of predictive, diagnostic, and prognostic biomarkers for complex NDDs. However, some aspects to consider when studying twins is that they have a shorter mean gestational age than singletons and have higher chances of preterm delivery. This becomes an issue when factors associated with gestational age at birth influences health outcomes of interest, as is the case for CP.[82,86] Lower birth weight is linked to preterm birth as well, which might indicate that this factor also likely increases the risk of developing a NDD.[102] Utilizing twins as a model to study NDDs has many advantages but care needs to be taken when extrapolating findings in twins to singletons such as comparing similar gestational ages in singleton cohorts or adjusting for gestational ages.

30.9.3 Choice and availability of tissue samples

The biologically relevant tissue type in epigenetic analysis involving NDDs is brain tissue; however, obtaining high-quality brain tissue samples is often not feasible and includes its own challenges. Most often, studies that include brain tissues are obtained from postmortem samples. A common alternative is to use peripheral tissues such as blood, buccal or saliva, which are generally considered to be good indicators of biological mechanisms in the brain.

Studies have shown that peripheral tissues can be used effectively to identify biomarkers of NDDs that may or may not mirror mechanisms in the brain. In many cases, blood has been used to detect differentially methylation patterns between affected and unaffected individuals in disorders such as schizophrenia,[103] bipolar disorder,[104] and Parkinson's disease.[105] The epigenetic profiles from blood tissue have been shown to have a large overlap with the profiles detected from brain samples.[103,105] Moreover, tissues used for predicting biomarkers do not necessarily have to be from the brain. The utility of a biomarker is the indication of a dysfunctional biological process that can be measured easily and noninvasively. A predictive biomarker could mirror a specific pathway in the brain or be a cumulative effect of several biological processes being affected in a disease.

Recently, the use of buccal tissues as an alternative to blood has been reported to be effective in EWAS. Lowe and colleagues compared the methylome of buccal versus blood and found a higher association of DNA methylation to disease phenotype in buccal cells compared to that of blood.[106] Buccal tissues are also reported to be a better proxy to study brain-related disorders as they exhibit closer similarities

to brain DNA methylation patterns than blood.[106,107] Many studies also identified strong correlations between the differentially methylated CpG sites identified in brain tissue compared with buccal tissues in both diseased population as well as healthy cohorts looking at childhood stress and adversity.[108,109] Buccal cells are also considered by some a better tissue to use to study NDDs as they originate from ectodermal cell lineage that is the same as the brain, and have been used to identify potential epigenetic biomarkers for neurological outcomes.[110,111] This is especially relevant for studies looking at early life exposures that take place before buccal and brain cells differentiate from a common germinal epithelium.

Therefore, given the difficulty to sample brain tissues for studies from living individuals, the most practical solution for epigenetic studies is to make use of the appropriate peripheral tissues such as blood or buccal epithelium, or both.

30.9.4 Study sample sizes and power of epigenetic analyses

Although the power of epigenetic studies depends on large sample sizes, as with other -omic studies, it is known that the proportion of variance explained by single epigenetic variants is often larger than with genetic variants.[112,113] The power of an EWAS study depends on several factors such as study design and study sample sizes. Typical sample sizes have grown from two to four-digit numbers over the past few years and the necessary sample size is dependent on effect size.[114] Recent evidence has shown that sample size of 500 cases and 500 controls can detect an effect of 2% with greater than 80% power in 81% of sites.[115] EWAS involving MZ twins offer greater power than studies of singletons, especially discordant MZ cotwin studies. Discordant MZ cotwin studies allow for smaller sample sizes because within-pair analysis controls for sex, age, parents, family environment, and genetics. The comparison of discordant MZ twins offers an alternative to the traditional case-control study. Tsai and Bell[116] have shown that sample sizes of 25 twin pairs or more are preferable to detect a mean effect size of 8% methylation with a statistical significance of 0.05 after adjusting for multiple testing.

30.10 The future of twin studies in contributing to understanding the role of epigenetics in neurodevelopmental disorders

During the last few years, it has become apparent that most chronic health conditions, from heart disease to psychiatric disorders, originate early in life. The studies discussed in this chapter shed a light on the importance of twins to further carry out epigenome studies of larger cohorts and warrants replication of results in nontwin population. An essential follow-up to these EWAS, to enhance the specificity and interpretability of such studies, is to integrate parallel multiomic data within the same cohort.[117–119] Combining data from multiomics studies has the potential to give insight into the biological pathways underlying the disease as well as provide disease markers to

facilitate early diagnosis. Complex disorders such as NDDs can benefit from such studies by assisting with a clear diagnosis, prognosis, and progression and guiding personalized treatments. A challenging aspect of multiomic studies, however, is the reproducibility of results as most neurodevelopmental conditions are compounded by data heterogeneity, a lack of standard clinical assessments and insufficient clinical data linked to molecular data. With recent technological advances, omic datasets are typically large and complex. Analysis of such omic datasets requires tailored statistical approaches and incorporating twin study designs into omic analyses will allow us to understand the effects of environmental and stochastic factors in human disease.

References

1. Harris J. New terminology for mental retardation in DSM-5 and ICD-11. *Current Opinion Psychiatry*. 2013;26:260–262.
2. Salvador-Carulla LRG, Vaez-Azizi LM, Cooper SA, et al. Intellectual developmental disorders: towards a new name, definition and framework for "mental retardation/ intellectual disability" in ICD-11. *World Psychiatry*. 2011;10:175–180.
3. Shen JMD. Advances in genetic diagnosis of autism spectrum disorders. *Current Pediatrics Reports*. 2014;2:71–81.
4. Niemi MEK, Martin HC, Rice DL, et al. Common genetic variants contribute to risk of rare severe neurodevelopmental disorders. *Nature*. 2018;562:268–271.
5. Waddington CH. Genetic assimilation of the bithorax phenotype. *Evolution; Internation Journal of Organic Evolution*. 1956;10:1–13.
6. Wu C, Morris JR. Genes, genetics, and epigenetics: a correspondence. *Science*. 2001;293:1103–1105.
7. Bird A. Perceptions of epigenetics. *Nature*. 2007;447:396–398.
8. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet*. 1993;341:938–941.
9. Burdge GCLK. Bridging the gap between epigenetics research and nutritional public health interventions. *Genome Med*. 2010;2:80.
10. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. 1992. *International Journal of Epidemiology*. 2013;42:1215–1222.
11. McCormick MCGS, Sobol AM. Very low birth weight children: behavior problems and school difficulty in a national sample. *J Ped*. 1990;117:687–693.
12. Burnett ACSS, Lee KJ, Cheong J, et al. Executive function in adolescents born <1000 g or <28 weeks: a prospective cohort study. *Pediatrics*. 2015;135:e826–e834.
13. Raznahan AGD, Lee NR, Clasen LS, Giedd JN. Prenatal growth in humans and postnatal brain maturation into late adolescence. *Pnas*. 2012;109:11366–11371.
14. Neel J. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *American Journal of Human Genetics*. 1962;14:353–362.
15. Gibney ERNC. Epigenetics and gene expression. *Heredity*. 2010;105:4–13.
16. Vaissière TSC, Herceg Z. Epigenetic interplay between histone modifications and DNA methylation in gene silencing. *Mutation Research*. 2008;659:40–48.
17. Gallinari P, Di Marco S, Jones P, Pallaoro M, Steinkuhler C. HDACs, histone deacetylation and gene transcription: from molecular biology to cancer therapeutics. *Cell Research*. 2007;17:195–211.

18. Wada T, Kikuchi J, Nishimura N, Shimizu R, Kitamura T, Furukawa Y. Expression levels of histone deacetylases determine the cell fate of hematopoietic progenitors. *Journal of Biological Chemistry*. 2009;284:30673–30683.
19. Gluckman PDHM, Mitchell MD. Developmental origins of health and disease: reducing the burden of chronic disease in the next generation. *Genome Med*. 2010;2:14.
20. Godfrey KM, Costello PM, Lillycrop KA. The developmental environment, epigenetic biomarkers and long-term health. *J Dev Orig Health Dis*. 2015;6:399–406.
21. Heijmans BT, Tobi EW, Lumey LH, Slagboom PE. The epigenome: archive of the prenatal environment. *Epigenetics*. 2014;4:526–531.
22. Weaver IC, Champagne FA, Brown SE, et al. Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. *Journal of Neuroscience*. 2005;25:11045–11054.
23. Petronis A. Epigenetics as a unifying principle in the aetiology of complex traits and diseases. *Nature*. 2010;465:721–727.
24. Loke YJ, Craig JM. The role of epigenetic change in Autism spectrum disorders. *Front Neurol.* 2015;6:107.
25. Mikeska T, Craig JM. DNA methylation biomarkers: cancer and beyond. *Genes (Basel)*. 2014;5:821–864.
26. Mehler MF. Epigenetic principles and mechanisms underlying nervous system functions in health and disease. *Progress in Neurobiology*. 2008;86:305–341.
27. van Dongen J, Slagboom PE, Draisma HH, Martin NG, Boomsma DI. The continuing value of twin studies in the omics era. *Nature Reviews Genetics*. 2012;13:640–653.
28. Wong AH, Gottesman II, Petronis A. Phenotypic differences in genetically identical organisms: the epigenetic perspective. *Human Molecular Genetics*. 2005;14(Spec No 1):R11–R18.
29. McNamara HC, Kane SC, Craig JM, Short RV, Umstad MP. A review of the mechanisms and evidence for typical and atypical twinning. *American Journal of Obstetrics and Gynecology*. 2016;214:172–191.
30. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nature Reviews Genetics*. 2002;3:872–882.
31. Martin NBD, Machin G. A twin-pronged attack on complex traits. *Nature Genetics*. 1997;17:387–392.
32. Gordon L, Joo JHE, Andronikos R, et al. Expression discordance of monozygotic twins at birth effect of intrauterine environment and a possible mechanism for fetal programming. *Epigenetics*. 2011;6:579–592.
33. Gordon L, Joo JE, Powel JE, et al. Neonatal DNA methylation profile in human twins is specified by a complex interplay between intrauterine environmental and genetic factors, subject to tissue-specific influence. *Genome Research*. 2012;22:1395–1406.
34. Ollikainen M, Smith KR, Joo EJ, et al. DNA methylation analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome. *Human Molecular Genetics*. 2010;19:4176–4188.
35. Jacob S, Wolff JJ, Steinbach MS, Doyle CB, Kumar V, Elison JT. Neurodevelopmental heterogeneity and computational approaches for understanding autism. *Transl Psychiatry*. 2019;9:63.
36. van Loo KMJMG. Genetic and environmental factors in complex neurodevelop disorders. *Current Genomics*. 2007;8:429–444.
37. Bailey ALCA, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M. Autism as a strongly genetic disorder: evidence from a British twin study. *Psychological Medicine*. 1995;25:63–77.

38. Kanner L. Autistic disturbances of affective contact. *Nervous Child*. 1943;2:217–250.
39. American Psychiatric Association D-TF. *Diagnostic and Statistical Manual of Mental Disorders: DSM-5™*. 5th ed. Arlington, VA 22209-3901: American Psychiatric Publishing, Inc.; 2013.
40. Matson JL, Shoemaker M. Intellectual disability and its relationship to autism spectrum disorders. *Research in Developmental Disabilities*. 2009;30:1107–1114.
41. Mannion ALG. Comorbidity in autism spectrum disorder: A literature review. *Res Autism Spectrum Disorders*. 2013;7:1595–1616.
42. (CDC) CfDCaP. Data & statistics on autism spectrum disorder 2015. https://www.cdcgov/ncbddd/autism/data.html. (Accessed on 12 July 2021).
43 Werling DM, Geschwind DH. Sex differences in autism spectrum disorders. *Current Opinion in Neurology*. 2013;26:146–153.
44. Idring S, Lundberg M, Sturm H, et al. Changes in prevalence of autism spectrum disorders in 2001-2011: findings from the Stockholm Youth Cohort. *J Autism Develop Disorders*. 2014;45:1766–1773.
45. Bourgeron T. Current knowledge on the genetics of autism and propositions for future research. *Comptes Rendus Biologies*. 2016;339:300–307.
46. Hallmayer JCS, Torres A, Phillips J, et al. Genetic heritability and shared environmental factors among twin pairs with autism. *Archives of General Psychiatry*. 2011;68:1095–1102.
47. Wong C, Meaburn EL, Ronald A, et al. Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits. *Molecular Psychiatry*. 2014;19:495–503.
48. Liang S, Li Z, Wang Y, et al. Genome-wide DNA Methylation analysis reveals epigenetic pattern of SH2B1 in Chinese monozygotic twins discordant for autism spectrum disorder. *Front Neurosci.* 2019;13:712.
49. Maillard AM, Ruef A, Pizzagalli F, et al. The 16p11.2 locus modulates brain structures common to autism, schizophrenia and obesity. *Molecular Psychiatry*. 2015;20:140–147.
50. Shaw PEK, Sharp W, Blumenthal J, et al. Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. *PNAS*. 2007;104:19649–19654.
51. Polanczyk G, de Lima MS, Horta BL, Biederman J, Rohde LA. The worldwide prevalence of ADHD: a systematic review and metaregression analysis. *American Journal of Psychiatry*. 2007;164:942–948.
52. Willcutt EG. The prevalence of DSM-IV attention-deficit/hyperactivity disorder: a meta-analytic review. *Neurotherapeutics*. 2012;9:490–499.
53. Kessler RC, Adler L, Barkley R, et al. The prevalence and correlates of adult ADHD in the United States: results from the National Comorbidity Survey Replication. *American Journal of Psychiatry*. 2006;163:716–723.
54. Franke B, Faraone SV, Asherson P, et al. The genetics of attention deficit/hyperactivity disorder in adults, a review. *Molecular Psychiatry*. 2012;17:960–987.
55. Moffitt TEHR, Asherson P, Belsky DW, et al. Is adult ADHD a childhood-onset neurodevelopmental disorder? Evidence from a four-decade longitudinal cohort study. *American Journal of Psychiatry*. 2015;172:967–977.
56. Caye A, Rocha TB, Anselmi L, et al. Attention-deficit/hyperactivity disorder trajectories from childhood to young adulthood: evidence from a birth cohort supporting a late-onset syndrome. *JAMA Psychiatry*. 2016;73:705–712.
57. Burt S. Rethinking environmental contributions to child and adolescent psychopathology: a meta-analysis of shared environmental influences. *Psychol Bulletin*. 2009;135:608–637.

58. Faraone SV, Perlis RH, Doyle AE, et al. Molecular genetics of attention-deficit/hyperactivity disorder. *Biological Psychiatry*. 2005;57:1313–1323.
59. van Dongen J, Zilhao NR, Sugden K, et al. Epigenome-wide association study of attention-deficit/hyperactivity disorder symptoms in adults. *Biological Psychiatry*. 2019;86:599–607.
60. Sekar A, Bialas AR, de Rivera H, et al. Schizophrenia risk from complex variation of complement component 4. *Nature*. 2016;530:177–183.
61. Chen YC, Sudre G, Sharp W, et al. Neuroanatomic, epigenetic and genetic differences in monozygotic twins discordant for attention deficit hyperactivity disorder. *Molecular Psychiatry*. 2018;23:683–690.
62. Kates WR, Burnette CP, Eliez S, et al. Neuroanatomic variation in monozygotic twin pairs discordant for the narrow phenotype for autism. *American Journal of Psychiatry*. 2004;161:539–546.
63. Rossi R, Pievani M, Jarvenpaa T, et al. Voxel-based morphometry study on monozygotic twins discordant for Alzheimer's disease. *Acta Neurologica Scandinavica*. 2016;133:427–433.
64. Suddath RL, Christison GW, Torrey EF, Casanova MF, Weinberger DR. Anatomical abnormalities in the brains of monozygotic twins discordant for schizophrenia. *New England Journal of Medicine*. 1990;322:789–794.
65. Pol HESH, Mandl RC, Brans RG, et al. Gray and white matter density changes in monozygotic and same-sex dizygotic twins discordant for schizophrenia using voxel-based morphometry. *Neuroimage*. 2006;31:482–488.
66. Lasky-Su J, Neale BM, Franke B, et al. Genome-wide association scan of quantitative traits for attention deficit hyperactivity disorder identifies novel associations and confirms candidate gene associations. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*. 2008;147B:1345–1354.
67. Wilmot BFR, Smeester L, Musser ED, Mill J, Nigg JT. Methylomic analysis of salivary DNA in childhood ADHD identifies altered DNA methylation in VIPR 2. *Journal of Child Psychology and Psychiatry and Allied Disciplines*. 2016;57:152–160.
68. Harvey P. Domains of cognition and their assessment. *Dialogues Clin Neurosci*. 2019;21:227–237.
69. Starnawska ATQ, Lenart A, McGue M, et al. Epigenome-wide association study of cognitive functioning in middle-aged monozygotic twins. *Front Aging Neurosci*. 2017;9(413):1–10.
70. Soerensen MH-RD, Nygaard M, Larsen MJ, et al. A genome-wide integrative association study of DNA methylation and gene expression data and later life cognitive functioning in monozygotic twins. *Front Neurosci*. 2020;14:233.
71. Skytthe ACL, Kyvik KO, Bødker FL, et al. The Danish twin registry: linking surveys, national registers, and biological information. *Twin Research Human Genetics*. 2013;16:104–111.
72. DJ RK, MM M, Bosc C, et al. A family of protein-deglutamylating enzymes associated with neurodegeneration. *Cell*. 2010;143:564–578.
73. Blalock EMGJ, Chen KC, Porter NM, Markesbery WR, Landfield PW. Incipient Alzheimer's disease: Microarray correlation analyses reveal major transcriptional and tumor suppressor responses. *PNAS*. 2004;101:2173–2178.
74. Sachdev PSLT, Wen W, Ames D, et al. The contribution of twins to the study of cognitive ageing and dementia: the older Australian twins study. *International Review of Psychiatry (Abingdon, England)*. 2013;25:738–747.

75. Lazarus JMK, Armstrong N, Song F, et al. DNA methylation in the apolipoprotein-A1 gene is associated with episodic memory performance in healthy older individuals. *J Alzheimer's Disease*. 2015;44:175–182.
76. Song FPA, Kochan NA, Raftery M, Brodaty H, Smythe GA, Sachdev PS. Plasma protein profiling of mild cognitive impairment and Alzheimer's disease using iTRAQ quantitative proteomics. *Proteome Science*. 2014;12:5.
77. Córdova-Palomera AF-VM, Falcón C, Bargalló N, et al. Birth weight and adult IQ, but not anxious depressive psychopathology, are associated with cortical surface area: a study in twins. *PLoS One*. 2015;10(6): e0129616: 1–14.
78. Lewis CRH-SA, Breitenstein RS, Sowards HA, et al. Dopaminergic gene methylation is associated with cognitive performance in a childhood monozygotic twin study. *Epigenetics*. 2019;14:310–323.
79. Nieoullon A. Dopamine and the regulation of cognition and attention. *Progress in Neurobiology*. 2002;67:53–83.
80. Rosenbaum NP P, Leviton A, Goldstein M, Martin B. A report: the definition and classification of cerebral palsy April 2006. *Developmental Medicine and Child Neurology Supplement*. 2007;109:8–14.
81. Novak I, Hines M, Goldsmith S, Barclay R. Clinical prognostic messages from a systematic review on cerebral palsy. *Pediatrics*. 2012;130:e1285–e1312.
82. Elovitz MA, Mrinalini C, Sammel MD. Elucidating the early signal transduction pathways leading to fetal brain injury in preterm birth. *Pediatric Research*. 2006;59:50–55.
83. Murphy DJ, Hope PL, Johnson A. Neonatal risk factors for cerebral palsy in very preterm babies: case-control study. *BMJ*. 1997;314:404–408.
84. O'Callaghan ME, MacLennan AH, Gibson CS, et al. Epidemiologic associations with cerebral palsy. *Obstetrics and Gynecology*. 2011;118:576–582.
85. Pinto-Martin JARS, Cnaan A, Holzman C, Susser MW, Paneth N. Cranial ultrasound prediction of disabling and nondisabling cerebral palsy at age two in a low birth weight population. *Pediatrics*. 1995;95:249–254.
86. Arpino C, D'Argenzio L, Ticconi C, et al. Brain damage in preterm infants: etiological pathways. *Ann Ist Super Sanita*. 2005;41:229–237.
87. Tollanes MC, Wilcox AJ, Lie RT, Moster D. Familial risk of cerebral palsy: population based cohort study. *BMJ*. 2014;349:g4294.
88. Fleiss B, Gressens P. Tertiary mechanisms of brain damage: a new hope for treatment of cerebral palsy? *Lancet Neurology*. 2012;11:556–566.
89. Schoendorfer NC, Obeid R, Moxon-Lester L, et al. Methylation capacity in children with severe cerebral palsy. *European Journal of Clinical Investigation*. 2012;42:768–776.
90. Crowgey EL, Marsh AG, Robinson KG, Yeager SK, Akins RE. Epigenetic machine learning: utilizing DNA methylation patterns to predict spastic cerebral palsy. *Bmc Bioinformatics [Electronic Resource]*. 2018;19:225.
91. Mohandas N, Bass-Stringer S, Maksimovic J, et al. Epigenome-wide analysis in newborn blood spots from monozygotic twins discordant for cerebral palsy reveals consistent regional differences in DNA methylation. *Clin Epigenetics*. 2018;10:25.
92. Bahado-Singh RO, Vishweswaraiah S, Aydas B, Mishra NK, Guda C, Radhakrishna U. Deep learning/artificial intelligence and blood-based DNA epigenomic prediction of cerebral palsy. *International Journal of Molecular Sciences*. 2019;20(9):2075: 1–15.
93. Sillanpaa M, Jalava M, Kaleva O, Shinnar S. Long-term prognosis of seizures with onset in childhood. *New England Journal of Medicine*. 1998;338:1715–1722.

94. Helbig IMN, Vadlamudi L, Lawrence KM, et al. Gene expression analysis in absence epilepsy using a monozygotic twin design. *Epilepsia*. 2008;49:1546–1554.
95. Vadlamudi L, Milne RL, Lawrence K, et al. Genetics of epilepsy: the testimony of twins in the molecular era. *Neurology*. 2014;83:1042–1048.
96. Kjeldsen MJ, Kyvik KO, Christensen K, Friis ML. Genetic and environmental factors in epilepsy: a population-based study of 11900 Danish twin pairs. *Epilepsy Research*. 2001;44:167–178.
97. Kobow K, Kaspi A, Harikrishnan KN, et al. Deep sequencing reveals increased DNA methylation in chronic rat epilepsy. *Acta Neuropathologica*. 2013;126:741–756.
98. Miller-Delaney SF, Das S, Sano T et al. Differential DNA methylation patterns define status epilepticus and epileptic tolerance. *J. Neurosci*. 2012;32(5):1577–1588.
99. Zhu, Q, Wang, L, Zhang, Y, et al. Increased expression of DNA methyltransferase 1 and 3a in human temporal lobe epilepsy. *J Mol Neurosci*, 2012;46(2):420–426. doi:10.1007/s12031-011-9602-7.
100. Long, HY, Feng, L, Kang, J, et al. Blood DNA methylation pattern is altered in mesial temporal lobe epilepsy. *Sci Rep*. 2017;7:43810. doi:10.1038/srep43810.
101. Mohandas N, Loke YJ, Hopkins S, et al. Evidence for type-specific DNA methylation patterns in epilepsy: a discordant monozygotic twin approach. *Epigenomics*. 2019;11:951–968.
102. A L. Preterm birth and cerebral palsy: is tumor necrosis factor the missing link? *Develop Med Child Neurol*. 1993;35:553–558.
103. Wockner LF, Noble EP, Lawford BR, et al. Genome-wide DNA methylation analysis of human brain tissue from schizophrenia patients. *Transl Psychiatry*. 2014;4:e339.
104. Teroganova N, Girshkin L, Suter CM, Green MJ. DNA methylation in peripheral tissue of schizophrenia and bipolar disorder: a systematic review. *Bmc Genetics*. 2016;17:27.
105. Masliah E, Dumaop W, Galasko D, Desplats P. Distinctive patterns of DNA methylation associated with Parkinson disease: identification of concordant epigenetic changes in brain and peripheral blood leukocytes. *Epigenetics*. 2013;8:1030–1038.
106. Lowe RGC, Beyan H, Hawa MI, et al. Buccals are likely to be a more informative surrogate tissue than blood for epigenome-wide association studies. *Epigenetics*. 2013;8:445–454.
107. Smith AK, Kilaru V, Klengel T, et al. DNA extracted from saliva for methylation studies of psychiatric traits: evidence for tissue specificity and relatedness to brain. *Am J Med Genet Part B-Neuropsychiatric Genet*. 2015;168:36–44.
108. Essex MJ, Boyce WT, Hertzman C, et al. Epigenetic vestiges of early developmental adversity: childhood stress exposure and DNA methylation in adolescence. *Child Development*. 2013;84:58–75.
109. Hagerty SL, Bidwell LC, Harlaar N, Hutchison KE. An Exploratory association study of alcohol use disorder and DNA methylation. *Alcoholism, Clinical and Experimental Research*. 2016;40:1633–1640.
110. Francois M, Leifert W, Martins R, Thomas P, Fenech M. Biomarkers of Alzheimer's disease risk in peripheral tissues; focus on buccal cells. *Curr Alzheimer Res*. 2014;11:519–531.
111. Sabine AS, Langie MM, Declerck K, et al. Salivary DNA methylation profiling: aspects to consider for biomarker identification. *Basic & Clinical Pharmacology & Toxicology*. 2017;121:93–101.
112. Godfrey KM, Inskip HM, Hanson MA. The long-term effects of prenatal development on growth and metabolism. *Seminars in Reproductive Medicine*. 2011;29:257–265.
113. Relton CL, Groom A, St Pourcain B, et al. DNA methylation patterns in cord blood DNA and body size in childhood. *PLoS One*. 2012;7:e31821.

114. Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome-wide association studies for common human diseases. *Nature Reviews Genetics*. 2011;12:529–541.
115. Mansell G, Gorrie-Stone TJ, Bao Y, et al. Guidance for DNA methylation studies: statistical insights from the Illumina EPIC array. *Bmc Genomics [Electronic Resource]*. 2019;20:366.
116. Pei-Chien Tsai JTB. Power and sample size estimation for epigenome-wide association scans to detect differential DNA methylation. *International Journal of Epidemiology*. 2015;44:1429–1441.
117. Higdon, R, Earl, RK, Stanberry, L, et al. The promise of multi-omics and clinical data integration to identify and target personalized healthcare approaches in autism spectrum disorders. *OMICS*. 2015;19(4):197–208. doi:10.1089/omi.2015.0020.
118. Wang, ZT, Tan, CC, Tan, L, Yu, JT. Systems biology and gene networks in Alzheimer's disease. *Neurosci Biobehav Rev*. 2019;96:31–44. doi:10.1016/j.neubiorev.2018.11.007.
119. Zhao, Y, Jhamb, D, Shu, L, Arneson, D, Rajpal, DK, & Yang, X. Multi-omics integration reveals molecular networks and regulators of psoriasis. *BMC Syst Biol*, 2019;13(1):8. doi:10.1186/s12918-018-0671-x.

CHAPTER

31

Contributions of twin research to the study of Alzheimer's disease and related dementias

Matthew S. Panizzon[a,b], Jeremy A. Elman[a,b], Eero Vuoksimaa[c]

[a]*Department of Psychiatry, University of California San Diego*

[b]*Center for Behavior Genetics of Aging, University of California San Diego*

[c]*Institute for Molecular Medicine Finland, University of Helsinki, Finland*

Broadly defined, dementia is major a neurocognitive disorder that involves the deterioration of cognitive functioning across multiple domains.[1] By far the most prominent form of dementia is Alzheimer's disease (AD), which is estimated to currently impact 47 million people worldwide.[2] In the present chapter we will focus on Alzheimer's disease and related forms of dementias (ADRD), consistent with the current research agenda proposed by the National Institutes of Health.[3] Alzheimer's disease and related dementias (ADRD) encompasses multiple forms of dementia including AD, frontotemporal dementia, Lewy body dementia, and vascular dementia. The use of this classification recognizes that it is often difficult to distinguish between different types of dementia and that a "pure" clinical presentation of any form of dementia is relatively rare, as many people will present with pathology and risk factors consistent with more than one type of dementia.[4]

It is estimated that by the year 2030 more than 60 million people worldwide will be living with ADRD.[5,6] This anticipated upsurge in the prevalence of ADRD represents a major public health crisis and will have a substantial economic impact, as even now ADRD is the most costly disease in the United States in terms of years of life lost and years lived with disability.[7] Twin studies of ADRD and related phenotypes—that is, biological markers or intermediate clinical syndromes such as mild cognitive impairment (MCI)—offer unique opportunities to examine the etiology of ADRD, providing insights not only into the genetic and environmental determinants of the disease but also illuminating in broad strokes the mechanisms by which risk and protective factors are associated with ADRD. In the present chapter, we will review how twin studies have contributed to our understanding of ADRD, discuss knowledge gaps in the literature that have yet to be filled, as well as outline directions for future research where the study of twins may further advance ADRD-focused research.

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00031-3

31.1 Genetic and environmental influences of ADRD

Perhaps the most common contribution of twin research to the study of any disease is the estimation of heritability—the degree to which genetic factors contribute to variance in disease risk.[8] It is important to note that, in most cases, genetic factors in twin studies are unmeasured, and instead represent latent constructs that are estimated based on the degree of twin resemblance. Heritability is a fundamental statistic in genetic epidemiology, providing a crucial jumping-off point for gene discovery efforts, and for understanding the dynamics of gene-environment interplay (i.e., gene–environment interaction and gene–environment correlation). To date, four distinct twin studies (see Table 31.1) have been conducted that demonstrate that the risk for AD/ADRD is under partial genetic influence.[9–12] The reader should note that we intentionally list only one study from each of the four twin cohorts, and that in some cases, as in the Swedish Twin Registry, multiple studies that corroborate these findings have been published as the samples and the data relevant to ADRD continued to develop. Results are provided by means of either twin pair concordance/discordance (i.e., the comparison of concordance rates in twin pairs with an identified proband) or the more robust estimation of heritability via structural equation modeling. These studies have varied substantially in terms of sample sizes and methods of participant ascertainment; nevertheless, despite being relatively few in number, they provide consistent support for genetic influences for ADRD.

The most robust heritability estimate of ADRD comes from the Swedish Twin Registry, one of the longest running and largest twin registries in the world.[13–15] As part of the HARMONY study,[16] 14,435 individuals aged 65 and older underwent a telephone cognitive screening, and subsequent cognitive testing for those who indicated possible cognitive impairment. The result was one of the largest, population-based, genetically informative cohorts for dementia research ever assembled. In

TABLE 31.1 Twin studies of Alzheimer's disease and related dementia.

First author	Year	Sample	Sample Size	Heritability
Gatz	2006	Swedish Twin Registry	11,884 pairs	58% (full model) 79% (reduced model)
Räihä	1996	Finnish Twin Cohort	13,888 pairs	[a]Not reported
Meyer	1998	NAS-NRC Registry	5,699 pairs	37% (full model) 74% (reduced model)
Bergem	1997	Norwegian Twin Registry	72 pairs	55% to 61%

[a] *Heritability was not reported, but comparison of twin concordance rates suggests the presence of genetic influences on ADRD.*
NAS-NRC = National Academy of Science-National Research Council. Full model indicates that the heritability estimate was derived in a model that allowed for additive genetic influences, common environmental influences, and unique environmental influences. Reduced model indicates that common environmental influences were fixed at zero.

analyses that adjusted for the ages of the twins, ADRD was found to have a heritability of 0.58, meaning that 58% of the risk liability could be attributed to additive genetic influences.[12] The heritability increased to 0.79 (79%) if the nonsignificant estimate of common environmental influences (0.19 in the full model) was fixed at zero. While now over a decade old, the heritability of ADRD generated from the Swedish Twin Registry remains the most widely cited estimate in the literature due in part to the population-based nature of the sample utilized, the comprehensive approach to dementia assessment, the evaluation of male, female, and opposite-sex twin pairs, as well as the data analytic approach employed by the researchers.

31.1.1 Sex differences

At an estimated rate of nearly 2–1, women are expected to represent the majority of new ADRD cases over the coming decade.[5] There is a well-established sex difference in the prevalence of ADRD, one that is not accounted for by greater female longevity; however, the mechanisms behind this difference remain poorly understood. This represents a significant knowledge gap in our understanding of the etiology of ADRD.[17–22] Twin studies of ADRD, specifically studies that utilize data from male, female, and opposite-sex dizygotic twins, offer a unique opportunity to test whether ADRD is equally heritable in men and women, as well as whether the same genetic and environmental influences underlie variation in the risk of developing ADRD among men and women.

Here again, results from the Swedish Twin Registry provide the best data to date on whether sex differences in ADRD extend to the level of the underlying genetic and environmental influences.[12,23] In the previously described study from the Swedish Twins[12] heritability estimates differed between men and women, with values of 0.58 for men and 0.45 for women; however, these estimates did not significantly differ from one another. A more recent study, utilizing a larger sample of participants from the Swedish Twin Registry has further addressed the issue.[23] Combining data from the HARMONY study,[16] the Aging in Men and Women study (also known as the GENDER study),[24] the Origins of Variance in the Oldest Old: Octogenarian Twins (OCTO-Twin),[25] and the Swedish Adoption/Twin Study of Aging (SATSA),[26] investigators replicated the sex difference in ADRD risk and once again found slight differences in the heritability between men and women; however, these heritability differences were not significantly different from one another. Moreover, by leveraging the presence of opposite-sex twin pairs and fitting what is referred to as a sex limitation model[a], the study found no indication that the underlying genetic influences of ADRD differed between men and women.

Thus, current results from twin studies suggest that the established sex difference in ADRD risk is not due to differential effects of genetic and environmental

[a] For a detailed explanation of the use of opposite-sex twin pairs in "sex-limitation" models, the reader is recommended to review Chapter 9 of Neale and Maes (2004), *Methodology for Genetic Studies of Twins and Families.* Kluwer Academic Publishers, The Netherlands.

influences, nor sex-specific genetic influences. The absence of differences at this level of analysis raises the question of why sex differences are present in ADRD? As we will discuss later in this chapter, alternative uses of twin data provide insights into possible mechanisms.

31.1.2 Age at onset

Any genetic analysis of ADRD, whether a classical twin study or a genome-wide association study (GWAS), is complicated by the fact that the relative risk of ADRD varies dramatically by age.[27,28] In other words, an individual's risk for developing ADRD is not consistent over time but increases with increasing age. The increase in risk can be thought of in similar terms as an increase in the phenotypic/overall variance of a continuous trait over time. As the phenotypic/overall variance increases, the opportunity arises for changes in the relative contributions of genetic and environmental influences to that variance. The potential impact of this variability in risk can be seen in the age-sensitive effects the apolipoprotein E (*APOE*) ε4 allele, the leading genetic risk factor for ADRD, which appears to show stronger associations with ADRD earlier in life.[29,30] Because the risk of ADRD changes with age the traditional case-control design for determining genetic contribution to the risk of ADRD will provide an imprecise representation of reality, since one cannot be certain if individuals identified as not having ADRD are "true" cases, or simply have not developed ADRD yet.

While the studies that have provided heritability estimates for ADRD have each made efforts to account for the effects of age, there has been little examination to date of whether the heritability of ADRD changes as a function of age. Findings from the Swedish Twin Registry suggest that the heritability of ADRD may be greater prior to age 80 (heritability was estimated at 0.59 for incident dementia with onset prior to age 80 and 0.40 if onset occurred after age 80); however, the observed heritability estimates of ADRD in the younger versus older age groups were not significantly different from one another.[31] An analysis of data from the National Academy of Sciences-National Research Council (NAS-NRC) Twin Registry,[32,33] the only United Stated based twin registry to have examined ADRD, found that if analyses account for the risk of ADRD being conditional on age (in technical terms the data are right-censored), then the estimate of heritability of ADRD is 0.37.[9] Similar work conducted using data from the Swedish Twin Registry and analyzing risk for ADRD in the context of a survival function has resulted in heritability estimates consistent with conventional methods (heritability estimates ranged from 0.57 to 0.78 depending on model parameters) and noted a substantial reduction in the contribution of common environmental influences.[34,35] While these studies better accounted for the age-dependent nature of ADRD, they nevertheless did not fully address whether the heritability of ADRD changes with age.

In addition to the findings from the NAS-NRC and Swedish Twin Registries, we are aware of only one study that has empirically demonstrated that the age-varying risk for ADRD has implications for understanding the heritability of the phenotype.

Silverman and colleagues,[28] using an extended family design, showed that the familial nature of ADRD decreased with increasing age. Since this study was not conducted using twins, the authors could not distinguish between the contributions of genetic and shared environmental factors to ADRD risk. If one assumes that a significant portion of the familial nature of ADRD is due to genetic factors, as the reported heritability estimates strongly suggest, and that the risk of ADRD increases with age, then is it possible that that the heritability of ADRD will be lower at later ages of onset.

To date, no twin study has sufficiently tested the hypothesis that the heritability of ADRD changes as a function of age, what would be referred to as an age-by-heritability interaction. Such an analysis is complicated by the previously mentioned censoring of dementia data, the need to account for the competing risk of mortality (i.e., accounting for individuals who die prior to dementia onset), limited statistical power (due to disease prevalence and limited cohort size), and the dichotomous nature of the dementia phenotype (which inherently limits statistical modeling options). The successful demonstration of such an effect would substantially improve ongoing gene discovery efforts by highlighting limitations in the current statistical models (i.e., the assumption of invariant or constant disease risk by age) and redirecting focus onto age at onset of ADRD rather than presence or absence of the condition. The recent success of efforts to use single nucleotide polymorphisms (SNPs) identified from ADRD GWAS and weight them according to participant age, an approach known as the polygenic hazard score, in order to improve disease and biomarker prediction highlights the relevance of this issue to genetically informed ADRD research.[27,36–39]

31.1.3 Intermediate ADRD phenotypes

Along with the examination of ADRD, twin studies have also been used to estimate the heritability of several intermediate ADRD phenotypes. Similar to the concept of the endophenotype,[40] a biological marker that is strongly correlated with a disease, an intermediate phenotype represents a mid-way point between normal functioning, and the severe impairment that is observed in ADRD. Cognitive dysfunction, for example, assessed via a telephone administration of a mental status exam, in nondemented participants from the Swedish Twin Registry was found to have a heritability of 0.35.[41] This heritability estimate was substantially lower than the heritability of ADRD (0.58) generated from the same cohort.

Results from the Vietnam Era Twin Study of Aging (VETSA),[42,43] a longitudinal study of cognitive and brain aging in male twins, provide perhaps the most comprehensive genetic examination of a key intermediate phenotype for ADRD, that being MCI. The increased emphasis on the early identification of ADRD has made MCI a major focal point for researchers interested in the trajectories of cognitive deterioration and potential interventions. Using a data-driven approach to MCI classification, rather than relying on subjective reports of cognitive difficulties, these investigators examined the heritability of MCI across multiple definitions of impairment (e.g., a participant is considered impaired if two tests within a cognitive domain

are 1.0 standard deviations below the mean, or one test within a domain is 1.5 standard deviations below the mean) in participants who were 55 years of age on average.[44] For any MCI (i.e., cognitive impairment in any domain, not just memory), heritability estimates ranged from 0.37 to 0.63, and there was little evidence of shared/common environmental influences. Adjusting for early life general cognitive ability did little to reduce the heritability estimates. Subsequent studies from the VETSA have continued to validate this approach to MCI classification, demonstrating that the diagnosis is associated with hippocampal atrophy during midlife,[45] and that is it associated with polygenic risk for AD[46].

31.2 Evaluating ADRD risk and protective factors

In addition to the estimation of the genetic and environmental influences of ADRD, twin data provide other novel opportunities to investigate ADRD etiology. The use of twins where members of a pair are discordant for ADRD, or are discordant for a risk or protective factor, is one such example. Another is the comparison of the risk of ADRD in opposite-sex twin pairs relative to same-sex twin pairs. These quasi-experimental designs (described below) allow for the rare opportunity to test causal hypotheses from cross-sectional observational data. Furthermore, their results can have important implications for how one conceptualizes ADRD and its the relationship with known risk and protective factors.

31.2.1 Co-twin control studies

Many potentially modifiable risk factors for ADRD have been identified, including education, hearing loss, traumatic brain injury, hypertension, alcohol consumption, obesity, smoking, depression, social isolation, physical activity, air pollution, and diabetes.[47] Since it is not possible to randomly assign research participants to one or more of these risk factors, it is difficult to draw causal inferences from their associations with ADRD. The co-twin control study is a unique example of a case-control design that enables researchers to account for the confounding effects of genetic and environmental factors, and greatly strengthens the case for causal inference of any observed associations[48].

Co-twin analyses test if the individual level (between-family) associations among ADRD and known risk factors are evident in twin pairs (i.e., within-family) discordant for ADRD. The degree to which associations are stronger or weaker within monozygotic (MZ) and dizygotic (DZ) twins relative to individual-level effects allows inferences to be made regarding the degree of genetic and environmental confounding present in an association (see Fig. 31.1). In a scenario where the point estimates (e.g., odds ratios, OR) are similar in magnitude in between- and within-family analyses, both in MZ and DZ pairs, there is no evidence of genetic or environmental confounding. Thus, if the OR for the association between a given risk factor and ADRD is 1.5 in an individual level analysis, conditional co-twin analyses of ADRD

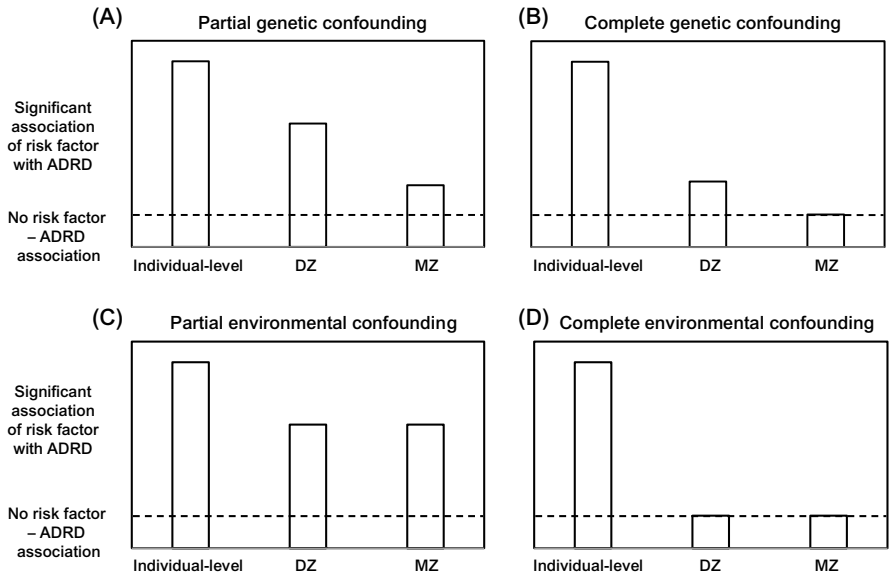


FIG. 31.1 Different scenarios of genetic and environmental confounding in co-twin control analyses. Genetic confounding (A and B) occurs when estimates from MZ twins are smaller than estimates from DZ twins, whereas environmental confounding (C and D) occurs when estimates from MZ and DZ are similar but smaller than estimates obtained from individual level analysis. Partial confounding occurs when a risk factor is associated with ADRD discordance both in DZ and MZ pairs (A and C) and complete confounding occurs when a risk factor is not associated with ADRD discordance in MZ pairs (B and D). The dashed line in each panel represents the threshold where the risk factor is associated with higher risk of ADRD. Greater bar height indicates larger effect size of the risk factor-ADRD association.

discordant pairs would results in OR's of 1.5 both in MZ and DZ pairs. The effect of the risk factor on ADRD would be independent of genetic liability to ADRD or shared environmental effects. If point estimates are attenuated in co-twin analyses compared to those observed in individual-level analyses (as shown in Fig. 31.1), there can occur either environmental (point estimates from co-twin analyses of DZ and MZ twins are similar in magnitude) or genetic confounding (point estimates are smaller in magnitude in MZ co-twin analyses compared to DZ co-twin analyses).

Studies of discordant twin pairs have investigated a wide variety of medical, socioeconomic, and behavioral predictors of ADRD. Indeed, a comprehensive review of all discordant twin studies relevant to ADRD exceeds to the scope of the present chapter. Here we review selected studies that highlight the insights that can be gained from this approach. Results from the NAS-NRC Twin Registry, for example, suggest that cardiovascular risk factors differ in terms of their potential causal relationships with cognitive decline.[49] Cognitive change, as assessed by a telephone interview of cognitive status, over a 12-year period did not differ between twins discordant

for hypertension ($N = 326$ pairs), hypercholesterolemia ($N = 282$ pairs), or obesity ($N = 166$) pairs. However, co-twins with diabetes ($N = 177$) showed greater decline compared to their co-twins without diabetes. These findings are supported by similar co-twin control studies using data from Finnish and Swedish twin samples. In the older Finnish Twin Cohort study, a well-validated middle-age dementia risk score, the Cardiovascular Risk Factors, Aging and Dementia (CAIDE), which included multiple cardiovascular risk factors were related to old age cognitive function in between-family (individual level) analysis, but this association was not evident in co-twin analyses neither in DZ or MZ pairs.[50] Moreover, mid-life diabetes was related to dementia risk in co-twin analyses in Swedish twins.[51] Together, results from co-twin control studies suggest that diabetes has a causal influence on old age cognition, while associations observed for the other cardiovascular risk factors are confounded by shared genetic and environmental factors.

The co-twin control design has also been used to illuminate the nature of the relationships among depression, anxiety, and ADRD. In data from the Swedish Twin Registry, depression at old age, but not earlier, was associated with ADRD.[52] This association was also evident in twins discordant for ADRD, suggesting that depression is a prodromal feature of ADRD rather than a risk factor. Also in the Swedish Twin Registry, a study with 28-years of follow-up indicated higher anxiety—independently of depression—as a risk factor for ADRD.[53] Moreover, the relationship between anxiety and ADRD was evident in DZ but not in MZ pairs indicating genetic mediation of the association.

Education is a potential risk/protective factor for later-life cognitive impairment and ADRD that has garnered much attention. Indeed, completion of primary and secondary education has been suggested to be one of the most effective strategies in preventing ADRD.[47] While the effects of education on ADRD have been closely studied, only a small number of studies have been conducted that leverage the discordant twin design. An analysis of 8,190 individuals from the Swedish Twin Registry compared the risk of ADRD in those with compulsory education (high school or less) to the risk in those with more education and found those with only compulsory education had increased risk of ADRD (odds ratio = 1.77).[54] A discordant monozygotic twin pair analysis yielded an increased risk of ADRD in co-twins with lower education (odds ratio = 3.17), indicating that the association between lower education and ADRD is not confounded by shared genetic effects. Because MZ's have similar genetic propensity for educational attainment, and they, by definition, share environmental effects that make twins similar in educational attainment, educational differences are due to environmental effects unique to each member of a twin pair. Thus, the MZ co-twin analysis suggests that higher educational attainment *per se* has protective effects against ADRD. However, this association could be still accounted for by a third variable not measured or included in the model. One such a factor could be general cognitive ability.

Indeed, the association between education and ADRD becomes less clear once the effect of early life cognitive ability is taken into consideration. A large study of over 660,000 Danish men found that cognitive ability assessed at the time of induction

into the military was significantly associated with ADRD later in life, and that adjusting for education had little effect on this relationship.[55] A co-twin analysis indicated that a 1 standard deviation unit lower cognitive ability was related to a 9% higher risk of dementia, but this association was nonsignificant and was much smaller than the 33% higher risk observed in individual-level analyses. A similar study from the previously mentioned VETSA project suggests that the protective effect of education is primarily explained by differences in cognitive ability.[56] Co-twin analyses reveal that higher educational attainment was associated with better verbal fluency and episodic memory after accounting for a measure of general cognitive ability obtained at military induction (roughly age 20); however, this effect accounted for less than 1% of the variance in functioning. For both verbal fluency and episodic memory, within DZ pair associations were less similar than individual level (between-family) associations. As with the previously described Danish study,[55] the associations in co-twin analyses were smaller in magnitude in MZ twins than in DZ twins, indicating at least partial genetic confounding[50].

The co-twin control design is a versatile and conceptually powerful tool for examining risk and protective factors for ADRD. Unlike biometrical analyses based on structural equation models, the co-twin design yields risk estimates that are comparable to risk estimates from conventional epidemiological research. Risk ratios for any factor tell if the associations are evident when controlling for environmental and genetic effects and risk ratios are can be interpreted in a way similar to those generated through an analysis of unrelated individuals. The method is, however, not without its limitations. Due to the relatively high heritability of ADRD, only a small proportion of monozygotic twins are discordant for dementia. Consequently, even large-scale studies have resulted in relatively small numbers of discordant monozygotic pairs. As the statistics (standard errors, confidence intervals, and *P*-values) in the models depend on the sample size, the effects observed in MZ twins can be nonsignificant even if the estimated risks are similar to those based on estimates from nontwin samples (i.e., unrelated individuals). Thus, these analyses have to be interpreted cautiously, keeping the sample size in mind. It is also important to keep in mind that the quasi-experimental co-twin design cannot replace true intervention trials. Even if there is no association between the risk factor and ADRD in discordant twins, interventions could still result in differences in cognitive functioning or other indicators of ADRD progression. Ultimately, multidomain lifestyle intervention studies in monozygotic twins would show how much cognition can be improved or how much cognitive decline or cognitive impairment can be delayed independently of genetic risk of ADRD.

31.2.2 Opposite sex twins

A variant of the co-twin control design is the examination of opposite-sex (male–female) dizygotic twin pairs. Opposite-sex twin pairs are as genetically related as typical siblings, sharing on average 50% of their segregating genes.[8] What most distinguishes these twins from other types of twins is the presence of an opposite-sex counterpart in the prenatal environment. Evidence from animal studies suggests

that the presence of a male fetus can result in an increase in the androgen levels present in the prenatal environment. This effect on the hormonal milieu of the prenatal environment may in turn result in a masculinization of a female fetus, known as the testosterone transfer hypothesis.[57,58] Thus, the comparison of opposite-sex twins relative to same-sex twins provides an opportunity to test the prenatal organization effects (i.e., effects on early neurodevelopmental that are long-standing if not permanent) of endocrine factors on physical and behavioral traits, as well as disease risk. It should be noted, however, that skepticism exists regarding the validity of the testosterone transfer hypothesis, as there is no direct of evidence of prenatal testosterone transfer in human opposite-sex twins. Moreover, postnatal socialization may contribute to differences between twins from opposite- and same-sex pairs.

The opposite-sex twin comparison has been used to examine a wide array of physiological and behavioral phenotypes with mixed success.[57,58] With respect to cognition, studies suggested that sex differences in expressive vocabulary and visual-spatial ability are driven in part by variation in the prenatal endocrine environments.[59,60] Recently, Luo and colleagues,[61] utilizing data from the Swedish Twin Registry, demonstrated that women from same-sex twin pairs had significantly higher ADRD risk relative to women from opposite-sex twin pairs. In contrast, male twins from opposite-sex pairs showed no differences in ADRD risk relative to men from same-sex pairs. This result provides intriguing evidence that the well-established sex difference in lifetime ADRD prevalence may in part be due to early endocrine factors (specifically androgens) that have long-lasting organization effects on cognitive functioning and the brain.

31.3 A new conceptualization of Alzheimer's disease and related dementias

In 2018, the National Institute on Aging-Alzheimer's Association (NIA-AA) introduced a new research framework for studying ADRD.[62] This new framework marked a dramatic shift away from conceptualizing ADRD as a disorder of cognitive functioning and has steered researchers toward a biologically based definition of ADRD.

The NIA-AA framework includes a classification system (termed A/T/N) that categorizes individuals based on whether they have abnormal levels of amyloid (A), tau (T), or neurodegeneration (N), and unlike previous criteria for AD/ADRD diagnoses, these categories are independent of cognitive status. In other words, the diagnosis focuses on the underlying pathology of ADRD rather than the clinical symptoms of the disease. This distinction is of particular importance because the pathology of AD may appear years, or even decades, prior to clinical symptom onset.[63–65] It is hoped that the emphasis on early identification of ADRD pathology will ultimately improve therapeutic interventions, which have thus far been ineffective at slowing or stopping the progression of AD once symptoms emerge. Importantly, the A/T/N classification is flexible in that any biomarker of these pathologies can be used, including those

derived from cerebro-spinal fluid (CSF), positron emission tomography (PET), magnetic resonance imaging, or blood.

This new conceptualization of ADRD offers many opportunities for the continued contribution of twin research to the study of dementia. For example, relatively little is known about the genetic and environmental influences of ADRD biomarkers, or the degree to which they are genetically and/or environmentally related to one another. The TwinsUK study, a sample of adult twins based in the UK, conducted an examination of multiple plasma-based ADRD biological markers and found the average heritability estimate to be only 0.26.[66] It should be noted that few of the biomarkers examined easily fit into the A/T/N framework with the exception of tau, which was found not to be heritable.

To date, very few GWAS analyses of ADRD biomarkers have been conducted,[67–70] and those that have been done had relatively small sample sizes (hundreds to a few thousand participants) in comparison to GWAS efforts for ADRD clinical diagnosis,[71–73] which range in the tens of thousands of participants. Thus, genetic studies of ADRD biomarkers have been dramatically underpowered to detect meaningful genetic influences. Twin-based heritability estimates can be used to inform gene discovery efforts by determining which markers are under the greatest genetic influence and therefore most suitable for further investigation. Estimates of shared and unique environmental influences may also provide information regarding the relative influence of ADRD-related environmental factors, as well as the effects of preanalytic sample handling (e.g., what temperature a sample is stored at, what material container is used to store the sample, time between sample collection and analysis, etc.) and measurement error. Biomarkers with very high estimates of E could potentially have high measurement error, and thus unlikely to represent reliable measures for both genetic and phenotypic analyses.

In addition, the application of the twin design to this new conceptualization of ADRD may help to identify new biomarkers that better capture the pathophysiological processes that are captured by current "gold standard" methods (i.e., CSF sampling or PET imaging). The ability of the A/T/N classification system to incorporate biomarkers of many kinds is a highly desirable feature of the framework because this flexibility allows for application across a wide variety of studies. However, biomarkers of the same pathology derived from different sources (e.g., PET, CSF, or blood) may measure different forms of a given pathological protein or have different levels of sensitivity. The result is that biomarkers from different modalities can have varying degrees of agreement, from quite high to extremely low. In the context of the twin design, genetic correlations can be tested to determine whether two biomarkers share genetic influences in common. If not, the biomarker may be measuring the outcomes of different biological processes and should not be considered interchangeable but rather complementary. This is of particular interest with the increased availability of blood-based biomarkers. Long sought for their relative ease of collection and cost efficiency, such analyses will clarify the extent to which peripheral markers of pathology are mediated by the same genetic influences as pathology located in the brain or CSF.

31.4 Summary and future directions

Twin studies have made great contributions to our understanding of ADRD; providing information regarding the heritability of ADRD, the nature and origins of observed sex differences, and the causal inferences that can be drawn regarding associations with many of the identified risk and protective factors for ADRD. There remain, however, numerous other ways in which the twin design can continue to contribute to the study of ADRD. For example, multivariate applications of the classical twin design provide the opportunity to not only estimate the heritability of a trait or disorder but also estimate genetic and environmental correlations with other phenotypes.[8] As with the co-twin control design, estimation of genetic and environmental correlations provides invaluable information for understanding how risk and protective factors are associated with ADRD—establishing whether associations are driven by shared genetic factors or are instead due to shared environmental risks. Multivariate applications of the twin design have rarely been applied to ADRD. Indeed, the analysis of ADRD and education conducted by the Swedish Twin Registry is one of the few we could identify.[54] Similarly, twin studies have yet to be used to examine the role of gene-environment interactions in ADRD. Genetic determinants of a trait or disease are not fixed within a population, but can fluctuate as a function of specific environmental factors.[74] In other words, the heritability of ADRD can differ based on other factors. Establishing what these factors are has great potential for the development of more targeted interventions that acknowledge that risk and protective factors for ADRD may not be equivalent across different subgroups of the population.

Additional contributions of twin studies to ADRD research can come in the form of a greater emphasis on acquiring diverse samples to study. The reader will have likely noticed that many of the studies referenced in this chapter originate from the Swedish Twin Registry. This fact is not the result of the authors' preference for this cohort or the studies that have originated from it, rather this reflects the limited number of large-scale twin studies that have focused on ADRD. As existing younger twin cohorts continue to age, one expects that a greater diversity of twin samples that can adequately examine ADRD will become available and contribute greatly to our understanding of sex differences, and the impact of socioeconomic factors to ADRD. Greater diversity of twin samples will hopefully also allow for a greater exploration racial and ethnic differences in ADRD, as nearly all samples to date have consisted of participants of European ancestry. The Interplay of Genes and Environment across Multiple Studies (IGEMS) consortium represents one effort to pool existing twin studies from multiple countries in order to increase sample size and sample diversity.[75,76] More efforts are clearly needed in this area if investigators are to adequately address one of the most prominent knowledge gaps that persist in ADRD research[77].

ADRD research is entering a remarkable new phase, one where the conceptualization of the disease is shifting dramatically. For decades, ADRD has been thought of as a neurocognitive disorder, and the majority of studies discussed here were conducted under that model. The A/T/N framework has shifted the focus on ADRD research from the clinical outcome (i.e. dementia) to the pathological processes that cause that outcome. The next phase of ADRD-focused twin studies will likely adhere

to this new framework, and in doing so provide novel insights into the genetic and environmental factors that contribute at ADRD pathology.

References

1. Association AP. *Diagnostic and Statistical Manual of Mental Disorders*. Fifth ed. Arlington, VA: American Psychiatric Association; 2013.
2. Arvanitakis Z, Shah RC, Bennett DA. Diagnosis and management of dementia: review. *JAMA*. 2019;322:1589–1599.
3. Corriveau RA, Koroshetz WJ, Gladman JT, et al. Alzheimer's disease-related dementias summit 2016: national research priorities. *Neurology*. 2017;89:2381–2391.
4. Kawas CH, Kim RC, Sonnen JA, et al. Multiple pathologies are common and related to dementia in the oldest-old: the 90+ study. *Neurology*. 2015;85:535–542.
5. Association As. 2019. Alzheimer's disease facts and figures. *Alzheimer's Dementia*. 2019;15:321–387.
6. Prince M, Bryce R, Albanese E, et al. The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimers Dement*. 2013;9:63–75 e2.
7. Murray CJ, Atkinson C, Bhalla K, et al. The state of US health, 1990-2010: burden of diseases, injuries, and risk factors. *JAMA*. 2013;310:591–608.
8. Neale MC, Maes HHM. *Methodology for Genetic Studies of Twins and Families*. Dordrecht: Klwer Academic Publisher B.V.; 2004.
9. Meyer JM, Breitner JC. Multiple threshold model for the onset of Alzheimer's disease in the NAS-NRC twin panel. *American Journal of Medical Genetics*. 1998;81:92–97.
10. Bergem AL, Engedal K, Kringlen E. The role of heredity in late-onset Alzheimer disease and vascular dementia. A twin study. *Arch General Psychiatry*. 1997;54:264–270.
11. Raiha I, Kaprio J, Koskenvuo M, et al. Alzheimer's disease in Finnish twins. *Lancet*. 1996;347:573–578.
12. Gatz M, Reynolds CA, Fratiglioni L, et al. Role of genes and environments for explaining Alzheimer disease. *Arch General Psychiatry*. 2006;63:168–174.
13. Lichtenstein P, De Faire U, Floderus B, et al. The Swedish twin registry: a unique resource for clinical, epidemiological and genetic studies. *Journal of Internal Medicine*. 2002;252:184–205.
14. Lichtenstein P, Sullivan PF, Cnattingius S, et al. The Swedish twin registry in the third millennium: an update. *Twin Res Hum Genet*. 2006;9:875–882.
15. Magnusson PK, Almqvist C, Rahman I, et al. The Swedish twin registry: establishment of a biobank and other recent developments. *Twin Res Hum Genet*. 2013;16:317–329.
16. Gatz M, Fratiglioni L, Johansson B, et al. Complete ascertainment of dementia in the Swedish twin registry: the HARMONY study. *Neurobiology of Aging*. 2005;26:439–447.
17. Mazure CM, Swendsen J. Sex differences in Alzheimer's disease and other dementias. *Lancet Neurology*. 2016;15:451–452.
18. Pike CJ. Sex and the development of Alzheimer's disease. *Journal of Neuroscience Research*. 2017;95:671–680.
19. Nebel RA, Aggarwal NT, Barnes LL, et al. Understanding the impact of sex and gender in Alzheimer's disease: a call to action. *Alzheimer's Dementia*. 2018;14:1171–1183.
20. Laws KR, Irvine K, Gale TM. Sex differences in Alzheimer's disease. *Curr Opin Psychiatry*. 2018;31:133–139.

21. Ferretti MT, Iulita MF, Cavedo E, et al. Sex differences in Alzheimer disease—the gateway to precision medicine. *Nature reviews Neurology*. 2018;14:457–469.
22. Medeiros AM, Silva RH. Sex differences in Alzheimer's disease: where do we stand? *J Alzheimers Disease*. 2019;67:35–60.
23. Beam CR, Kaneshiro C, Jang JY, et al. A twin study of sex differences in genetic risk for all dementia, Alzheimer's Disease (AD), and non-AD dementia. *Journal of Alzheimer's Disease*. 2020;76:539–551.
24. Gold CH, Malmberg B, McClearn GE, et al. Gender and health: a study of older unlike-sex twins. *Journals of Gerontology. Series B, Psychological Sciences and Social Sciences*. 2002;57:S168–S176.
25. McClearn GE, Johansson B, Berg S, et al. Substantial genetic influence on cognitive abilities in twins 80 or more years old. *Science*. 1997;276:1560–1563.
26. Gatz M, Pedersen NL. Study of dementia in Swedish twins. *Twin Res Hum Genet*. 2013;16:313–316.
27. Desikan RS, Fan CC, Wang Y, et al. Genetic assessment of age-associated Alzheimer disease risk: development and validation of a polygenic hazard score. *Plos Medicine*. 2017;14:e1002258.
28. Silverman JM, Ciresi G, Smith CJ, et al. Variability of familial risk of Alzheimer disease across the late life span. *Archives of General Psychiatry*. 2005;62:565–573.
29. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer disease meta analysis consortium. *J Am Med Assoc*. 1997;278:1349–1356.
30. Liu L, Caselli RJ. Age stratification corrects bias in estimated hazard of APOE genotype for Alzheimer's disease. *Alzheimers Dement*. 2018;4:602–608.
31. Pedersen NL, Gatz M, Berg S, et al. How heritable is Alzheimer's disease late in life? Findings from Swedish twins. *Annals Neurol*. 2004;55:180–185.
32. Breitner JC, Welsh KA, Robinette CD, et al. Alzheimer's disease in the NAS-NRC registry of aging twin veterans. II. Longitudinal findings in a pilot series. *Nat Acad Sci, Nat Res Council Registry. Dementia.* 1994;5:99–105.
33. Gallo JJ, Breitner JC. Alzheimer's disease in the NAS-NRC Registry of aging twin veterans, IV. Performance characteristics of a two-stage telephone screening procedure for Alzheimer's dementia. *Psychological Medicine*. 1995;25:1211–1219.
34. Pedersen NL, Posner SF, Gatz M. Multiple-threshold models for genetic influences on age of onset for Alzheimer disease: findings in Swedish twins. *American Journal of Medical Genetics*. 2001;105:724–728.
35. Posner SF, Pedersen NL, Gatz M. Application of life table analysis to the onset of dementia in a genetically informative design. *American Journal of Medical Genetics*. 1999;88:207–210.
36. Kauppi K, Fan CC, McEvoy LK, et al. Combining polygenic hazard score with volumetric MRI and cognitive measures improves prediction of progression from mild cognitive impairment to Alzheimer's disease. *Front Neurosci*. 2018;12:260.
37. Tan CH, Bonham LW, Fan CC, et al. Polygenic hazard score, amyloid deposition and Alzheimer's neurodegeneration. *Brain*. 2019;142:460–470.
38. Tan CH, Fan CC, Mormino EC, et al. Polygenic hazard score: an enrichment marker for Alzheimer's associated amyloid and tau deposition. *Acta Neuropathologica*. 2018;135:85–93.
39. Tan CH, Hyman BT, Tan JJX, et al. Polygenic hazard scores in preclinical Alzheimer disease. *Annals of Neurology*. 2017;82:484–488.

40. Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *American Journal of Psychiatry*. 2003;160:636–645.
41. Reynolds CA, Fiske A, Fratiglioni L, et al. Heritability of an age-dependent categorical phenotype: cognitive dysfunction. *Twin Res Hum Genet*. 2006;9:17–23.
42. Kremen WS, Franz CE, Lyons MJ. VETSA: the Vietnam era twin study of aging. *Twin Research Human Genet*. 2013;16:399–402.
43. Kremen WS, Thompson-Brenner H, Leung YJ, et al. Genes, environment, and time: the Vietnam era twin study of aging (VETSA). *Twin Res Hum Genet*. 2006;9:1009–1022.
44. Kremen WS, Jak AJ, Panizzon MS, et al. Early identification and heritability of mild cognitive impairment. *International Journal of Epidemiology*. 2014;43:600–610.
45. Jak AJ, Panizzon MS, Spoon KM, et al. Hippocampal atrophy varies by neuropsychologically defined MCI among men in their 50s. *American Journal of Geriatric Psychiatry*. 2015;23:456–465.
46. Logue MW, Panizzon MS, Elman JA, et al. Use of an Alzheimer's disease polygenic risk score to identify mild cognitive impairment in adults in their 50s. *Molecular Psychiatry*. 2019;24:421–430.
47. Livingston G, Huntley J, Sommerlad A, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet.* 2020;396:413–446.
48. McGue M, Osler M, Christensen K. Causal Inference and observational research: the utility of twins. *Perspect Psychol Sci*. 2010;5:546–556.
49. Xiong GL, Plassman BL, Helms MJ, et al. Vascular risk factors and cognitive decline among elderly male twins. *Neurology*. 2006;67:1586–1591.
50. Iso-Markku P, Kaprio J, Lindgren N, et al. Middle-age dementia risk scores and old-age cognition: a quasi-experimental population-based twin study with over 20-year follow-up. *Journal of Neurology, Neurosurgery, and Psychiatry*. 2021;92:323–330.
51. Xu W, Qiu C, Gatz M, et al. Mid- and late-life diabetes in relation to the risk of dementia: a population-based twin study. *Diabetes*. 2009;58:71–77.
52. Brommelhoff JA, Gatz M, Johansson B, et al. Depression as a risk factor or prodromal feature for dementia? Findings in a population-based sample of Swedish twins. *Psychology and Aging*. 2009;24:373–384.
53. Petkus AJ, Reynolds CA, Wetherell JL, et al. Anxiety is associated with increased risk of dementia in older Swedish twins. *Alzheimers Dement*. 2016;12:399–406.
54. Gatz M, Mortimer JA, Fratiglioni L, et al. Accounting for the relationship between low education and dementia: a twin study. *Physiology & Behavior*. 2007;92:232–237.
55. Osler M, Christensen GT, Garde E, et al. Cognitive ability in young adulthood and risk of dementia in a cohort of Danish men, brothers, and twins. *Alzheimers Dement*. 2017;13:1355–1363.
56. Kremen WS, Beck A, Elman JA, et al. Influence of young adult cognitive ability and additional education on later-life cognition. *Proc Natl Acad Sci USA*. 2019;116:2021–2026.
57. Tapp AL, Maybery MT, Whitehouse AJ. Evaluating the twin testosterone transfer hypothesis: a review of the empirical evidence. *Hormones Behavior*. 2011;60:713–722.
58. Ahrenfeldt LJ, Christensen K, Segal NL, et al. Opposite-sex and same-sex twin studies of physiological, cognitive and behavioral traits. *Neuroscience and Biobehavioral Reviews*. 2020;108:322–340.
59. Galsworthy MJ, Dionne G, Dale PS, et al. Sex differences in early verbal and non-verbal cognitive development. *Developmental Sci*. 2000;3:206–215.
60. Vuoksimaa E, Kaprio J, Kremen WS, et al. Having a male co-twin masculinizes mental rotation performance in females. *Psychological Sci*. 2010;21:1069–1071.

61. Luo J, Beam CR, Karlsson IK, et al. Dementia risk in women higher in same-sex than opposite-sex twins. *Alzheimers Dement (Amst)*. 2020;12:e12049.
62. Jack Jr CR, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 2018;14:535–562.
63. Villemagne VL, Burnham S, Bourgeat P, et al. Amyloid beta deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *Lancet Neurology*. 2013;12:357–367.
64. Beason-Held LL, Goh JO, An Y, et al. Changes in brain function occur years before the onset of cognitive impairment. *Journal of Neuroscience*. 2013;33:18008–18014.
65. Bateman RJ, Xiong C, Benzinger TL, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *New England J Med*. 2012;367:795–804.
66. Kiddle SJ, Sattlecker M, Proitsi P, et al. Candidate blood proteome markers of Alzheimer's disease onset and progression: a systematic review and replication study. *J Alzheimer's Disease*. 2014;38:515–531.
67. Scelsi MA, Khan RR, Lorenzi M, et al. Genetic study of multimodal imaging Alzheimer's disease progression score implicates novel loci. *Brain*. 2018;141:2167–2180.
68. Yan Q, Nho K, Del-Aguila JL, et al. Genome-wide association study of brain amyloid deposition as measured by Pittsburgh Compound-B (PiB)-PET imaging. *Molecular Psychiatry*. 2018;26:309–321.
69. Hong S, Prokopenko D, Dobricic V, et al. Genome-wide association study of Alzheimer's disease CSF biomarkers in the EMIF-AD multimodal biomarker discovery dataset. *Transl Psychiatry*. 2020;10:403.
70. Deming Y, Li Z, Kapoor M, Harari O, et al. Genome-wide association study identifies four novel loci associated with Alzheimer's endophenotypes and disease modifiers. *Acta Neuropathologica*. 2017;133:839–856.
71. Kunkle BW, Grenier-Boley B, Sims R, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nature Genet*. 2019;51:414–430.
72. Lambert JC, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nature Genetics*. 2013;45:1452–1458.
73. Jansen IE, Savage JE, Watanabe K, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nature Genetics*. 2019;51:404–413.
74. Martin N. Gene-environment interaction and twin studies. In: Spector TD, Snieder H, MacGregor AJ, eds. *Advances in Twin and Sib-pair Analysis*. London: Greenwich Medical Media Ltd; 2000:143–150.
75. Pedersen NL, Christensen K, Dahl AK, et al. IGEMS: the consortium on interplay of genes and environment across multiple studies. *Twin Res Hum Genet*. 2013;16:481–489.
76. Pedersen NL, Gatz M, Finch BK, et al. IGEMS: the consortium on interplay of genes and environment across multiple studies—an update. *Twin Research and Human Genet*. 2019;22:809–816.
77. Brewster P, Barnes L, Haan M, et al. Progress and future challenges in aging and diversity research in the United States. *Alzheimers Dement*. 2019;15:995–1003.

SECTION

7 Twin Research: Genetics, Epigenetics, Microbiome, and Environmental Adaptation

CHAPTER

Twins and omics: the role of twin studies in multi-omics

32

Fiona A. Hagenbeek[a,b], Jenny van Dongen[a,b,c], René Pool[a,b], Dorret I. Boomsma[a,b,c]

[a]*Netherlands Twin Register, Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*

[b]*Amsterdam Public Health Research Institute, Amsterdam, The Netherlands*

[c]*Amsterdam Reproduction and Development Research Institute, Amsterdam, The Netherlands*

32.1 Introduction

The "-omics" suffix denotes a discipline in biology, while the related suffix "-ome" signifies the object of study in this field.[1] Genomics, transcriptomics, proteomics, and metabolomics, referring to the study of the genome (DNA), transcriptome (RNA), proteome (proteins), and small molecules involved in metabolism, respectively,[2] cover the core molecules in the central dogma of biology.[3] The central dogma of biology describes how proteins are formed by the transcription and translation of genetic information (genomics → transcriptomics → proteomics).[3] Metabolomics, the study of the metabolites, that is, all small-molecules in an organism,[2] and the central dogma together describe the omics cascade from genes to metabolites (Fig. 32.1).[4] In addition to the linear, unidirectional oriented connections in the omics cascade, more complex relationships exist between and within the different omics layers, including feedback loops among omics levels.[3] Increasingly, other omics layers, such as the epigenome, microbiome, glycome, phosphoproteome, lipidome, fluxome, or exposome, are added to the omics cascade.[5] Many of these, such as the glycome or phosphoproteome, reflect regulatory and modulatory processes,[5] others, such as the exposome, reflect exposures to the environment.[6]

Large-scale omics studies are often carried out in cohorts of unrelated individuals. This is, in part, because many statistical models originally designed to study omics data rely on standard techniques for association and regression. In the field of genomics, particularly for genome-wide association (GWA) studies, it was quickly recognized that leveraging the information contained within the many twin registries around the world would result in many advantages, if we properly account for the clustering of observations.[7] This recognition spurred efforts to apply approaches, such as mixed models and generalized estimating equations, to account for relatedness among participants in twin and family studies.[8] Approaches that allow for the inclusion of related individuals led to the inclusion of large numbers of samples of

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00029-5

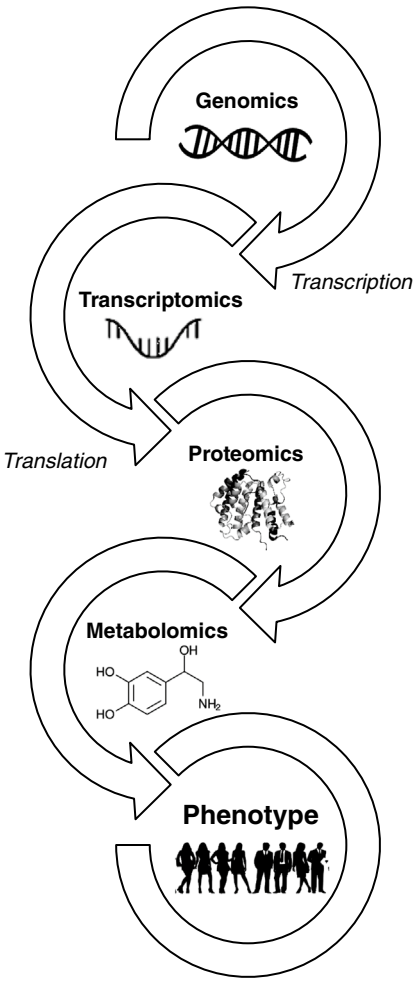


FIG. 32.1 The omics cascade—the omics cascade describes the cascade from genotype to phenotype.

well-phenotyped participants from twin registries in e.g., GWA studies of migraine, major depression, educational attainment.[9–11] However, twin designs themselves are powerful analytical tools for omics data beyond contributing to association studies.[12] In this chapter, we will first introduce some often studied omics domains: genomics, epigenomics, transcriptomics, and metabolomics. Next, for each of these domains, we outline the contributions made by twin studies and consider the added value of twin research in omics. We illustrate some designs such as the discordant twin design, in some detail and consider a combination of the classical twin design with genome-wide genotype data.

32.2 Genomics

32.2.1 What is genomics and how do we measure the genome?

Deoxyribonucleic acid (DNA) polymer molecules contain the hereditary information of the organism. DNA consists of two polynucleotide chains ("strands") that form a double-helical structure that is stabilized by hydrogen bonding between the nucleotides of both strands. These hydrogen bonds are formed between complementary nucleotides. There are four nucleotide types, where adenine (A) pairs with thymine (T) and guanine (G) pairs with cytosine (C). Segments of DNA contain genes, that consist of a few hundred to more than two million base pairs.[13] Genes consist of multiple long noncoding regions called introns and shorter coding regions called exons.[14] By coding, we mean coding for a function in the next omics layer(s). Originally it was believed that all genes contain the instructions to encode proteins, however, we now know that many genes are not protein coding. Almost all DNA molecules are contained in the nucleus of each cell. The cell nucleus is approximately 5–8 μm in diameter. By contrast, unfolded human DNA is approximately 2 m in length. To fit DNA in the cell nucleus, DNA is packed into highly condensed structures called chromosomes, each of which comes in two copies (one inherited from each parent). Humans have 23 pairs of chromosomes: 22 autosomal chromosome pairs and a sex chromosome pair.[13]

With genome we refer to the complete set of hereditary information, where the word "genome" is a conjunction "gene" and "chromosome." Therefore, genomics has been coined to refer to the study of the structure, function, and mapping of genomes.[15] In this chapter, we focus on genomic studies characterizing the DNA sequence variants between individuals. We can distinguish various types of sequence variants, spanning from a single nucleotide to dozens of base pairs and even entire chromosomes. The single nucleotide variants (SNVs), also called point mutations, are variations (substitutions, insertions, or deletions) in a single base pair. When these occur in more than 1% of individuals we refer to them as single nucleotide polymorphisms (SNPs). Small insertions or deletions that affect several[2–50] base pairs are called indels and substitutions of several base pairs are called block substitutions.[16] Differences in copy number (deletions, insertions, duplications), orientation (inversions; i.e. stretches of flipped DNA sequence), or location (translocations, i.e., stretches of DNA that have migrated within the genome) between individuals that span more than 50 base pairs are called structural variants (SVs).[17] The largest SVs can affect whole chromosomes, as such, they are also referred to as chromosomal aberrations.

To characterize nucleotide sequence variations in DNA, two techniques are commonly used: DNA microarrays and sequencing. Microarrays typically measure up to 1 million SNPs, while whole-genome sequencing yields nearly 100% of the (structural) information of the genome. Due to the correlation structure of the DNA sequence, the genomic information in DNA microarray data often suffices when studying the relation between the genome and biological (dys)function. DNA microarrays use a technology comprised of a collection of single-stranded oligonucleotide

probes covalently linked to a flat surface, often times on a medium analogous to a microscope slide. For these probes, their locations in the genome are known. Synthetic oligonucleotide probes interact with highly specific genomic sequences via complementary base pairing (hydrogen bonding between the probe and target DNA sequences) in a process termed hybridization. The probes are typically designed to hybridize to the target sequence immediately upstream of the polymorphic nucleotide. Following hybridization, fluorescently labeled nucleotides are utilized in an extension or ligation reaction to discriminate between the different alleles known to occur at that locus and are subsequently imaged utilizing a laser-powered scanner. After the raw intensity data for samples processed on the DNA microarray are generated; next steps involve genotype calling, quality control of genotypes, including tests of Hardy–Weinberg (HE) equilibrium of alleles, of Mendelian transmission (in family data), and comparison of allele frequencies to reference sets.[24] DNA microarrays can be designed to target SNPs, either in small numbers for dedicated purposes such as arrays targeting (rare) exonic variants,[20] or SNPs of interest for particular traits[21] or contain genome-wide common genetic variants, such as present on the global screening array or the Axiom UK Biobank Array.[22,23]

DNA sequencing technologies allow for the measurement of most variants in the genome. DNA sequencing was first developed by Sanger in 1975, and this technique is now referred to as Sanger sequencing. Sanger sequencing has high accuracy, low throughput (it only produces a single DNA fragment at a time), the maximum sequence length is 1000 base pairs, is relatively expensive, and is not suitable for large-scale sequencing projects.[25] Because of its high accuracy, it is often used as a follow-up of findings that result from other sequencing techniques. Several technological advances have contributed to the development of high-throughput sequencing. One advance was the development of polymerase chain reaction (PCR), which allows for massive amplification of small DNA samples, a development that improved the scalability of sequencing as this could be applied in multiwell plates.[26] These and other developments led to the newer next-generation sequencing (NGS) techniques. A complete overview of all types of NGS techniques is outside the scope of this chapter, the reader is referred to, for example, a review by Goodwin, McPherson, and McCombie.[27]

32.2.2 Sequence differences between monozygotic twins

Because monozygotic (MZ) twins arise from one fertilized oocyte they are taken to be genetically identical; a key assumption in the classic twin design.[28] While MZ twins are genetically identical at conception, somatic mutations can arise during cell division (mitosis).[29] Such somatic mutations cause differences in the DNA sequence across different cells of the body. An individual with different populations of cells with different DNA sequences originating from the same zygote is called a mosaic[29] and mosaic mutations can differ between MZ twins from the same pair. Mutations can also arise in germ cells (germline mutations), and be transmitted to the offspring resulting in a constitutional mutation that is present in all cells.[30] Germline mutations,

or pretwinning *de novo* mutations, are therefore shared between MZ twins, but not between the twins and their parents. By contrast, somatic mutations, or post-twinning *de novo* mutations, are present in only one MZ twin or even only in some of the cells of one twin (mosaicism). The genetic (dis)similarity of MZ twins, therefore, depends on the moment in life at which mutations occur. Multiple genetically different cell lineages within one person can also originate from different zygotes. This is referred to as chimerism and can arise for example if dizygotic (DZ) twin zygotes merge early in development.[29] In contrast to gross chimerism, which is present in the majority of the cells in the total population of cells of a particular cell type, microchimerism is present in less than 1% of the total cell population. It occurs frequently, for example as a result of the passage of blood between mother and child during pregnancy, with twin chimerism as a special case and can be a source of discordance in MZ twin pairs.[31]

DNA sequencing studies suggest that the *de novo* SNV mutation rate in somatic cells is approximately 0.82×10^{-8} to 1.70×10^{-8} mutations per base per generation.[32–34] Study designs utilizing MZ twins allow for distinguishing between prezygotic (present in both twins of a pair) and postzygotic (present in only one twin of a pair) *de novo* mutations and to estimate the postzygotic mutation rate. A whole-genome sequencing study of a healthy MZ twin pair and their parents obtained a rate of 0.97×10^{-8} per base per generation for *de novo* SNVs shared by the twin pair. For twin-specific *de novo* SNVs, rate of 0.34×10^{-8} base pair per generation was calculated for one twin and 0.04×10^{-8} base pair per generation for the other twin,[34] that is, an overall *de novo* SNV rate of 1.01 and 1.31×10^{-8}. A comparison of whole-genome DNA sequence data of two monozygotic twin pairs, 40 and 100 years old, was carried out to detect somatic mosaicism and identified 1720 putative postzygotic mutations in blood cells from the 40-year-old MZ twin pair and 1739 in the 100-year-old pair.[35] The identified postzygotic mutations were nonrandomly distributed across the genome, with enrichment for regulatory elements such as coding exons or genes involved in GTPase activity.

Discordances in MZ twin pairs have also been reported for chromosomal abnormalities, particularly for aneuploidy, where one or more chromosomes are missing or present in an extra copy,[29] such as monosomy X (missing sex chromosome; e.g., Turner Syndrome), or trisomy 21 (gain of extra chromosome 21, e.g., Down Syndrome).[36] Postzygotic *de novo* CNVs have been observed in for example a sample of 1097 unselected MZ twin pairs. One hundred fifty-three putative *de novo* CNVs were detected in peripheral blood and buccal epithelium cells, of which 58.8% were located in the same 15q11.2 region.[37] Replication of 20 candidate CNVs with qPCR validated two CNVs in the same 13-year-old MZ twin pair. The twins had no large phenotypic discordances. The twin with three copies of both CNVs outperformed its cotwin (with 1 and 2 copies, respectively, for each of the CNVs) on school achievement. This study also compared CNVs derived from peripheral blood or buccal epithelium cells in the complete group of 1097 MZ twin pairs. While more CNVs were found in DNA from blood, buccal epithelium DNA CNVs had higher concordance rates per twin pair.

As *de novo* postzygotic mutations may arise at each cell division, it is believed that somatic mutations accumulate with age and that aging might even be a consequence of the accelerated accumulation of somatic mutations.[38] A study of twins and singletons investigated CNV accumulation with age.[39] In a healthy cohort of 159 MZ twin pairs and 296 singletons, CNVs were compared in a younger (≤55) and older (≥60) age group. In contrast to the younger group, where no large CNVs were detected, 3.4% of subjects in the older age group had large CNVs, indicating a relationship between age and CNV occurrence in peripheral blood DNA. In addition, for 18 MZ twin pairs (50.7–72.6 years of age at baseline), data on small CNVs were available longitudinally, measured ten years apart. The longitudinal data showed that both increases and decreases in the number of CNVs can be observed. Thus, CNVs appear to accumulate with age, but the populations of peripheral blood cells with CNVs are not stable.

The discordant MZ design also is a tool to identify trait- or disorder-associated genetic variants. An early study of whole-genome sequencing in MZ discordant twins was published in 2010.[40] In addition to whole-genome DNA sequencing, this study also included data on mRNA sequencing, genome-wide SNP microarrays, and DNA methylation profiles with the objective to identify genetic, transcriptomic, and epigenetic differences between $CD4^+$ T cells of three pairs of MZ twins discordant for multiple sclerosis (MS). Differences in SNPs, indels, CNVs, viral genome sequences, gene expression levels and CpG methylation levels could not be reproducibly detected in $CD4^+$ T cells to explain MS discordance. While this early study on MS showed no clear differences within the MZ discordant pairs, this design has been applied with clearer results for CNVs. For example, comparison of CNVs in peripheral blood in a sample of 19 adult MZ twin pairs, of which 9 pairs were discordant for neurodegenerative disorders and 10 pairs were phenotypically unselected, found a larger number of CNVs in the disease discordant than in the other MZ twin pairs.[41] While some of the CNVs reported in the discordant MZ twins might be pathogenic for the neurodegenerative disorders, the authors stressed that replication in larger samples across multiple (relevant) tissues is necessary. As the last example, a study investigating the contribution of the number and the size of CNVs in attention problems identified 8 pre- and 18 post-twinning CNVs in 50 MZ twin pairs. In this group, for 25 MZ pairs both parents were genotyped so that pretwinning *de novo* CNV events could be detected.[42] Of the three possible pretwinning *de novo* CNVs that were included in a qPCR replication study, one pretwinning *de novo* CNV mutation was confirmed, where both MZ twins had a duplication on chromosome 15q11.2. This region contains the gene *HERC2P3*, which is expressed in the human brain. However, both twins scored in the normal range for attention problems.

32.2.3 Sequence differences between dizygotic twins

Classical twin models assume that MZ twins are genetically identical and that dizygotic (DZ) twin pairs and full siblings share on average 50% of their DNA sequence.[28] This last assumption can be tested empirically by estimating the amount

of genetic material that DZ twins or full siblings have inherited identical-by-descent (IBD). DNA segments are IBD if they are inherited from a common ancestor without recombination. This is in contrast to identity-by-state sharing, where DNA segments are identical between pairs of individuals, but do not need to derive from a common ancestor.[43] Genome-wide microsatellite markers data and SNP data indicated that the proportion of IBD sharing between DZ twins and full siblings ranges from 42% to 58%, and confirmed that the average is indeed close to 50%.[44,45]

32.3 Epigenomics

32.3.1 What is epigenomics and how do we measure the epigenome?

With the exception of *de novo* somatic mutations, all cells in the body have the same DNA sequence (except for red blood cells that do not contain DNA), and differences between cell functions are mainly due to differences in which parts of the DNA sequence are expressed in different cells. Gene expression also is modified in response to developmental and environmental cues[46] and is under tight control through multiple regulating mechanisms.[47] Gene expression occurs in regions of the DNA where the chromatin permits transcription.[48] Chromatin is the macromolecular complex that is responsible for condensing DNA into smaller packages of chromosomes and is built up of nucleosomes; a segment of DNA wound around eight histone proteins.[13] Approximately 99% of a cell's genome is located in so-called heterochromatin, a highly compact state where the DNA is not accessible for transcription.[48] At present, 15 distinct chromatin states have been characterized.[49]

Epigenomics is the comprehensive study of the mechanisms that control gene expression by influencing the accessibility of the genome for transcription and/or the ability of the transcription machinery to adhere to accessible DNA segments.[48] Multiple systems cooperate in epigenetic control: DNA methylation (addition of a methyl group to DNA), histone modification (e.g., methylation or acetylation of histone proteins), nucleosome remodeling (change the position of the DNA wrapped around the nucleosomes), and noncoding RNAs (ncRNAs; which are functional RNA molecules that are transcribed from DNA but not translated into proteins and which can influence DNA methylation and histone modifications).[46] Here our focus is mainly on DNA methylation, which is the best-studied epigenomic mechanism in human studies including twin studies and is currently the only one that is suited for assessment in large-scale human epidemiological studies. The relationship between DNA methylation and transcription depends on the genomic context: whereas DNA methylation at gene promoters is usually associated with transcriptional repression, gene body methylation is a feature of actively transcribed genes. Methylation occurs at the C5 position of the aromatic rings of cytosines (5-methylcytosine). This can occur at any cytosine, but in humans, DNA methylation happens almost exclusively at regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide (CpGs). CpG sites tend to cluster in so-called CpG-islands, regions of at least 200 base pairs consisting of 55% or more CG sites.[50]

Several methods for the analysis of epigenomics are available, of which microarrays and sequencing are the main ones. The most frequently used technologies make use of a bisulfite treatment step of the DNA. Unmethylated cytosines are converted to uracil by sodium bisulfite treatment.[51] In PCR amplification uracil is recognized as thymine, as methylated cytosines are immune to the bisulfate conversion they remain cytosines, therefore methylated cytosines can be distinguished from unmethylated cytosines.[52] The bisulfite-treated DNA is then introduced to a methylation microarray which typically includes several hundreds of thousands of probes. The most commonly used Illumina microarrays return, for each interrogated site, the methylation level (proportion of methylated alleles).[53,54] In DNA that is derived from a mixture of cells, such as found in whole blood, the methylation level represents a continuous variable with values that may range between zero and one. For example, a methylation level of 1 means that all DNA strands had a methyl group attached at this position and a value of 0.5 that 50% of all DNA strands had a methyl group attached at this position. Intermediate values arise when a position is methylated in a fraction of cells or on one of the two chromosomes.

32.3.2 Causes of epigenetic variation

The epigenome is often discussed in the context of environmental explanations for diseases, but the epigenome is also shaped by genetic influences. In fact, the epigenome may be a key mediator of the effects of common genetic variants on complex traits and disease, because these variants usually reside in regulatory regions (rather than protein-coding regions) of the genome.[55] Disease-associated SNPs are often associated with expression levels of transcription factors, which in turn drive variation in the DNA methylation level of distal binding sites.[56] Large-scale methylation Quantitative Trait Loci (mQTL) analyses can map associations between genetic variants (typically, SNPs) and DNA methylation levels across the genome.[57] As MZ twins share their genomes, such mQTLs contribute to their epigenetic similarity. In 49 MZ twin pairs from the Netherlands Twin Register,[58] DNA methylation was measured at ~850.000 sites in the genome with the Illumina EPIC array in buccal samples, which consist for about 80% of epithelial cells and about 20% of white blood cells. After adjusting for cellular composition, the methylation levels of MZ twins were more similar at CpG sites whose methylation level was strongly influenced by SNPs than at CpG sites for which no significant mQTLs were detected.

DNA methylation profiles can be seen as complex traits, or phenotypes, and differences between individuals may be analyzed by the classical twin design to estimate heritability. Data from a large cohort of twins and family members from the Netherlands Twin Register were analyzed to estimate the overall heritability for DNA methylation levels at multiple sites. As the participants were genotyped, the variance explained by genome-wide SNPs could also be estimated. In follow-up analyses, interactions of genetic and environmental influences with age and sex were examined.[59] All results are described in a catalog (http://bbmri.researchlumc.nl/atlas/). In 2603,

genotyped adult individuals (mean age 37.2, sd = 13.3, 66% females), DNA methylation was measured at ~450.000 sites in the genome with the Illumina 450 k array. Based on the twin data, the total heritability was 19% on average across the genome. On average 7% (s.d. = 12%) of the variance of DNA methylation was explained by common genetic variants in the genome (h^2_{SNPs}). Thus, the proportion of the total heritability that can be explained by SNPs, i.e., h^2_{SNPs}/h^2 was 0.37 (s.d. = 0.40).

Epigenetic differences between MZ twins are observed in tissues collected at birth,[60] but may also emerge postnatally: results from both cross-sectional studies and longitudinal studies of adult twins suggest that the epigenomes of MZ twins diverge as they age.[59,61,62] This means that the differences between individuals in a population become larger as a function of age; older individuals show more variation in DNA methylation level at these loci. With data from MZ and DZ twins, the causes of age-related changes in variance (genetic and environmental) can be examined by adding a moderator variable to the classical twin model[63] to test the interaction between age and the genetic and environmental effects. Such models have found that age-interaction effects were widespread: 10.4% of all measured sites showed a significant interaction effect of age and genetic or environmental effects on DNA methylation level.[59] At 82% of sites, the unique environmental variance changed with age. These sites typically showed an increase in the unique environmental variance and total variance with age, and a decrease of the heritability. At 90% of sites with significant age interaction, the heritability was lower at age 50 than at age 25, although the difference in heritability between younger and older people was usually modest.

The average heritability of DNA methylation in blood is almost the same in males (mean $h^2 = 0.199$) and females (mean $h^2 = 0.198$), but a small percentage (0.7% of all measured sites) showed a significant interaction effect of sex and genetic or environmental effects on DNA methylation level. At 59% of these sites, the heritability was lower in women. At 76% of all sites with significant sex interaction, the unique environmental variance (rather than the additive genetic variance) differed between the sexes. At sites with a lower heritability in females, the variance of DNA methylation due to environmental influences was usually larger in females. Such methylation sites with sex-specific variation in epigenetic regulation can be studied in future epigenetic studies of diseases with a sex-specific etiology.

32.3.3 MZ discordant design applied to epigenomics studies

Differences in DNA methylation and histone modifications within MZ twin pairs have been reported for multiple tissues and cell types, including blood cells, buccal cells, and fat.[64] The distinct methylomes of MZ twins are even studied by forensic scientists to develop tools to distinguish MZ twins in forensic settings.[65–70] Epigenetic differences between MZ twins can arise from stochastic (random) events, different environmental exposures of cotwins, and genetic mutations. Here, we highlight a few studies investigating epigenetic differences in (MZ) twins, for more detail, we refer the reader to review articles on this topic.[64,71,72]

Stochastic variation can result from the imperfect molecular control of gene expression. For example, the maintenance of DNA methylation in dividing cells by DNA methyltransferases (DNA MTase, DNMT) enzymes is not 100% accurate. Differences in exposures and lifestyle, such as smoking behavior impact of on the epigenome of circulating cells. MZ twin pairs who are discordant for smoking show DNA methylation differences at several loci in white blood cells.[73] This study of 20 MZ pairs of which one twin smoked regularly and the cotwin never smoked or had stopped smoking more than 10 years ago confirmed several loci identified previously in epigenome-wide association studies (EWAS) that compared unrelated smokers to nonsmokers. Note that a key strength of the MZ twin design is that many alternative explanations are ruled out, because MZ twins are genetically identical. For example, one of the most strongly associated genetic variants for nicotine dependence is located in the DNA methyltransferase gene DNMT3B,[74] which might lead to differences in genome-wide DNA methylation between people with different genotypes at this gene, regardless of their smoking behavior. This would be an example of a pleiotropic genetic effect, where a genotype influences genome-wide DNA methylation as well as smoking behavior. Because MZ twins carry the same genetic predisposition for nicotine dependence, potential pleiotropic effects of genetic variants that influence multiple traits independently are not an issue in MZ twin studies.

Epigenetic differences between MZ twins may cause different usage of the identical DNA code. This can lead to extreme phenotypic differences,[36] as illustrated by the study of one MZ pair, in which one twin had a severe congenital caudal duplication malformation and the other did not.[75] There was a very strong candidate gene for the disorder, for which no DNA sequence differences were found. However, this gene showed strong epigenetic differences between the two girls.

Not all epigenetic differences that are observed in monozygotic twin pairs lead to phenotypic discordance. If the two twins are measured on, for example, different days, on different arrays, technical variation can lead to dissimilarity. Differences between twins in the cellular composition of blood samples can also contribute to differences in DNA methylation between MZ twins. Epigenetic differences can of course arise as a result of a disease of one twin, can represent a marker of a disease-causing event, or can be caused by medication use of the affected twin. This opens up possibilities for the identification of dynamic epigenetic biomarkers (those that indicate the emergence and progression of a disease or that indicate current exposure to a risk factor) and persistent epigenetic biomarkers of environmental exposures in the past.[76] A study of 45 MZ twin pairs discordant for MS measured genome-wide DNA methylation in peripheral blood mononuclear cells and identified disease-associated methylation sites, loci where differences between twins in methylation level reflect whether a person is currently receiving interferon-beta treatment, and a locus whose methylation level reflected prior glucocorticoid treatment.[77] Epigenomic studies in MZ twins can have more power than traditional case-control EWA studies[78] and can contribute to our understanding of the underlying pathways and consequences of disease and to the identification of biomarkers.

32.4 Transcriptomics

32.4.1 What is transcriptomics and how do we measure the transcriptome?

The mechanism by which cells copy DNA information into ribonucleic acid (RNA) is called transcription. In contrast to DNA, RNA is single-stranded and it contains uracil (U) bases instead of the thymine (T) bases found in DNA.[14] During transcription one of the two DNA strands acts as a template for RNA synthesis. The sequence of the RNA is synthesized complementary to the nucleotides of the antisense DNA strand and is therefore a copy of the sense strand (with exception for the substitution of U for T). The entire length of a gene, both introns and exons, is transcribed. Next, RNA splicing removes the introns and combines the exons. Not all exons of a gene need be included in the final RNA transcript. Through alternative splicing, different combinations of exons allow for the production of different proteins from the same gene.[14] Such protein-encoding RNA transcripts are referred to as messenger RNA (mRNA). Other types of RNA include ribosomal (rRNA; which forms the core of ribosomes where mRNA is translated to proteins), transfer RNA (tRNA; which is involved in the process of translation of mRNA into proteins by connecting amino acids for incorporation into the protein), and microRNA (miRNA; which is involved in regulation of gene expression).[14,79] The analysis of the complete set of transcripts (RNAs) in a cell or the study of RNA or RNA variants is known as transcriptomics, often also referred to as gene expression studies. Similar to other omics, two techniques are common to study the transcriptome: microarrays and RNA sequencing (RNA-Seq).[19]

32.4.2 Causes of variation in gene expression levels

Like DNA methylation profiles, transcriptome profiles can be regarded as complex phenotypes, and differences between individuals may be analyzed by the classical twin design to decompose variation into genetic and nongenetic variance components. These analyses provide heritability estimates of gene expression which gives an indication of the extent to which the DNA sequence regulates its own expression. Below we give two illustrations of how twin studies shed light on the causes of variation in gene expression. Both studies derive from the Netherlands Twin Register. Wright et al. (2014) employed several methods to analyze variation in RNA microarray data. These included a classical twin design with MZ and DZ twin pairs, and a design with genotyped DZ twin pairs to obtain SNP-heritability estimates.[80] Gene expression of 18,392 genes was assessed in peripheral blood samples obtained from 2752 twins, including 690 complete MZ and 618 complete DZ twin pairs. Twin-based heritability across all RNA probes was 0.10 (sd = 0.14). To assess the contribution of heritability attributable to local genetic variation, SNPs were selected that were located 1 mega base upstream of a transcription start site and 1 mega base downstream of a transcription end site. Estimates of IBD sharing in DZ-twin pairs for these SNPs were used to estimate the ratio of $h^2_{\text{local IBD}}$ (that is, the variance in

gene expression level explained by all local genetic variants; both common and rare) to overall narrow-sense heritability of gene expression levels. The mean and median for the $h^2_{\text{local IBD}}/h^2$ ratio were 0.11 and 0.30, respectively, across all RNA probes. Second, local $r^2_{\text{Socal SNP}}$ (that is, the variance in gene expression level explained by most significant local SNP within 1 Mb) was estimated in unrelated participants using the GCTA software.[82] The ratio of $r^2_{\text{local SNP}}$ to h^2 had a mean = 0.04 and median = 0.09. These 2 sets of estimates are consistent with a higher explained variation from the total local contribution of a region.

The second study by Ouwens et al. (2020) focused on RNA sequence data and included a subsample of these same twin pairs. Classical twin and GRM- (Genetic Relatedness Matrices, based on SNP data) approaches were used to decompose transcriptome variation from RNA sequence data into genetic and nongenetic variance components.[81] Peripheral blood gene expression was obtained for 52844 genes in 1497 twins, including 459 complete MZ and 150 complete DZ twin pairs.[81] Heritability of gene expression profiles based the classic twin design, was 0.20 on average. The mean contribution of the shared environment was 0.05 and the mean contribution of the unique (unshared) environment was 0.75. Next, this total (twin-based) heritability was compared to the heritability which could be attributed to genome-wide SNP data. This was accomplished by creating two GRMs: one GRM containing all SNPs in a 250 kb window of a gene (referred to as *cis*), and one GRM including all autosomal SNPs for closely-related individuals in the dataset. Because of the large number of related individuals this last GRM captures genetic variance tagged by substantial IBD sharing, with the sum of the two effects being roughly equal to the total heritability, which contains the genetic variation in the *cis*-window a gene (h^2_{cis}) and the residual heritability (h^2_{res}). With this approach, an average total heritability of 0.26 was found, which correlated 0.98 with the h^2 estimate obtained from twin modeling. The mean *cis*-heritability ($h^2_{\text{cis;}}$ that is, the variance in gene expression level explained by local SNPs) was 0.06, and a mean residual heritability of 0.20.

Both these studies were conducted in peripheral blood samples; however, gene expression can be tissue specific.[83] For example, a study in 856 female twins (154 complete MZ and 232 complete DZ twin pairs) investigated the heritability of expressed transcripts and performed *cis*- and *trans*-eQTL analysis of adipose and skin tissue and lymphoblastoid cell lines (LCL).[84] Average heritability for these three tissue-types was 0.26 for adipose, 0.16 for skin, and 0.21 for LCL-based gene expression. The study also reported 3529, 2796, and 4625 adipose, skin and LCL *cis*-eQTLs, and 639, 609, and 557 adipose, skin and LCL *trans*-eQTLs, respectively.

Multivariate extensions of the classic twin design are valuable to characterize the genetic and environmental correlations between multiple outcome traits, for example, between expression levels of different genes, or between gene expression levels and complex traits or diseases. A significant genetic correlation between multiple outcome traits indicates that the observed phenotypic correlation between those traits is to a significant extent caused by overlapping genetic influences. An array-based transcriptome-wide analysis of blood pressure in peripheral leukocytes for 391 twins (193 complete same-sex pairs) identified that expression of the *MOK* gene was

significantly associated with systolic blood pressure and this finding was replicated in an independent population cohort.[85] Additionally, out of 40 genes whose expression levels were previously associated with blood pressure, this study replicated the effects of 12 genes. Heritability for the expression levels of these 12 genes ranged from 6% to 65%. Bivariate models estimated the contribution of genes and environment to the association of blood pressure and gene expression levels. The association of blood pressure with *CD97*, *TIPARP*, and *TPP3* expression levels was determined completely by shared genetic factors. By contrast, the association with *LMNA*, *SLC31A2*, *TSC22D3*, and *TAGLN2* expression levels was determined completely by the environment. The association of *CRIP1*, *F12*, *S100A10*, *TAGAP*, and *MOK* expression levels with blood pressure were determined by both genetic and environmental factors.

After the successes of GWA studies for complex traits and disorders, it became clear that common genetic variants often did not fully account for the heritability of these traits as observed in twin-family studies.[86,87] Gene finding for omics phenotypes have been very successful, but for these traits we also observe a gap between the variance explained by omics QTLs and twin-based heritability estimates. Several explanations for the "missing heritability" problem have been proposed.[88] Most omics QTL studies have focused on common SNPs, while it is likely that rare genetic variants also contribute to the heritability of omics phenotypes. Gene–gene (GxG, or epistasis) and gene–environment (GxE) interactions have also been listed as possible reasons for "missing heritability."[88] With twin designs, both GxG and GxE effects have been identified for gene expression levels.[89] For example, gene-by-body mass index (GxBMI) interactions on gene expression regulation were identified in a cohort of 856 female twin individuals with multitissue RNA sequencing data.[90] In adipose tissue, this study found 16 *cis* and 53 *trans* GxBMI interactions. However, recent findings now strongly suggest that the "still missing heritability" of complex phenotypes is accounted for by rare variants, in particular those in regions of the genome of low linkage disequilibrium.[91]

32.4.3 MZ discordant design applied to transcriptomics studies

The MZ discordant design has been frequently used to identify differentially expressed genes for various traits and disorders. Such differentially expressed genes may provide insight in the underlying biology of traits and disorders and could shed light on disease mechanisms. Below, we give two examples to illustrate the strength of the MZ discordant design for transcriptomics studies. Examples of other traits and diseases for which gene expression has been investigated in discordant MZ twin pairs include Type I Diabetes,[92,93] Rheumatoid Arthritis,[94] treatment of Childhood Primary Myelofibrosis,[95] hormone replacement therapy,[96] Parkinson's Disease,[97] Schizophrenia and Schizophrenia treatment,[98,99] Bipolar Disorder,[100] sleep duration,[101] and neurodevelopmental disorders due to trisomy's, such as Down Syndrome.[102,103]

Our first example concerns obesity. A study of mitochondrial DNA gene expression in subcutaneous fat and peripheral leukocytes in 14 obesity-discordant

MZ twin pairs detected upregulation of genes involved in inflammatory pathways and downregulation of genes in mitochondrial branched-chain amino acid catabolism in obese twins as compared to their lean cotwins.[104] Additional evidence that obesity is associated with dysregulation of cellular metabolism and mitochondrial function comes from a BMI-discordant MZ study on the role of sirtuin (SIRT) and NAD^+ biosynthesis gene expression pathways in obesity.[105] The NAD^+/SIRT pathway is involved in sensing energy levels within cells, with the SIRT proteins involved in, for example, mitochondrial oxidation, lipid oxidation, lipolysis, and adipogenesis. This study found that, compared to their leaner cotwins, heavier MZ twins had reduced expression of genes involved in mitochondrial unfolded protein responses and SIRT and NAD^+ biosynthesis and increased poly-ADP ribose polymerase (PARP) activity in subcutaneous adipose tissue (SAT). Transcriptomics studies in obesity-discordant MZ twins also identified obesity subtypes based on transcriptomic profiles and correlations with clinical characteristics. A study in 26 BMI-discordant MZ twin pairs revealed three distinct subgroups based on their molecular profiles and showed that for subgroup one the transcriptional differences between the heavy and leaner cotwins were benign, transcriptional differences between the MZ twins in subgroup two appeared to be characterized by downregulation of mitochondrial function in the heavy twins, and subgroup three showed a clear inflammation pattern in addition to the downregulated mitochondrial function in the heavy twins.[106]

The second example of the MZ discordant design involves multiple phenotypes. In order to identify differentially expressed genes for multiple phenotypes and integrate mean expression differences across phenotypes, Tangirala and Patel (2018) performed a meta-analysis of MZ discordant studies for seven phenotypes, based on studies from public repositories including ten or more MZ twin pairs.[107] These studies focused on ulcerative colitis, chronic fatigue syndrome, physical activity, intelligence quotient (IQ), intermittent allergic rhinitis, major depressive disorder (MDD), and obesity, with gene expression data measured in different tissues, including peripheral blood, lymphoblastoid cell lines, adipose tissue, muscle tissue, and colon tissue. For each of the seven phenotypes, differential gene expression analysis was performed and results were meta-analyzed per phenotype at the gene level. In total, 5% of the genes in the datasets were significantly differentially expressed between discordant MZ twins across all phenotypes. Little overlap in the differentially expressed genes was observed among the phenotypes, with an average overlap of 0.009%. Meta-analysis of each gene across the seven phenotypes identified no genes that were both overall significant and significant for the individual phenotypes. Differential gene expression for most genes was not heterogeneous across the multiple phenotypes. Overall, this study found a small common gene expression signature across the seven phenotypes, where 0.08% of the full list of differentially expressed genes (across all seven phenotypes) were in fact differentially expressed across all seven phenotypes in discordant MZ twins. The study concluded that the majority of differentially expressed genes are phenotype specific.

32.4.4 Other applications of twin research in transcriptomics studies

The discordant MZ design is often expanded to include discordant DZ twin pairs or case-control groups of unrelated individuals. Effects in this last group represent associations at the population level. A comparison between the unaffected MZ twins from discordant pairs with healthy unrelated controls provides information regarding whether these two groups have comparable transcription levels, or whether unaffected twins exhibit a disease-related profile that is more similar (although perhaps milder) to that of their affected cotwin. Gene expression studies in peripheral blood samples for systemic autoimmune diseases, such as rheumatoid arthritis or systemic lupus erythematosus, reported 92–537 differentially expressed genes between probands and unrelated matched controls.[108,109] They also reported that both human and viral gene expression levels of the unaffected twins were intermediate between the expression levels of their affected cotwin and the healthy unrelated controls. Therefore, they concluded that the unaffected MZ twins may be in a transitional or intermediate state of immune regulation.[108]

MZ twin pairs concordant for a disorder may still present discordant phenotypes with unique transcriptomics profiles. For example, miRNA expression of placenta samples in mono-chorionic twin pairs with ($N = 17$) and without ($N = 16$) selective intrauterine growth restriction (sIUGR) identified seven upregulated and seven downregulated miRNAs among the larger sIUGR twins as compared to their smaller cotwins.[110] This study showed that pathogenesis of sIUGR is associated with miRNA pathways involved in organ size, cell differentiation, cell proliferation, and cell migration. Longitudinal designs can be strengthened by inclusion of MZ twin pairs, as these designs are robust for changes in gene expression profiles due to genetic liabilities. A longitudinal MZ design in 235 MZ twin pairs was used to assess the transcriptional changes in the blood associated with cognitive ability differences over a 10-year interval.[111] While this study found no significant transcripts associated with cognitive level or cognitive change over time, it reported two suggestive transcripts; *POU6F1* was negatively associated with cognitive level and *MAD2L1* was positively associated with cognitive change. In addition, gene set enrichment analyses indicated that genes involved in protein metabolism, translation, RNA metabolism, the immune system, and infectious diseases were correlated with lower cognitive levels and cognitive decline. Similar results had previously been observed in individuals with cognitive impairments, indicating these pathways could play a role in aging and cognitive aging in general.

The discordant MZ twin pair design is a valuable tool to examine causality,[112] as illustrated by an example study that aimed to identify gene expression profiles for smoking behavior and to elucidate whether such gene expression profiles are cause or consequent of smoking.[113] In two Dutch population-based cohorts peripheral gene expression microarray data were available for 743 current smokers, 1686 never smokers, and 890 former smokers (age range: 18–88 years). The study identified 220 gene expression probes (of 132 genes) differentially expressed between current and never smokers, that were enriched for immune system, natural killer cells, blood coagulation, and cancer pathways. The expression levels of the 132 smoking-related

genes were compared between current and former smokers and between former and never smokers, as this comparison informs on the reversibility of gene expression levels. Six out of 132 smoking-related genes smoking had irreversible effects on gene expression levels, 31 out of 132 genes were slowly reversible (expression patterns differ between current and former smokers and between former and never smokers) and 94 out of 132 were reversible. Comparisons of gene expression levels of the 132 smoking-related genes in MZ twin pairs discordant for smoking behavior ($N = 56$ pairs) identified 6 differentially expressed genes, indicating these expression levels changed as a consequence of smoking behavior. Successful look-up of *cis*-eQTLs of the smoking-related genes in a GWA for number of cigarettes smoked per day suggested that *GPR56* and *RARRES3* expression are causative for smoking behavior. Thus, the majority of gene expression differences in smoking behavior are a consequence rather than a cause of smoking, which can be largely reversed after cessation of smoking.

32.5 Metabolomics

32.5.1 What is metabolomics and how do we measure the metabolome?

Metabolites are the small molecules, with low molecular weight (<1 kDa), that are involved in cellular metabolism.[114] In the human body, metabolites have numerous functions, including structure formation, signaling, and energy storage.[115] Metabolites can be endogenous (i.e., originate from within an organism) or exogenous (i.e., originate from outside of an organism, e.g., toxins, drugs, and nutrients)[116] and are a highly diverse set of molecules that include amino acids, keto acids, sugars, and lipids.[117] The metabolome is the complete set of metabolites that can be measured within a specific biofluid (e.g., serum, plasma, urine, cerebrospinal fluid, or saliva) or tissue sample.[118] Metabolomics is the study of the metabolome of a biological system, for example, a tissue, cell, or entire organism.[119] As the field of metabolomics includes a broad spectrum of molecular species of different (physical) chemical nature, many metabolomics subtypes focusing on specific molecule types have arisen. One can think of subtypes that are aimed at exogenous molecules taken up by the organism (drugs, nutrients), or molecules involved in specific biological pathways or systems (hormones, lipids). Among the most studied metabolomics subtypes is lipidomics, the study of lipids.[120] Metabolomics strategies focusing on known metabolites, often of similar chemical structures, are called targeted metabolomics and are common in hypothesis testing. Nontargeted metabolomics aims for global detection of a wide range of metabolites and are commonly used to identify changes in metabolites between conditions without *a priori* knowledge of relevant biological pathways.[121] The number and variety of measured metabolites for targeted and nontargeted strategies depend on the sensitivity of the chosen analytical chemical technology.

Different combinations of separation and detection methods are applied in metabolomics.[116] Nuclear magnetic resonance (NMR) spectroscopy, liquid-chromatography mass spectrometry (LC-MS), and gas-chromatography mass spectrometry (GC-MS) are the most widespread platforms.[122] Most NMR metabolomics studies focus on proton (^{1}H) NMR spectroscopy, because it has higher sensitivity then carbon (^{13}C) NMR spectroscopy due to the low natural abundance of carbon (~1.1%).[123] The identification of metabolites with ^{1}H-NMR is based on the so-called "chemical shifts" of the signals and the relative intensity of these signals. The chemical shift in NMR is the variation in resonance frequencies of protons due to different compositions of the surrounding molecules, with respect to a reference frequency or sample.[124] Like in nuclear magnetic imaging (MRI), an NMR signal is produced by aligning the spin states of all protons via a strong magnetic field. Next, an electromagnetic pulse in the radio frequency range is applied to the sample, causing the proton spin states to resonate. The energy emitted from the protons as they relax from the excited spin state to the one before the pulse is measured.[125]

MS determines the molecular weight of metabolites by measuring the mass to charge ratio (m/z).[126] Prior to MS, separation is important to separate analytes with identical m/z values, to prevent high-abundance metabolites to dominate the MS spectrum, or to select which metabolites may pass into the mass spectrometer. GC- and LC-MS are most commonly applied in metabolomics studies. In GC-MS, metabolites injected into the chromatographic device are heated to approximately 300°C to convert them to a gaseous state. Separation of the metabolites depends on their volatility, as more easily evaporated metabolites are driven through the chromatographic column, and subsequently to the detector, faster than less volatile metabolites.[127] LC-MS setups can be distinguished by separation on hydrophobicity or polarity. In reversed-phase chromotography, dissolved metabolites bind to the column (the stationary phase) based on their hydrophobic interactions with the hydrophilic liquid (the mobile phase) in the column. By making the mobile–mobile phase more hydrophobic, the metabolites are eluded from the column, toward the entrance of the mass spectrometer, by use of a strong hydrophobic solvent.[128,129] Normal phase LC-MS is based on the polarity of the metabolites rather than their hydrophobicity.[130] After separation metabolites are destructed into charged fragments. The fragment composition after destruction serves as a fingerprint for the molecule type and hence enables identification of a given metabolite. The gas-phase ionic fragments are generated by the mass spectrometer at its ionization source where molecules are charged by the removal of electrons. After ionization, the ions enter the mass analyzer through which the ions travel based on its m/z ratio. The ionized sample hits the detector, where the number of separated ions with particular m/z values is recorded (mass spectrum).[128]

32.5.2 Causes of variation in metabolite levels

Differences in metabolite levels among individuals reflect individual differences in genetic make-up, physiology, lifestyle, and behavior or responses to environmental factors.[131] Similarities in genetic and environmental backgrounds between individuals

result in more similar lipid profiles, as shown through hierarchical clustering of plasma lipids (LC-MS) in young adult twins and nontwin siblings.[132,133] Twin-family studies estimated the heritability of metabolite levels from approximately 0% to 80%.[134–139] The average heritability observed for metabolite levels differs among metabolites classes. For example, one study estimated the total and SNP-based heritability of 1097 metabolites (UPLC-MS/MS) in plasma for 1111 individuals, and reported that the median total heritability for lipids was 37% and for amino acids 40%.[140] This is in contrast to heritability estimates derived from a study in 221 MZ and 340 DZ twin pairs, that found higher heritability estimates for NMR-measured lipids (range: 0.48–0.62) and lipoproteins (range: 0.50–0.76) than for amino acids and other small molecules (range: 0.23–0.55).[141] A higher heritability for LC-MS measured amino acids than lipids was also seen in a family cohort.[142] The same study reported higher heritability levels for essential amino acids than for nonessential amino acids. Heritability differences among lipid species were also found in twin and family studies of lipidomics data that reported that sphingolipids and glycerolipids tended to have higher heritability estimates than phospholipids.[140,143,144]

The influence of genetic factors on metabolites levels has also been substantiated through genetic association studies that successfully identified metabolite QTLs.[145] For example, in serum samples from 79 MZ twin pairs, 215 DZ twin pairs, and 413 unrelated individuals, the genetic influence on metabolite levels as obtained from two metabolomics platforms were compared,[146] with 160 metabolites measured on a targeted platform (FIA-MS/MS) and 488 metabolites on a nontargeted platform (combination UHPLC-MS and GC-MS), with 43 metabolites measured on both platforms. The mean correlation between these 43 overlapping metabolites was 0.44, and 29 of these 43 metabolites were heritable on both platforms, with heritability estimates ranging from 0.29 to 0.72. For all metabolites on both platforms, GWA identified 61 significant metabolite-SNP associations at 26 independent loci. Of these 26 loci, 19 loci were associated with metabolites measured on one platform, and 7 loci were associated with six metabolites measured on both platforms. This study observed moderate heritability ($h^2 > 0.26$) and correlation ($r > 0.38$) among five of the metabolites associated with the seven loci. Here, the main message is that genetic influences on metabolite concentrations can be observed from data generated by different platforms, possibly utilizing different techniques (NMR vs MS). Even when concentrations of the same metabolite measured by different platforms correlate only moderately (due to e.g. experimental differences) and have only moderate heritability, the interaction with genetic variants may remain detectable. This enables combining/extending studies based on different platforms.

Metabolite QTL information can be used to obtain additional insights into the genetic architecture of metabolite classes. A recent study investigated the heritability of 361 metabolites, in a cohort of 5117 twin-family members (mean age: 42.1), with an extended GRM-based approach.[147] Four GRMs were obtained based on twin and SNP information: two GRMs defined the total (h^2_{total}) and SNP-based heritability (h^2_{SNP}), and two GRMs defined the contribution of metabolite QTLs of the same or of different metabolite classes. These last two GRMs included all loci from GWA and

(exome-) sequencing studies published between November 2008 and October 2018, which identified >800 loci associated with metabolite levels. In this study, the 361 metabolites could be classified as 309 lipids and 52 organic acids and were measured on four different metabolomics platforms (NMR and MS). The mean and median h^2_{total} for lipids both were 0.47. For the organic acids mean and median heritability were 0.41 and 0.40. The median heritability captured by all metabolite QTLS ($h^2_{\text{metabolite-hits}}$) was 0.06 for lipids and 0.01 for organic acids and was mainly attributable to with class-specific hits. Differences in heritability estimates among subclasses of organic acids, lipids, and among lipid species were investigated with mixed-effect meta-regression models. These analyses demonstrated that subclasses of lipids and organic acids differed significantly in $h^2_{\text{metabolite-hits}}$ and that higher degrees of unsaturation in phosphatidylcholines is associated with higher estimates of $h^2_{\text{metabolite-hits}}$.

Unlike the influence of genetic factors on metabolite levels, contributions of the environment shared by family members has been less well characterized and here the classical twin design is of substantial value. An NMR metabolomics twin study in 221 MZ and 340 DZ twin pairs (aged 22–25 years) for 216 metabolites reported that a model including shared environment was the best one for only 31 metabolites (variance explained by shared environment ranged between 15% and 38%).[141] For 6 of these 31 metabolites shared environment explained all familial resemblance. Thus, shared environment influences metabolite levels for a minority of metabolites in a young adult population. In contrast, a family-based FIA-MS/MS metabolomics study in 48 individuals from 16 families (12 parents [mean age = 42] and 26 children aged 8–18 years) reported shared environmental influences for 55 out of 147 measured metabolites.[148] A study from the Netherlands Twin Register estimated the contribution of genetic and shared environmental influences on 237 metabolite levels measured on three platforms (NMR, FIA-MS/MS, and LC-MS) in 886 MZ and 601 DZ adult twin pairs (mean age = 35).[149] A significant contribution of shared environment was reported for 6 out of 237 metabolites (25% explained variance, range 17%–43%) only. Together these studies indicate that the common environment does not play a large role in adult metabolite levels and that substantial effects are mostly found in studies that include younger participants or small sample sizes.

The value of multivariate extensions of the classic twin design for multiple metabolites was highlighted in a study of 221 MZ and 340 DZ young adult twin pairs that explored the association of serum n-6 and n-3 polyunsaturated (PUFAs), monosaturated (MUFAs), and saturated (SFAs) fatty acids with NMR-measured lipoprotein particle concentrations.[150] Bivariate models were applied to those metabolites with a phenotypic correlation of ≥0.3. The bivariate analysis of total n-6 PUFAs and Linoleic Acid (LA) with triglyceride and VLDL particles showed that approximately half (44%–56%) of the phenotypic covariance between the metabolites pairs was due to genetic factors. For MUFAs genetic factors explained more than half of the phenotypic variance between the metabolites, with bivariate heritability estimates of ~80% of MUFAs and HDL-related metabolites and of 58% to 66% for MUFAs and triglyceride and VLDL subclasses. Thus, shared genetic factors play a large role in explaining the associations of PUFAs and MUFAs with lipoprotein particle concentrations.

32.5.3 MZ discordant design applied to metabolomics studies

In contrast to epigenomics or transcriptomics studies, in metabolomics studies the MZ discordant design is less frequently applied. One example concerns an application to schizophrenia. An ^{1}H-NMR metabolomics study in plasma samples of 21 schizophrenia discordant MZ pairs and 8 pairs of matched unaffected MZ pairs showed that signals for VLDL and LDL lipoproteins and aromatic metabolites were the most important to differentiate affected, unaffected and control twins.[151] The differentiation between affected and unaffected twins was more pronounced for female twin pairs. In discordant pairs, MZ twins with schizophrenia had a 23% increase in plasma VLDL signals and a 14% reduction in plasma aromatic metabolites as compared to their unaffected cotwin.

While the MZ discordant design has not often been applied as the main analysis in metabolomics studies, a design with discordant MZ twin pairs to test for replication has gained popularity. Examples include blood metabolomics profiles of food preference and nutrition[136,152–154] and a recent study of urinary metabolites and neurotransmitter ratios, as measured with LC-MS and GC-MS, and childhood aggression.[155] The discovery sample in the aggression study had 783 MZ and DZ twins, the replication sample 189 MZ twin pairs discordant for aggression, and had an additional validation sample of 183 unrelated children who had been referred to a child psychiatry clinic. Positive associations were reported for two metabolites and childhood aggression in the discovery phase. The study did not replicate or validate its findings, but provided suggestive evidence linking childhood aggression to metabolic dysregulation in energy metabolism, oxidative stress, and neurotransmission pathways.

32.5.4 Other application of twin research in metabolomics studies

Discordant DZ twin pairs control for shared environmental factors and partially for genetic factors. While such a design weakens the ability to control for genetic factors, inclusion of DZ pairs would increase statistical power as discordant MZ twin pairs, particularly longitudinally discordant MZ twin pairs, are relatively scarce. A study investigating the long-term effect of physical activity on the serum NMR metabolome selected 16 same-sex twin pairs (7 MZ and 9 DZ pairs; age range: 50–74 years) longitudinally discordant (32 years) for leisure-time physical activity in addition to three independent population cohorts with longitudinally (>5 years) active and inactive participants (N = 1037, mean ages: 31–52 years).[156] Compared to persistently inactive individuals, the serum metabolome of persistently active individuals was characterized by lower concentrations of very-low-density lipoprotein particles, α1-acid glycoprotein, glucose, isoleucine, and polyunsaturated fatty acids and by higher concentrations of large and very large high-density lipoprotein particles and saturated fatty acids.

A discordant MZ twin pair design is suited for dichotomous traits such as presence or absence of disorders. For continuous traits, paired differences between MZ twins also inform about associations of omics profiles with such traits, adjusted for shared genetic, and environmental factors. A recent paper incorporated this strategy

to elucidate plasma metabolite profiles for metabolic risk factors.[157] For 40 MZ twin pairs (mean age 30.7 years) 111 plasma UPLC-MS metabolites were measured as well as blood lipids, fasting glucose, fasting insulin, C-reactive protein (CRP), adiposity measures and homeostasis model assessment (HOMA). First, the 93 metabolites that survived quality control were regressed against the adiposity and blood biochemistry measures, while accounting for twin relatedness. After correction for multiple testing, 18 metabolites were significantly associated with adiposity measures (BMI, percentage of body fat, abdominal visceral adipose tissue, and liver fat) and 24 with blood biochemistry measures (HOMA, CRP, triglycerides, and high-density lipoprotein cholesterol [HDL-C]). Next, follow-up with within-twin pair moderated *t*-tests (this type of *t*-tests uses the square root of the moderated variance as the SD instead of the sample variance) showed that the associations of 9 metabolites with adipose measures and of 10 with blood biochemistry measures (only HDL-C) were independent of confounding factors shared by twins.

32.6 Twin studies in other omics domains

We have considered in some detail the value of twin studies in genomics, epigenomics, transcriptomics, and metabolomics, but other omics domains also benefited from twin research. Proteomics is the large-scale study of the entire range of proteins, the vital molecules that have direct involvement in cellular function,[158] in a cell type (proteome).[159] Protein synthesis is accomplished by converting the information contained in the mRNA sequence to amino acids, a process called translation. Decoding of mRNA is done by the ribosomes where mRNA travels through the ribosome to translate one codon (block of three mRNA nucleotides) at a time to an amino acid, in this process, tRNA is responsible for forming the covalent peptide bonds between the amino acids.[14] As proteins are three-dimensional structures, folding forms the final protein structure. Some proteins fold spontaneously while they are released from the ribosome, while most others require molecular chaperones to help them fold correctly.[160] Large-scale high-throughput proteomics studies predominantly employ two types of analytical strategies. The first uses analytical protein microarrays that rely on antigen-antibody pairing.[161] While protein microarrays have good sensitivity and reproducibility,[161] they are limited in the number of proteins, and the specific group of proteins or molecular pathways they can assess. Therefore, MS-based proteomics provides a more versatile analytical strategy.

Regardless of the analytical strategy, sample preparation for proteomics experiments are labor-intensive, often involving multiple steps such as purification, enzymatic digestion, cell lysis, and solid-phase extraction.[162] The challenges in sample preparation, combined with those in protein and peptide identification, means that large-scale proteomics studies remain relatively expensive and proteomics has not been as extensively studied in twins. The discordant MZ design has been applied to characterize proteomic profiles for BMI,[163] ischemic stroke,[164] bipolar disorder,[165] fatigue,[166,167] hormone replacement therapy,[168] strabismus,[169] and multiple

autoimmune disorders.[170] Twin studies using various other designs have also been applied to proteomics studies. For example, in 15 pairs of opposite-sex DZ twins, sex-specific differences in LC-MS proteins of human endothelial cells were investigated.[171] This study reported small (average fold difference of 1.1–1.2) sex-specific differences in protein levels for approximately 10% of the measured proteins.

Another omics type that has benefitted from twin studies is the microbiome, which is the total ecological community of microorganism such as bacteria, fungi, and viruses that live on and inside our body.[172] Techniques to examine the human microbiome assess both structure and function of the microbiome. The most common application is structural, aimed at cataloging which microbes are present and what their relative abundance is.[173] This can be done by sequencing the gene that encodes the RNA component of the small ribosomal subunit (16S rRNA), followed by taxonomy of the 16S rRNA sequences.[174] Twin studies suggest a greater similarity for measures of relative abundance in MZ than in DZ twins.[175–178] Environmental factors, ranging from pre- and perinatal conditions to household sharing, may be important contributors to the microbiome composition.[178] Twin studies confirm that cohabiting MZ twin pairs have more similar microbiota communities than noncohabiting MZ twin pairs,[179] and that cohabitation can make microbial strains more similar between twins.[180] Rare SNVs in a fecal metagenomes sequencing study were assessed in a cohort of family members, including some twin families.[181] Strain persistence and within-family strain transmissions were analyzed from birth into adulthood. Strong evidence of transmission of maternal strains was seen for vaginally born infants. Later in childhood there was replacement by strains from the environment, including those from family members, with fathers appearing to be more frequently donors of novel strains to other family members. Twins generally did not have more similar rare SNV profiles than nontwin siblings, consistent with findings from abundance studies.

Other omics domains can often be considered subtypes of the traditional omics domains. Subtypes of proteomics include for example glycomics (i.e., the study of glycosylation, or the attachment of glycans or carbohydrates to proteins),[182] or phosphoproteomics (i.e., the study of proteins containing a phosphate group as a post-translational modification).[183] Fluxomics (i.e., the study of the rate of metabolite conversion or transportation in biochemical reaction networks),[184] can be seen as a subtype of metabolomics. Many of these subtypes currently are not optimized for application on a large scale, and twin studies are scarce.

Finally, the exposome has been defined as the totality of exposure individuals experience over their lives.[185] The exposome "summarizes" all environmental influences and is the accumulation of a person's environmental exposures from conception onward. It characterizes the environmental exposures in space and time on omics and on other phenotypes or phenotypic development. The exposome comprises of three domains: (1) internal, (2) specific external, and (3) general external.[6] The internal exposome refers to processes within the body, for example, body morphology or physical activity, but also encompasses the other omics layers such as the interactions between host and (gut) microflora (i.e., the microbiome). Specific external exposures

are the target of classic epidemiology studies and include exposure to environmental pollutants, diet, or lifestyle. General external exposures may include more general economic or social influences. An overview of twin studies in this research domain would go beyond the scope of the current chapter, but we note that twin studies indicate that exposures that are commonly labeled "environment" may show substantial heritability.[186,187]

32.7 Discussion

We have considered and reviewed the value of multiple twin analytical designs in omics research, from the classical twin design which relies on the comparison of resemblance in mono- and dizygotic twin pairs to the discordant twin design. The classic twin design is still invaluable to determine the contribution of genetic and environmental factors on variation in omics levels, with one of its strengths being the possibility to distinguish shared and unique environment. The classic twin design can be extended in multiple ways. A particular strength is combining the twin design with genome-wide SNP data. A recent example of such a combined analysis investigated the heritability of blood metabolites.[147] Based on the twin and SNP information, four genetic relatedness matrices (GRMs) among participants were obtained. Two GRMs defined the total and the SNP heritability. With the addition of two extra GRMs a distinction was made in the contribution of metabolite SNPs of the same or of different metabolite classes. Thus, this method relies on four GRMs: (1) a GRM including all autosomal SNPs for all closely-related individuals in the pedigree (h^2_{ped}); (2) a GRM including all autosomal SNPs (excluding all metabolite QTLs ± 50 kb) for all individuals in the dataset (h^2_{g}); (3) a GRM including the metabolite QTLs of a specific metabolite class for all individuals in the dataset ($h^2_{\text{class-hits}}$); and (4) a GRM including all metabolite QTLs (excluding all QTLs ± 50 kb as included in the third GRM) for all other metabolite classes for all individuals in the dataset ($h^2_{\text{notclass-hits}}$). In this model, the total heritability (h^2_{total}) is obtained by summing across all four heritabilities, SNP-based heritability is obtained by summing across the variance components obtained from the other 3 GRMs and the variance explained by all metabolite QTLs ($h^2_{\text{metabolite-hits}}$) can be obtained by summing $h^2_{\text{class-hits}}$ and $h^2_{\text{notclass-hits}}$. By specifying separate variance components for $h^2_{\text{class-hits}}$ and $h^2_{\text{notclass-hits}}$ metabolite QTLs of the same metabolite class were found to have higher heritability than metabolite QTLs of all other metabolite classes. The study reported nonzero median $h^2_{\text{notclass-hits}}$ estimates, suggesting that metabolite QTLs of other metabolite classes contribute to variance in metabolite levels. This may mean that more powerful GWA or sequencing studies will find associations of these QTLs for the relevant metabolites or this could be a reflection of metabolic networks which can span across distinct metabolite classes. This example and similar studies demonstrate the versatility of combining twin data with genome-wide SNP data. Thus, joining new omics analytical strategies with twin data will be of great benefit to omics research.

Multiple popular analytical strategies in omics research may benefit from including twin data. First, GWA studies have demonstrated that most complex traits and disorders have a highly polygenic nature. To capture polygenic signatures at the individual level, polygenic scores can be constructed.[188] Polygenic scores are calculated by computing the sum of the risk alleles an individual carries at a particular locus, weighted by the locus effect size, as obtained from a GWA. Similar scores can now be constructed from other omics data, for example, DNA methylation scores,[76,189] the epigenetic equivalent of polygenic scores. DNA methylation scores have been explored for traits such as BMI[190] and smoking.[191] DNA methylation scores hold promise as disease biomarkers that, in contrast to polygenic scores, can capture the cumulative and long-term effects of lifetime environmental exposures and the disease process itself. The MZ twin design offers a unique opportunity to examine if prediction of disease risk can be improved by combining polygenic scores with epigenetic scores. MZ twins have identical polygenic scores, yet their discordance rate for many diseases is high, illustrating that the accuracy of polygenic scores will never be perfect. Future studies can examine if epigenetic scores can aid further stratification of disease risk in individuals with identical polygenic scores.

Second, omics data can be used to construct predictors of biological aging and mortality. Well-established predictors rely on epigenetic markers to create the so-called epigenetic clocks.[192] Epigenetic clocks have also been investigated in twins. These studies indicated that the rate of epigenetic aging of MZ cotwins age tends to be similar but is often not identical and these differences in epigenetic aging between MZ cotwins have been associated with traits such as the cerebroplacental ratio (reflects fetal adaptation to hypoxic conditions),[193] and grip strength.[194] No differences in epigenetic aging between MZ cotwins were reported for studies investigating, for example, the association with leisure-time physical activity,[195] depression symptomatology in elderly twins,[196] or cognitive functioning.[197] While epigenetic clocks are frequently used to determine biological aging, clocks based on data from other omics domains are also being developed. For example, with microarray gene expression of T cells in a sample of 27 MZ twins (age range: 22–98) a transcriptomic signature of 125 genes could be constructed to estimate chronological age.[198] This gene expression clock could be replicated in gene expression datasets of T cells, but had poor performance when calculating it using gene expression data of human muscle, indicating that the gene expression clock is likely tissue-specific. Similarly, a metabolomics predictor for chronological age has been constructed using 56 ^{1}H-NMR blood metabolites as measured in 22 cohorts (N = 18,716).[199] A large, positive, difference between an individual's metabolomic and chronological age (ΔmetaboAge) indicates that, for a given chronological age, this individual has a relatively "old" blood metabolome. This has been associated with poor cardio-metabolic health in Dutch BBMRI (Biobanking and BioMolecular Resources Research Infrastructure) cohorts, and with an increased risk for future cardiovascular disease, higher mortality and lower functionality in independent cohorts of older individuals.

Third, in order to establish causal relationships randomized controlled trials often are the preferred method. However, for many research questions RTCs are not

feasible or ethical. Twin models, such as the discordant MZ twin design or methods investigating intra-pair differences, may serve as alternatives to assess causality.[112] Yet, the MZ discordant design does have a caveat, as *de novo* sequence differences between MZ twin pairs can occur. Furthermore, differences between MZ twins could be inflated by measurement error, as this introduces random divergence within twin pairs.

Based on cross-sectional data from MZ and DZ pairs the direction of causation between two traits can be assessed (Direction of Causation model) if the pattern of heritability and shared environmental influences is not too similar for the two traits.[200,201] Mendelian randomization (MR) employs genetic variants as instrumental variables to detect a causal effect of a risk factor on a complex trait or disease.[202] MR requires strong instrumental variables, and as most genetic variants have small effect sizes it has been proposed to combine them into polygenic scores. However, many genetic variants are pleiotropic, and polygenic scores may violate the "no pleiotropy" assumption (instrumental variables may not have direct effects on the outcome) of MR. Several methods are available to include multiple genetic variants that are robust for the "no pleiotropy" assumption.[203] When integrating MR with the Direction of Causation twin model (MR-DoC), the "no pleiotropy" assumption can be relaxed and polygenic scores can serve as instrumental variables.[204]

Twin studies are also valuable in providing information on the reliability of omics traits and profiles, as illustrated by a study of DNA methylation profiles.[205] Reliable methylation probes, defined as probes with a large correlation between replicate measures of the same DNA, have a higher heritability. In general, unreliable traits cannot be highly correlated in monozygotic twin pairs, and therefore the MZ correlation offers a lower bound for the reliability of a trait.

The majority of the twin omics studies described here tended to focus on a single omics domain. However, while each of the different omics layers provides us with a unique picture of the underlying biology of complex traits and disorders, this is an incomplete picture.[206] Because the multiple omics domains are interrelated and interact, we need to study the omics domains collectively to fully understand biological processes.[207] Studies combining multiple omics domains are becoming more frequent, often including multiple omics layers with the purpose of providing biological or functional interpretation of the results for the first omics domain through study of a second (or more) omics domain. Such a strategy is applied in many GWA or EWA studies, were follow-up analyses investigate colocalization of the top SNPs/CpGs with eQTLs. This type of multiomics integration is called sequential integration, when simultaneously analyzing multiple omics domains this is called parallel integration.[208] Many methods for parallel integration of multiomics data have been developed in order to aid in disease classification or subtyping, biomarker prediction, or obtaining insight into disease biology. Most of the studies in twin samples to date have focused on sequential integration of multiomics data. We anticipate that combining twin designs with parallel multiomics integration strategies will be of benefit in disease classification or subtyping and biomarker prediction.

32.8 Conclusion

We have described the value of twin studies in genomics, epigenomics, transcriptomics, and metabolomics. We have discussed the application of the classical twin design and highlighted the benefits of the MZ discordant twin design for identifying omics profiles for complex traits and disorders and to inform on the causal role of omics domains. Much of the twin research has focused on elucidating the causes of variation in omics data, demonstrating the strength of the classical twin design. We also provided a brief overview of other omics domains that can benefit from more twin research in the future and have suggested analytical designs for omics studies that may benefit from the inclusion of twin data. Due to the wide availability of omics data and the methodological advances in multiomics analyses, twin studies with multiomics designs will likely see substantial growth in the coming years.

Acknowledgments

This work was performed within the framework of BBMRI-NL, a research infrastructure financed by the Dutch government (NWO, nos. 184.021.007 and 184.033.111); NWO-funded X-omics project (184.034.019); and the European Union Seventh Framework Program (FP7/2007–2013) ACTION Consortium (Aggression in Children: Unraveling gene–environment interplay to inform Treatment and InterventiON strategies; Grant number 602768). We thank drs Erik Ehli, Jeff Beck, and Jennifer Harris for their critical review and help with the manuscript.

References

1. Zhang XD. Precision medicine, personalized medicine, omics and big data: concepts and relationships. *J Pharmacogenomics Pharmacoproteomics*. 2015;06:1–2.
2. Kim M, Tagkopoulos I. Data integration and predictive modeling methods for multi-omics datasets. *Mol Omi*. 2018;14:8–25.
3. Buescher JM, Driggers EM. Integration of omics: More than the sum of its parts. *Cancer Metab [Internet]*. 2016;4:1–8. https://doi.org/10.1186/s40170-016-0143-y.
4. Franklin S, Vondriska TM. Genomes, proteomes, and the Central Dogma. *Circ Cardiovasc Genet [Internet]*. 2011;4:576. https://www.ahajournals.org/doi/10.1161/CIRCGENETICS.110.957795.
5. Giera M, Wuhrer M. Recent developments in clinical omics. *Chromatographia*. 2015;78:305–306.
6. Wild CP. The exposome: From concept to utility. *International Journal of Epidemiology*. 2012;41:24–32.
7. Visscher PM, Andrew T, Nyholt DR. Genome-wide association studies of quantitative traits with related individuals: little (power) lost but much to be gained. *European Journal of Human Genetics*. 2008;16:387–390.
8. Sul JH, Martin LS, Eskin E. Population structure in genetic studies: Confounding factors and mixed models. *Plos Genetics*. 2018;14:1–22.

9. Lee JJ, Wedow R, Okbay A, et al. Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. *Nature Genetics*. 2018;50:1112–1121.
10. Wray NR, Ripke S, Mattheisen M, et al. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nat Genet [Internet]*. 2018;50:668–681. https://linkinghub.elsevier.com/retrieve/pii/S0924977X17304960.
11. Gormley P, Anttila V, Winsvold BS, et al. Meta-analysis of 375,000 individuals identifies 38 susceptibility loci for migraine. *Nature Genetics*. 2016;48:856–866.
12. van Dongen J, Slagboom PE, Draisma HHM, et al. The continuing value of twin studies in the omics era. *Nat Rev Genet [Internet]*. 2012;13:640–653. http://www.ncbi.nlm.nih.gov/pubmed/22847273.
13. Alberts B, Bray D, Hopkin K, et al.DNA and chromosomes *Essential Cell Biology*. Third ed. New York, NY: Garland Science; 2010:171–195.
14. Alberts B, Bray D, Hopkins K, et al. From DNA to proteins: how cells read the genome*Essential Cell Biology*. Third ed. New York, NY: Garland Science; 2010:231–268.
15. Singh DD, Datta M. Genomics*Omics Approaches, Technologies And Applications [Internet]*. Singapore: Springer Singapore; 2018:11–38. http://link.springer.com/10.1007/978-981-13-2925-8_2.
16. Haraksingh RR, Snyder MP. Impacts of variation in the human genome on gene regulation. *J Mol Biol [Internet]*. 2013;425:3970–3977. https://doi.org/10.1016/j.jmb.2013.07.015.
17. Escaramís G, Docampo E, Rabionet R. A decade of structural variants: description, history and methods to detect structural variation. *Brief Funct Genomics*. 2015;14:305–314.
18. Bumgarner R. Overview of DNA microarrays: types, applications, and their future*Current Protocols in Molecular Biology [Internet]*. Hoboken, NJ: John Wiley & Sons, Inc.; 2013:1–11. http://doi.wiley.com/10.1002/0471142727.mb2201s101.
19. Rajawat J. Transcriptomics*Omics Approaches, Technologies And Applications [Internet]*. Singapore: Springer Singapore; 2018:39–56. http://link.springer.com/10.1007/978-981-13-2925-8_3.
20. Guo Y, He J, Zhao S, et al. Illumina human exome genotyping array clustering and quality control. *Nature Protocols*. 2014;9:2643–2662.
21. Voight BF, Kang HM, Ding J, et al. The Metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. In: Gibson G, ed.; 2012. *PLoS Genet [Internet]*. 8:e1002793. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3410907&tool=pmcentrez&rendertype=abstract.
22. Ehli EA, Abdellaoui A, Fedko IO, et al. A method to customize population-specific arrays for genome-wide association testing. *Eur J Hum Genet [Internet]*. 2017;25:267–270. http://dx.doi.org/10.1038/ejhg.2016.152.
23. Beck JJ, Hottenga J-J, Mbarek H, et al. Genetic similarity assessment of twin-family populations by custom-designed genotyping array. *Twin Res Hum Genet [Internet]*. 2019;22:210–219. https://www.cambridge.org/core/product/identifier/S1832427419000410/type/journal_article.
24. Marees AT, de Kluiver H, Stringer S, et al. A tutorial on conducting genome-wide association studies: quality control and statistical analysis. *International Journal of Methods in Psychiatric Research*. 2018;27:1–10.
25. Petersen B-S, Fredrich B, Hoeppner MP, et al. Opportunities and challenges of whole-genome and -exome sequencing. *BMC Genet [Internet]*. 2017;18:14. http://bmcgenet.biomedcentral.com/articles/10.1186/s12863-017-0479-5.

26. Mardis ER. Next-generation sequencing platforms. *Annual Review of Analytical Chemistry*. 2013;6:287–303.
27. Goodwin S, McPherson JD, McCombie WR. Coming of age: Ten years of next-generation sequencing technologies. *Nature Reviews Genetics*. 2016;17:333–351.
28. Boomsma DI, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nat Rev Genet [Internet]*. 2002;3:872–882. http://www.nature.com/doifinder/10.1038/nrg932.
29. Strachan T, Read A. Chromosome structure and function*Human Molecular Genetics*. 4th edition. New York, NY, USA: Garland Science; 2011:29–60.
30. Griffiths AJF, Miller JH, Suzuki DT. Somatic versus germinal mutation. In: Freeman WH, (ed.). An Introduction to Genetic Analysis [Internet]. 7th edition. New York, NY; 2000. https://www.ncbi.nlm.nih.gov/books/NBK21894/
31. Johnson BN, Ehli EA, Davies GE, et al. Chimerism in health and potential implications on behavior: a systematic review. *Am J Med Genet Part A*. 2020;182(6):1513–1529.
32. Campbell CD, Chong JX, Malig M, et al. Estimating the human mutation rate using autozygosity in a founder population. *Nature Genetics*. 2012;44:1277–1281.
33. Kong A, Frigge ML, Masson G, et al. Rate of de novo mutations and the importance of father's age to disease risk. *Nature*. 2012;488:471–475. http://dx.doi.org/10.1038/nature11396.
34. Dal GM, Ergüner B, Sağiroğlu MS, et al. Early postzygotic mutations contribute to de novo variation in a healthy monozygotic twin pair. *Journal of Medical Genetics*. 2014;51:455–459.
35. Ouwens KG, Jansen R, Tolhuis B, et al. A characterization of postzygotic mutations identified in monozygotic twins. *Human Mutation*. 2018;39:1393–1401.
36. Zwijnenburg PJG, Meijers-Heijboer H, Boomsma DI. Identical but not the same: the value of discordant monozygotic twins in genetic research. *Am J Med Genet Part B Neuropsychiatr Genet*. 2010;153:1134–1149.
37. Abdellaoui A, Ehli EA, Hottenga JJ, et al. CNV concordance in 1,097 MZ twin pairs. *Twin Res Hum Genet*. 2015;18:1–12.
38. Melzer D, Pilling LC, Ferrucci L. The genetics of human ageing. *Nat Rev Genet [Internet]*. 2020;21:88–101. http://dx.doi.org/10.1038/s41576-019-0183-6.
39. Forsberg LA, Rasi C, Razzaghian HR, et al. Age-related somatic structural changes in the nuclear genome of human blood cells. *Am J Hum Genet [Internet]*. 2012;90:217–228. http://dx.doi.org/10.1016/j.ajhg.2011.12.009.
40. Baranzini SE, Mudge J, Van Velkinburgh JC, et al. Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis. *Nature*. 2010;464:1351–1356.
41. Bruder CEG, Piotrowski A, Gijsbers A, et al. Phenotypically concordant and discordant monozygotic twins display different DNA copy-number-variation profiles. *American Journal of Human Genetics*. 2008;82:763–771.
42. Ehli EA, Abdellaoui A, Hu Y, et al. De novo and inherited CNVs in MZ twin pairs selected for discordance and concordance on Attention Problems. *European Journal of Human Genetics*. 2012;20:1037–1043.
43. Strachan T, Read A. Mapping genes conferring susceptibility to complex diseases. *Human molecular genetics*. 4th edition. New York, NY, USA: Garland Science; 2011:467–496.
44. Visscher PM, Macgregor S, Benyamin B, et al. Genome partitioning of genetic variation for height from 11,214 sibling pairs. *American Journal of Human Genetics*. 2007;81:1104–1110.
45. van Dongen J, Jansen R, Smit D, et al. The contribution of the functional IL6R polymorphism rs2228145, eQTLs and other genome-wide SNPs to the heritability of plasma sIL-6R levels. *Behav Genet [Internet]*. 2014;44:368–382. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4283105&tool=pmcentrez&rendertype=abstract.

46. Jones PA, Liang G. The human epigenome*Epigenetic Epidemiology [Internet]*. Dordrecht: Springer; 2012:5–20. http://link.springer.com/10.1007/978-94-007-2495-2_2.
47. Hawkins LJ, Al-attar R, Storey KB. Transcriptional regulation of metabolism in disease: from transcription factors to epigenetics. *PeerJ*. 2018;6:e5062. doi: 10.7717/peerj.5062.
48. Carlberg C, Molnár F. What is epigenomics?*Human Epigenomics [Internet]*. Singapore: Springer; 2018:3–18. http://link.springer.com/10.1007/978-981-10-7614-5_1.
49. Kundaje A, Meuleman W, Ernst J, et al. Integrative analysis of 111 reference human epigenomes. *Nature [Internet]*. 2015;518:317–330. http://www.nature.com/articles/nature14248.
50. Carlberg C, Molnár F. DNA methylation*Human Epigenomics [Internet]*. Singapore: Springer; 2018:57–73. http://link.springer.com/10.1007/978-981-10-7614-5_4.
51. Carlberg C, Molnár F. Methods and applications of epigenomics*Human Epigenomics [Internet]*. Singapore: Springer; 2018:19–38. http://link.springer.com/10.1007/978-981-10-7614-5_2.
52. Li Y, Tollefsbol TO. DNA methylation detection: bisulfite genomic sequencing analysis*Methods in Molecular Biology (Clifton, NJ) [Internet]*; 2011:11–21. http://www.ncbi.nlm.nih.gov/pubmed/21913070.
53. Bibikova M, Barnes B, Tsan C, et al. High density DNA methylation array with single CpG site resolution. *Genomics [Internet]*. 2011;98:288–295. http://dx.doi.org/10.1016/j.ygeno.2011.07.007.
54. Pidsley R, Zotenko E, Peters TJ, et al. Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. *Genome Biol [Internet]*. 2016;17:1–17. http://dx.doi.org/10.1186/s13059-016-1066-1.
55. Maurano MT, Humbert R, Rynes E, et al. Systematic localization of common disease-associated variation in regulatory DNA. *Science*. 2012;337(6099):1190–1195. doi:10.1126/science.
56. Bonder MJ, Luijk R, Zhernakova DV, et al. Disease variants alter transcription factor levels and methylation of their binding sites. *Nat Genet*. 2017;49:131–138.
57. Min JL, Hemani G, Hannon E, et al. Genomic and phenotypic insights from an atlas of genetic effects on DNA methylation. *Nat Genet*. 2021;53:1311–1321. https://doi.org/10.1038/s41588-021-00923-x.
58. Van Dongen J, Ehli EA, Jansen R, et al. Genome-wide analysis of DNA methylation in buccal cells: a study of monozygotic twins and mQTLs. *Epigenetics Chromatin [Internet]*. 2018;11:1–14. https://doi.org/10.1186/s13072-018-0225-x.
59. van Dongen J, Nivard MG, Willemsen G, et al. Genetic and environmental influences interact with age and sex in shaping the human methylome. *Nat Commun [Internet]*. 2016;7:11115. http://www.nature.com/doifinder/10.1038/ncomms11115.
60. Martino D, Loke YJ, Gordon L, et al. Longitudinal, genome-scale analysis of DNA methylation in twins from birth to 18 months of age reveals rapid epigenetic change in early life and pair-specific effects of discordance. *Genome biol.*. 2013;14(5):R42. doi: 10.1186/gb-2013-14-5-r42.
61. Fraga MF, Ballestar E, Paz MF, et al. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci U.S.A*. 2005.
62. Talens RP, Christensen K, Putter H, et al. Epigenetic variation during the adult lifespan: cross-sectional and longitudinal data on monozygotic twin pairs. *Aging Cell*. 2012;11:694–703.
63. Purcell S. Variance components models for gene-environment interaction in twin analysis. *Twin Res*. 2002;5(6):554–571. doi:10.1375/136905202762342026.

64. Castillo-Fernandez JE, Spector TD, Bell JT. Epigenetics of discordant monozygotic twins: implications for disease. *Genome Med*. 2014;6:60.
65. Vidaki A, Daniel B, Court DS. Forensic DNA methylation profiling—potential opportunities and challenges. *Forensic Science International: Genetics*. 2013;7:499–507.
66. Vidaki A, Kayser M, Nothnagel M. Unsupported claim of significant discrimination between monozygotic twins from multiple pairs based on three age-related DNA methylation markers. *Forensic Science International: Genetics*. 2019;39:e1–e2.
67. Li C, Zhang S, Que T, Li L, Zhao S. Identical but not the same: the value of DNA methylation profiling in forensic discrimination within monozygotic twins. *Forensic Sci Int Genet Suppl Ser*. 2011;3:e337–e338.
68. Xu J, Fu G, Yan L, et al. LINE-1 DNA methylation: a potential forensic marker for discriminating monozygotic twins. *Forensic Science International: Genetics*. 2015;19:136–145.
69. Stewart L, Evans N, Bexon KJ, Van Der Meer DJ, Williams GA. Differentiating between monozygotic twins through DNA methylation-specific high-resolution melt curve analysis. *Analytical Biochemistry*. 2015;476:36–39.
70. Du Q, Zhu G, Fu G, et al. A Genome-Wide Scan of DNA Methylation Markers for Distinguishing Monozygotic Twins. *Twin Res Hum Genet*. 2015;18:670–679.
71. Tan Q. The epigenome of twins as a perfect laboratory for studying behavioural traits. *Neuroscience Biobehavioral Rev*. 2019;107:192–195.
72. Palma-Gudiel H, Córdova-Palomera A, Navarro V, et al. Twin study designs as a tool to identify new candidate genes for depression: a systematic review of DNA methylation studies. *Neurosci Biobehavioral Rev*. 2020;112:345–352.
73. Allione A, Marcon F, Fiorito G, et al. Novel epigenetic changes unveiled by monozygotic twins discordant for smoking habits. *PLoS One*. 2015;10:e0128265.
74. Hancock DB, Guo Y, Reginsson GW, et al. Genome-wide association study across European and African American ancestries identifies a SNP in DNMT3B contributing to nicotine dependence. *Mol Psychiatry*. 2017;23(9):1911–1919. doi:10.1038/mp.2017.193.
75. Oates NA, Van Vliet J, Duffy DL, et al. Increased DNA methylation at the AXIN1 gene in a monozygotic twin from a pair discordant for a caudal duplication anomaly. *Am J Hum Genet*. 2006;79(1):155–162. doi:10.1086/505031.
76. Nwanaji-Enwerem JC, Colicino E. DNA methylation–based biomarkers of environmental exposures for human population studies. *Curr Environ Health Rep*. 2020;7(2):121–128. doi:10.1007/s40572-020-00269-2.
77. Souren NY, Gerdes LA, Lutsik P, et al. DNA methylation signatures of monozygotic twins clinically discordant for multiple sclerosis. *Nat Commun*. 2019;10:2094. https://doi.org/10.1038/s41467-019-09984-3.
78. Tsai PC, Bell JT. Power and sample size estimation for epigenome-wide association scans to detect differential DNA methylation. *International Journal of Epidemiology*. 2015;44:1429–1441.
79. Hu Y, Ehli EA, Boomsma DI. MicroRNAs as biomarkers for psychiatric disorders with a focus on autism spectrum disorder: current progress in genetic association studies, expression profiling, and translational research. *Autism Res*. 2017;10:1184–1203.
80. Wright FA, Sullivan PF, Brooks AI, et al. Heritability and genomics of gene expression in peripheral blood. *Nature Genetics*. 2014;46:430–437.
81. Ouwens KG, Jansen R, Nivard MG, et al. A characterization of cis- and trans-heritability of RNA-Seq-based gene expression. *Eur J Hum Genet [Internet]*. 2019;10. http://www.nature.com/articles/s41431-019-0511-5.

82. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet [Internet]*. 2011;88:76–82. http://dx.doi.org/10.1016/j.ajhg.2010.11.011.
83. Aguet F, Ardlie KG. Tissue specificity of gene expression. *Curr Genet Med Rep*. 2016;4:163–169.
84. Grundberg E, Small KS, Hedman ÅK, et al. Mapping cis-and trans-regulatory effects across multiple tissues in twins. *Nature Genetics*. 2012;44:1084–1089.
85. Huang Y, Ollikainen M, Sipil P, et al. Genetic and environmental effects on gene expression signatures of blood pressure: a transcriptome-wide twin study. *Hypertension*. 2018;71:457–464.
86. Maher B. Personal genomes: the case of the missing heritability. *Nature [Internet]*. 2008;456:18–21. http://www.nature.com/doifinder/10.1038/456018a.
87. A MT, FS C, Cox NJ, et al. Finding the missing heritability of complex diseases. *Nature [Internet]*. 2009;461:747–753. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2831613&tool=pmcentrez&rendertype=abstract.
88. Génin E. Missing heritability of complex diseases: case solved? *Hum Genet [Internet]*. 2020;139:103–113. https://doi.org/10.1007/s00439-019-02034-4.
89. Buil A, Brown AA, Lappalainen T, et al. Gene-gene and gene-environment interactions detected by transcriptome sequence analysis in twins. *Nat Genet [Internet]*. 2015;47:88–91. http://www.nature.com/articles/ng.3162.
90. Glastonbury CAA, Viñuela A, Buil A, et al. Adiposity-dependent regulatory effects on multi-tissue transcriptomes. *American Journal of Human Genetics*. 2016;99:567–579.
91. Wainschtein P, Jain DP, Yengo L, et al. Recovery of trait heritability from whole genome sequence data. *bioRxiv*. 2019.
92. Caramori ML, Kim Y, Moore JH, et al. Gene expression differences in skin fibroblasts in identical twins discordant for type 1 diabetes. *Diabetes*. 2012;61:739–744.
93. Beyan H, Drexhage RC, Nieuwenhuijsen L, et al. Monocyte gene-expression profiles associated with childhood-onset type 1 diabetes and disease risk: a study of identical twins. *Diabetes*. 2010;59:1751–1755.
94. Haas CS, Creighton CJ, Pi X, et al. Identification of genes modulated in rheumatoid arthritis using complementary DNA microarray analysis of lymphoblastoid B cell lines from disease-discordant monozygotic twins. *Arthritis and Rheumatism*. 2006;54:2047–2060.
95. Ding N, Zhang Z, Yang W, et al. Transcriptome analysis of monozygotic twin brothers with childhood primary myelofibrosis. *Genomics, Proteomics Bioinforma [Internet]*. 2017;15:37–48. http://dx.doi.org/10.1016/j.gpb.2016.12.002.
96. Ronkainen PHA, Pöllänen E, Alén M, et al. Global gene expression profiles in skeletal muscle of monozygotic female twins discordant for hormone replacement therapy. *Aging Cell*. 2010;9:1098–1110.
97. Alieva AK, Rudenok MM, Novosadova EV, et al. Whole-transcriptome analysis of dermal fibroblasts, derived from three pairs of monozygotic twins, discordant for Parkinson's Disease. *Journal of Molecular Neuroscience*. 2020;70:284–293.
98. Kakiuchi C, Ishiwata M, Nanko S, et al. Up-regulation of ADM and SEPX1 in the lymphoblastoid cells of patients in monozygotic twins discordant for schizophrenia. *Am J Med Genet Part B Neuropsychiatr Genet*. 2008;147:557–564.
99. Nakazawa T, Kikuchi M, Ishikawa M, et al. Differential gene expression profiles in neurons generated from lymphoblastoid B-cell line-derived iPS cells from monozygotic twin cases with treatment-resistant schizophrenia and discordant responses to clozapine. *Schizophr Res [Internet]*. 2017;181:75–82. http://dx.doi.org/10.1016/j.schres.2016.10.012.

100. Matigian N, Windus L, Smith H, et al. Expression profiling in monozygotic twins discordant for bipolar disorder reveals dysregulation of the WNT signalling pathway. *Molecular Psychiatry*. 2007;12:815–825.
101. Watson NF, Buchwald D, Delrow JJ, et al. Transcriptional signatures of sleep duration discordance in monozygotic twins. *Sleep*. 2017;40(1):zsw019. doi:10.1093/sleep/zsw019.
102. Stamoulis G, Garieri M, Makrythanasis P, et al. Single cell transcriptome in aneuploidies reveals mechanisms of gene dosage imbalance. *Nature Communication*. 2019;10:1–11.
103. You S-H, Lee Y-S, Chang Y-J, et al. Gene expression profiling of amniotic fluid mesenchymal stem cells of monozygotic twins discordant for trisomy 21. *Gene [Internet]*. 2020;738:144461. https://doi.org/10.1016/j.gene.2020.144461.
104. Pietiläinen KH, Naukkarinen J, Rissanen A, et al. Global transcript profiles of fat in monozygotic twins discordant for BMI: Pathways behind acquired obesity. *Plos Medicine*. 2008;5:0472–0483.
105. Jukarainen S, Heinonen S, Rämö JT, et al. Obesity is associated with low nad+/sirt pathway expression in adipose tissue of BMI-discordant monozygotic twins. *Journal of Clinical Endocrinology and Metabolism*. 2016;101:275–283.
106. Muniandy M, Heinonen S, Yki-Järvinen H, et al. Gene expression profile of subcutaneous adipose tissue in BMI-discordant monozygotic twin pairs unravels molecular and clinical changes associated with sub-types of obesity. *International Journal of Obesity*. 2017;41:1176–1184.
107. Tangirala S, Patel CJ. Integrated analysis of gene expression differences in twins discordant for disease and binary phenotypes. *Sci Rep [Internet]*. 2018;8:1–9. http://dx.doi.org/10.1038/s41598-017-18585-3.
108. O'Hanlon TP, Rider LG, Gan L, et al. Gene expression profiles from discordant monozygotic twins suggest that molecular pathways are shared among multiple systemic autoimmune diseases. *Arthritis Res Ther [Internet]*. 2011;13:R69. http://arthritis-research.biomedcentral.com/articles/10.1186/ar3506.
109. Gan L, O'Hanlon TP, Lai Z, et al. Gene expression profiles from disease discordant twins suggest shared antiviral pathways and viral exposures among multiple systemic autoimmune diseases. *PLoS One*. 2015;10:1–15.
110. Wen H, Chen L, He J, Lin J. MicroRNA expression profiles and networks in placentas complicated with selective intrauterine growth restriction. *Mol Med Rep*. 2017;16:6650–6673.
111. Nygaard M, Larsen MJ, Thomassen M, et al. Global expression profiling of cognitive level and decline in middle-aged monozygotic twins. *Neurobiol Aging [Internet]*. 2019;84:141–147. https://doi.org/10.1016/j.neurobiolaging.2019.08.019.
112. Vitaro F, Brendgen M, Arseneault L. The discordant MZ-twin method: one step closer to the holy grail of causality. *Int J Behav Dev [Internet]*. 2009;33:376–382. http://jbd.sagepub.com/cgi/doi/10.1177/0165025409340805.
113. Vink JM, Jansen R, Brooks A, et al. Differential gene expression patterns between smokers and non-smokers: cause or consequence?. *Addiction Biology*. 2017;22:550–560.
114. Patti GJ, Yanes O, Siuzdak G. Innovation: Metabolomics: the apogee of the omics trilogy. *Nature Reviews Molecular Cell Biology*. 2012;13:263–269.
115. Dunn WB, Broadhurst DI, Atherton HJ, et al. Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chem Soc Rev [Internet]*. 2011;40:387–426. http://www.ncbi.nlm.nih.gov/pubmed/20717559.
116. Schrimpe-Rutledge AC, Codreanu SG, Sherrod SD, et al. Untargeted metabolomics strategies—challenges and emerging directions. *J Am Soc Mass Spectrom [Internet]*. 2016;27:1897–1905. https://linkinghub.elsevier.com/retrieve/pii/S0031938416312148.

117. Adamski J, Suhre K. Metabolomics platforms for genome wide association studies-linking the genome to the metabolome. *Current Opinion in Biotechnology*. 2013;24:39–47.
118. Dunn WB, Ellis DI. Metabolomics: Current analytical platforms and methodologies. *TrAC Trends Anal Chem [Internet]*. 2005;24:285–294. http://linkinghub.elsevier.com/retrieve/pii/S0165993605000348.
119. Fiehn O. Metabolomics—the link between genotypes and phenotypes. *Plant Molecular Biology*. 2002;48:155–171.
120. Wenk MR. The emerging field of lipidomics. *Nat Rev Drug Discov*. 2005;4:594–610.
121. Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chem Soc Rev*. 2011;40:387–426.
122. Li B, He X, Jia W, Li H. Novel applications of metabolomics in personalized medicine: a mini-review. *Molecules [Internet]*. 2017;22:1173. http://www.mdpi.com/1420-3049/22/7/1173.
123. Clendinen CS, Lee-McMullen B, Williams CM, et al. 13 C NMR metabolomics: applications at natural abundance. *Anal Chem [Internet]*. 2014;86:9242–9250. https://pubs.acs.org/doi/10.1021/ac502346h.
124. Lenz EM, Wilson ID. Analytical strategies in metabonomics. *Journal of Proteome Research*. 2007;6:443–458.
125. Berger A. How does it work? Magnetic resonance imaging. *Br Med J*. 2002;324:35.
126. Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics. *Mass Spectrom Rev [Internet]*. 2007;26:51–78. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1904337&tool=pmcentrez&rendertype=abstract.
127. Issaq HJ, Abbott E, Veenstra TD. Utility of separation science in metabolomic studies. *J Sep Sci [Internet]*. 2008;31:1936–1947. http://www.ncbi.nlm.nih.gov/pubmed/18348322.
128. Griffiths WJ, Wang Y. Mass spectrometry: from proteomics to metabolomics and lipidomics. *Chem Soc Rev*. 2009;38:1882–1896.
129. Vaughan AA, Dunn WB, Allwood JW, et al. Liquid chromatography-mass spectrometry calibration transfer and metabolomics data fusion. *Anal Chem [Internet]*. 2012;84:9848–9857. http://www.ncbi.nlm.nih.gov/pubmed/23072438.
130. Trivedi DK, Iles RK. Do not just do it, do it right: urinary metabolomics -establishing clinically relevant baselines. *Biomed Chromatogr [Internet]*. 2014;28:1491–1501. http://www.ncbi.nlm.nih.gov/pubmed/24788800.
131. Fiehn O. Metabolomics—the link between genotypes and phenotypes. *Plant Mol Biol [Internet]*. 2002;48:155–171. http://www.ncbi.nlm.nih.gov/pubmed/11860207.
132. Draisma HHM, Reijmers TH, Bobeldijk-Pastorova I, et al. Similarities and differences in lipidomics profiles among healthy monozygotic twin pairs. *OMICS [Internet]*. 2008;12:17–31. http://www.ncbi.nlm.nih.gov/pubmed/18266560.
133. Draisma HHM, Reijmers TH, Meulman JJ, van der Greef J, Hankemeier T, Boomsma DI. Hierarchical clustering analysis of blood plasma lipidomics profiles from mono- and dizygotic twin families. *Eur J Hum Genet [Internet]*. 2013;21:95–101. http://www.nature.com/articles/ejhg2012110.
134. Nicholson G, Rantalainen M, Maher AD, et al. Human metabolic profiles are stably controlled by genetic and environmental variation. *Mol Syst Biol [Internet]*. 2011;7:525. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3202796&tool=pmcentrez&rendertype=abstract.

135. Alul FY, Cook DE, Shchelochkov OA, et al. The heritability of metabolic profiles in newborn twins. *Heredity (Edinb) [Internet]*. 2013;110:253–258. http://dx.doi.org/10.1038/hdy.2012.75.
136. Menni C, Zhai G, MacGregor A, et al. Targeted metabolomics profiles are strongly correlated with nutritional patterns in women. *Metabolomics [Internet]*. 2013;9:506–514. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3608890&tool=pmcentrez&rendertype=abstract.
137. van 't Erve TJ, Doskey CM, Wagner BA, et al. The heritability of glutathione and related metabolites in, stored red blood cells. *Free Radic Biol Med [Internet]*. 2014;76:107–113. http://www.sciencedirect.com/science/article/pii/S0891584914003578.
138. van 't Erve TJ, Wagner BA, Martin SM, et al. The heritability of metabolite concentrations in stored human red blood cells. *Transfusion [Internet]*. 2014;54:2055–2063. http://www.ncbi.nlm.nih.gov/pubmed/24601981.
139. Shin S-Y, Fauman EB, Petersen A-K, et al. An atlas of genetic influences on human blood metabolites. *Nat Genet [Internet]*. 2014;46:543–550. http://www.ncbi.nlm.nih.gov/pubmed/24816252.
140. Darst BF, Koscik RL, Hogan KJ, et al. Longitudinal plasma metabolomics of aging and sex. *Aging (Albany NY) [Internet]*. 2019;11:1262–1282. http://www.aging-us.com/article/101837/text.
141. Kettunen J, Tukiainen T, Sarin A-P, et al. Genome-wide association study identifies multiple loci influencing human serum metabolite levels. *Nat Genet [Internet]*. 2012;44:269–276. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3605033&tool=pmcentrez&rendertype=abstract.
142. Rhee EP, Ho JE, Chen M-H, et al. A genome-wide association study of the human metabolome in a community-based cohort. *Cell Metab [Internet]*. 2013;18:130–143. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3973158&tool=pmcentrez&rendertype=abstract.
143. Bellis C, Kulkarni H, Mamtani M, et al. Human plasma lipidome is pleiotropically associated with cardiovascular risk factors and death. *Circ Cardiovasc Genet [Internet]*. 2014;7:854–863. https://www.ahajournals.org/doi/10.1161/CIRCGENETICS.114.000600.
144. Frahnow T, Osterhoff MA, Hornemann S, et al. Heritability and responses to high fat diet of plasma lipidomics in a twin study. *Science Reports*. 2017;7:1–11.
145. Kastenmüller G, Raffler J, Gieger C, et al. Genetics of human metabolism: an update. *Hum Mol Genet [Internet]*. 2015;24:R93–101. http://www.hmg.oxfordjournals.org/lookup/doi/10.1093/hmg/ddv263.
146. Yet I, Menni C, Shin SY, et al. Genetic influences on metabolite levels: a comparison across metabolomic platforms. *PLoS One [Internet]*. 2016;11(4). http://dx.doi.org/10.1371/journal.pone.0153672.
147. Hagenbeek FA, Pool R, van Dongen J, et al. Heritability estimates for 361 blood metabolites across 40 genome-wide association studies. *Nat Commun [Internet]*. 2020;11:39. http://www.nature.com/articles/s41467-019-13770-6.
148. Tremblay BL, Guénard F, Lamarche B, et al. Familial resemblances in human plasma metabolites are attributable to both genetic and common environmental effects. *Nutr Res [Internet]*. 2019;61:22–30. https://doi.org/10.1016/j.nutres.2018.10.003.
149. Pool R, Hagenbeek FA, Hendriks A, et al. Genetics and not shared environment explains familial resemblance in adult metabolomics data. *Twin Res Hum Genet*. 2020;23:145–155.
150. Jelenkovic A, Bogl LH, Rose RJ, et al. Association between serum fatty acids and lipoprotein subclass profile in healthy young adults: exploring common genetic and

environmental factors. *Atherosclerosis [Internet]*. 2014;233:394–402. http://www.ncbi.nlm.nih.gov/pubmed/24530769.
151. Tsang TM, Huang JTJ, Holmes E, et al. Metabolic profiling of plasma from discordant schizophrenia twins: Correlation between lipid signals and global functioning in female schizophrenia patients. *Journal of Proteome Research*. 2006;5:756–760.
152. Pallister T, Sharafi M, Lachance G, et al. Food Preference Patterns in a UK Twin Cohort. *Twin Res Hum Genet*. 2015;18:793–805.
153. Pallister T, Jennings A, Mohney RP, et al. Characterizing blood metabolomics profiles associated with self-reported food intakes in female twins. *PLoS One*. 2016;11:1–16.
154. Pallister T, Jackson MA, Martin TC, et al. Untangling the relationship between diet and visceral fat mass through blood metabolomics and gut microbiome profiling. *Int J Obes [Internet]*. 2017;41:1106–1113. http://dx.doi.org/10.1038/ijo.2017.70.
155. Hagenbeek FA, Roetman PJ, Pool R, et al. Urinary amine and organic acid metabolites evaluated as markers for childhood aggression: the ACTION biomarker study. *Front Psychiatry [Internet]*. 2020;11(165). https://www.frontiersin.org/article/10.3389/fpsyt.2020.00165/full.
156. Kujala UM, Mäkinen V-P, Heinonen I, et al. Long-term Leisure-time Physical Activity and Serum Metabolome. *Circulation [Internet]*. 2013;127:340–348. https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.112.105551.
157. Muniandy M, Velagapudi V, Hakkarainen A, et al. Plasma metabolites reveal distinct profiles associating with different metabolic risk factors in monozygotic twin pairs. *Int J Obes [Internet]*. 2019;43:487–502. http://dx.doi.org/10.1038/s41366-018-0132-z.
158. Vaz C, Tanavde V. Proteomics*Omics Approaches, Technologies And Applications [Internet]*. Singapore: Springer; 2018:57–73. http://link.springer.com/10.1007/978-981-13-2925-8_4.
159. Aslam B, Basit M, Nisar MA, et al. Proteomics: Technologies and their applications. *Journal of Chromatographic Science*. 2017;55:182–196.
160. Alberts B, Bray D, Hopkin K, et al. Protein structure and function. *Essential Cell Biology*. 2010:119–170.
161. Sutandy FXR, Qian J, Chen C-S, et al. Overview of Protein Microarrays. *Curr Protoc Protein Sci [Internet]*. 2013;72. 27.1.1-27.1.16. http://doi.wiley.com/10.1002/0471140864.ps2701s72.
162. Aebersold R, Mann M. Mass spectrometry-based proteomics. *Nature [Internet]*. 2003;422:198–207. http://www.nature.com/articles/nature01511%0Ahttp://www.nature.com/nature/journal/v422/n6928/abs/nature01511.html.
163. Sahebekhtiari N, Saraswat M, Joenväärä S, et al. Plasma proteomics analysis reveals dysregulation of complement proteins and inflammation in acquired obesity—a study on rare BMI-discordant monozygotic twin pairs. *Proteomics Clin Appl*. 2019;13(4):e1800173. doi:10.1002/prca.201800173.
164. Vadgama N, Lamont D, Hardy J, et al. Distinct proteomic profiles in monozygotic twins discordant for ischaemic stroke. *Mol Cell Biochem [Internet]*. 2019;456:157–165. http://dx.doi.org/10.1007/s11010-019-03501-2.
165. Kazuno A-a, Ohtawa K, Otsuki K, Usui M, Sugawara H, Okazaki Y, et al. Proteomic analysis of lymphoblastoid cells derived from monozygotic twins discordant for bipolar disorder: a preliminary study. *PLoS One*. 2013;8(2):e53855. https://doi.org/10.1371/journal.pone.0053855.
166. Ciregia F, Giusti L, Da Valle Y, et al. A multidisciplinary approach to study a couple of monozygotic twins discordant for the chronic fatigue syndrome: A focus on potential

salivary biomarkers. *J Transl Med*. 2013;11:243. https://doi.org/10.1186/1479-5876-11-243.
167. Ciregia F, Kollipara L, Giusti L, et al. Bottom-up proteomics suggests an association between differential expression of mitochondrial proteins and chronic fatigue syndrome. *Transl Psychiatry*. 2016;6(9):e904..
168. Laakkonen EK, Soliymani R, Karvinen S, et al. Estrogenic regulation of skeletal muscle proteome: a study of premenopausal women and postmenopausal MZ cotwins discordant for hormonal therapy. *Aging Cell*. 2017;16:1276–1287.
169. Liu G, Bai H, Yan Z, et al. Differential expression of proteins in monozygotic twins with discordance of infantile esotropic phenotypes. *Molecular Vision*. 2011;17:1618–1623.
170. O'Hanlon TP, Li Z, Gan L, et al. Plasma proteomic profiles from disease-discordant monozygotic twins suggest that molecular pathways are shared in multiple systemic autoimmune diseases. *Arthritis Res Ther*. 2011;13(2):R69. doi:10.1186/ar3330.
171. Witt E, Lorenz M, Völker U, et al. Sex-specific differences in the intracellular proteome of human endothelial cells from dizygotic twins. *J Proteomics [Internet]*. 2019;201:48–56. https://doi.org/10.1016/j.jprot.2019.03.016.
172. Peterson J, Garges S, Giovanni M, et al. The NIH Human Microbiome Project. *Genome Res [Internet]*. 2009;19:2317–2323. http://genome.cshlp.org/cgi/doi/10.1101/gr.096651.109.
173. Young VB. The role of the microbiome in human health and disease: An introduction for clinicians. *BMJ*. 2017;356:831. doi:10.1136/bmj.j831.
174. Handelsman J. Metagenomics: application of genomics to uncultured microorganisms. *Microbiol Mol Biol Rev [Internet]*. 2004;68:669–685. http://www.ncbi.nlm.nih.gov/pubmed/15590779.
175. Stewart JA, Chadwick VS, Murray A. Investigations into the influence of host genetics on the predominant eubacteria in the faecal microflora of children. *J Med Microbiol [Internet]*. 2005;54:1239–1242. https://www.microbiologyresearch.org/content/journal/jmm/10.1099/jmm.0.46189-0.
176. Goodrich JK, Waters JL, Poole AC, et al. Human genetics shape the gut microbiome. *Cell [Internet]*. 2014;159:789–799. http://dx.doi.org/10.1016/j.cell.2014.09.053.
177. Goodrich JK, Davenport ER, Beaumont M, et al. Genetic determinants of the gut microbiome in UK Twins. *Cell Host Microbe [Internet]*. 2016;19:731–743. http://dx.doi.org/10.1016/j.chom.2016.04.017.
178. Rothschild D, Weissbrod O, Barkan E, et al. Environment dominates over host genetics in shaping human gut microbiota. *Nature*. 2018;555:210–215.
179. Finnicum CT, Beck JJ, Dolan CV, et al. Cohabitation is associated with a greater resemblance in gut microbiota which can impact cardiometabolic and inflammatory risk. *Bmc Microbiology*. 2019;19:1–10.
180. Koo H, Hakim JA, Crossman DK, et al. Sharing of gut microbial strains between selected individual sets of twins cohabitating for decades. *PLoS One*. 2019;14:1–13.
181. Korpela K, Costea P, Coelho LP, et al. Selective maternal seeding and environment shape the human gut microbiome. *Genome Res [Internet]*. 2018;28:561–568. http://genome.cshlp.org/lookup/doi/10.1101/gr.233940.117.
182. Raman R, Raguram S, Venkataraman G, et al. Glycomics: an integrated systems approach to structure-function relationships of glycans. *Nat Methods [Internet]*. 2005;2:817–824. http://www.nature.com/articles/nmeth807.
183. Cohen P. The origins of protein phosphorylation. *Nat Cell Biol*. 2002;4(5):E127–30. doi:10.1038/ncb0502-e127.

184. Cortassa S, Caceres V, Bell LN, et al. From metabolomics to fluxomics: a computational procedure to translate metabolite profiles into metabolic fluxes. *Biophys J [Internet]*. 2015;108:163–172. http://dx.doi.org/10.1016/j.bpj.2014.11.1857.
185. Wild CP. Complementing the genome with an "exposome": The outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiology and Prevention Biomarkers*. 2005;14:1847–1850.
186. Kendler KS, Baker JH. Genetic influences on measures of the environment: a systematic review. *Psychol Med [Internet]*. 2007;37:615. http://www.journals.cambridge.org/abstract_S0033291706009524.
187. Vinkhuyzen AAE, van der Sluis S, de Geus EJC, et al. Genetic influences on 'environmental' factors. *Genes, Brain Behav [Internet]*. 2010;9:276–287. http://doi.wiley.com/10.1111/j.1601-183X.2009.00554.x.
188. Wray NR, Lee SH, Mehta D, et al. Research review: polygenic methods and their application to psychiatric traits. *J Child Psychol Psychiatry Allied Discip*. 2014;55:1068–1087.
189. Hüls A, Czamara D. Methodological challenges in constructing DNA methylation risk scores. *Epigenetics [Internet]*. 2020;15:1–11. https://doi.org/10.1080/15592294.2019.1644879.
190. Shah S, Bonder MJ, Marioni RE, et al. Improving phenotypic prediction by combining genetic and epigenetic associations. *Am J Hum Genet*. 2015;97(1):75–85. doi:10.1016/j.ajhg.2015.05.014. PMID: 26119815; PMCID: PMC4572498.
191. Elliott HR, Tillin T, McArdle WL, et al. Differences in smoking associated DNA methylation patterns in South Asians and Europeans. *Clin Epigenet*. 2014;6:4. https://doi.org/10.1186/1868-7083-6-4.
192. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet*. 2018;19(6):371–384. doi:10.1038/s41576-018-0004-3.
193. Palma-Gudiel H, Eixarch E, Crispi F, et al. Prenatal adverse environment is associated with epigenetic age deceleration at birth and hypomethylation at the hypoxia-responsive EP300 gene. *Clin Epigenetics*. 2019;11:1–10.
194. Sillanpaä E, Laakkonen EK, Vaara E, et al. Biological clocks and physical functioning in monozygotic female twins. *BMC Geriatr*. 2018;18:1–7.
195. Sillanpää E, Ollikainen M, Kaprio J, et al. Leisure-time physical activity and DNA methylation age—a twin study. *Clin Epigenetics*. 2019;11:1–8.
196. Starnawska A, Tan Q, Soerensen M, et al. Epigenome-wide association study of depression symptomatology in elderly monozygotic twins. *Transl Psychiatry [Internet]*. 2019;9. http://doi.org/10.1038/s41398-019-0548-9.
197. Starnawska A, Tan Q, Lenart A, et al. Blood DNA methylation age is not associated with cognitive functioning in middle-aged monozygotic twins. *Neurobiol Aging [Internet]*. 2017;50:60–63. http://dx.doi.org/10.1016/j.neurobiolaging.2016.10.025.
198. Remondini D, Intrator N, Sala C, et al. Identification of a T cell gene expression clock obtained by exploiting a MZ twin design. *Science Reports*. 2017;7:1–8.
199. Akker van EB, Trompet S, Wolf J, et al. Predicting biological age based on the BBMRI-NL 1H-NMR metabolomics repository. *bioRxiv*. 2019.
200. Heath AC, Kessler RC, Neale MC, et al. Testing hypotheses about direction of causation using cross-sectional family data. *Behavior Genetics*. 1993;23:29–50.
201. Duffy DL, Martin NG. Inferring the direction of causation in cross-sectional twin data: theoretical and empirical considerations. *Genetic Epidemiology*. 1994;11:483–502.

202. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet [Internet]*. 2014;23:R89–R98. https://academic.oup.com/hmg/article-lookup/doi/10.1093/hmg/ddu328.
203. Burgess S, Bowden J, Fall T, et al. Sensitivity analyses for robust causal inference from mendelian randomization analyses with multiple genetic variants. *Epidemiology (Cambridge, Mass.)*. 2017;28:30–42.
204. Minică CC, Dolan CV, Boomsma DI, et al. Extending causality tests with genetic instruments: an integration of mendelian randomization with the classical twin design. *Behav Genet [Internet]*. 2018;48:337–349. http://dx.doi.org/10.1007/s10519-018-9904-4.
205. Sugden K, Hannon EJ, Arseneault L, et al. Patterns of reliability: assessing the reproducibility and integrity of DNA methylation measurement. Patterns [Internet]. 2020;1:100014. https://doi.org/10.1016/j.patter.2020.100014
206. Olivier M, Asmis R, Hawkins GA, et al. The need for multi-omics biomarker signatures in precision medicine. *International Journal of Molecular Sciences*. 2019;20(19).
207. Hillmer RA. Systems Biology for Biologists. In: True-Krob HL, ed.; 2015. *PLOS Pathog [Internet]*. 11:e1004786. https://dx.plos.org/10.1371/journal.ppat.1004786.
208. Subramanian I, Verma S, Kumar S, et al. Multi-omics data integration, interpretation, and its application. *Bioinform Biol Insights [Internet]*. 2020;14:117793221989905. https://doi.org/10.1177/1177932219899051.

CHAPTER

Epigenetics 33

Aino Heikkinen, Sara Lundgren, Miina Ollikainen
Institute for Molecular Medicine Finland FIMM, HiLIFE, University of Helsinki, Helsinki, Finland

33.1 Introduction to epigenetics and epigenomics

Epigenetics is a field of study on changes in gene expression or gene function that are caused without alterations in the underlying DNA sequence and are maintained through cell divisions. It originates from the discovery of how the cells in an organism are able to develop into the different cell types that form the various tissues in our bodies even though these cells have identical DNA sequences. The term epigenetics was first introduced by Conrad Waddington back in 1942, originally to explain the link between developmental processes and genetics.[1] This definition has evolved over the course of time and today it is often used broadly to describe those cellular processes that affect gene expression without changing the DNA. However, there is still no universally accepted definition for epigenetics to date.[2] The first epigenetic mark, a modified cytosine in the DNA, was detected in the late 1940s.[3] This modification became known as DNA methylation. A later study found that these methylated cytosines could also affect the expression of genes.[4] After these discoveries, human epigenetics began to be investigated extensively. Later, along with the development of high-throughput methods, the focus on epigenetic research has begun to shift from studying the mechanistic properties of a single epigenetic modification to investigating the whole epigenetic state of a cell—the epigenome.

The most prominent function of epigenetic mechanisms is to maintain and regulate gene expression patterns in cells. Epigenetic mechanism are highly dynamic in nature, acting at the interface of the genetic code and phenotype. They respond to external environmental exposures like diet, stress, exercise, as well as internal environmental exposures such as gender and different medical conditions. Indeed, a growing body of literature shows that epigenetic modifications associate with numerous human diseases and phenotypes by modifying gene activity or chromatin structure in response to the environment, guided by the underlying genetic code. Monozygotic (MZ) twins have virtually identical genomes and are consequently more similar in their epigenetic profiles compared to dizygotic (DZ) twins or unrelated individuals. Therefore, studies involving MZ and DZ twin pairs provide an important avenue for

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00033-7

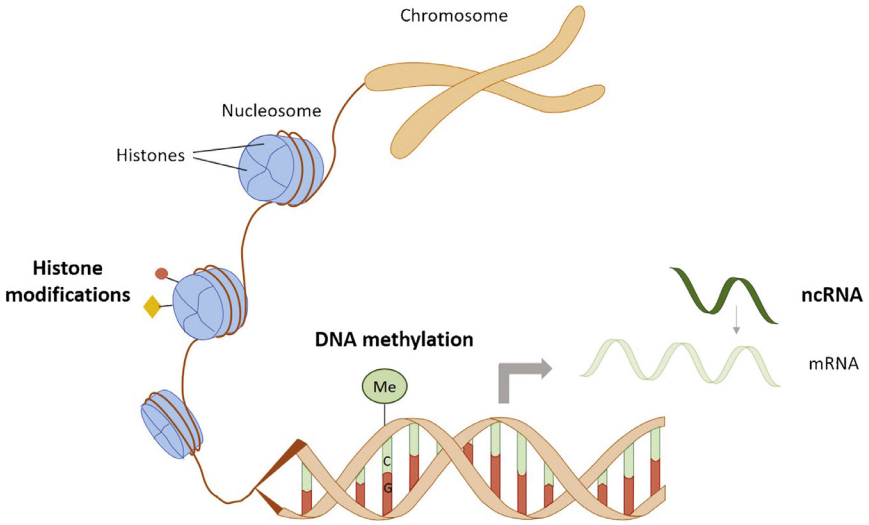


FIG. 33.1 A schematic representation of the three main epigenetic mechanisms.

The basic component of chromatin structure is the nucleosome, which consists of DNA wrapped around histone proteins. The N-terminal tail of these histones can be covalently modified constituting the first class of epigenetic mechanisms. The most common histone modifications include acetylation, methylation, phosphorylation, ubiquitination, and ribosylation. The second class of epigenetic mechanisms is DNA methylation, which takes place primarily at CpG dinucleotides. The reaction is catalyzed by DNA methyltransferases (DMNTs). Methylation in CpG-rich gene promoter areas are usually associated with gene repression. Finally, noncoding RNAs that bind to their complementary mRNAs, lead to the degradation or translational inhibition of the mRNAs, which results in gene silencing. CpG, cytosine-guanine dinucleotide; mRNA, messenger RNA; ncRNA, noncoding RNA.

epigenetic research as the relative contributions of environment and genome to the epigenome can be estimated, and moreover, the influence of genomes and shared environment can be eliminated when comparing MZ cotwins to each other. In the following sections, three main classes of epigenetic mechanisms are introduced: DNA methylation, histone modifications, and noncoding RNAs (Fig. 33.1).

33.1.1 DNA methylation

DNA methylation refers to the addition of a methyl (CH_3) group to the fifth carbon of a cytosine residue in a DNA strand to form 5-methylcytosine (5mC). The transition is catalyzed by DNA methyltransferases (DNMTs) for which S-adenosylmethionine (SAM) is the main methyl donor.[5] There are three classes of DNMTs that possess catalytic activity: *de novo* methyltransferases DNMT3a and DNMT3b, which add methyl groups to previously unmethylated cytosines,[6] and maintenance methyltransferase DNMT1, which ensures that DNA methylation patterns are transmitted to the daughter cells in cell division.[7]

Methylation in human cells occurs predominantly, but not exclusively, at cytosine-guanine dinucleotides (CpGs), which are distributed over the genome.[8] The majority of all CpGs are constantly methylated,[9] showing cell and tissue-specific patterns. However, there are regions in the DNA with higher density of CpG-sites than expected, which are referred to as CpG islands (CPIs). 70% of the CPIs lie within promoter regions. Unlike other CpG sites, CPIs remain mostly unmethylated.[10] As hypomethylation is generally associated with the expression of genes rather than suppression, it may not be surprising that many of the unmethylated promoters belong to genes essential to the function of any cell. In addition to CpG-methylation, recent evidence suggests that DNA methylation occurs in non-CpG context, too, and that non-CpG methylation may impact some cellular processes and biological traits.[11] In addition to gene expression and suppression, DNA methylation is the driving factor in many other cellular events like genomic imprinting,[12] silencing of retroviral elements,[13] and X-chromosome inactivation.[14]

Two main explanations of the mechanisms through which methylated cytosine nucleotides affect gene transcription have been forwarded. The first proposes that the addition of a methyl-group to the cytosines in CPIs may physically block the binding of transcription factors (TFs) and thereby block gene expression. The alternative posits that DNA methylation may recruit additional proteins important in gene suppression, including some histone-modifying proteins. The effect of DNA methylation on gene expression is dependent on the genomic context; for example, methylation in gene bodies often results in increased gene expression[15] whereas methylation in promoter regions is associated with gene silencing. While some generalizations can be made, the relationship between gene activity and DNA methylation is complex, and gene expression levels cannot be predicted purely from DNA methylation patterns.[16]

33.1.1.1 DNA methylation in diseases and traits

Cancer was the first human disease linked to epigenetic aberrations, and genome-wide hypomethylation is currently a well-established hallmark of cancer. To date, DNA methylation alterations have been observed in numerous other human diseases and traits. The extent and patterns of DNA methylation across the genome differ between healthy individuals and individuals with more serious conditions such as neurodegenerative diseases and type 2 diabetes,[17,18] but site-specific DNA methylation levels are also associated with life-style related phenotypes, such as physical activity,[19] smoking,[20,21] and obesity.[22,23] Additionally, aging is closely linked with epigenomic changes; this pertains to many CpGs across the genome that become hypomethylated with increasing age, with certain CpG-sites being exceptions.[24,25] Aging also increases the variation in epigenetic profiles within MZ twin pairs, suggesting that distinct environments shape epigenetics over life course.[25,26] Certain CpG sites in the human genome seem to respond to aging uniformly between individuals, which have led to the development of so-called epigenetic clocks; algorithms that predict chronological or biological age of an individual based on DNA methylation levels.[27]

33.1.1.2 Genetic effects on DNA methylation

It has become evident that the regulation of DNA methylation patterns is not fully independent of the DNA sequence. Current technological updates have led to a discovery that multiple methylation sites appear to be under strict genetic control.[28–30] The genomic variants that associate with DNA methylation levels are referred to as methylation quantitative trait loci (meQTLs), suggesting that the state of a methylome is partly explained by genetic influences. The genome works together with environmental factors and sporadic factors to modify DNA methylation profiles throughout life, which is seen as increased discordance within MZ twin pairs' methylomes as time passes.

33.1.2 Histone modifications

Histone modifications constitute another class of epigenetic mechanisms. In eukaryotic cells, DNA is wrapped around histone proteins, which help compactly pack the DNA. Units of eight histones and approximately two turns of DNA form nucleosomes which are the building blocks of chromatin. Histone proteins can be chemically modified with a diverse group of reactions, in which a functional group is covalently attached to the histone protein tails. The reactions are catalyzed by specific histone-modifying enzymes. One of the most common histone modifications is histone acetylation: an acetyl group from acetyl-CoA is transferred to a lysine residue in the N-terminal end of a histone. Other common modifications include histone methylation, phosphorylation, ubiquitination, and ribosylation. As there are tens of possible modifiable sites in a histone,[31] and a single amino acid residue may be a target for more than one type of reaction, there are numerous possible combinations of modifications (histone codes).

Two main ways histone modifications affect gene expression have been suggested: by changing the nucleosome structure, or by attracting DNA and chromatin-modifying enzymes in place. The linking of a charged chemical group, such as acetyl or phosphorus, potentially changes the nucleosome structure by decreasing the positive charge of a histone, thereby weakening the electrochemical interaction between the histone and negatively charged DNA resulting in loosened chromatin structure and making the genome more accessible to TFs and other DNA-binding proteins. Alternatively, specific histone codes are recognized by proteins belonging to chromatin-associated protein families, activating pathways that result in changes in chromatin structure and gene activity. In many cases, the outcome for gene activity is not a result of a single histone modification, but rather an extensive crosstalk between different histone marks (and other epigenetic mechanisms). Similar to DNA methylation, aberrant function of histone-modifying enzymes and modification patterns are seen in human diseases such as cancer,[32] autoimmune diseases,[33] and type 2 diabetes.[34]

33.1.3 Noncoding RNAs

Noncoding RNAs (ncRNAs) are functional RNAs that do not code for proteins. NcRNAs are sometimes considered to be an epigenetic mechanism since they modify gene expression without altering the DNA sequence. However, there is not a uniform view in the field whether or not ncRNAs should be classified as an epigenetic process, probably due to the ambiguous definition of epigenetics. Two main ncRNAs have attracted researchers because of their ability to regulate gene expression: microRNAs (miRNA) and small interfering RNAs (siRNA) which have somewhat overlapping functions. These RNA molecules are transcribed in the nucleus and transferred to the cytoplasm where they are cleaved from the precursors to around 20 nucleotide-long RNA strands. In the cytoplasm, ncRNAs are able to bind to a complementary mRNA molecule, activating a mRNA degradation process or inhibiting the mRNA translation. The major difference between miRNA and siRNA is that a single miRNA may have multiple mRNA targets, the binding can be only partially complementary, whereas siRNAs have only one, fully complementary target mRNA.[35] ncRNAs have a major role in developmental processes. Similarly to DNA methylation and histone modifications, alterations in miRNA functions and expression have been suggested as a contributing factor in many human diseases, such as cancer[36] and cardiovascular diseases.[37]

33.1.4 Complex interactions between epigenetic marks

Additionally, epigenetic marks interact with each other, creating a complex network of signaling pathways, which translate genetic and environmental cues into biological functions. The close relationship between histone modifications and DNA methylation has been documented in the literature. For instance, both CpG methylation and histone lysine methylation are required for establishing and maintaining heterochromatin (inactive DNA segments).[38] These functions are also interrelated, as shown in model organisms: histone methylation can help in targeting DNA methylation in heterochromatin.[39] In addition, methyl-CpG binding proteins (MBDs) directly link DNA methylation to histone modifications by recognizing methylated CpGs in the genome and binding to certain histone-modifying enzymes.[40] Noncoding RNAs, too, are seen to interact with other epigenetic modifications; miRNAs can affect the expression of DNMTs or histone-modifying enzymes by binding to their mRNA molecules and inhibiting translation. Conversely, DNA methylation has the potential to regulate the transcription of miRNAs (similar to any other gene).[41,42]

33.2 Challenges in epigenetic research

Epigenetic studies often face similar challenges to other omics-based research or epidemiological studies, such as selection of an appropriate study cohort, reproducibility of obtained results, and difficulties in addressing causality. However, certain challenges are particularly relevant to epigenetic studies.

The epigenome is highly tissue and even cell-type-specific, which makes it hard to generalize findings across tissues and distinct populations. The epigenome also develops over the lifetime of an individual as a response to environmental stimuli. Therefore, a large number of studies conducted in different tissues, age groups, and ethnicities are required to obtain a comprehensive picture of the human epigenome. Additionally, most common methods measure the mean value of an epigenetic mark at a particular locus for all the cells in a sample, which introduces the possibility that the results may reflect changes in cell-type proportions rather than epigenetic changes within all cells or in a single cell-type. Methods for single-cell epigenetic sequencing are emerging, but the costs are high and sufficient tools for the data analysis are still lacking. The biggest challenge, however, is that the relevant tissue for a particular condition is not often available. This additionally limits one's ability to interpret the results.

As described above, epigenetic mechanisms are dependent on both the genome and environmental signals. Therefore, disentangling the contribution of either one may be challenging especially in cross-sectional studies. MZ twins, sharing practically identical DNA sequences and childhood environment, offer an exceptional design for investigating the effects of the later life environmental factors unique to one of the twins in a pair.

A number of studies look for associations between certain changes in epigenetic mechanisms and traits of interest. However, a commonly encountered phenomenon in this type of study is reverse causation, a term to describe an observed association in which the direction of causation is the opposite of what is expected, or a two-way relationship. Longitudinal study designs, randomized trials, or analytical methods such as Mendelian randomization,[43] which exploit known genetic variants, are thus required to infer causality. Twin and family studies provide another causal inference approach, which is based on the assessment of familial confounding to DNA methylation data.[44]

To overcome the challenges described above, and to characterize the dynamic interplay of different epigenetic mechanisms, there is a need for highly powerful sequencing technologies and bioinformatic tools. Fortunately, the field advances fast and we are currently reaching the era where single-strand sequencing technologies, bioinformatic methods, and deep learning algorithms have become more developed and cost-effective. When coupling high-quality data with suitable study designs, such as twin and family designs that overcome many of the described challenges, we are one step closer to characterizing the role of varying epigenomes on human diseases and phenotypes.

33.3 Value of twins in epigenetic research

Epigenetic data on twins can be helpful in dissecting the genetic and environmental components of epigenetic regulation. In addition to the classical twin modeling using both MZ and DZ twin pairs, MZ twin pairs discordant for a phenotype are of special interest for epigenetics research. This study design increases the power to detect reliable associations, helps in identifying important environmental risk factors that

affect epigenetic marks associated with health outcomes. Twin data can also help in the identification of epigenetic marks that could be used as molecular targets for interventions or therapies, through assessing causality of the observed disease-associated epigenetic marks. Fig. 33.2 provides a simplified overview of the sources of similarities and differences in MZ and DZ twin pairs, and unrelated individuals, and the effects of those in the epigenome.

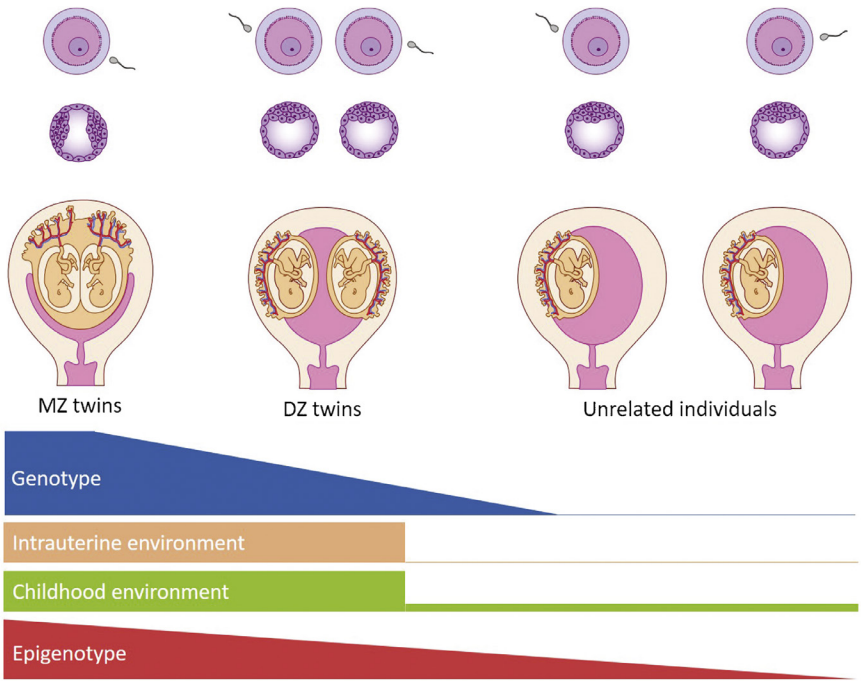


FIG. 33.2 MZ twins originate when a single egg is fertilized by a single sperm forming a zygote that splits and develops into two genetically identical individuals, who are same sex and age.

DZ twins are formed when two eggs are fertilized each by a different sperm. Two individuals who share, on average 50% of their genes, start to develop in the same womb. DZ twins are always the same age but can be of different or same sex. Unrelated individuals are obviously originated from different eggs and sperms, they are different for their genetic polymorphisms, develop in different wombs, can be of any age (compared to each other) or either sex. Because of differences in the degree to which MZ, DZ, and unrelated individuals share in common their genes, epigenomes, intrauterine, childhood environments, MZ twins in a pair are more similar than DZ twins, which, in turn, are more similar than pairs of unrelated individuals. The great similarity between MZ twin siblings, and the relative differences between MZ versus DZ twin pairs provide advantages for epigenetic studies over those conducted using unrelated individuals. The figure is an oversimplification and the relative sizes of each triangle should not be taken as being proportional to each other.

The same methodological approaches that are undertaken in epidemiological twin studies can be used for studying the epigenome (see Part IV. Twin Methodology for details). Below we discuss the most common twin study designs and the rationale for them.

33.3.1 Classical twin model

The classical twin study design, in which the additive genetic (A), common/shared environmental exposures (C), and unique environmental exposures (E) are estimated using structural equation modeling[45] can be applied to epigenetic data. This is to estimate the proportions of genetic and environmental variation that explain variation in epigenetic differences between twins, for example, during development and aging, or in the context of disease progression. This classical twin method makes use of the genetic differences between MZ and DZ twins; while MZ twins are thought to be genetically identical or nearly identical[46] to their cotwin, DZ twins in a pair share on average 50% of their segregating alleles. This model assumes that shared environmental influences contribute equally to twin resemblance in MZ and DZ pairs. The within-pair differences in the epigenome will accumulate due to differences in environmental effects (i.e., unique environmental effects) and stochastic factors that exert effects starting early in development and accumulate throughout life. Therefore longitudinal studies of twins that allow the evaluation of genetic and environmental contributions to the stability and change of the epigenome during the life course are especially interesting.

The classical twin model assumes that the greater differences within DZ twin pairs compared with MZ pairs are mainly due to genetic factors, however, it is likely that DZ twins in a pair are also epigenomically more dissimilar than MZ twins in a pair. This is because each DZ twin pair originates from different zygotes (formed from different sperm and egg) that are potentially already epigenetically very different. In contrast, each MZ pair originates from a single zygote and thus from a single zygotic epigenome. For these reasons, heritability estimates of phenotypes that are epigenetically regulated may be overestimated in twin studies.[47]

33.3.2 Within-pair comparisons

The epigenetic state of a given genomic region is determined by the genotype, the environment, and stochastic processes. Therefore epigenetic studies involving unrelated individuals are at risk of confounding by unmeasured heterogeneous genetic and environmental factors in the study population. Instead, within-pair comparisons of twins control for the effects of genotype on epigenotype by approximately 50% in DZ pair and 100% in MZ pair comparisons. In addition, confounding due to environmental and other factors shared by the twin siblings is greatly reduced in the twin design. Epigenetic analyses conducted on within-pair differences thus increase the power to detect true associations while reducing the possibility for false-positive findings.

Epigenetic differences within twin pairs may arise through the processes of establishment and maintenance of epigenetic marks from very early development

throughout the life. For example, DNA methyltransferases that lay down DNA methylation and maintain the marks in cell divisions may make mistakes that are carried on in the subsequent cell divisions as epimutations. This may result in within-pair epigenetic discordance. Another factor that may result in epigenetic differences in twins of a pair already at early life is the hypothesized differences in the epigenetic profiles of cells contributing to the MZ twinning event; however, precise mechanisms of MZ twinning is still not known. In addition, differences in environmental exposures may result in epigenetic differences within the twin pairs. This may occur already in utero where the microenvironments of the cotwins may differ. If and when the postnatal lifestyles of the cotwins diverge, it is likely that their epigenomes also become less similar.

Discordant MZ twin pairs have proven especially valuable for identifying trait, disease, or treatment-associated epigenetic marks because MZ cotwins are the same age and sex, and in general, identical by their genotypes. They also share most of their intrauterine and rearing environments during their childhood and adolescent years. Therefore, using the case cotwin control design, where the cotwins with the phenotype of interest (cases) are compared with their cotwin without the given phenotype (controls), completely eliminates (for age, sex, genotype), or significantly reduces the known and unknown confounding in the epigenetic analyses. Simulation studies demonstrate that the trait discordant MZ twin pair study design increases the power in epigenome wide association study (EWAS) if the heritability of the trait is at least moderate (>0.3), while for traits with very low heritability, this study design does not provide power advantage over the usual case-control design of unrelated individuals.[48] This suggests that the power of the discordant MZ twin designs is mainly attributed to the complete removal of genetic effects that mask some of the signals in EWAS of unrelated individuals.

Epigenetics may provide a molecular-level solution for identifying MZ twin siblings from one another. Differentiating MZ twin siblings in, for example, forensics has remained challenging as MZ cotwins share, in practice, an identical DNA sequence. As described above; however, MZ cotwins may differ by their DNA methylation profiles, which opens new avenues for forensics applications.[49,50]

33.3.3 Inferring causality

Epigenetic marks identified in an EWAS can be used to predict risk or as a biomarker. However, the within-twin pair designs, although powerful, cannot rule out reverse causation when using cross-sectional data, or unmeasured confounding even in longitudinal study designs. Association does not imply causation because the association can arise between two variables both in the presence or absence of a causal relationship. Assessing causality in epigenetic studies is essential if the aim is to identify targets for therapies or interventions, rather than establishing epigenetic biomarkers.

Twin data can be useful for inferring causality in observed epigenetic associations. Li and colleagues applied a causal inference method, called ICE FALCON, based on

the assessment of familial confounding in DNA methylation data in order to address this.[44,51] The basic idea behind this approach is that if DNA methylation measured in one twin associates with the trait of their cotwin, and this association remains unchanged after conditioning on the trait of the first twin, then familial factors act on both the trait of interest and DNA methylation. However, if the association disappears after conditioning on the trait of the first twin, then the trait causes the change in DNA methylation. Here, the existence of familial factors (shared genetic and environmental factors) is essential, as otherwise ICE FALCON could not make explicit causal inference based on cross-twin cross-trait correlation for the trait of interest and DNA methylation.

33.4 Key findings from epigenetic studies involving twins

Twin cohorts and registries across the world have collected longitudinal phenotypic data and biological samples on tens of thousands of twins, which has proven useful and increased our knowledge on the genetic and environmental determinants of epigenetic variation between individuals and across time. The importance of genetic factors and both prenatal and postnatal environments to the establishment and maintenance of the human epigenome has been demonstrated by twin studies. Twin studies have also generated clear evidence linking epigenetic marks to the disease-associated risk in humans. In this section, we will describe what we have learned from epigenetics studies involving twins.

33.4.1 The contribution of the genome and environment to the establishment and maintenance of DNA methylation

Epigenetic variation between individuals results from genetic differences and from environmental and stochastic variation. Twin study designs and heritability analyses have been employed to improve our understanding of the regulation of epigenetic variation (reviewed in, e.g.,),[52] while studying MZ twins has provided insights into the extent of which epigenetic variation is due to environmental factors or stochastic events. Both have been further linked to the role of epigenetic variation in complex phenotypes.[53–59]

Many studies have demonstrated that MZ cotwins are more similar in their epigenetic marks compared to DZ cotwins[53,60–62] already at birth.[63,64] Like in the traditional epidemiological twin studies, this has been attributed to the genetic identity of twins in MZ pairs vs the (on average) 50% genetic similarity of twins in DZ pairs. In addition, the phenotypic discordance of twins in MZ pairs has been considered to result from both stochastic events and environmental factors unique to each twin in a pair. As cotwins can differ in their DNA methylation at birth,[63,64] it implies that both stochastic and intrauterine factors can influence the epigenome during pregnancy. Interestingly, one twin study showed that at birth, some unrelated individuals are more similar in their overall methylation profile than some cotwins, providing

support for the importance of stochastic and prenatal nonshared environmental factors in determining the epigenetic makeup of an individual.[63]

The average heritability of DNA methylation across the genome is low, and varies from 5% to 12%, depending on tissue, in newborns.[63] The heritabilities of DNA methylation are higher in adulthood, (17–86 years) and vary from 16% to 24%,[53,61,62,65] but decline with increasing age[53] and during aging of individuals.[65] Studies of twins have increased our knowledge of the heritability of complex traits including DNA methylation. In addition, they provide valuable information on interindividual epigenetic variation established already during early embryonic development, and its potential impact on future health and disease. More than 10 years ago, it was already proposed that epigenetic similarity at the time of the twinning event at the blastocyst stage may also contribute to the phenotypic similarities observed between MZ cotwins.[60] Moreover, greater phenotypic discordance among DZ than MZ cotwins may not only reflect that they are less similar genetically, but could also reflect that they originated from two epigenetically different zygotes. This may increase heritability estimates from twin studies, especially for those traits that have epigenetic contribution.

33.4.1.1 *Super similarity of MZ twins*

More recently, additional support has been provided to the theory that epigenetic similarity contributes to the phenotypic similarity between MZ twins, by showing that regions of epigenetic supersimilarity within MZ twin pairs exist. Supersimilarity of epigenetic loci results from the establishment of the epigenome prior to the twinning event.[47] These supersimilar loci show systemic interindividual epigenetic variation, regardless of genetic variation, in response to the periconceptional environment and stochastic events. Importantly, these epigenetic loci that are established at the time of the major epigenetic reprogramming during early embryonic development, are variable between individuals (and supersimilar within MZ twin pairs), and may provide a link between the early development and adult health and disease. At the same time, these findings suggest that part of the missing heritability in complex traits may be attributed to the overestimation of heritability in twin studies for traits at least partially under epigenetic control.

33.4.1.2 *MZ twinning*

MZ twins are formed when a single fertilized oocyte splits into two separate zygotes, occurring mostly within the first week after fertilization, as early as the first cell division. The reason for this splitting event is not known, but it has been hypothesized that it is a random process, because MZ twinning rates are highly similar across the world,[66,67] or that it results from an epigenetic event[68] since MZ twinning occurs at the time of early epigenetic reprogramming essential for normal embryonic development.[69] A very recent study has found evidence for the putative role of DNA methylation in MZ twinning,[70] showing that MZ twins differ from DZ twins in DNA methylation at a large number of CpG sites. Many of these sites are metastable epialleles that are supersimilar within MZ twin pairs.[47] These sites have

established methylation around the MZ twinning event, resulting in more similar DNA methylation patterns in those MZ pairs that split later (and share placenta) vs those who split earlier (have individual placentas) in development.

33.4.2 The contribution of epigenetic variation to phenotypic variation

Since within-pair analysis of epigenetic data increases the power to detect true associations, especially for traits that are at least moderately heritable,[48] the cotwin control design has been applied in many studies aiming to identify epigenetic associations in various complex diseases and traits (Table 33.1). The first epigenetic studies on discordant MZ twin pairs concentrated on carefully selected candidate genes, while later studies are conducted mainly on a genome-wide level. Table 33.1 lists such studies from the last 5 years. These studies show that within-pair differences in DNA methylation are associated with phenotypic discordance, and may in some cases explain the phenotypic differences in these genetically identical individuals. DNA methylation at the CpG sites associated with a phenotype could be used for developing disease- or trait-specific biomarkers (see Table 33.1).

An obvious challenge faced by many of these studies is that discordant MZ twin pairs are rather rare, limiting the number of pairs in each of the studies, which clearly has implications for generalization of the results to the population level, especially if the obtained results are not validated in an independent sample.

33.4.3 Stability and drift of methylation in time

Longitudinal epigenetic studies in twins have increased our understanding of the relationship between genetics and other factors that influence temporal changes in DNA methylation profiles in humans. The very first larger-scale twin study in epigenetics demonstrated that young MZ twin siblings display more similar epigenetic marks compared to older twin siblings, and that the epigenetic discordance increased in relation with an increase in lifestyle differences between twin siblings.[26] Although this study was cross-sectional, investigating different pairs at different ages, it provided the first clues about epigenetic differences arising during the lifetime of MZ twins, and prompted other researchers to investigate this further. A longitudinal twin study conducted during early childhood later confirmed that MZ cotwins are already in early life discordant for DNA methylation at three different regions of the genome, and that the discordance is mainly attributable to environmental factors.[71] The same study also revealed that different genomic regions show varying levels of epigenetic divergence over time and that the dynamic changes in DNA methylation are influenced by a range of shared and nonshared environmental factors present already in early life.[71] Another study, also concentrating on candidate regions, showed that the epigenetic divergence between MZ cotwins begins already in utero, and that this drift is tissue-specific.[64] The first longitudinal genome-wide DNA methylation twin study, which followed the first 18 months of life, interestingly reported that some

TABLE 33.1 Recent epigenome-wide studies within twin pairs.

Platform	Study sample	Trait of interest	Tissue	Conclusions from the study	Publication Year	Citation
Reduced representation bisulphite sequencing (RRBS)	4 discordant MZ twin pairs	Cerebral palsy	Whole blood	Discordance for CP in MZ twins is likely associated with DNA methylation alterations that contribute to the development of CP.	2017	114
450K BeadChip	52 MZ twin pairs	Birth weight	Saliva	DNA methylation may serve as the mediator in the relationship between birth weight and cortical morphology that may be attributed to intrauterine environment.	2017	115
RRBS	5 discordant MZ twin pairs	Amyotrophic lateral sclerosis (ALS)	Leukocytes	These findings add information to ALS pathogenesis, provide a reference for future ALS DNA methylation studies, and could perhaps be used to develop ALS biomarkers.	2017	116
450K BeadChip	62 discordant siblings including 12 discordant MZ twin pairs, and two unrelated sets of 221 cases vs. 227 controls and 472 cases vs. 487 controls	Parkinson's disease (PD)	Peripheral blood mononuclear cells	DNA methylation signature in blood cells can be used to separate PD from controls reasonably well, and it holds a potential for future biomarker development.	2017	117

(Continued)

TABLE 33.1 Cont'd

Platform	Study sample	Trait of interest	Tissue	Conclusions from the study	Publication Year	Citation
450K BeadChip	169 MZ twin pairs	Lung function	Leukocytes	Epigenetic regulation of immunological-, cancer - and TGF-β-receptor-related genes may be involved in lung function and its change in time.	2017	118
450K BeadChip	14 discordant MZ twin pairs	Attention deficit hyperactivity disorder (ADHD)	Whole blood	Neuroimaging may be helpful in the search for epigenetic mechanisms in neurodevelopmental disorders.	2018	119
450K BeadChip	6 discordant, 4 concordant affected, and 7 concordant unaffected MZ twin pairs	Depression	Whole blood	Individuals with risk for depression may show epigenetic outlier profiles.	2018	120
450K BeadChip	15 discordant MZ twin pairs	Cerebral palsy (CP)	Blood spots	Immune dysfunction may have a role in CP.	2018	121
450K BeadChip, MeDIP-seq	28 discordant MZ twin pairs, and 8 non- diabetic human adipose tissue donors and 5 human pancreatic islet donors	Type 2 Diabetes (T2D)	Peripheral blood (preadipocytes and adipocytes, and pancreatic beta cells from unrelated individuals)	T2D associated blood DNA methylation serves as a proxy for the epignetic status in adipose and pancreatic tissue. The findings could be developed in to epigenetic biomarker.	2018	122
450K BeadChip	79 discordant MZ twin pairs	Rheumatoid arthritis (RA)	Whole blood	Differentially variable DNA methylation rather than differential methylation associate with RA.	2018	58

Platform	Study sample	Trait of interest	Tissue	Conclusions from the study	Publication Year	Citation
450K BeadChip	5 discordant and 4 concordant MZ twin pairs, and 30 unrelated cases and 30 matched controls	Autism spectrum disorder (ASD)	Whole blood	SH2B1 gene associates with ASD suggesting that neurtrophin signaling pathway should be investigated further in ASD.	2019	123
EPIC BeadChip	79 discordant MZ twin pairs	Major depression (MD)	Peripheral blood monocytes	If validated by another study, the identified peripheral blood epigenetic marks could serve as novel MD biomarkers or drug targets.	2019	124
RRBS	30 discordant MZ twin pairs	BMI	Whole blood	New candidate genomic regions for epignetic regulation of obesity were identified.	2019	125
EPIC BeadChip	45 discordant MZ twin pairs	Multiple sclerosis (MS)	Peripheral blood mononuclear cells	MS treatments and genetic background of individuals are major confounders in epigenetic studies of MS.	2019	57
450K BeadChip	18 discordant MZ and 8 discordant DZ twin pairs	Systemic sclerosis (Ssc)	Whole blood	DNA methylation may have a functional role in Ssc etiology.	2019	126
EPIC BeadChip	15 discordant MZ twin pairs	Epilepsy	Whole blood and buccal cells	Different types of epilepsy may have distinct DNA methylation profiles.	2019	127
450K BeadChip	1004 twins of which 185 MZ pairs with within-pair differences and 44 significantly discordant MZ pairs	Alcohol consumption	Whole blood	Alcohol consumption associate with DNA methylation, and the greater the alcohol consumption, the higher the age acceleration of an individual.	2020	128

(Continued)

TABLE 33.1 Cont'd

Platform	Study sample	Trait of interest	Tissue	Conclusions from the study	Publication Year	Citation
450K BeadChip	75 MZ twin pairs of which 27 discordant, 42 concordant unaffected and 6 concordant affected pairs.	Early onset major depression	Whole blood	Early onset major depression associate with DNA methylation variation, and call for further studies to establish causal pathways.	2020	129
450K BeadChip	226 MZ twin pairs	Later life congnitive function	White blood cells	This study replicated previous candidates and identified novel cognition genes and pathways.	2020	130
RRBS	60 MZ twin pairs discordant for FEV1, 59 MZ twin pairs discordant for FVC, and 44 MZ twin pairs discordant for FEV1/FVC	Lung function	Whole blood	Several CpG sites, differentially methylated regions and biological pathways associated with lung function, and may serve as clues for further studies.	2021	131
EPIC BeadChip	14 MZ and 32 DZ pairs discordant for tic spectrum disorder	Tic spectrum disorder	Whole blood	This study suggests that altered mTOR signalling may play a role in the complex pathogenesis of tic spectrum disorders.	2021	132
RRBS	58 discordant MZ twin pairs	Depression	Whole blood	DNA methylation is weakly associated with depression, and its value as a biomarker or therapy target for depression should be investigated in the future.	2021	133

Platform	Study sample	Trait of interest	Tissue	Conclusions from the study	Publication Year	Citation
EPIC BeadChip	6 post traumatic stress disorder (PTSD) discordant MZ pairs and 15 migraine discordant MZ pairs	PTSD and migraine	Whole blood	This study suggest that PTSD and migraine share common genes and pathways.	2021	134
EPIC BeadChip	62 discordant, 28 concordant unexposed and 28 concordant exposed MZ twin pairs	Adolescense victimization	Whole blood and buccal cells	This longitudinal study provided preliminary evidence for adolescence victimization -associated DNA methylation, and warrant futher studies for drawing any firm conclusions.	2021	135
scBS-seq, scATAC-seq	A MZ discordant pair, and 10 unrelated cases and 10 unrelated controls	Common variable immunodeficiency (CVID)	Naïve and memory B-cells	NaïveB-cell populations are almost identical in co-twins in terms of their DNA methylation, chromatin accessibility and transcription profile, while the CVID epigenetic and gene expression defects are established during naïve B-cell activation and memory B-cell generation resulting in defective cell-cell communication in immune responses.	2022	136
450K and EPIC BeadChip	1720 MZ and 1107 DZ twin pairs	Early life effects	Cord blood, peripheral blood and adipose tissue	Early life strongly influences DNA methylation variation across the lifespan, and could affect late-life health through affecting DNA methylation.	2022	137

twin pairs become more discordant in their DNA methylation profile while others become more similar in their DNA methylation profile in early postnatal life, and that the rate of change over time is strongly affected by the regional genomic context.[72] The reason why some cotwins become epigenetically more similar could be because there were larger differences in their prenatal than in their postnatal environments. This study further confirmed the suspected complex interplay between environment, nonshared environment, and stochastic factors in forming the epigenome in early life.[72] The stability of DNA methylation across time has been shown to be mainly due to genetic factors, while an individual's unique experiences and exposures result in DNA methylation discordance within twin pairs in later life.[65] Multiple longitudinal studies of twins have confirmed that epigenetic change over time is regulated mainly by environmental and stochastic factors, in a tissue- and genome context-dependent manner,[53,71,72] and that the role of the environment in epigenetic drift becomes increasingly important during aging.[65,73]

33.4.4 Inferring causality for epigenetic associations by using twins

Twin data can be useful for inferring causality in observed epigenetic associations in EWAS. The linear regression-based ICE FALCON method[51] has been employed to infer causality in the association between BMI and DNA methylation in a twin family study.[44] This study showed that BMI has a causal effect on DNA methylation, rather than DNA methylation having an effect on BMI. These findings mean that most of the BMI-associated DNA methylation observed in multiple studies is likely caused by BMI or some BMI-associated clinical measures. Thus, DNA methylation at these BMI-associating sites may be used as biomarkers rather than targets for obesity therapies.

33.4.5 Epigenetic aging

Aging is strongly associated with DNA methylation changes. Therefore, biological aging and age acceleration can be inferred from DNA methylation and are called epigenetic aging and epigenetic age acceleration (see section "DNA methylation as a surrogate measure" below). Twin studies have contributed to the field of epigenetic aging by demonstrating that DNA methylation at age-related CpG sites show much stronger heritabilities than DNA methylation at CpG sites across the genome, ranging from 36% to 52%,[74] with the heritabilities decreasing as age increases. In line with this, heritability estimates for epigenetic age acceleration are the highest in newborns (100%,[27]) and decrease with age, with values of 74% at age 23 and 53% at age 62.[73] Correspondingly, the unique environment, not shared by the cotwins, has a stronger effect on epigenetic aging in older compared with younger twin pairs. These findings suggest that while genetic factors are important in determining biological aging in early life, nongenetic factors become more relevant contributors to biological aging in later life. Within-pair analysis of MZ twins at older ages shows

that epigenetic age acceleration, independent of genetic and other shared factors, correlates with grip strength, which is a strong predictor of the development of older age disabilities and mortality.[75] Another recent study investigated within-pair differences in epigenetic aging of twin pairs discordant for physical activity level and revealed that the active twins were, on average, 3 years younger compared to their inactive cotwins.[76] This suggests that leisure-time physical activity may slow epigenetic aging. The results from the twin studies encourage further investigations of the impact of various environmental and lifestyle factors on the progression of human aging over the life course, and the development of therapies that can influence the aging process and thereby target functional impairments and diseases commonly related to aging.

33.5 Technical and statistical methods in epigenetics

This section focuses on technical methods used to measure DNA methylation in humans and the statistical methods implemented in the analysis of the resultant data. These methods apply to study populations of both related and unrelated individuals. Here, we focus on DNA methylation since it is the most commonly used epigenetic measure in population-scale studies due to its stability and ease of measurement.

33.5.1 Methods to measure DNA methylation

DNA methylation can be measured using multiple methods, which can be divided into different categories based on whether it relies on an array or sequencing technology, and if bisulfite conversion of DNA or methylation-specific and sensitive restriction enzymes are required. The most common platform used to assess DNA methylation in population-based studies is one of the Illumina Human Methylation Bead Chip arrays, currently the EPIC array,[77] which measures DNA methylation at approximately 850,000 CpGs across the genome, and historically the 450k[78] or 27k[79] arrays. Sodium bisulfite conversion is used to distinguish a methylated cytosine from an unmethylated cytosine. After DNA extraction, treatment with sodium bisulfite deaminates unmethylated cytosine to uracil, while 5-methylcytosine is protected and remains unconverted. During subsequent PCR, the uracil is propagated as thymine, thus distinguishing 5-methylcytosine from cytosine is a simple genotyping problem of cytosine versus thymine.

The Illumina methylation arrays employ two probe types, which are linked with allele-specific oligonucleotides of 50 nucleotides in length; bisulfite-converted and amplified DNA is hybridized to the array, followed by single-base extension. Type I probes use two probe sequences for measuring the methylated and unmethylated signal, respectively, while Type II probes have only one probe sequence which measures both the methylated and unmethylated signal.[77] The level of methylation at each probe is called a beta value and ranges between 0 and 1. Since a CpG site in a cell can only be methylated or unmethylated, a beta value around 0.5, for example,

would indicate that the CpG is methylated in around half of the cells in the sample, or that the observed methylation is specific to one allele as is the case at imprinted sites. Most CpGs have beta values close to 0 or close to 1, which translates to a distinct bimodal distribution of beta values for CpGs across the genome.

Other methods for measuring genome-wide DNA methylation include whole-genome bisulfite sequencing, reduced-representation bisulfite sequencing, nanopore sequencing, and Pacific Biosciences (PacBio) sequencing. Nanopore and PacBio sequencing are unique in that there is no requirement for treatment with sodium bisulfite prior to sequencing. In Nanopore sequencing, 5-methylcytosine is instead distinguished from cytosine by changes to the electrical current produced when nucleotides pass through a protein nanopore. Computational and statistical methods such as convolutional neural networks in DeepSignal,[80] hidden Markov models in Nanopolish,[81] or the Kolmogorov–Smirnov test in NanoMod[82] are used to convert the raw electrical signal to features which can be analyzed. PacBio sequencing is a parallelized single-molecule DNA sequencing method known as single-molecule real-time sequencing, which distinguishes different DNA bases via the detection of a fluorescent signal released as fluorescently labeled nucleotides are incorporated by DNA polymerase; DNAm is detected by differences in DNA polymerase kinetics, measured via the duration of and interval between the fluorescence signals.[83] One important advantage of nanopore and PacBio sequencing is that they can detect not only 5mC but also 5-hydroxymethylcytosine (5hmC).[84,85] 5hmC can be distinguished from 5mC also using the more traditional Illumina array technology with the OxyBS method;[86] however, this requires two arrays per sample and an extra oxidative bisulfite treatment step making the procedure costly.

33.5.2 Methods to analyze DNA methylation data

In order to identify associations between traits or diseases of interest and genome-wide DNA methylation, we perform an EWAS, in which each measured CpG is tested individually with relation to the trait of interest.[87] The bimodal distribution of the beta value can result in heteroscedasticity, or unequal variance of residuals over the range of an independent variable, which violates the assumption of homoscedasticity in a linear model. Therefore, beta values are transformed to M-values using a logit transformation.[88] One of the most common methods of linear modeling for an EWAS is implemented in the R package limma,[89,90] a method that was originally developed for microarray-based gene expression data. Limma builds on a simple linear model by borrowing information from the modeling of each feature (in this case each CpG) using parametric empirical Bayes in order to calculate moderated t-statistics for each CpG,[91] which increases the effective degrees of freedom. Additionally, limma allows adjustment of models by relevant covariates to control for confounding, inclusion of information of sample quality for weighting of samples, and incorporation of random effects for correlated or longitudinal data, amongst other options.[90] More recently, the differential expression for repeated measures (dream) pipeline builds on limma to improve performance for repeated measures designs by increasing statistical power

and controlling the false positive rate.[92] This also has utility for twin studies, as having a sample from each twin in a pair creates observations that are not independent, similar to having multiple longitudinal samples from a single individual.

Statistical analysis of epigenetic data in twin pairs requires a few modifications from typical studies with independent samples. Twin data can be modeled in multiple ways, either with each twin pair treated as an observation, or with twins as individuals. When each twin pair is considered an observation, the values of the independent and dependent variables are subtracted from each other so that the difference in the predictor is tested against the difference in the outcome. When each twin is considered as a separate observation, a random effect term must be included in the statistical model in order to account for the correlation between twins in a pair.

As in any EWAS, we can learn more about the epigenetic dysregulation that occurs in a particular disease or in response to an exposure by performing a variety of follow-up analyses including assessing enrichment of genomic pathways amongst significantly differentially methylated CpGs and identifying regions of differential methylation (differentially methylated regions, DMRs). Among the most common databases used for enrichment analysis are the gene ontology database, which is a large collection of molecular functions, cellular components, and biological processes related to gene products,[93] the molecular signature database (MSigDB), a collection of annotated gene sets,[94] and the Kyoto Encyclopedia of Genes and Genomes.[95]

33.5.3 DNA methylation as a surrogate measure

DNA methylation can also be used as a surrogate measure for numerous things in addition to the primary use of the data in an EWAS. Two of the most common measures to estimate from DNA methylation data are the proportions of cell types comprising a sample, and the so-called "epigenetic age," intended to estimate an individual's biological age (as opposed to their chronological age). DNA methylation plays an important role in cell identity,[96] and thus each cell type and tissue has a distinct epigenetic profile.[97,98] Differences in cell-type proportions between samples can confound associations between an exposure and outcome since DNA methylation is both highly related to cell identity, and cell-type proportions also differ between phenotypes and exposures of interest. Reference-based methods of cell-type deconvolution in samples comprised of cell-type mixtures refer to models that are developed using reference epigenomes created for each cell type (for example, after employing cell sorting) and DNA methylation data from a mixed sample with a known composition of cell types, while reference-free methods estimate "putative" cell types in a sample with the assumption that the largest source of variation in a sample is due to its cell-type composition. Currently, reference-based methods exist mainly for estimating immune cell proportions in whole blood, while reference-free methods are used most commonly for solid tissues and other nonblood tissues which lack cell-type-specific reference epigenomes. The accuracy for estimation of blood cell types is extremely high, with the true proportion of a cell type explaining at lowest 95.4% of the variation in the predicted cell-type proportion for CD4 T cells

up to 100% for B cells.[99] Multiple reference-based methods for estimation of cell types exist, with some of the most widely used including the original Houseman method,[100] epiDISH,[101] and IDOL.[99] Estimated cell-type proportions can be utilized in multiple ways. As of yet, there are several reference-free methods of cell type estimation for solid tissues. The most widely used methods for this purpose are RefFreeEWAS[102] and MeDeCom,[103] both of which are based on non-negative matrix factorization. However, since both of these methods operate on the assumption that the most significant source of variation in DNA methylation between samples is due to cell-type differences, which may not always be the case, these can lead to over-adjustment of the statistical analysis.[104] In addition to controlling confounding by cell-type composition in an EWAS, estimation of cell types allows the computation of informative metrics such as the neutrophil-lymphocyte ratio, which is associated with many diseases[105] even when flow cytometry is no longer possible (for example in archived blood samples). The advent of single-cell sequencing technology has already begun to increase our knowledge of cell types in different tissues and will allow for the development of better reference-based methods for solid tissues as is already occurring with gene expression data.[106,107]

Epigenetic age, sometimes referred to as biological age, can also be inferred from DNA methylation in multiple tissues, as aging is strongly associated with epigenetic alterations. The discrepancy between biological age and chronological age is called age acceleration, which can be either positive or negative indicating an older or younger biological age than expected given chronological age, respectively. The earliest epigenetic clocks were developed solely on DNA methylation data and chronological age. The most well-known is the pan-tissue Horvath clock, which was developed using elastic net regression of chronological age on genome-wide CpG methylation to accurately predict age in multiple tissues and cell types.[27] Likewise, the Hannum clock is based on the relationship of DNA methylation with chronological age, with elastic net regression highlighting CpGs predictive of age in blood leucocytes.[108] Given that models based on chronological age alone are inherently limited for detecting true biological acceleration in aging, more recently developed epigenetic clocks employ multi-step approaches based on a range of phenotypic data in addition to survival analysis. The PhenoAge clock is based first on penalized Cox proportional hazards regression to identify associations of blood biomarkers including C-reactive protein, lymphocyte percent, and glucose with age-related-mortality, and a second step using a parametric proportional hazards model with the selected biomarkers and chronological age to create a scaled mortality score, the PhenoAge value.[109] Similarly, the GrimAge clock is based on a 2-step process, first identifying associations of different blood proteins including adrenomedullin and cystatin C with DNA methylation data using elastic net models, and next using elastic net Cox proportional hazards regression on predicted blood protein levels, predicted smoking pack-years, and chronological age to construct the predicted epigenetic age.[110] Both PhenoAge and GrimAge are more strongly associated with survival and multiple morbidities than either the Horvath pan-tissue clock or the HannumAge.[109,110] Due to the differences in the way each clock was developed, their usefulness depends on

multiple factors including the tissue of interest and the phenotype being studied. By comparing differences in age acceleration within twin-pairs, we can learn a lot about exposures that affect the epigenome and aging. Growing evidence suggests that accelerated epigenetic age strongly associates with common diseases and occurs in response to a number of environmental factors.

Epigenetic clocks have also been developed for estimating age early in life, including gestational age. Two of these were developed using a penalized regression model with DNA methylation measured in cord blood to predict clinically estimated gestational age.[111,112] Gestational age acceleration, a measure analogous to age acceleration in adults, has been found to be positively associated with birthweight percentile, with infants born at the 50th percentile of weight having age acceleration of approximately zero.[111] More recently, a set of three epigenetic clocks based on placental tissue samples were developed, also using penalized regression;[113] these clocks were robust, providing accurate estimates of gestational age regardless of common pregnancy complications. Of note, the clocks developed in cord blood do not provide accurate estimations of gestational age when applied to placental tissue. Epigenetic clocks that estimate gestational age are useful for numerous purposes, including determining the developmental stage of infants with unknown gestational age or the assessment of the impact of prenatal exposures on gestational age acceleration.

33.6 Future of epigenetic twin studies

Within the last 20 years, twin studies have proven to be highly valuable in providing new insights into the role of epigenetic regulation in the development and progression of complex diseases and traits. We foresee that epigenetic twin studies will continue to contribute importantly to this field.

Improved technologies to map epigenetic variation along with reduced costs of high-resolution epigenetic assays in the future will enable twin researchers to provide more accurate information of the relative contributions of the genome sequence and environmental effects on each CpG site methylation, or histone modification. Thus, epigenetic research on twins will continue to increase our understanding of the functional human genome.

Although many twin cohorts are relatively large, more active collaboration between twin cohorts will be needed to detect small epigenetic alterations and precise epigenetic profiles associated with complex diseases and traits, and to infer causality of the observed associations. These large-scale international research efforts are especially crucial for studies involving trait discordant MZ twin pairs, as existence of such pairs is relatively rare. In addition, more longitudinal twin cohorts starting from the prenatal period, and collaboration between such cohorts, are needed. These would aid in establishing the importance of genetic and environmental effects on early-onset intermediate phenotypes potentially contributing to disease risk in adulthood, and to identify important biomarkers. Additionally, this would help to pinpoint

the timing of epigenetic alterations with respect to phenotype appearance starting from infancy. Obviously longitudinal studies in later life will also be needed to investigate the role of epigenetics in disease susceptibility and progression.

References

1. Waddington CH. The epigenotype. *Endeavour*. 1942;1:18–20.
2. Deans C, Maggert KA. What do you mean, 'Epigenetic'? *Genetics*. 2015;199(4):887–896. http://doi.org/10.1534/genetics.114.173492.
3. Hotchkiss RD. The quantitative separation of purines, pyrimidines, nucleosides by paper chromatography. *Journal of Biological Chemistry*. 1948;175(1):315–332.
4. Holliday R, Pugh JE. DNA modification mechanisms and gene activity during development. *Science (80-.)*. 1975;187(4173):226–232.
5. Anderson OS, Sant KE, Dolinoy DC. Nutrition and epigenetics: an interplay of dietary methyl donors, one-carbon metabolism and DNA methylation. *J Nutr Biochem*. 2012;23(8):853–859. http://doi.org/10.1016/j.jnutbio.2012.03.003.
6. Okano M, Bell DW, Haber DA, et al. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*. 1999;99(3):247–257.
7. Hermann A, Goyal R, Jeltsch A. The Dnmt1 DNA-(cytosine-C5)-methyltransferase methylates DNA processively with high preference for hemimethylated target sites. *J Biol Chem*. 2004;279(46):48350–48359. http://doi.org/10.1074/jbc.M403427200.
8. Bird A. DNA methylation patterns and epigenetic memory. *Genes & development*. 2002;16(1):6–21.
9. Ehrlich M, Gama-Sosa MA, Huang LH, et al. Amount and distribution of 5-methylcytosine in human DNA from different types of tissues of cells. *Nucleic Acids Res*. 1982;10(8):2709–2721. http://doi.org/10.1093/nar/10.8.2709.
10. Saxonov S, Berg P, Brutlag DL. A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. *Proc Natl Acad Sci U S A*. 2006;103(5):1412–1417. http://doi.org/10.1073/pnas.0510310103.
11. Patil V, Ward RL, Hesson LB. The evidence for functional non-CpG methylation in mammalian cells. *Epigenetics*. 2014;9(6):823–828. http://doi.org/10.4161/epi.28741.
12. Barlow DP, Bartolomei MS. Genomic imprinting in mammals. *Cold Spring Harb Perspect Biol*. 2014;6(2):a018382. http://doi.org/10.1101/cshperspect.a018382.
13. Jansz N. DNA methylation dynamics at transposable elements in mammals. *Essays in Biochemistry*. 2019;63(6):677–689. http://doi.org/10.1042/EBC20190039.
14. Sharp AJ, Stathaki E, Migliavacca E, et al. DNA methylation profiles of human active and inactive X chromosomes. *Genome Research*. 2011;21(10):1592–1600. http://doi.org/10.1101/gr.112680.110.
15. Anastasiadi D, Esteve-Codina A, Piferrer F. Consistent inverse correlation between DNA methylation of the first intron and gene expression across tissues and species. *Epigenetics & chromatin*. 2018;11(1):37. http://doi.org/10.1186/s13072-018-0205-1.
16. Zhong H, Kim S, Zhi D, et al. Predicting gene expression using DNA methylation in three human populations. *PeerJ*. 2019;7:e6757. http://doi.org/10.7717/peerj.6757.
17. Martínez-Iglesias O, Carrera I, Carril JC, et al. DNA methylation in neurodegenerative and cerebrovascular diseases disorders. *Int J Mol Sci*. 2020;21(6). http://doi.org/10.3390/ijms21062220.

18. Ahmed SAH, Ansari SA, Mensah-Brown EPK, et al. The role of DNA methylation in the pathogenesis of type 2 diabetes mellitus. *Clin. Epigenetics*. 2020;12(1):1–23. http://doi.org/10.1186/s13148-020-00896-4.
19. Sailani MR, Halling JF, Moller DH, et al. Lifelong physical activity is associated with promoter hypomethylation of genes involved in metabolism, myogenesis, contractile properties and oxidative stress resistance in aged human skeletal muscle. *Sci Rep*. 2019;9(1):1–11. http://doi.org/10.1038/s41598-018-37895-8.
20. Breitling LP, Yang R, Korn B, et al. Tobacco-smoking-related differential DNA methylation: 27K discovery and replication. *Am J Hum Genet*. 2011;88(4):450–457. http://doi.org/10.1016/j.ajhg.2011.03.003.
21. Joehanes R, Just AC, Marioni RE, et al. Epigenetic signatures of cigarette smoking. *Circ. Cardiovasc. Genet*. 2016;9(5):436–447. http://doi.org/10.1161/CIRCGENETICS.116.001506.
22. He F, Berg A, Kawasawa YI, et al. Association between DNA methylation in obesity-related genes and body mass index percentile in adolescents. *Sci Rep*. 2019;9(1):2079. http://doi.org/10.1038/s41598-019-38587-7.
23. Pietiläinen KH, Ismail K, Järvinen E, et al. DNA methylation and gene expression patterns in adipose tissue differ significantly within young adult monozygotic BMI-discordant twin pairs. *Int J Obes*. 2016;40(4):654–661. http://doi.org/10.1038/ijo.2015.221.
24. Bollati V, Schwartz J, Wright RO, et al. Decline in genomic DNA methylation through aging in a cohort of elderly subjects. *Mech Ageing Dev*. 2009;130(4):234–239. http://doi.org/10.1016/j.mad.2008.12.003.
25. Talens RP, Christensen K, Putter H, et al. Epigenetic variation during the adult lifespan: cross-sectional and longitudinal data on monozygotic twin pairs. *Aging Cell*. 2012;11(4):694–703. http://doi.org/10.1111/j.1474-9726.2012.00835.x.
26. Fraga MF, Ballestar E, Paz MF, et al. Epigenetic differences arise during the lifetime of monozygotic twins. *PNAS*. 2005;102(30):10604–10609.
27. Horvath S. DNA methylation age of human tissues and cell types. *Genome biology*. 2013;14(10). http://doi.org/10.1186/gb-2013-14-10-r115.
28. Gibbs JR, van der Brug MP, Hernandez DG, et al. Abundant quantitative trait loci exist for DNA methylation and gene expression in human brain. *Plos Genetics*. 2010;6(5):29. http://doi.org/10.1371/journal.pgen.1000952.
29. Zhang D, Cheng L, Badner JA, et al. Genetic control of individual differences in gene-specific methylation in human brain. *Am J Hum Genet*. 2010;86(3):411–419. http://doi.org/10.1016/j.ajhg.2010.02.005.
30. Smith AK, Kilaru V, Kocak M, et al. Methylation quantitative trait loci (meQTLs) are consistently detected across ancestry, developmental stage, tissue type. *BMC Genomics*. 2014;15(1):145. http://doi.org/10.1186/1471-2164-15-145.
31. Kurdistani SK, Tavazoie S, Grunstein M. Mapping global histone acetylation patterns to gene expression. *Cell*. 2004;117(6):721–733. https://doi.org/10.1016/j.cell.2004.05.023.
32. LeRoy G, DiMaggio PA, Chan EY, et al. A quantitative atlas of histone modification signatures from human cancer cells. *Epigenetics and Chromatin*. 2013;6(1):1–14. http://doi.org/10.1186/1756-8935-6-20.
33. Gillespie J, Savic S, Wong C, et al. Histone deacetylases are dysregulated in rheumatoid arthritis and a novel histone deacetylase 3-selective inhibitor reduces interleukin-6 production by peripheral blood mononuclear cells from rheumatoid arthritis patients. *Arthritis and Rheumatism*. 2012;64(2):418–422. http://doi.org/10.1002/art.33382.

34. Miao F, Gonzalo IG, Lanting L, et al. In Vivo chromatin remodeling events leading to inflammatory gene transcription under diabetic conditions. *J Biol Chem*. 2004;279(17):18091–18097. http://doi.org/10.1074/jbc.M311786200.
35. Lam JKW, Chow MYT, Zhang Y, et al. siRNA versus miRNA as therapeutics for gene silencing. *Mol. Ther. - Nucleic Acids*. 2015;4:e252. http://doi.org/10.1038/mtna.2015.23.
36. Peng Y, Croce CM. The role of MicroRNAs in human cancer. *Signal Transduct. Target. Ther.*. 2016;1(1):15004. http://doi.org/10.1038/sigtrans.2015.4.
37. Ikeda S, Kong SW, Lu J, et al. Altered microRNA expression in human heart disease. *Physiol Genomics*. 2007;31(3):367–373. http://doi.org/10.1152/physiolgenomics.00144.2007.
38. Richards EJ, Elgin SC. Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. *Cell*. 2002;108(4):489–500.
39. Honda S, Selker EU. Direct interaction between DNA methyltransferase DIM-2 and HP1 is required for DNA methylation in Neurospora crassa. *Mol Cell Biol*. 2008;28(19):6044–6055. http://doi.org/10.1128/MCB.00823-08.
40. Fuks F, Hurd PJ, Deplus R, et al. The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. *Nucleic Acids Res*. 2003;31(9):2305–2312.
41. Fabbri M, Garzon R, Cimmino A, et al. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci U S A*. 2007;104(40):15805–15810. http://doi.org/10.1073/pnas.0707628104.
42. Glaich O, Parikh S, Bell RE, et al. DNA methylation directs microRNA biogenesis in mammalian cells. *Nat Commun*. 2019;10(1):1–11. http://doi.org/10.1038/s41467-019-13527-1.
43. Relton CL, Smith GD. Mendelian randomization: applications and limitations in epigenetic studies. *Epigenomics*. 2015;7(8):1239–1243. http://doi.org/10.2217/epi.15.88.
44. Li S, Wong EM, Bui M, et al. Inference about causation between body mass index and DNA methylation in blood from a twin family study. *Int J Obes*. 2019;43(2):243–252. http://doi.org/10.1038/s41366-018-0103-4.
45. Tan Q, Christiansen L, Von Bornemann Hjelmborg J, et al. Twin methodology in epigenetic studies. *J Exp Biol*. 2015;218(1):134–139. http://doi.org/10.1242/jeb.107151.
46. Jonsson H, Magnusdottir E, Eggertsson HP, et al. Differences between germline genomes of monozygotic twins. *Nat Genet*. 2021;53(1):27–34. http://doi.org/10.1038/s41588-020-00755-1.
47. van Baak TE, Coarfa C, Dugue P-A, et al. Epigenetic supersimilarity of monozygotic twin pairs. *Genome biology*. 2018;19(1):1–20. http://doi.org/10.1186/s13059-017-1374-0.
48. Li W, Christiansen L, Hjelmborg J, et al. On the power of epigenome-wide association studies using a disease-discordant twin design. *Bioinformatics*. 2018;34(23):4073–4078. http://doi.org/10.1093/bioinformatics/bty532.
49. Vidaki A, Kayser M. Recent progress, methods and perspectives in forensic epigenetics. *Forensic Sci Int Genet*. 2018;37:180–195. August. http://doi.org/10.1016/j.fsigen.2018.08.008.
50. Vidaki A, Diez Lopez C, Carnero-Montoro E, et al. Epigenetic discrimination of identical twins from blood under the forensic scenario. *Forensic Sci Int Genet*. 2017;31:67–80. http://doi.org/10.1016/j.fsigen.2017.07.014.
51. Hopper JL, Bui QM, Erbas B, et al. Does eczema in infancy cause hay fever, asthma, or both in childhood? Insights from a novel regression model of sibling data. *J Allergy Clin Immunol*. 2012;130(5):1117–1122. e1. http://doi.org/10.1016/j.jaci.2012.08.003.

52. Bell JT, Saffery R. The value of twins in epigenetic epidemiology. *Int J Epidemiol*. 2012;41(1):140–150. http://doi.org/10.1093/ije/dyr179.
53. Van Dongen J, Nivard MG, Willemsen G, et al. Genetic and environmental influences interact with age and sex in shaping the human methylome. *Nat Commun*. 2016;7:1–13. http://doi.org/10.1038/ncomms11115.
54. Van Dongen J, Nivard MG, Baselmans BML, et al. Epigenome-wide association study of aggressive behavior. *Twin Res. Hum. Genet.*. 2015;18(6):686–698. http://doi.org/10.1017/thg.2015.74.
55. Paul DS, Teschendorff AE, Dang MAN, et al. Increased DNA methylation variability in type 1 diabetes across three immune effector cell types. *Nat Commun*. 2016;7. http://doi.org/10.1038/ncomms13555.
56. Young PE, Jew SK, Buckland ME, et al. Epigenetic differences between monozygotic twins discordant for amyotrophic lateral sclerosis (ALS) provide clues to disease pathogenesis. *PLoS One*. 2017;12(8):1–19. http://doi.org/10.1371/journal.pone.0182638.
57. Souren NY, Gerdes LA, Lutsik P, et al. DNA methylation signatures of monozygotic twins clinically discordant for multiple sclerosis. *Nat Commun*. 2019;10(1):1–12. http://doi.org/10.1038/s41467-019-09984-3.
58. Webster AP, Plant D, Ecker S, et al. Increased DNA methylation variability in rheumatoid arthritis-discordant monozygotic twins. *Genome Med*. 2018;10(1):1–12. http://doi.org/10.1186/s13073-018-0575-9.
59. Ollikainen M, Ismail K, Gervin K, et al. Genome-wide blood DNA methylation alterations at regulatory elements and heterochromatic regions in monozygotic twins discordant for obesity and liver fat. *Clin. Epigenetics*. 2015;7(1). 39-015-0073–5eCollection2015doi: http://doi.org/10.1186/s13148-015-0073-5 [doi].
60. Kaminsky ZA, Tang T, Wang S-C, et al. DNA methylation profiles in monozygotic and dizygotic twins. *Nature Genetics*. 2009;41(2):240–245.
61. Bell JT, Tsai P-C, Yang T-P, et al. Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. *Plos Genetics*. 2012;8(4). http://doi.org/10.1371/journal.pgen.1002629.
62. Hannon E, Knox O, Sugden K, et al. Characterizing genetic and environmental influences on variable DNA methylation using monozygotic and dizygotic twins. *Plos Genetics*. 2018;14(8):1–27. http://doi.org/10.1371/journal.pgen.1007544.
63. Gordon L, Joo J-HE, Powell JE, et al. Neonatal DNA methylation profile in human twins is specified by a complex interplay between intrauterine environmental and genetic factors, subject to tissue-specific influence. *Genome Research*. 2012;22(8):1395–1406. http://doi.org/10.1101/gr.136598.111.
64. Ollikainen M, Smith KR, Joo EJ-H, et al. DNA methylation analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome. *Hum Mol Genet*. 2010;19(21):4176–4188. http://doi.org/10.1093/hmg/ddq336.
65. Reynolds CA, Tan Q, Munoz E, et al. A decade of epigenetic change in aging twins: Genetic and environmental contributions to longitudinal DNA methylation. *Aging Cell*. 202019(8):e13197. http://doi.org/10.1101/778555.
66. Hoekstra C, Zhao ZZ, Lambalk CB, et al. Dizygotic twinning. *Hum Reprod Update*. 2008;14(1):37–47. http://doi.org/10.1093/humupd/dmm036.
67. Smits J, Monden C. Twinning across the developing world. *PLoS One*. 2011;6(9):8–10. http://doi.org/10.1371/journal.pone.0025239.

68. Weksberg R. Discordant KCNQ1OT1 imprinting in sets of monozygotic twins discordant for Beckwith-Wiedemann syndrome. *Hum Mol Genet*. 2002;11(11):1317–1325. http://doi.org/10.1093/hmg/11.11.1317.
69. Smith ZD, Chan MC, Humm KC, et al. DNA methylation dynamics of the human preimplantation embryo. *Nature*. 2014;511:611–615. doi:10.1038/nature13581.
70. Van Dongen J, Gordon SD, McRae AF, et al. Identical twins carry a persistent epigenetic signature of early genome programming. *Nat Commun*. 2021;12:5618. https://doi.org/10.1038/s41467-021-25583-7.
71. Wong CCY, Caspi A, Williams B, et al. A longitudinal study of epigenetic variation in twins. *Epigenetics*. 2010;5(6):516–526. http://doi.org/10.4161/epi.5.6.12226.
72. Martino D, Loke YJ, Gordon L, et al. Longitudinal, genome-scale analysis of DNA methylation in twins from birth to 18 months of age reveals rapid epigenetic change in early life and pair-specific effects of discordance. *Genome biology*. 2013;14(5):R42. http://doi.org/10.1186/gb-2013-14-5-r42.
73. Sillanpää E, Ollikainen M, Kaprio J, et al. Leisure-time physical activity and DNA methylation age—a twin study. *Clin. Epigenetics*. 2019;11(1):1–8. http://doi.org/10.1186/s13148-019-0613-5.
74. Jylhävä J, Hjelmborg J, Soerensen M, et al. Longitudinal changes in the genetic and environmental influences on the epigenetic clocks across old age: Evidence from two twin cohorts. *EBioMedicine*. 2019;40:710–716. http://doi.org/10.1016/j.ebiom.2019.01.040.
75. Sillanpaä E, Laakkonen EK, Vaara E, et al. Biological clocks and physical functioning in monozygotic female twins. *BMC Geriatr*. 2018;18(83). http://doi.org/10.1186/s12877-018-0775-6.
76. Kankaanpää A, Tolvanen A, Bollepalli S, et al. Leisure-time and occupational physical activity associates differently with epigenetic aging. *Aging. Med Sci Sports Exerc*. 2021;53(3):487–495. doi:10.1249/MSS.0000000000002498.
77. Pidsley R, Zotenko E, Peters TJ, et al. Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. *Genome Biology*. 2016;17(208). doi: https://doi.org/10.1186/s13059-016-1066-1.
78. Bibikova M, Barnes B, Tsan C, et al. High density DNA methylation array with single CpG site resolution. *Genomics*. 2011;98(4):288–295. http://doi.org/10.1016/j.ygeno.2011.07.007.
79. Bibikova M, Le J, Barnes B, et al. Genome-wide DNA methylation profiling using Infinium® assay. *Epigenomics*. 2009;1(1):177–200. doi:10.2217/epi.09.14.
80. Ni P, Huang N, Zhang Z, et al. DeepSignal: detecting DNA methylation state from Nanopore sequencing reads using deep-learning. *Bioinformatics*. 2019;35(22):4586–4595. http://doi.org/10.1093/bioinformatics/btz276.
81. Simpson JT, Workman RE, Zuzarte PC, et al. Detecting DNA cytosine methylation using nanopore sequencing. *Nat Methods*. 2017;14(4):407–410. http://doi.org/10.1038/nmeth.4184.
82. Liu Q, Georgieva DC, Egli D, et al. NanoMod: a computational tool to detect DNA modifications using Nanopore long-read sequencing data. *Bmc Genomics [Electronic Resource]*. 2019;20. http://doi.org/10.1186/s12864-018-5372-8.
83. Flusberg BA, Webster DR, Lee JH, et al. Direct detection of DNA methylation during single-molecule, real-time sequencing. *Nat Methods*. 2010;7(6):461–465. http://doi.org/10.1038/nmeth.1459.Direct.

84. Rand AC, Jain M, Eizenga JM, et al. Mapping DNA methylation with high-throughput nanopore sequencing. *Nat Methods*. 2017;14(4):411–413. http://doi.org/10.1038/nmeth.4189.
85. Liu Y, Cheng J, Siejka-Zielinska P, et al. Accurate targeted long-read DNA methylation and hydroxymethylation sequencing with TAPS. *Genome biology*. 2020;21(54):1–9. http://doi.org/10.1186/s13059-020-01969-6.
86. Houseman EA, Johnson KC, Christensen BC. OxyBS: Estimation of 5-methylcytosine and 5-hydroxymethylcytosine from tandem-treated oxidative bisulfite and bisulfite DNA. *Bioinformatics*. 2016;32(16). http://doi.org/10.1093/bioinformatics/btw158.
87. Rakyan VK, Down TA, Balding DJ, et al. Epigenome-wide association studies for common human diseases. *Nature Reviews Genetics*. 2011;12(8):529–541. http://doi.org/10.1038/nrg3000.
88. Du P, Zhang X, Huang C-C, et al. Comparison of beta-value and M-value methods for quantifying methylation levels by microarray analysis. *BMC Bioinform*. 2010;11(587). http://doi.org/10.1186/1471-2105-11-587.
89. Law CW, Chen Y, Shi W, et al. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol*. 2014;15:R29. https://doi.org/10.1186/gb-2014-15-2-r29.
90. Ritchie ME, Phipson B, Wu D, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47. http://doi.org/10.1093/nar/gkv007.
91. Smyth GK. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.*. 2004;3(1):1–26.
92. Hoffman GE, Roussos P. Dream: Powerful differential expression analysis for repeated measures designs. *Bioinformatics*. 2021;37(2):192–201. https://doi.org/10.1093/bioinformatics/btaa687.
93. Blake JA, Christie KR, Dolan ME, et al. Gene Ontology Consortium: Going forward. *Nucleic Acids Res*. 2015;43(D1):D1049–D1056. http://doi.org/10.1093/nar/gku1179.
94. Liberzon A, Subramanian A, Pinchback R, et al. Molecular signatures database (MSigDB) 3.0. *Bioinformatics*. 2011;27(12):1739–1740. http://doi.org/10.1093/bioinformatics/btr260.
95. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;28(1):27–30.doi:10.1093/nar/28.1.27.
96. Bogdanović O, Lister R. DNA methylation and the preservation of cell identity. *Current Opinion in Genetics and Development*. 2017;46. http://doi.org/10.1016/j.gde.2017.06.007.
97. Reinius LE, Acevedo N, Joerink M, et al. Differential DNA methylation in purified human blood cells: implications for cell lineage and studies on disease susceptibility. *PLoS One*. 2012;7(7):e41361. http://doi.org/10.1371/journal.pone.0041361.
98. Christensen BC, Houseman EA, Marsit CJ, et al. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. *Plos Genetics*. 2009;5(8):e1000602. http://doi.org/10.1371/journal.pgen.1000602.
99. Salas LA, Koestler DC, Butler RA, et al. An optimized library for reference-based deconvolution of whole-blood biospecimens assayed using the Illumina HumanMethylationEPIC BeadArray. *Genome biology*. 2018;19(64). http://doi.org/10.1186/s13059-018-1448-7.

100. Houseman EA, Accomando WP, Koestler DC, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinform*. 2012;13:86. https://doi.org/10.1186/1471-2105-13-86.
101. Teschendorff AE, Breeze CE, Zheng SC, et al. A comparison of reference-based algorithms for correcting cell-type heterogeneity in Epigenome-Wide Association Studies. *BMC Bioinform*. 2017;18(1). http://doi.org/10.1186/s12859-017-1511-5.
102. Houseman EA, Kile ML, Christiani DC, et al. Reference-free deconvolution of DNA methylation data and mediation by cell composition effects. *BMC Bioinform*. 2016;17(1). http://doi.org/10.1186/s12859-016-1140-4.
103. Lutsik P, Slawski M, Gasparoni G, et al. MeDeCom: discovery and quantification of latent components of heterogeneous methylomes. *Genome biology*. Nov. 2017;18(1). http://doi.org/10.1186/s13059-017-1182-6.
104. Hattab MW, Shabalin AA, Clark SL, et al. Correcting for cell-type effects in DNA methylation studies: reference-based method outperforms latent variable approaches in empirical studies. *Genome biology*. 2017;18(24). http://doi.org/10.1186/s13059-017-1148-8.
105. Koestler DC, Usset J, Christensen BC, et al. DNA methylation-derived neutrophil-tolymphocyte ratio: an epigenetic tool to explore cancer inflammation and outcomes. *Cancer Epidemiol Biomarkers Prev*. 2017;26(3):328–338. http://doi.org/10.1158/1055-9965.EPI-16-0461.
106. Wang X, Park J, Susztak K, et al. Bulk tissue cell type deconvolution with multi-subject single-cell expression reference. *Nat Commun*. 2019;10(1). http://doi.org/10.1038/s41467-018-08023-x.
107. Clark SJ, Lee HJ, Smallwood SA, et al. Single-cell epigenomics: powerful new methods for understanding gene regulation and cell identity. *Genome biology*. 2016;17(1). http://doi.org/10.1186/s13059-016-0944-x.
108. Hannum G, Guinney J, Zhao L, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell*. 2013;49(2):359–367. http://doi.org/10.1016/j.molcel.2012.10.016.
109. Levine ME, Lu AT, Quach A, et al. An epigenetic biomarker of aging for lifespan and healthspan. *Aging (Albany, NY)*. 2018;10(4):573–591. http://doi.org/10.18632/aging.101414.
110. Lu AT, Quach A, Wilson JG, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging (Albany, NY)*. 2019;11(2):303–327. http://doi.org/10.18632/aging.101684.
111. Bohlin J, Håberg SE, Magnus P, et al. Prediction of gestational age based on genome-wide differentially methylated regions. *Genome biology*. 2016;17(207). http://doi.org/10.1186/s13059-016-1063-4.
112. Knight AK, Craig JM, Thelda C, et al. An epigenetic clock for gestational age at birth based on blood methylation data. *Genome biology*. 2016;17(206). http://doi.org/10.1186/s13059-016-1068-z.
113. Lee Y, Choufani S, Weksberg R, et al. Placental epigenetic clocks: Estimating gestational age using placental DNA methylation levels. *Aging (Albany, NY)*. 2019;11(12):4238–4253. http://doi.org/10.18632/aging.102049.
114. Jiao Z, Jiang Z, Wang J, et al. Whole-genome scale identification of methylation markers specific for cerebral palsy in monozygotic discordant twins. *Molecular Medicine Reports*. 2017;16:9423–9430. https://doi.org/10.3892/mmr.2017.7800.

115. Casey KF, Levesque ML, Szyf M, et al. Birth weight discordance, DNA methylation, and cortical morphology of adolescent monozygotic twins. *Hum Brain Mapp*. 2017;38(4):2037–2050. doi:10.1002/hbm.23503.
116. Young PE, Kum Jew S, Buckland ME, et al. Epigenetic differences between monozygotic twins discordant for amyotrophic lateral sclerosis (ALS) provide clues to disease pathogenesis. *PLoS One*. 2017;12(8):e0182638. https://doi.org/10.1371/journal.pone.0182638.
117. Kaut O, Schmitt I, Tost J, et al. Epigenome-wide DNA methylation analysis in siblings and monozygotic twins discordant for sporadic Parkinson's disease revealed different epigenetic patterns in peripheral blood mononuclear cells. *Neurogenetics*. 2017;18:7–22. https://doi.org/10.1007/s10048-016-0497-x.
118. Bolund ACS, Starnawska A, Miller MR, et al. Lung function discordance in monozygotic twins and associated differences in blood DNA methylation. *Clin Epigenet*. 2017;9:132. https://doi.org/10.1186/s13148-017-0427-2.
119. Chen YC, Sudre G, Sharp W, et al. Neuroanatomic, epigenetic and genetic differences in monozygotic twins discordant for attention deficit hyperactivity disorder. *Mol Psychiatry*. 2018;23:683–690. https://doi.org/10.1038/mp.2017.45.
120. Córdova-Palomera A, Palma-Gudiel H, Forés-Martos J, Tabarés-Seisdedos R, Fañanás L. Epigenetic outlier profiles in depression: A genome-wide DNA methylation analysis of monozygotic twins. *PLoS One*. 2018;13(11):e0207754. https://doi.org/10.1371/journal.pone.0207754.
121. Mohandas N, Bass-Stringer S, Maksimovic J, et al. Epigenome-wide analysis in newborn blood spots from monozygotic twins discordant for cerebral palsy reveals consistent regional differences in DNA methylation. *Clin Epigenet*. 2018;10:25. https://doi.org/10.1186/s13148-018-0457-4.
122. Hwang JY, Lee HJ, Go MJ, et al. Genome-wide methylation analysis identifies ELOVL5 as an epigenetic biomarker for the risk of type 2 diabetes mellitus. *Sci Rep*. 2018;8:14862. https://doi.org/10.1038/s41598-018-33238-9.
123. Liang S, Li Z, Wang Y, et al. Genome-Wide DNA Methylation Analysis Reveals Epigenetic Pattern of SH2B1 in Chinese Monozygotic Twins Discordant for Autism Spectrum Disorder. *Front Neurosci*. 2019;13:712. https://www.frontiersin.org/article/10.3389/fnins.2019.00712.
124. Zhu Y, Strachan E, Fowler E, et al. Genome-wide profiling of DNA methylome and transcriptome in peripheral blood monocytes for major depression: A Monozygotic Discordant Twin Study. *Transl Psychiatry*. 2019;9:215. https://doi.org/10.1038/s41398-019-0550-2.
125. Li W, Zhang D, Wang W, et al. DNA methylome profiling in identical twin pairs discordant for body mass index. *Int J Obes*. 2019;43:2491–2499. https://doi.org/10.1038/s41366-019-0382-4.
126. Ramos PS, Zimmerman KD, Haddad S, et al. Integrative analysis of DNA methylation in discordant twins unveils distinct architectures of systemic sclerosis subsets. *Clin Epigenet*. 2019;11:58. https://doi.org/10.1186/s13148-019-0652-y.
127. Mohandas N, Loke YJ, Hopkins S, et al. Evidence for type-specific DNA methylation patterns in epilepsy: a discordant monozygotic twin approach. *Epigenomics*. 2019;11(8):951–968. doi:10.2217/epi-2018-0136.
128. Stephenson M, Bollepalli S, Cazaly E, et al. Associations of Alcohol Consumption With Epigenome-Wide DNA Methylation and Epigenetic Age Acceleration: Individual-Level

and Co-twin Comparison Analyses. *Alcohol Clin Exp Res*. 2021;45(2):318–328. doi:10.1111/acer.14528.

129. Roberson-Nay R, Lapato DM, Wolen AR, et al. An epigenome-wide association study of early-onset major depression in monozygotic twins. *Transl Psychiatry*. 2020;10(1):301. doi:10.1038/s41398-020-00984-2.
130. Soerensen M, Hozakowska-Roszkowska DM, Nygaard M, et al. A Genome-Wide Integrative Association Study of DNA Methylation and Gene Expression Data and Later Life Cognitive Functioning in Monozygotic Twins. *Front Neurosci*. 2020;14:233. doi:10.3389/fnins.2020.00233.
131. Wang T, Wang W, Li W, et al. Genome-wide DNA methylation analysis of pulmonary function in middle and old-aged Chinese monozygotic twins. *Respir Res*. 2021;22(1):300. doi:10.1186/s12931-021-01896-5.
132. Hildonen M, Levy AM, Hansen CS, et al. EWAS of Monozygotic Twins Implicate a Role of mTOR Pathway in Pathogenesis of Tic Spectrum Disorder. *Genes (Basel)*. 2021;12(10):1510. doi:10.3390/genes12101510.
133. Wang W, Li W, Wu Y, et al. Genome-wide DNA methylation and gene expression analyses in monozygotic twins identify potential biomarkers of depression. *Transl Psychiatry*. 2021;11(1):416. doi:10.1038/s41398-021-01536-y.
134. Bainomugisa CK, Sutherland HG, Parker R, et al. Using Monozygotic Twins to Dissect Common Genes in Posttraumatic Stress Disorder and Migraine. *Front Neurosci*. 2021;15:678350. doi:10.3389/fnins.2021.678350.
135. Kandaswamy R, Hannon E, Arseneault L, et al. DNA methylation signatures of adolescent victimization: analysis of a longitudinal monozygotic twin sample. *Epigenetics*. 2021;16(11):1169–1186. doi:10.1080/15592294.2020.1853317.
136. Rodríguez-Ubreva J, Arutyunyan A, Bonder MJ, et al. Single-cell Atlas of common variable immunodeficiency shows germinal center-associated epigenetic dysregulation in B-cell responses. *Nat Commun*. 2022;13(1):1779. doi:10.1038/s41467-022-29450-x.
137. Li S, Ye Z, Mather KA, et al. Early life affects late-life health through determining DNA methylation across the lifespan: A twin study. *EBioMedicine*. 2022;77:103927. doi:10.1016/j.ebiom.2022.103927.

CHAPTER

34

An experiment in cotwin control: Adaptation to space travel[1]

Carole Tafforin[a], **Nancy L. Segal**[b]

[a]*Ethospace, Research and Study Group in Human and Space Ethology, Toulouse, France*

[b]*Department of Psychology, California State University, Fullerton, CA, United States*

34.1 Introduction

In twin research, monozygotic (MZ or identical) cotwins exposed to different environmental effects offer an informative, naturally occurring cotwin control design. Given their genetic identity, cotwin differences in physical, medical, and behavioral outcomes can be linked to their experiential differences. This scientific advantage helps identify factors that either exacerbate or mitigate cognitive ability, mental health status, disease risk, and other human traits.[2] This information is critical for its potential applicability to managing social isolation and occupational stress.

Studies of MZ and DZ (dizygotic or fraternal) reared-apart twins show that individual differences in special mental skills are influenced by genetic factors, ranging from 0.67 (perceptual speed) to 0.79 (verbal abilities), with the exception of content memory for which genetic influence is estimated to be 0.33.[3] Twin studies also show genetic effects (0.48) on loneliness.[4] Prior studies of nontwins suggest that radiation and microgravity associated with space travel negatively affect some cognitive abilities in both humans and nonhumans[5–8] and also increase stress-related disease risk.[9] However, a twin-based approach to these space-related questions that includes an ideal genetic control has never been conducted.

34.2 Twins reared apart and together

As indicated above, reared-apart twins are a powerful research design because the same genotypes are exposed to different environments, revealing possible differences in how genes are expressed. In contrast, reared-together twins experience the same environments. The experimental exposure of MZ cotwins to different environments

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00015-5

can be conducted in a relatively controlled manner, as in the case of the Kelly twins, Scott and Mark (SK and MK). SK spent an extended period of time in the International Space Station (ISS), while his cotwin stayed on earth. It is unlikely that such a rare opportunity involving MZ twins will ever arise again. Interestingly, however, in April 1972, MZ twin astronaut Charles Duke participated in Apollo 16, becoming the tenth individual (and only twin) to ever reach the moon's surface. His twin brother, William, was born with a heart defect, so was physically incapable of pursuing a career in space travel; William became a physician.[10] A formal comparison of these twins was never undertaken, but would have been far less informative than that of the Kelly brothers, due to their different health histories, and because the Apollo 16 mission lasted for only eleven days.

34.3 Space travel: effects on adaptive systems

In space research, a wide range of ethological studies has been conducted on the effects of microgravity on humans during orbital flights (Space shuttle and Mir station) and overtime. The first investigations focused on changes in spatial and motor skills within short sequences of reduced gravity from parabolic flights. Experimental protocols were designed for comparing novices, experienced subjects, and astronauts. Further analyses revealed meaningful modifications in movement, posture, and orientation throughout mid-term mission, from ground training to postflight periods.[11]

With respect to these behavioral domains, investigating MZ twin astronauts, SK who spent nearly 1-year aboard the ISS and his twin brother MK who remained on earth as a control subject, was a rare opportunity to gather multidisciplinary data on long-term processes. In spring 2015, SK launched to the ISS, serving as the Flight Engineer for the 43rd and 44th expeditions, and as the Commander for the 45th and 46th expeditions. During his 340-day mission, nearly 400 experiments were conducted on the station. SK landed on March 1, 2016. During that mission time, MK was living and working in a usual 1-g environment, while SK had to adapt to unusual living and working conditions in 0-g environment, as shown in Fig. 34.1. In space, SK had a continuous global view of the earth, whereas his twin had a distant view of the ISS (i.e., a light point at night). Consequently, their own sensory-motor and cognitive experiences were quite different, constituting a core factor in the behavioral outcomes.

This chapter reviews some of the discoveries of factors affecting SK's global health, and the extent to which a space mission modifies different adaptive systems from biochemical to cognitive functions. Perspectives regarding functions impacted by epigenetic effects over the long-term and upon shifting environments, in space or on earth, are summarized.

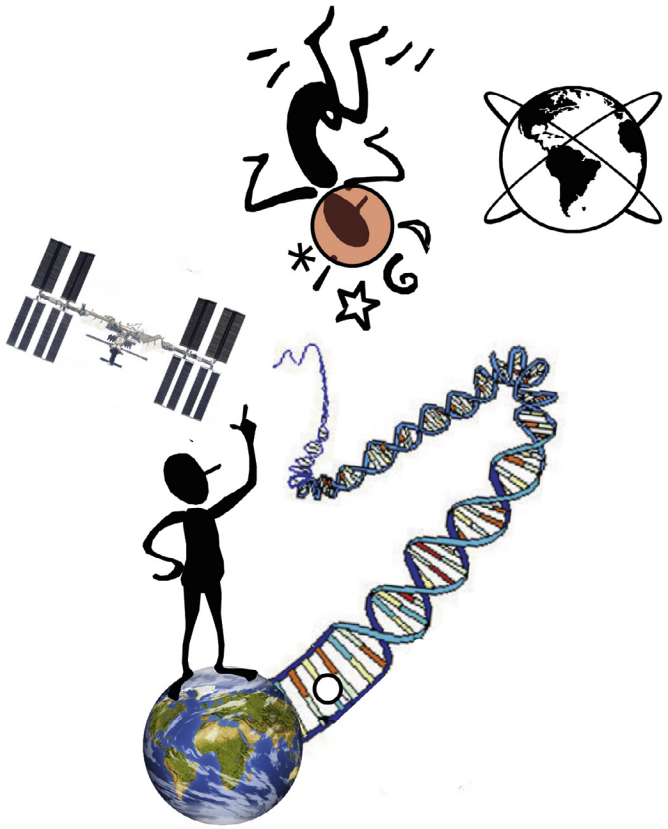


FIG. 34.1 Experience of a twin on earth and a twin in space.

34.4 Review of findings: a twin in space

Ten groups of investigators (Susan Bailey, Brenda Rana, Suart Lee, Fred Turek, Emmanuel Mignot, Scott M. Smith, Andy Feinberg, Chris Mason, Mathias Basner, and Mike Synder) have examined a wide variety of data concerning the Kelly twins' health. The health-related domains of interest include their biomedical profile, cognitive performance, immunological response, bone formation, multiomics markers, gut microbiome, and how DNA might be affected by microgravity and by living in space. Some preliminary findings from NASA's Investigators Workshop (IWS) have been validated and show changes as a result.[12,13] An integrated publication of the findings offered an overview of the NASA Twin Study.[14]

One of the most interesting findings is that genetic expression is sensitive to changing environments in space and on earth. Specifically, SK's telomeres showed elongation while in space, but decreased in length within 48 h after returning to earth,

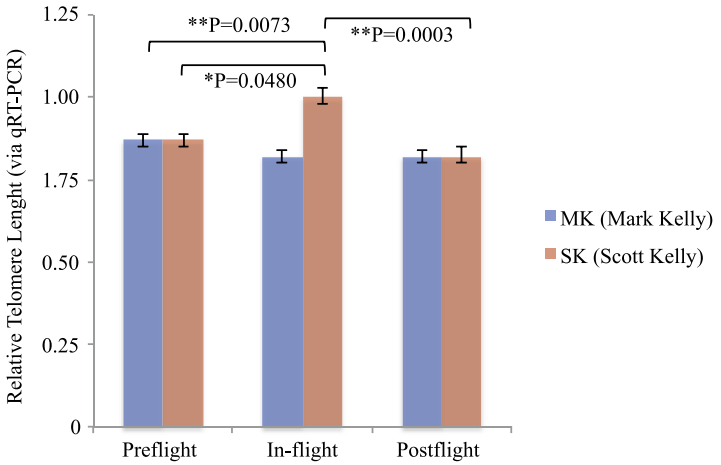


FIG. 34.2 Relative average telomere length in cotwin control before, during, and after spaceflight (adapted from Garrett-Bakelman et al. (2019)[14]).

displayed in Fig. 34.2. It is estimated that 7% of his genes may have altered their expression in space and according to time. However, the question of whether or not real genetic change occurred has been a matter of some debate. Bailey, an investigator of the Twin Astronaut Study Consortium Project, had predicted that SK's telomeres would shorten, given that this is a sign of aging and would reflect the physical stressors of space travel.[15] However, the opposite occurred and the telomeres of other astronauts have shown similar responses.[16] This finding led Bailey to speculate that short telomeres, sensitive to environments in space, might have disappeared, artificially raising the number of longer telomeres present. Alternatively, she proposed that microgravity may have stimulated the release of the enzyme telomerase that could have added length to the telomeres. However, she noted that the lengthening of telomeres has never been demonstrated in humans and telomere length dynamics reflect the cumulative effects of genetics, lifestyle, and environmental factors.[17]

Changes were also observed in genes that control functions related to DNA repair, bone formation, gut bacteria digestion, and the immune system. It appears that astronauts' global health is also affected as a result of long-term space travel. The 10 most important findings are presented in Fig. 34.3. Epigenetic alterations between the twins were not pronounced. Cardiovascular changes that are characteristic of other astronauts were found in SK, but not in MK, such as a 10% increase in cardiac output, and a modest decrease in blood pressure. SK's carotid artery thickness also increased and remained that way for four days after his return to earth. SK's cognitive skills were affected mostly during his postflight period, in that his performance speed declined for all mental ability tests, excerpt for Digit Symbol Substitution. In addition, his accuracy dropped in all areas except for spatial orientation; these declines persisted for a 6-month period following his return to earth.

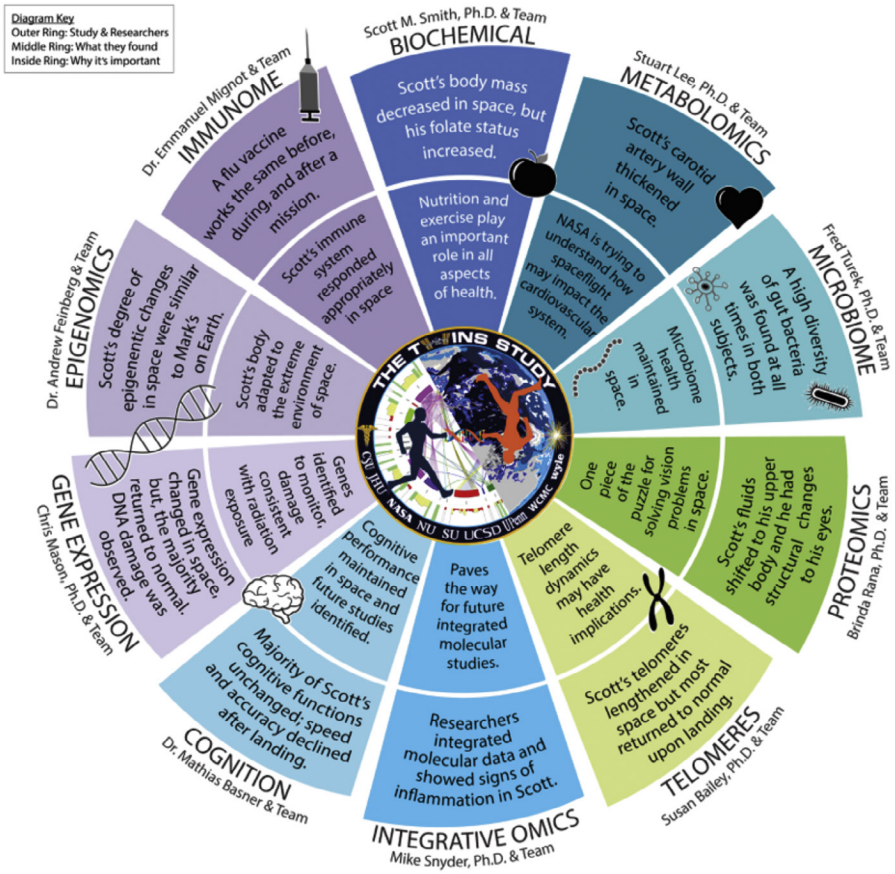


FIG. 34.3 Twins study at a glance—What they found and what it is important (image©NASA).

https://www.nasa.gov/sites/default/files/thumbnails/image/pinwheel_041119_me-01_0.png.

34.5 Discussion

Subsequent research on multisystem effects of extended space travel will offer significant new perspectives and insights as scientists look toward a long-term journey to Mars. The experiment on cotwin control from the Twin Astronaut Study offered state-of-the-art investigations focused on -omics (metabolomics, proteomics, epigenomics, integrative omics). A round-trip journey will minimally require a 6-month outbound trip and a 6-month return trip, while a stay on the red planet could last for one year and beyond. Synergies of genetic and epigenetic changes will impact human behavior at both the individual level and at the social level.

The cultural value of the international team-members having to live in micro-society far from earth may shed light on some aspects of human behavior in an evolutionary context.[18] (The assumption is that genetic expression affected by a weightlessness environment, through adaptive behavioral strategies, will yield a new cognitive representation of the space team-members and of the space habitat. This has been shown in previous research.) For the last quarter of a century, a wide range of ethological studies has been conducted on the effects of microgravity on humans during orbital flights (Space shuttle and Mir station) for short-term and mid-term missions. Changes in spatial and motor skills as behavioral strategies, affected by gravity variations from parabolic flights, were key goals of these studies.[11] In the theoretical model, the adaptive process began with a spontaneous phase showing sensorimotor reflexes, followed by preliminary and integrative phases enhancing specific cognitive functions over the course of space travel with the prevalence of visual cues.

Tying this line of work to twin studies can prove very valuable. MZ twin comparisons involving differential exposure to space environment factors will further our knowledge of spaceflight outcomes, as demonstrated above. Observations were made during a 12-month polar mission (Concordia station), then during the Mars-500 experiment with reference to the effects of a 520-day period of isolation and confinement.[19] In a long-term adaptive process, analyses revealed time effects, cultural preferences and individual differences in crew behavior, simulating a Mars mission.[20] Such data constitute a comprehensive database against which to evaluate the results from future studies involving long-term space travel, such as the recent experience of SK.

The fact that two identical twin brothers (SK and MK) were separated for nearly a year and SK was in a risky environment raises key questions for these twins, in particular, and for all astronauts and their family members, in general. In particular, how does prolonged isolation from close family members and socialization with unrelated team members affect the psychological and physiological systems? The current ethological answers support the hypothesis that the space traveler, with his or her own neuro-physiological system, a psycho-social system, a sensory-motor system, and genetic identity, organizes a relationship to the space environment in a positive way.[21] It is a salutogenic adaptation (i.e., ability to promote human health and well-being), based on optimization of the relationship. Most importantly, twin data can further the goal of assessing genetic factors underlying physical, biological, and behavioral aspects of space exploration missions from an evolutionary perspective.

Acknowledgments

The space ethological research of Carole Tafforin as a whole is supported by the French Space Agency. The research on twins of Nancy L. Segal is supported, in part, by the Wang Family Excellence Award (2016) and senior faculty research awards from California State University, Fullerton.

References

1. Tafforin C, Segal NL. Twins in space: review and perspectives. *Human Ethol*. 2019;34:123–129. http://ishe.org/volume-34-2019/twins-in-space-review-and-perspectives/.
2. Segal NL. Born together-reared apart: The landmark Minnesota twin study. Cambridge, MA: Harvard University Press; 2012.
3. Johnson W, Bouchard Jr TJ, McGue M, et al. Genetic and environmental influences on the verbal-perceptual-image rotation (VPR) model of the structure of mental abilities in the Minnesota study of twins reared apart. *Intelligence*. 2007;35:542–562.
4. Boomsma DI, Willemsen G, Dolan CV, et al. Genetic and environmental contributions to loneliness in adults: The Netherlands Twin Register Study. *Behavior Genetics*. 2005;35:745–752.
5. Tafforin C. Initial moments of adaptation to microgravity of human orientation behavior, in parabolic flight conditions. *Acta Astronautica*. 1996;38:963–971.
6. Grabherr L, Mast FW. Effects of microgravity on cognition: The case of mental imagery. *J Vestibular Res*. 2010;20:53–60.
7. Jandial R, Hoshide R, Waters JD, et al. Space–brain: the negative effects of space exposure on the central nervous system. *Surgical Neurol Int*. 2018;9:9.
8. Shuryak I, Brenner DJ, Blattnig SR, et al. Modeling space radiation induced cognitive dysfunction using targeted and non-targeted effects. *Scientific Reports*. 2021;11:8845.
9. Pierson DL, Stowe RP, Phillips TM, et al. Epstein–Barr virus shedding by astronauts during space flight. *Brain Behavior Immunity*. 2005;19:235–242.
10. Segal NL. *Entwined Lives: Twins and What They Tell us About Human Behavior*. New York NY: Plume; 2000.
11. Tafforin C. Synthesis of ethological studies on behavioural adaptation of the astronaut to space flight conditions. *Acta Astronautica*. 1994;32:131–142.
12. IWS. *Annual NASA Human Research Program Investigators' Workshop*, Galveston,TX; 2017:1–108. https://three.jsc.nasa.gov/iws/SRIW-Cvent-Program-2017.pdf (accessed January 23–26, 2017).
13. IWS. *Annual NASA Human Research Program Investigators' Workshop*, Galveston, Texas; 2018:1–94. https://three.jsc.nasa.gov/iws/FINAL_2018_HRP_IWS_program.pdf (accessed January 22–25, 2018).
14. Garrett-Bakelman FE, Darshi M, Green SJ, et al. The NASA twins study: a multidimensional analysis of a year-long human spaceflight. *Science*. 2019;364:1–20.
15. Bailey SM, Luxton JJ, McKenna LE, et al. Ad astra - Telomeres in Space!. *International Journal of Radiation Biology*. 2022;98:395–403.
16. Luxton JJ, McKenna MJ, Lewis A, et al. Telomere length dynamics and DNA damage responses associated with long-duration spaceflight. *Cell Reports*. 2020;33:108457.
17. Koren M. What's different about astronaut DNA? *The Atlantic. Science*; 2017. https://www.theatlantic.com/science/archive/2017/03/nasa-twins-scott-kelly/518145/. [Accessed 3 March 2017].
18. Tafforin C, Abati FG. Cultural ethology as a new approach of interplanetary crew's behavior. *Acta Astronautica*. 2017;139:102–110.
19. Tafforin C. The Mars-500 crew in daily life activities: an ethological study. *Acta Astronautica*. 2013;91:69–76.
20. Tafforin C. Time effects, cultural influences, and individual differences in crew behavior during the Mars-500 experiment. Aviation, Space, Environ. Med. 2013;84:1082-6.
21. Tafforin C. Humans' 3R-adaptation for Space Colonization. *J Humanities Social Sci Studies*. 2020;2:72–82.

CHAPTER

35

Environmental risk factors for neurodevelopmental disorders: Evidence from twin studies

Garth Stephenson[a], **Jeffrey M. Craig**[a,b]

[a]*IMPACT Institute, School of Medicine, Deakin University, Geelong, VIC, Australia*

[b]*Environmental & Genetic Epidemiology Research, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria, Australia*

35.1 Rationale and aims

The aim of this chapter is to discuss the normal and abnormal prenatal development in twins and its relationship with environmental factors in twin pregnancy. Here we will discuss the relationship of both shared and nonshared environmental influences and the risk for compromised neurodevelopment, in the short and long term. Although neurodevelopmental disorders can occur in any pregnancy, the additional demands and stress experienced in a multiple gestation pregnancy and increased occurrence of prenatal and perinatal factors presents added risk beyond that of inherited genes, leading to the increased prevalence of neurodevelopmental disorders such as cerebral palsy[1] and autism (after adjustment for familial confounding advanced paternal age).[2,3] Additionally, most twin pregnancies have relatively shortened gestational periods and associated preterm births (PTB),[4] posing even greater physical risks to mothers and infants and increasing the probability of mental disability.[14] With this in mind, this chapter will discuss environmental factors that may occur in twin pregnancies and how these factors can contribute to neurodevelopmental disorders.

35.2 Introduction

The Developmental Origins of Health and Disease (DOHaD) hypothesis, previously referred to as the "Fetal Origins of Adult Disease,"[5] describes how the exposure of a developing fetus to adverse maternal conditions and /or uterine environment can cause abnormal outcomes.[6] Factors such as poor nutrition, infections, toxins, metabolites or hormonal perturbations[7] during critical periods of development and

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00037-4

growth have been related to birth weight and long term health consequences on adult organisms.[5,6]

Environmental factors during pregnancy primarily include external, social, and biological factors.[8] However, in a twin pregnancy, intrauterine factors can have a direct "nonshared" environmental influence on development of individual fetuses.[9] Furthermore, although the risks of developing chronic diseases are attributed to both genetic and environmental factors, computer generated data based in part on twins' which determined the fraction of diseases attributable to genetic versus non-genetic factors, has suggested that between 70% and 90% of disease risks within a population may be attributable to differences in shared and nonshared environments.[8,10] External influences such as air pollution, water and food contamination from microbes, toxic chemicals or metals, physical exposures (noise and radiation), anthropogenic changes (including climate change) and exposure to other hazardous materials and built environment are estimated to contribute to 21.2% of global deaths and 16.3% of global disability adjusted life years (DALYs) each year.[8]

Social factors including stress and low socioeconomic status (poverty) have been identified as risk factors for adverse outcomes for mothers and children during pregnancy. Stress is a feeling of being overwhelmed or unable to cope with mental or emotional pressure. This feeling will normally subside when the stimulus is removed or when an individual is able to adapt to the effect.[11] However, prolonged symptoms can lead to chronic stress, which in turn can manifest as neurological conditions such as anxiety and depression, in addition to having an adverse effect on the immune, endocrine, and cardiovascular systems.[11] In a recent study into levels of anxiety and depression in pregnancy it was noted that there was an increase in anxiety after infertility treatment (IT) compared to spontaneous conception, whereas IT parents of twins demonstrated higher anxiety at mid-pregnancy than IT parents of singletons.[12] In a separate study it was determined that one third of women expecting twins suffered from major depression and high levels of stress.[13] Anxiety and depression in pregnancy have been linked to shorter gestation periods, impaired fetal growth[14] and adverse implications for fetal neurodevelopment.[14,15]

Biological factors relating to preconception health and to poor maternal health have a direct effect on both mother and fetus. Pre-existing conditions such as stress may have significant effects on pregnancy, maternal health and the developing fetus.[16] It has been suggested that activation of the maternal stress response and the resulting changes in endocrine and inflammatory activity play a role in the etiology of prenatal stress-related physiological changes on the developing fetus.[16] Prenatal stress can also act indirectly on maternal health which can also affect infant health and development.[16] Intrauterine infection prior to 32 weeks gestation is a major cause of PTB and is associated with high rates of morbidity and mortality.[17–20] Current data from the USA Centre for Disease Control and Prevention for 2019 indicate a 7-fold higher PTB (less than 37wks gestation) rate of 60.87% in twins compared to 8.47% in singletons.[21] Furthermore, in twins there is the added risk of the nonshared intrauterine environment, which may not be equal for both twins in a pair. How the individual twin reacts to the intrauterine environment may be subject to the chorionicity (monochorionic or dichorionic) and hence zygosity, monozygotic (MZ) or dizygotic (DZ)[22]. We will therefore discuss this in more detail throughout the chapter.

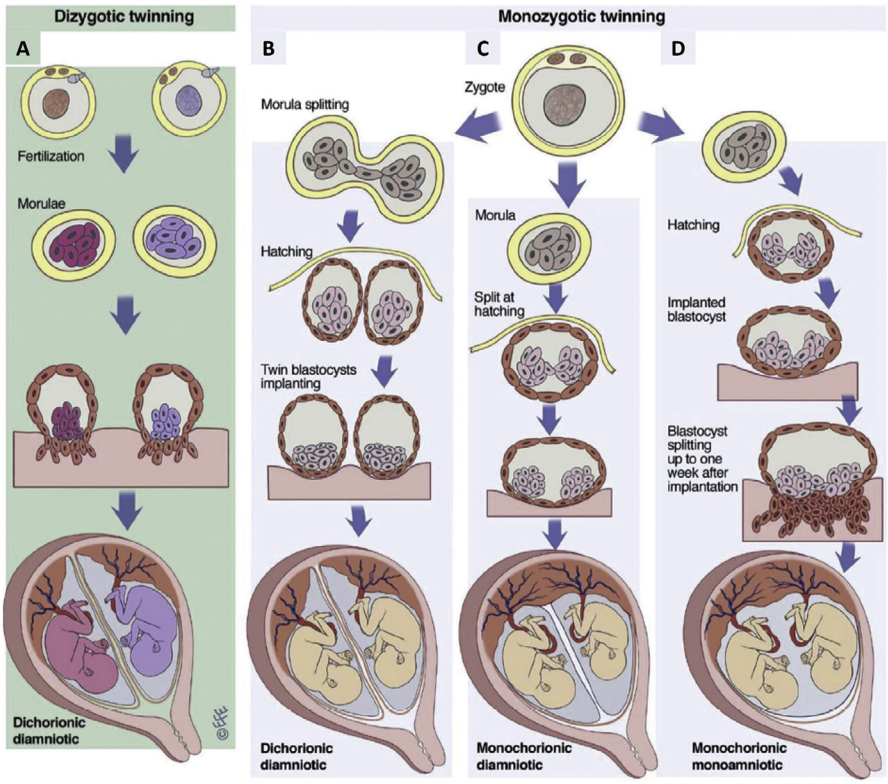


FIG. 35.1 Schematic representation of chorionic and amniotic development of dizygotic and monozygotic twins (Taken from Am J Obstet Gynecol with permission).[24]

35.3 Zygosity and chorionicity

Although twins can be classified into one of two zygosities, their developmental pathways are not always obvious. Dizygotic twins develop from two fertilization events (two zygotes) and occur in 4-8 per 1000 births or two thirds of twin pregnancies (Fig. 35.1A) whereas MZ twins arise from the splitting of a single early embryo and occur in 4 per 1000 births or one third of twin pregnancies[23] (Fig. 35.1B–D). MZ twins can occur in one of three scenarios based on the unproven hypothesis of postzygotic division of the conceptus.[24]

1. Splitting of the early zygote on days 1–3 to the morula stage leads to two separate blastula and results in MZ twins with individual placentae (dichorionic, DC), individual amnionic sacs (diamniotic, DA) and individual umbilical cords. This occurs in one-third of MZ twins[25] (Fig. 35.1B).
2. Splitting of the internal cell mass on days 3-8 (during which blastocyst hatching occurs) where the twins developing within the single blastula resulting in MZ twins with a single shared placenta (monochorionic, MC), individual

amnionic sacs (DA) and individual umbilical cords (Fig. 35.1C). This occurs in approximately two-thirds of MZ twins.[26]

3. Splitting of the internal cell mass on days 8–13, during implantation where the twins developing within the single blastula and results in MZ twins being MC, single shared amnionic sac (MA) and individual umbilical cords, occurring in only 2% of MC twins[26] (Fig. 35.1D).[24]

Each of these developmental pathways produces a different intrauterine environment where each twin may have either their own placenta and amnionic sac, a shared placenta and own amnionic sac, or shared placenta and shared amnionic sac.

Monozygotic twins share almost 100% of their DNA sequence whereas DZ twin share around 50%, the same as at that of singleton siblings.[27] All opposite sex twin pairs are DZ, and all MC pairs are MZ, with rare exceptions.[23,28–30]

35.4 Twins as a model for developmental variation

The classical twin design has been widely used to determine the genetic and environmental contribution to a wide variety of human traits.[31] Contrasting characteristics between monozygotic and dizygotic twins are easily determined based on their level of genetic similarity and shared and non-shared factors,[32,33] the latter including stochastic developmental variation and twin-specific environments.[33–35] As such, twin studies can be defined as being a special type of epidemiological design where the contribution of genetics is able to be measured as opposed to the environment, both shared and nonshared, for any given trait.[27] Historically, phenotypical discordance within pairs of MZ twins was accredited to non-shared environmental factors acting after birth.[36] However, more recent studies have also attributed these differences to such influences as genetic mosaicism and stochastic factors, in addition to the intrauterine environment[37] and factors it is influenced by. With this in mind, we will navigate some of the environmental influences experienced during pregnancy, both internal and external and discuss their influence on DZ and MZ twin development.

35.5 The intrauterine environment

MCMZ twins share the same source of nutrition, as opposed to DZ and DCMZ twins which each have their own placenta. Therefore, the placental vascular system of monochorionic twins must provide ample blood supply to address the needs both developing fetuses.[22] Although MC twins each have a defined share of the placenta, unequal placental sharing is a major cause of fetal growth discordance in MZ twins.[22,38–40] In addition, compensatory anatomical changes to placental blood vessels may exist. Here, MC twins are connected with each other through vascular anastomoses (VA).[41] VA is a connection between blood vessels to

ensure a continuous supply of blood to the connecting tissue. These can be either arterioarterial anastomosis (AA), venovenous anastomosis (VV), venoarterial anastomosis, or arteriovenous anastomosis (AV). In a study by Sun et al., 2015, of 60 uncomplicated MCDA pregnancies, vascular anastomoses were present in 100% of cases. Here the authors reported that 96.7% (58/60) presented with AA anastomoses while 28.3% presented with VV anastomosis (17/60) indicating that both AA and VV can be present in the shared placenta. In a separate study, in a group of 53 MC twins, it was determined that 88.7% of cases demonstrated VA, of which 71.1% presented with AA, 26.4% with VV and 75.4% with AV anastomoses.[42] In both studies it was determined that VA occurs in in the majority of MC twins.

Interestingly, it was also observed that the diameter of the AA in placentas which were unequally distributed between the developing twins were larger when compared to the equally shared placentas, (0.27 ± 0.12 cm versus 0.19 ± 0.1 cm, $p < 0.05$, respectively). Furthermore, the distance between cord insertions was shorter in the unequally shared group compared to the equally shared group, (14.5 ± 6.0 cm versus 18.3 ± 6.5 cm, $p < 0.05$, respectively). This suggests that although there appears to be differences in shared blood supply within MC twins due to cord location and shared placenta, in most cases, anatomical compensation ensures adequate nutrition and perfusion of the twin with the smaller placental part thus protecting them against growth restriction and other pathology.[43] However, unbalanced inter-twin blood transfusion such as that caused by arteriovenous (AV) anastomoses may lead to various complications, including twin-to-twin transfusion syndrome (TTTS), selective fetal growth restriction (sFGR) and twin anemia polycythemia sequence (TAPS).[44] Each of these conditions can result in increased risk for neurodevelopmental disorders NDD.

35.6 Twin to twin transfusion syndrome (TTTS)

In extreme cases, VA within the shared placenta of MCDA and MCMA twins may result in TTTS (1-3 per 10,000 births).[45] Research has shown that the most severe TTTS results from a single unidirectional deep arterial-venous (AV) anastomoses while milder cases are indicated by additional bidirectional superficial AA anastomosis or VA shunts (≤ 1mm diameter).[46] In addition, it has been demonstrated that AA anastomoses are thought to be protective against TTTS and are therefore decreased in twin gestations with TTTS.[47]

TTTS occurs in approximately 8%–15% of MCDA pregnancies compared to 2 to 3% of MCMA pregnancies.[45,48–50] TTTS is distinguished by the twin oligo–polyhydramnios sequence (TOPS).[51] Here the donor twin can experience hypovolemia (low extracellular fluid volume) and oligohydramnios (abnormal reduction in amniotic fluid) while the recipient twin produces hypervolemia (high extracellular fluid volume) and polyhydramnios (abnormal increase in amniotic fluid) and hepatosplenomegaly (a disorder where both the liver and spleen swell beyond their

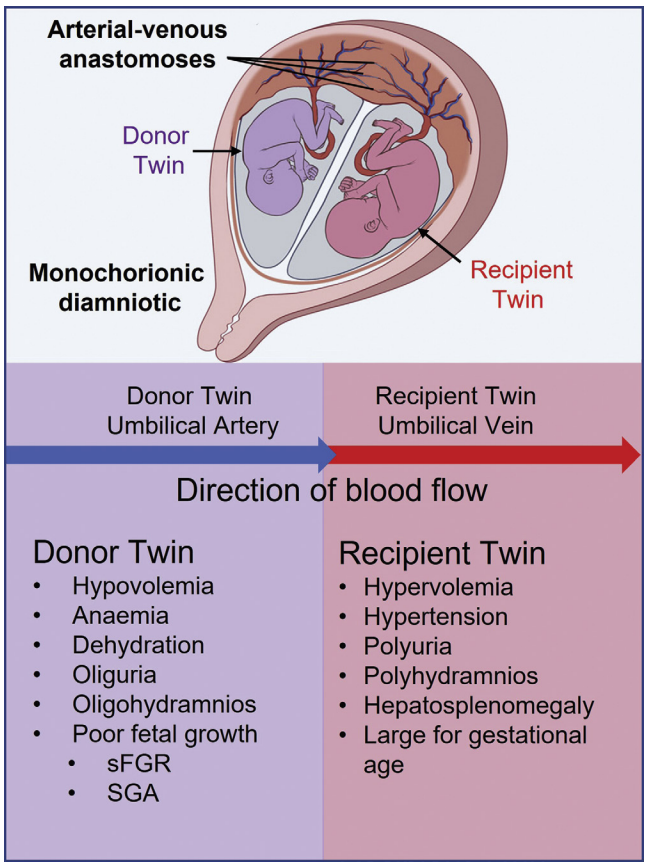


FIG. 35.2 Illustration of Twin-to-Twin Transfusion Syndrome (TTTS).

Figure demonstrates vascular anastomosis in monochorionic diamniotic twins. Twin 1(donor) donates blood and nutrients to twin 2 (recipient). Donor twin experiences reduced blood supply leading to poor fetal growth, low amnionic fluid, dehydration, and urine output. Recipient twin experiences increased bold supply resulting in high blood pressure and excessive urination and Polyhydramnios (excessive amniotic fluid) (Figure adapted from fig.3, Am J Obstet Gynecol with permission).[24].

normal size)[45] (Fig. 35.2). Continued progression of TTTS may lead to accelerated expansion of the uterine cavity and shortening of the cervix resulting in preterm labor or preterm rupture of the membranes[52] while early onset and non-treatment can result in high mortality rates, >90% for both twins[45] with fetal deaths usually due to cardiac failure.[48] Furthermore, twins are at risk of morbidity associated

with prematurity, such as respiratory distress syndrome, chronic lung disease, necrotizing enterocolitis, and cerebral injury, including intraventricular hemorrhage (IVH), major cerebral lesions and periventricular leukomalacia (PVL).[53] As such, TTTS often requires surgical correction by laser photocoagulation of communicating placental vessels which can reduce the perinatal mortality rate to 30%–50%.[48] The current survival rate of one or more fetuses following laser surgery is >90% with at least one in five survivors of TTTS have serious adverse neurodevelopmental outcomes (usually cerebral palsy).[54] In addition, almost two-thirds of pregnancies complicated by TTTS also present with selective fetal growth restriction (sFGR), suggesting an underlying association between the pathogeneses of both disorders.[55,56]

35.7 Selective fetal growth restriction (sFGR)

Selective fetal growth restriction (sFGR), also referred to as selective intrauterine growth restriction (sIUGR) is primarily due to unequal placental sharing and is observed in approximately 10%–15% of monochorionic twin pregnancies.[57,58] However, it has been reported to be as high as 26.5%.[59] Specifically, sFGR is identified if cases where one fetus has an estimated fetal weight (EFW) below the 10th percentile and where the within twin pair EFW discordance is greater than 25%.[58,60] Further classification of sFGR is determined by the level of end diastolic flow (EDF) in the umbilical artery of the smaller fetus and is designated as being Types 1–3. Type I is assigned where EDF is positive whereas in Type II the EDF is absent or reversed. In both cases the fetus is monitored to define the most appropriate time of delivery. If TTTS is present, laser photocoagulation surgery is recommended. Type III sFGR is defined where EDF is intermittent, and the two umbilical cords are closely adjacent resulting in the pregnancy behaving similar to that of monoamniotic twins. Type III rarely develops TTTS; however, unexpected death occurs in 20–30% of cases.[61] Both Type II and III have a high prevalence of perinatal morbidity and mortality.[58] In a study by Groene et al., 2019, investigating twins with TTTS and sFGR it was noted that the proportion of TTTS neonates born small for gestational age (SGA) was 21% (73/352) in the TTTS-only group and 49% (231/465) in the TTTS + sFGR group ($p < 0.0001$).[55] Furthermore, placentas in the TTTS + sFGR group more often presented with an AA anastomosis (51/296 (17%) – 19/203 (9%) $p = 0.013$), VCI (116/440 (26%) – 61/330 (19%), $p = 0.007$) and unequal placental sharing (33.6 (18.1–52.0) – 22.1 (10.7–37.2), $p < 0.0001$), when compared to TTTS-only, respectively. Severe neurodevelopmental impairment in long-term survivors between the TTTS-only and TTTS + sFGR groups was similar with no significant difference, 7% (13/198) and 9% (27/299), respectively ($p = 0.385$).[55] It is suggested that TTTS with coexistent sFRG prior to laser surgery results in a more severe initial presentation and decreased donor perinatal survival. Furthermore, sFGR is independently associated with decreased perinatal survival.[55]

35.8 Twin anemia–polycythemia sequence (TAPS)

TAPS is a chronic form of fetofetal transfusion in monochorionic twins through small anastomoses at the placental surface.[62,63] TAPS is characterized by a large intertwin hemoglobin difference without signs of TOPS and can occur spontaneously with an incidence of 1% and 5%. However, it is more frequently diagnosed after treatment of TTTS with fetoscopic laser surgery occurring in 1% to 16% of such cases.[62–64] In a study by Slaghekke et al. (2014), the authors found that 11% (33/306) of MC twin pairs developed TAPS following laser surgery for TTTS and a survival rate of 80% (53/66). A follow up study on 89% (47/53) of these children included neurological examination and an assessment of cognitive and motor development using the Dutch version of the Bayley Scales of Infant and Toddler Development (BSID). Results determined that 9% (4/47) of these children were identified as positive for neurodevelopmental impairment, comprising one donor (1/20; 5%) and three recipients (3/27; 11%) ($p = 0.63$). Furthermore, mild-to-moderate cognitive delay, i.e., scores below 85, were detected in 8/47 (17%) children. Risk factors for low cognitive scores were determined to be low gestational age at birth ($p = 0.02$) and low birth weight ($p < 0.01$) indicating reduced growth *in utero*. Moreover, the lowest cognitive scores (median score, 82.5) were detected in TAPS survivors who were treated with intrauterine transfusion.[62]

35.9 Neurodevelopmental disorders

Neurodevelopmental disorders (NDD) are multifactorial conditions characterized by impairments in cognition, communication, behavior, and/or motor skills resulting from abnormal brain development. NDDs includes conditions such as intellectual disability (Intellectual Developmental Disorder), communication disorders, attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), cerebral palsy (CP), schizophrenia,[65] and epilepsy.[66] NDD's (except epilepsy) require a diagnosis under the Diagnostic and Statistical Manual of Mental Disorders Version 5 (DSM-5) criteria. Epilepsy diagnosis requires an accurate historical description of an event suspected to be a seizure and the appropriate use of a confirmative test, such as electroencephalogram (EEG), neuroimaging, and genetic studies.[67]

35.10 Attention-deficit hyperactivity disorder

ADHD is a NDD classically characterized by symptoms of inattention, impulsivity, and hyperactivity with an onset which typically manifests in early childhood.[68] The current worldwide prevalence of ADHD is approximately 2.2%; however, it has been estimated in children and adolescents (aged <18 years) to be as high as 8.1% (USA) and as low as 0.1% (Iraq).[69] ADHD is a common childhood disorder which is estimated to occur more often in twins than singletons.[70,71]

35.11 Autism spectrum disorder

ASD is a complex developmental condition where a child must present with persistent deficits in each of three areas of social communication and interaction plus at least two of four types of restricted, repetitive behaviours.[72] ASD is characterized by some degree of impaired social behavior, communication and language, and a narrow range of interests and activities that are both unique to the individual and carried out repetitively. ASD has become more recognized since 2000 with the current worldwide prevalence estimated at 1 in 160 children[73] to as many as 1 in 54 children as determined in the USA.[74] Furthermore, although males are four to eight times more likely to be affected by a neurodevelopmental disorder than females,[75] the ratio of males to females for ASD is closer to 3:1.[76] According to a recent meta-analysis, correlations for MZ were almost perfect at 0.98 (95% Confidence Interval, 0.96–0.99). The DZ correlation, however, was 0.53 (95% CI 0.44–0.60) when ASD prevalence rate was set at 5% (in line with the Broad Phenotype of ASD) and increased to 0.67 (95% CI 0.61–0.72) when applying a prevalence rate of 1%.[77] Twin and family studies have demonstrated that both genetic and shared environmental effects contribute to ASD aetiology.[2,77–79]

35.12 Cerebral palsy

CP is the most common physical disability in children and originates from a non-progressive damage to the immature brain.[80] However, a diagnosis of CP cannot usually be made at birth and in some cases may be delayed until 2–3 years of age[81] with the etiology of CP being generally unknown.[80] Although the current prevalence of CP is around 2.1 per 1000 live births,[80,82] the prevalence in multiple births is almost four times that of singletons.[81,82] It has been reported that there are many factors that may be responsible for this increased risk in multiple birth pregnancies, with the most likely being low birth weight and preterm birth, both known risk factors for CP.[81] CP involved several comorbidities including increased risk of blindness, deafness, autism spectrum disorders, and ADHD with one of the most common comorbidities of CP being epilepsy.[80]

35.13 Schizophrenia

Schizophrenic is a serious mental disorder in which people interpret reality abnormally. Although the lifetime risk in the general population is <1%[83] it can rise to as much as 40% in MZ of affected people.[84] Estimated concordance rates of 50% in MZ twins and 10%–19% in DZ twins have previously been reported.[84,85] In one study, 31 MZ and 28 DZ schizophrenic probands and their co-twins were personally interviewed with structured diagnostic instruments and classified according to DSM-III-R criteria. The concordance rates of 48% for MZ twins and 4% for DZ twins indicating a strong genetic influence on schizophrenia.[86]

35.14 Epilepsy

Epilepsy is a neurological disorder associated with abnormal electrical activity in the brain which can result in sudden recurrent episodes of sensory disturbance, loss of consciousness, or seizures. The type of seizures, and severity of the epileptic condition, are strictly relate to the brain regions that are affected by the overactivity.[66] Epilepsy is the fourth most common neurological disorder and affects 1 in 26 people in the United States and 65 million people worldwide.[87] The causes of epilepsy are divided into the several categories: structural, genetic, infectious, metabolic, immune, and unknown. Although, epilepsy can be caused postnatally by cerebral trauma, stroke, neural infection and brain tumors, it can also manifest prenatal from causes such as brain damage from loss of oxygen (hypoxia/hypoxemia), trauma during birth or low birth weight, congenital abnormalities or genetic conditions with associated brain malformations.[88] Studies into the causes of epileptic seizures and syndromes in twins have confirmed significantly higher concordance rates for MZ twins compared to DZ twins for both epileptic seizures (0.56 for MZ and 0.21 for DZ pairs, $p < 0.001$) and for epilepsy (0.49 for MZ and 0.16 for DZ pairs, $p < 0.001$).[89] The results of this study found that genetic factors accounted for 80% of epilepsy. As epilepsy is associated with brain abnormalities it is commonly associated with other NDDs such as CP,[80] ASD,[90] ADHD,[91] and schizophrenia.[92]

35.15 Environmental influences on neurodevelopment in twins

In this section we will discuss both internal and external environmental influences on neurodevelopment in twins. Internal influences will focus on biological influences of maternal health conditions such as maternal infections and immune activation, obesity,[93] diabetes,[94] and hypertension[95] are all conditions which can affect fetal neurodevelopment. External influences will focus on maternal smoking and alcohol intake.

35.16 Maternal immune activation

Maternal immune activation (MIA) can be defined as measured levels of inflammatory markers, such as interleukin-6, exceeding normal range or more broadly defined as levels of these markers in the higher normal range.[96] Maternal inflammatory conditions that develop during pregnancy, such as infection or high body mass index, can lead to a state of MIA.[96] Medical conditions experienced during pregnancy can induce placental programming leading to changes in developmental trajectory and ultimately affecting the fetus.[97] Neurodevelopmental and psychiatric disorders such as ASD, cognitive impairment, CP, epilepsy, and schizophrenia have all been linked to early life inflammation[98] and more specifically MIA.[96] For example, studies have shown a

twofold increase in the risk of ASD in offspring associated with maternal influenza infection.[99,100] Furthermore, in pregnant rodents, injection with the viral immunostimulant polyinosinic:polycytidylic acid (poly I:C), resulted in the release of inflammatory cytokines.[101] Several of these cytokines, including IL-6 and IL-1β, in addition to type 1 interferons are currently implicated in MIA-induced neurodevelopmental impairment.[101] Inflammatory cytokines have the ability to pass through the placenta to the developing fetus and are essential for normal brain development.[102] However, immune-induced inflammation can result in increased occurrence of adverse neural events resulting in a direct effect on brain development. Further studies using MIA mice induced by polyI:C have produced offspring with abnormalities in behavior, cognition, and gene expression reminiscent of NDD including autism and schizophrenia.[103] Maternal exposure to influenza during early to mid-gestation has been associated with a threefold increased risk of schizophrenia.[104]

Although the occurrence of MIA is not directly influenced by multiple birth pregnancies, the increased susceptibility to infection in twin gestation may subsequently lead to MIA. In a recent study into immunological changes in pregnancy and increased susceptibility to infection for women with multiple versus singleton gestations, logistic regression analysis was used to determine the odds ratios and 95% confidence intervals for demographic data, pre-existing medical conditions, and acute medical and infectious complications. It was determined that for women with multiple gestation, 38.4 per 1000 women had an infectious complication compared to 12.8 per 1000 women with singletons (OR 3.12, CI 3.05, 3.18 for composite infection). Furthermore, the most significant infectious morbidities associated with multiple gestation were intestinal infections, pyelonephritis, influenza, and pneumonia.[105] Each of these conditions has reportingly been indicated in MIA.[106] In addition, in a recent Swedish population-based cohort study of children born between 1973 and 2014, the author identified an association between MIA and ASD risk when the maternal infection was severe (sepsis, pneumonia, pyelonephritis, meningitis, influenza, and chorioamnionitis).[106,107] Additional findings have reported that although mild cases of maternal influenza were not associated with an increased risk of having a child with ASD more severe cases requiring hospitalization were associated with an increased risk of ASD.[106,108,109]

35.17 Maternal obesity and gestational diabetes

Maternal obesity has been indicated to impact on brain development and cognitive function in offspring.[93,110] High fat diets and severe cases of obesity can induce low grade neural inflammation from chronic activation of the innate immune system.[111] In addition, increases in oxidative stress, dysregulated insulin, glucose, and leptin signaling; dysregulation in serotonergic and dopaminergic signaling; and perturbations in synaptic plasticity and DNA methylation patterns have been observed.[112–115] The risk of each of these outcomes are increased in the presence of diabetes.[99]

Pregnant mothers suffering from gestational diabetes mellitus (GDM), a condition associated with low grade chronic inflammation[116] demonstrate a significantly higher risk of producing a child with ASD (hazard ratio 3.91, 95% confidence interval 1.76–8.68).[116] However, the occurrence of GDM in twin pregnancies appears to be conflicting with multiple recent studies reporting no increase in the occurrence of GMD in twins pregnancies; however, the presence of GDM in twins pregnancy is a risk factor for adverse maternal outcomes which can lead to preterm birth.[117]

35.18 Maternal hypertension

Hypertensive disorders in pregnancy include gestational hypertension (GH) and preeclampsia (PE).[118,119] GH is defined as having a blood pressure greater than 140/90 on two separate occasions at least 6 hours apart whereas the appearance of proteinuria indicates that PE has developed. PE is the leading cause of maternal and perinatal morbidity.[118,120] Prolonged GH can initiate a series of events resulting in adverse *in utero* conditions leading to PE. The etiology of PE originates from abnormal remodeling of the maternal spiral arteries at the maternal-placental interface, leading to an ischemic placenta that releases factors that drive the pathophysiology.[121] The onset of PE results in the increase stimulation of placental and renal Toll-like receptor 4 (TLR4) receptors. TLRs are a critical component of the innate immune system where they function as rapid pathogen sensors.[122] Stimulation of TLR4 leads to the increased release of pro-inflammatory cytokines and placental/renal dysfunction.[123,124] Furthermore, PE is also associated with a decrease in anti-inflammatory cytokines[123,125,126] therefore amplifying the inflammatory state. PE can lead to altered fetal development and increase the risk of long term psychiatric and cognitive outcomes.[118] Mothers who are pregnant with multiple births are at an estimated 3-4 times higher risk for preeclampsia.[127,128] For example, twin births experience an overall rate of 9.5% being a 2-3-fold increase over singletons births with nulliparity (adjusted odds ratio (OR) 1.57, 95% confidence interval (CI) 1.02–2.41).[129] A study by Campbell, D.M and MacGillivray, I., (1999) determined that preeclampsia is more common in association with monochorionic placentation placing MZ twins at a greater risk.[130]

35.19 Maternal smoking

Smoking, both direct and passive,[131] has been long recognized to impact on fetal and infant development. Tobacco smoke contains thousands of health-threatening chemicals, many of which are potentially toxic and oxygen depriving, contributing to alterations in neurotransmitter activity in the developing brain.[132,133] Nicotine and carbon monoxide are two substances in tobacco smoke with the highest risk to the developing child.[134] Nicotine from both active and passive smokers has been shown to cross the placenta and accumulates in the fetal compartments from as early as

7 weeks of gestation.[132,133] Nicotine can cause preterm delivery, low birth weight, and poor physical growth due to its constrictive effect on uterine blood vessels.[135] Nicotine exposure early in fetal development in experimental animals has adverse effects on synaptic development and function of serotonin systems as well as those of other monoamines (dopamine, norepinephrine), resulting in neuronal damage and cell death.[136] Some of these negative effects including low birth weight, very low birth weight, and extreme premature delivery are significantly higher for women carrying twins.[137]

35.20 Alcohol

Alcohol is a teratogen, and it can cause lasting birth defects.[138] Alcohol intake during pregnancy has been linked to numerous forms of neurodevelopmental damage, from developmental delay, intellectual impairment, growth disturbance and behavioral changes. Where only some of the clinical signs of prenatal exposure to alcohol are present the condition is referred to as fetal alcohol effects (FAE) whereas fetal alcohol syndrome disorder (FASD) is typically indicated for heavy drinkers.[139] FASD demonstrates many of the symptoms from mild alcohol exposures but can include minor craniofacial anomalies and birth defects in addition to behavioral problems throughout life. FASD is prevalent in 0.77% of the global population with European/ North American rates ranging from 2% to 5%.[140] The outcome of alcohol intake, however, is influenced by the genetics of the fetus. In an early study, it was shown that DZ twins exposed to similar amounts of alcohol at the same time during gestation had differences in fetal susceptibility to ethanol-induced dysmorphogenesis with one child displaying FASD and the other FAE.[141] This was later confirmed by Hemingway, (2019) in addition to reporting that twins with identical DNA (MZ) experiencing identical alcohol exposure demonstrated identical fetal alcohol symptoms whereas four pairs of DZ twins demonstrate symptoms at opposite ends of the spectrum suggesting that fetal genetics may influence fetal vulnerability.[142]

35.21 The female reproductive microbiome

Microbial populations have previously been identified in the urogenital system including the vagina; however, recent advances in next generation sequencing technology have detected microorganisms residing in the uterus, fallopian tubes, ovaries, and placenta.[143] Along with the vagina, these areas are believed to be primarily populated from the gastrointestinal tract (GIT) and oral microbiota.[144] In female humans, the vagina and cervix harbor a microbiome designed to maintain a protective acidic barrier for the reproductive organ containing >95% Lactobacillus spp with the highest diversity of species located proximal to the cervical entrance. Dysbiosis (the imbalance of microbiota homeostasis) of the vaginal microbiota can result in conditions such as bacterial vaginosis (BV) causing severe reproductive health outcomes.[143]

A large proportion of PTB (40% in the USA) are associated with intrauterine infection, which triggers an inflammatory response.[145] It has been hypothesized that pathogenic bacteria or other microbes entering the lower genital tract during sexual intercourse,[146] times of altered cervical mucin confirmation[143] or when compromised during pregnancy[38] may pass by vertical ascension through the cervix leading to intrauterine infection. Maternal vaginal infections such as bacterial vaginosis (BV), a condition derived from dysbiosis of the virginal microbiome resulting in replacement of vaginal *lactobacilli* by an overgrowth of *Gardnerella vaginalis,* anaerobes, and mycoplasmas, may also contribute to an inflammatory state during or after conception.[147] It has therefore been hypothesized that dysbiosis of the reproductive microbiome may be associated with increased probability of ASD in offspring through MIA. Recent studies of dysbiosis in the GIT of mice have identified increased $CD4^+$ Th17 cells and inflammatory cytokines linked to autistic behaviour.[148] Studies on dysbiosis of the female reproductive tract (FRT) at the cervicovaginal interface have identified increased genital antigen presenting cell activation, bacteria induced $CD4^+$ Th17 cells and pro-inflammatory cytokines, which induce a state of MIA.[149] Excessive Th17 immunity may induce uncontrolled neutrophil infiltration at the maternal-fetal interface while excessive Th17 cells have been detected in the decidua and peripheral blood of aborted fetuses.[150–154] Epidemiological studies suggest that exposure of a fetus to maternal inflammation increases the chance of developing ASD[149] and multiple infections during pregnancy were associated with increased risk of ASD (OR adj = 1.36, 95% CI 1.05–1.78).[109] To date there has been no indication of increased prevalence of bacterial vaginosis in twin pregnancies.[155]

Conversely, intrauterine viral infections during pregnancy by pathogens such as Zika virus (ZV), Cytomegalovirus (CMV), Rubella, and Herpes Simplex virus (HSV) can increase the risk of prenatal as well as postnatal NDD.[157] For instance, ZV transmission can result in microcephaly, CMV can result in schizophrenia and HSV-2 infection has been implicated in ASD.[157] In a recent meta-analysis of five articles following at-risk pregnancies, it was determined that the rate of vertical transmission in twin pregnancies is 58.7% (95% CI 43.3%–72.3%) whereas in singleton pregnancies it is 31.4% (95% CI: 29.0%–34.0%) p = 0.0002. Furthermore, it was determined that discordance of congenital CMV in twins is not rare, by identifying 21 of 42 twin pairs with at least one twin infected (50.0%, 95% CI: 34.4%–65.6%).[158] A similar finding has also been identified for discordance in ZV transmission demonstrating that each twin should be evaluated independently for vertical transmission.[159] As with bacterial infections, it has been established that regardless of the virus, MIA and the subsequent inflammatory response may be a key determinant of viral induced neurological outcomes.[157] Therefore, the development of an accurate inflammatory profile for specific infections and subsequent treatment targeting multiple inflammatory indicators following viral or bacterial infection may lead to a successful NDD therapy during pregnancy.[157]

35.22 Conclusion

The prevalence of NDDs is reported to be consistently higher in twins than in singletons due to lower birth weights and gestational age at birth which are common traits in twins, and are two major factors associated with increased risk of neurodevelopmental disabilities.[160] However, environmental factors linked to MIA are also highly indicated as causes of NDD in twins and in many cases are more prevalent than in singleton births. In addition, studies have repeatedly shown that males have a higher incidence of NDD than females.[160,161] The classic twin model is ideal to estimate the proportion of variance of any phenotype due to genetics, shared and non-shared factors.[32,33] Future studies into NDD should focus on environmental factors relating to activation of the maternal immune response and the effect on genetic expression (epigenetics) (See Chapter 30). These studies may lead to a greater understanding of the causes of NDD and subsequent intervention.

References

1. Blickstein I. Cerebral palsy in multifoetal pregnancies. *Developmental Medicine and Child Neurology*. 2002;44(5):352–355.
2. Hallmayer J, Cleveland S, Torres A, et al. Genetic heritability and shared environmental factors among twin pairs with autism. *Archives of General Psychiatry*. 2011;68(11): 1095–1102.
3. Carlsson T, Molander F, Taylor MJ, et al. Early environmental risk factors for neurodevelopmental disorders - a systematic review of twin and sibling studies. *Development and Psychopathology*. 2021;33(4):1448–1495.
4. Costello-Harris VA, Segal NL. The unmet concerns of twins with special needs: diagnostic challenges and service recommendations. *Community Practitioner*. 2015;88(2):32–35.
5. Hales CN, Barker DJ, Clark PM, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ*. 1991;303(6809):1019–1022.
6. Mandy M, Nyirenda M. Developmental origins of health and disease: the relevance to developing nations. *Int Health*. 2018;10(2):66–70.
7. Gluckman PD, Hanson MA, Cooper C, et al. Effect of in utero and early-life conditions on adult health and disease. *New England Journal of Medicine*. 2008;359(1):61–73.
8. Gibson JM. Environmental determinants of health. In: Daaleman TPH MR, ed. *Chronic Illness Care*. Cham: Springer; 2018:451–467.
9. Salsberry PJ, Reagan PB. Effects of heritability, shared environment, and nonshared intrauterine conditions on child and adolescent BMI. *Obesity (Silver Spring)*. 2010;18(9):1775–1780.
10. Rappaport SM, Smith MT. Epidemiology. Environment and disease risks. *Science*. 2010;330(6003):460–461.
11. Khan S, Khan RA. Chronic stress leads to anxiety and depression. *Ann Psychiatry Ment Health*. 2017;5(1):4.
12. Tendais I, Figueiredo B. Parents' anxiety and depression symptoms after successful infertility treatment and spontaneous conception: does singleton/twin pregnancy matter? *Human Reproduction*. 2016;31(10):2303–2312.

13. Benute GR, Nozzella DC, Prohaska C, et al. Twin pregnancies: evaluation of major depression, stress, and social support. *Twin Res Hum Genet*. 2013;16(2):629–633.
14. Dunkel Schetter C, Tanner L. Anxiety, depression and stress in pregnancy: implications for mothers, children, research, and practice. *Curr Opin Psychiatry*. 2012;25(2):141–148.
15. O'Donnell K, O'Connor TG, Glover V. Prenatal stress and neurodevelopment of the child: focus on the HPA axis and role of the placenta. *Developmental Neuroscience*. 2009;31(4):285–292.
16. Coussons-Read ME. Effects of prenatal stress on pregnancy and human development: mechanisms and pathways. *Obstet Med*. 2013;6(2):52–57.
17. Goldenberg RL, Culhane JF, Iams JD, et al. Epidemiology and causes of preterm birth. *Lancet*. 2008;371(9606):75–84.
18. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. *New England Journal of Medicine*. 2000;342(20):1500–1507.
19. Payne MS, Newnham JP, Doherty DA, et al. A specific bacterial DNA signature in the vagina of Australian women in mid-pregnancy predicts high risk of spontaneous preterm birth (The Predict1000 Study). *American Journal of Obstetrics and Gynecology*. 2021;224(2):206.e1–206.e23. doi:10.1016/j.ajog.2020.08.034.
20. Stinson LF, Payne MS. Infection-mediated preterm birth: Bacterial origins and avenues for intervention. *Australian and New Zealand Journal of Obstetrics and Gynaecology*. 2019;59(6):781–790.
21. Martin BEH JA, Osterman MJK, Driscoll; AK. Births: Final Data for 2019. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2021. Contract No.: 2.
22. Marceau K, McMaster MT, Smith TF, et al. The prenatal environment in twin studies: a review on chorionicity. *Behavior Genetics*. 2016;46(3):286–303.
23. Cutler TL, Murphy K, Hopper JL, et al. Why accurate knowledge of zygosity is important to twins. *Twin Res Hum Genet*. 2015;18(3):298–305.
24. McNamara HC, Kane SC, Craig JM, et al. A review of the mechanisms and evidence for typical and atypical twinning. *American Journal of Obstetrics and Gynecology*. 2016;214(2):172–191.
25. Australia TR. What is the frequency of MZ and DZ twin births?: Twins Research Australia; 2021. Available from: https://www.twins.org.au/twins-and-families/frequently-asked-questions/63-twin-figures/23-frequency-of-twin-births.
26. Cordero L, Franco A, Joy SD, et al. Monochorionic diamniotic infants without twin-to-twin transfusion syndrome. *Journal of Perinatology*. 2005;25(12):753–758.
27. Sahu M, Prasuna JG. Twin studies: a unique epidemiological tool. *Indian J Community Med*. 2016;41(3):177–182.
28. Chen K, Chmait RH, Vanderbilt D, et al. Chimerism in monochorionic dizygotic twins: case study and review. *American Journal of Medical Genetics. Part A*. 2013;161A(7):1817–1824.
29. Umstad MP, Short RV, Wilson M, et al. Chimaeric twins: why monochorionicity does not guarantee monozygosity. *Australian and New Zealand Journal of Obstetrics and Gynaecology*. 2012;52(3):305–307.
30. Wachtel SS, Somkuti SG, Schinfeld JS. Monozygotic twins of opposite sex. *Cytogenetics and Cell Genetics*. 2000;91(1–4):293–295.
31. Polderman TJ, Benyamin B, de Leeuw CA, et al. Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nature Genetics*. 2015;47(7):702–709.

32. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nature Reviews Genetics*. 2002;3(11):872–882.
33. Craig JMS, E. K, Jahanfar S. The environmental differences between twins in utero and their importance for downstream development: a need for standardized monitoring in obstetric research. *Developmental and Fetal Origins of Differences in Monozygotic Twins*. 2020:320–343.
34. Knopik VS, Neiderhiser JM, de Geus E, et al. The importance of the prenatal environment in behavioral genetics: introduction to special issue. *Behavior Genetics*. 2016;46(3):281–285.
35. Stromswold K. Why aren't identical twins linguistically identical? Genetic, prenatal and postnatal factors. *Cognition*. 2006;101(2):333–384.
36. Wong AH, Gottesman II, Petronis A. Phenotypic differences in genetically identical organisms: the epigenetic perspective. *Human Molecular Genetics*. 2005;14:R11–R18 Spec No 1.
37. Czyz W, Morahan JM, Ebers GC, et al. Genetic, environmental and stochastic factors in monozygotic twin discordance with a focus on epigenetic differences. *Bmc Medicine [Electronic Resource]*. 2012;10:93.
38. Adams Waldorf KM, McAdams RM. Influence of infection during pregnancy on fetal development. *Reproduction (Cambridge, England)*. 2013;146(5):R151–R162.
39. Cleary-Goldman J, D'Alton ME. Growth abnormalities and multiple gestations. *Seminars in Perinatology*. 2008;32(3):206–212.
40. Nikkels PG, Hack KE, van Gemert MJ. Pathology of twin placentas with special attention to monochorionic twin placentas. *Journal of Clinical Pathology*. 2008;61(12): 1247–1253.
41. Lewi L, Deprest J, Hecher K. The vascular anastomoses in monochorionic twin pregnancies and their clinical consequences. *American Journal of Obstetrics and Gynecology*. 2013;208(1):19–30.
42. Lipa M, Kosinski P, Stanirowski P, et al. Vascular anastomoses in intrauterine growth in monochorionic twins. *Journal of Perinatal Medicine*. 2020;48(6):539–543 .abc.
43. Sun LM, Li Y, Zou G, et al. Monochorionic twins with unequal placental sharing: why can the outcome still be favorable? *The Journal of Maternal-Fetal & Neonatal Medicine*. 2016;29(8):1261–1264.
44. Tollenaar LS, Slaghekke F, Middeldorp JM, et al. Twin anemia polycythemia sequence: current views on pathogenesis, diagnostic criteria, perinatal management, and outcome. *Twin Res Hum Genet*. 2016;19(3):222–233.
45. Bautista TN, Krebs TL, Jnah A, et al. Twin-to-twin transfusion syndrome: a case report. *Neonatal Network*. 2018;37(5):292–302.
46. Bajoria R. Vascular anatomy of monochorionic placenta in relation to discordant growth and amniotic fluid volume. *Human Reproduction*. 1998;13(1O):2933–2940.
47. Borse V, Shanks AL. *Twin-to-Twin Transfusion Syndrome*. Treasure Island (FL): StatPearls; 2021.
48. Society for Maternal-Fetal M Simpson LL. Twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*. 2013;208(1):3–18.
49. Johnson A. Diagnosis and management of twin-twin transfusion syndrome. *Clinical Obstetrics and Gynecology*. 2015;58(3):611–631.
50. Mosquera C, Miller RS, Simpson LL. Twin-twin transfusion syndrome. *Seminars in Perinatology*. 2012;36(3):182–189.

51. Slaghekke F, Kist WJ, Oepkes D, et al. TAPS and TOPS: two distinct forms of feto-fetal transfusion in monochorionic twins. *Zeitschrift Fur Geburtshilfe Und Neonatologie*. 2009;213(6):248–254.
52. Medicine JH. *Twin-to-Twin Transfusion Syndrome (TTTS)*. Baltimore, Maryland: The Johns Hopkins University, The Johns Hopkins Hospital, and Johns Hopkins Health System; 2020. [Available from. https://www.hopkinsmedicine.org/health/conditions-and-diseases/twintotwin-transfusion-syndrome-ttts.
53. van Klink JMM, Koopman HM, Steggerda SJ. Cerebral injury and neurodevelopmental outcome in twin-twin transfusion syndrome. *Curr Obstet Gynecol Rep*. 2013;2:240–248.
54. Bolch C, Fahey M, Reddihough D, et al. Twin-to-twin transfusion syndrome neurodevelopmental follow-up study (neurodevelopmental outcomes for children whose twin-to-twin transfusion syndrome was treated with placental laser photocoagulation). *Bmc Pediatrics [Electronic Resource]*. 2018;18(1):256.
55. Groene SG, Tollenaar LSA, van Klink JMM, et al. Twin-twin transfusion syndrome with and without selective fetal growth restriction prior to fetoscopic laser surgery: short and long-term outcome. *J Clin Med*. 2019;8(7):969–980.
56. Van Winden KR, Quintero RA, Kontopoulos EV, et al. Perinatal survival in cases of twin-twin transfusion syndrome complicated by selective intrauterine growth restriction. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2015;28(13):1549–1553.
57. Lewi L, Jani J, Blickstein I, et al. The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. *American Journal of Obstetrics and Gynecology*. 2008;199(5):514 e1-8.
58. Monaghan C, Kalafat E, Binder J, et al. Prediction of adverse pregnancy outcome in monochorionic diamniotic twin pregnancy complicated by selective fetal growth restriction. *Ultrasound in Obstetrics & Gynecology: The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2019;53(2): 200–207.
59. Badr DA, Carlin A, Kang X, et al. Evaluation of the new expert consensus-based definition of selective fetal growth restriction in monochorionic pregnancies. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2020;35(12):2338–2344.
60. Khalil A, Rodgers M, Baschat A, et al. ISUOG Practice Guidelines: role of ultrasound in twin pregnancy. *Ultrasound in Obstetrics & Gynecology: The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2016;47(2):247–263.
61. Australian Bureau of Statistics; Disability, Ageing and Carers, Australia: Summary of Findings. 2018. Available from: https://www.abs.gov.au/ausstats/abs@.nsf/Latestproducts/4430.0Main%20Features102018?opendocument&tabname=Summary&prodno=4430.0&issue=2018&num=&view=.
62. Slaghekke F, van Klink JM, Koopman HM, et al. Neurodevelopmental outcome in twin anemia-polycythemia sequence after laser surgery for twin-twin transfusion syndrome. *Ultrasound in Obstetrics & Gynecology: The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2014;44(3):316–321.
63. Lopriore E, Middeldorp JM, Oepkes D, et al. Twin anemia-polycythemia sequence in two monochorionic twin pairs without oligo-polyhydramnios sequence. *Placenta*. 2007;28(1):47–51.
64. Slaghekke F, Kist WJ, Oepkes D, et al. Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. *Fetal Diagnosis and Therapy*. 2010;27(4):181–190.

65. Mullin AP, Gokhale A, Moreno-De-Luca A, et al. Neurodevelopmental disorders: mechanisms and boundary definitions from genomes, interactomes and proteomes. *Transl Psychiatry*. 2013;3:e329.
66. Bozzi Y, Casarosa S, Caleo M. Epilepsy as a neurodevelopmental disorder. *Front Psychiatry*. 2012;3:19.
67. Stafstrom CE, Carmant L. Seizures and epilepsy: an overview for neuroscientists. *Cold Spring Harbor perspectives in medicine*. 2015;5(6):1–18.
68. Matthews M, Nigg JT, Fair DA. Attention deficit hyperactivity disorder. *Curr Top Behav Neurosci*. 2014;16:235–266.
69. Fayyad J, Sampson NA, Hwang I, et al. The descriptive epidemiology of DSM-IV adult ADHD in the World Health Organization World Mental Health Surveys. *Atten Defic Hyperact Disord*. 2017;9(1):47–65.
70. In Hay DAS L. Adaptation and maladaptation in the multiple birth family editor. In: Blickstein IK, LG, ed. *Multiple Pregnancy: Epidemiology, Gestation and Perinatal Outcome*. London: Parthenon Press; 2005:845–855.
71. McDougall MR, Hay DA, Bennett KS. Having a co-twin with attention-deficit hyperactivity disorder. *Twin Res Hum Genet*. 2006;9(1):148–154.
72. Association AP. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Arlington, VA: American Psychiatric Association; 2013.
73. Organisation WH. Autism spectrum disorders: World Health Organization; 2019. Available from: https://www.who.int/news-room/fact-sheets/detail/autism-spectrum-disorders.
74. Disabilities NCoBDaD. Comunity report on autism 2020. USA: Autism and Developmental Disabilities Monitoring (ADDM) network; 2020, 2020.
75. Bale TL. The placenta and neurodevelopment: sex differences in prenatal vulnerability. *Dialogues Clin Neurosci*. 2016;18(4):459–464.
76. Loomes R, Hull L, Mandy WPL. What is the male-to-female ratio in autism spectrum disorder? a systematic review and meta-analysis. *Journal of the American Academy of Child and Adolescent Psychiatry*. 2017;56(6):466–474.
77. Tick B, Bolton P, Happe F, et al. Heritability of autism spectrum disorders: a meta-analysis of twin studies. *Journal of Child Psychology and Psychiatry and Allied Disciplines*. 2016;57(5):585–595.
78. Willfors C, Carlsson T, Anderlid BM, et al. Medical history of discordant twins and environmental etiologies of autism. *Transl Psychiatry*. 2017;7(1):e1014.
79. Sandin S, Lichtenstein P, Kuja-Halkola R, et al. The familial risk of autism. *JAMA*. 2014;311(17):1770–1777.
80. Tollanes MC, Wilcox AJ, Stoltenberg C, et al. Neurodevelopmental disorders or early death in siblings of children with cerebral palsy. *Pediatrics*. 2016;138(2):1–11.
81. Mohandas N, Bass-Stringer S, Maksimovic J, et al. Epigenome-wide analysis in newborn blood spots from monozygotic twins discordant for cerebral palsy reveals consistent regional differences in DNA methylation. *Clin Epigenet*. 2018;10:25.
82. Smithers-Sheedy H, McIntyre S, Gibson C, et al. A special supplement: findings from the Australian Cerebral Palsy Register, birth years 1993 to 2006. *Developmental Medicine and Child Neurology*. 2016;58(Suppl 2):5–10.
83. Braun S, Bridler R, Muller N, et al. Inflammatory processes and schizophrenia: two independent lines of evidence from a study of twins discordant and concordant for schizophrenic disorders. *European Archives of Psychiatry and Clinical Neuroscience*. 2017;267(5):377–389.

84. Narayan CL, Shikha D, Shekhar S. Schizophrenia in identical twins. *Indian J Psychiatry*. 2015;57(3):323–324.
85. Fischer M. Psychoses in the offspring of schizophrenic monozygotic twins and their normal co-twins. *British Journal of Psychiatry*. 1971;118(542):43–52.
86. Onstad S, Skre I, Torgersen S, et al. Twin concordance for DSM-III-R schizophrenia. *Acta Psychiatrica Scandinavica*. 1991;83(5):395–401.
87. Milligan TA. Epilepsy: a clinical overview. *American Journal of Medicine*. 2021;134(7):840–847.
88. World Health Organisation. Epilepsy: World Health Organisation; 2022 [25/01/2022]. Available from: https://www.who.int/news-room/fact-sheets/detail/epilepsy.
89. Kjeldsen MJ, Corey LA, Christensen K, et al. Epileptic seizures and syndromes in twins: the importance of genetic factors. *Epilepsy Research*. 2003;55(1–2):137–146.
90. Canitano R. Epilepsy in autism spectrum disorders. *European Child & Adolescent Psychiatry*. 2007;16(1):61–66.
91. Williams AE, Giust JM, Kronenberger WG, et al. Epilepsy and attention-deficit hyperactivity disorder: links, risks, and challenges. *Neuropsychiatr Dis Treat*. 2016;12:287–296.
92. Cascella NG, Schretlen DJ, Sawa A. Schizophrenia and epilepsy: is there a shared susceptibility? *Neuroscience Research*. 2009;63(4):227–235.
93. Leddy MA, Power ML, Schulkin J. The impact of maternal obesity on maternal and fetal health. *Rev Obstet Gynecol*. 2008;1(4):170–178.
94. Chen S, Zhao S, Dalman C, et al. Association of maternal diabetes with neurodevelopmental disorders: autism spectrum disorders, attention-deficit/hyperactivity disorder and intellectual disability. *International Journal of Epidemiology*. 2021;50(2):459–474.
95. Brand JS, Lawlor DA, Larsson H, et al. Association between hypertensive disorders of pregnancy and neurodevelopmental outcomes among offspring. *JAMA Pediatr*. 2021;175(6):577–585.
96. Boulanger-Bertolus J, Pancaro C, Mashour GA. Increasing role of maternal immune activation in neurodevelopmental disorders. *Front Behav Neurosci*. 2018;12:230.
97. Nunez Estevez KJ, Rondon-Ortiz AN, Nguyen JQT, et al. Environmental influences on placental programming and offspring outcomes following maternal immune activation. *Brain, Behavior, and Immunity*. 2020;83:44–55.
98. Jiang NM, Cowan M, Moonah SN, et al. The impact of systemic inflammation on neurodevelopment. *Trends in Molecular Medicine*. 2018;24(9):794–804.
99. Bolte S, Girdler S, Marschik PB. The contribution of environmental exposure to the etiology of autism spectrum disorder. *Cellular and Molecular Life Sciences*. 2019;76(7):1275–1297.
100. Atladottir HO, Henriksen TB, Schendel DE, et al. Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. *Pediatrics*. 2012;130(6):e1447–e1454.
101. Baines KJ, Hillier DM, Haddad FL, et al. Maternal immune activation alters fetal brain development and enhances proliferation of neural precursor cells in rats. *Frontiers in immunology*. 2020;11:1–16.
102. Ratnayake U, Quinn T, Walker DW, et al. Cytokines and the neurodevelopmental basis of mental illness. *Front Neurosci*. 2013;7:180.
103. Nakamura JP, Schroeder A, Hudson M, et al. The maternal immune activation model uncovers a role for the Arx gene in GABAergic dysfunction in schizophrenia. *Brain, Behavior, and Immunity*. 2019;81:161–171.

104. Brown AS, Meyer U. Maternal immune activation and neuropsychiatric illness: a translational research perspective. *American Journal of Psychiatry*. 2018;175(11): 1073–1083.
105. Dotters-Katz SK, Patel E, Grotegut CA, et al. Acute infectious morbidity in multiple gestation. *Infectious Diseases in Obstetrics and Gynecology*. 2015;2015:173261.
106. Bauman MD, Van de Water J. Translational opportunities in the prenatal immune environment: promises and limitations of the maternal immune activation model. *Neurobiology of Disease*. 2020;141:104864.
107. Al-Haddad BJS, Jacobsson B, Chabra S, et al. Long-term risk of neuropsychiatric disease after exposure to infection in utero. *JAMA Psychiatry*. 2019;76(6):594–602.
108. Zerbo O, Iosif AM, Walker C, et al. Is maternal influenza or fever during pregnancy associated with autism or developmental delays? Results from the CHARGE (CHildhood Autism Risks from Genetics and Environment) study. *Journal of Autism and Developmental Disorders*. 2013;43(1):25–33.
109. Zerbo O, Qian Y, Yoshida C, et al. Maternal infection during pregnancy and autism spectrum disorders. *Journal of Autism and Developmental Disorders*. 2015;45(12): 4015–4025.
110. Na X, Phelan NE, Tadros MR, et al. Maternal obesity during pregnancy is associated with lower cortical thickness in the neonate brain. *Ajnr American Journal of Neuroradiology*. 2021;42(12):2238–2244.
111. Macedo F, Dos Santos LS, Glezer I, et al. Brain innate immune response in diet-induced obesity as a paradigm for metabolic influence on inflammatory signaling. *Front Neurosci*. 2019;13:342.
112. Auyeung B, Lombardo MV, Baron-Cohen S. Prenatal and postnatal hormone effects on the human brain and cognition. *Pflugers Archiv: European journal of physiology*. 2013;465(5):557–571.
113. Rivera HM, Christiansen KJ, Sullivan EL. The role of maternal obesity in the risk of neuropsychiatric disorders. *Front Neurosci*. 2015;9:194.
114. Edlow AG. Maternal obesity and neurodevelopmental and psychiatric disorders in offspring. *Prenatal Diagnosis*. 2017;37(1):95–110.
115. Godfrey KM, Reynolds RM, Prescott SL, et al. Influence of maternal obesity on the long-term health of offspring. *Lancet Diabetes Endocrinol*. 2017;5(1):53–64.
116. Li M, Fallin MD, Riley A, et al. The association of maternal obesity and diabetes with autism and other developmental disabilities. *Pediatrics*. 2016;137(2):e20152206.
117. Egeland GM, Irgens LM. Is a multiple birth pregnancy a risk factor for gestational diabetes? *American Journal of Obstetrics and Gynecology*. 2001;185(5):1275–1276.
118. Alsnes IV, Vatten LJ, Fraser A, et al. Hypertension in pregnancy and offspring cardiovascular risk in young adulthood: prospective and sibling studies in the HUNT study (Nord-Trondelag Health Study) in Norway. *Hypertension*. 2017;69(4):591–598.
119. Roberts CL, Algert CS, Morris JM, et al. Hypertensive disorders in pregnancy: a population-based study. *Medical Journal of Australia*. 2005;182(7):332–335.
120. Roberts CL, Ford JB, Algert CS, et al. Population-based trends in pregnancy hypertension and pre-eclampsia: an international comparative study. *BMJ Open*. 2011;1(1):e000101.
121. Logue OC, George EM, Bidwell 3rd GL. Preeclampsia and the brain: neural control of cardiovascular changes during pregnancy and neurological outcomes of preeclampsia. *Clinical Science (London, England: 1979)*. 2016;130(16):1417–1434.
122. Reynolds JM, Martinez GJ, Chung Y, et al. Toll-like receptor 4 signaling in T cells promotes autoimmune inflammation. *Proc Natl Acad Sci U S A*. 2012;109(32): 13064–13069.

123. El-Sayed AAF. Preeclampsia: a review of the pathogenesis and possible management strategies based on its pathophysiological derangements. *Taiwan J Obstet Gynecol*. 2017;56(5):593–598.
124. Sun W, Cui B, Hong F, et al. Establishment of ApoE-knockout mouse model of preeclampsia and relevant mechanisms. *Exp Ther Med*. 2016;12(4):2634–2638.
125. Denney JM, Nelson EL, Wadhwa PD, et al. Longitudinal modulation of immune system cytokine profile during pregnancy. *Cytokine*. 2011;53(2):170–177.
126. Mihu D, Razvan C, Malutan A, et al. Evaluation of maternal systemic inflammatory response in preeclampsia. *Taiwan J Obstet Gynecol*. 2015;54(2):160–166.
127. Ros HS, Cnattingius S, Lipworth L. Comparison of risk factors for preeclampsia and gestational hypertension in a population-based cohort study. *American Journal of Epidemiology*. 1998;147(11):1062–1070.
128. Bergman L, Nordlof-Callbo P, Wikstrom AK, et al. Multi-fetal pregnancy, preeclampsia, and long-term cardiovascular disease. *Hypertension*. 2020;76(1):167–175.
129. Chantanahom N, Phupong V. Clinical risk factors for preeclampsia in twin pregnancies. *PLoS One*. 2021;16(4):e0249555.
130. Campbell DM, MacGillivray I. Preeclampsia in twin pregnancies: incidence and outcome. *Hypertension in Pregnancy*. 1999;18(3):197–207.
131. Triche EW, Hossain N. Environmental factors implicated in the causation of adverse pregnancy outcome. *Seminars in Perinatology*. 2007;31(4):240–242.
132. Ekblad M, Korkeila J, Lehtonen L. Smoking during pregnancy affects foetal brain development. *Acta Paediatrica*. 2015;104(1):12–18.
133. Luck W, Nau H, Hansen R, et al. Extent of nicotine and cotinine transfer to the human fetus, placenta and amniotic fluid of smoking mothers. *Developmental Pharmacology and Therapeutics*. 1985;8(6):384–395.
134. Dempsey DA, Benowitz NL. Risks and benefits of nicotine to aid smoking cessation in pregnancy. *Drug Safety*. 2001;24(4):277–322.
135. Loukas MTRS, Abrahams PH, Carmichael SW. 2nd edGray's Anatomy Review2016 Philadelphia: Elsevier; 2020.
136. Blood-Siegfried J, Elizabeth KR. The long-term effects of perinatal nicotine exposure on neurologic development. *Journal of Midwifery & Womens Health*. 2010;55(2): 143–152.
137. Pollack H, Lantz PM, Frohna JG. Maternal smoking and adverse birth outcomes among singletons and twins. *American Journal of Public Health*. 2000;90(3):395–400.
138. Cramer C, Davidhizar R. FAS/FAE: impact on children. *Journal of Child Health Care*. 1999;3(3):31–34.
139. Ornoy A, Ergaz Z. Alcohol abuse in pregnant women: effects on the fetus and newborn, mode of action and maternal treatment. *International Journal of Environmental Research and Public Health*. 2010;7(2):364–379.
140. Wozniak JR, Riley EP, Charness ME. Clinical presentation, diagnosis, and management of fetal alcohol spectrum disorder. *Lancet Neurology*. 2019;18(8):760–770.
141. Chasnoff IJ. Fetal alcohol syndrome in twin pregnancy. *Acta Geneticae Medicae Et Gemellologiae*. 1985;34(3–4):229–232.
142. Astley Hemingway SJ, Bledsoe JM, Brooks A, et al. Twin study confirms virtually identical prenatal alcohol exposures can lead to markedly different fetal alcohol spectrum disorder outcomes—fetal genetics influences fetal vulnerability. *Adv Pediatr Res*. 2019;5(23):19.
143. Chen C, Song X, Wei W, et al. The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. *Nature Communication*. 2017;8(1):875.

144. Walker RW, Clemente JC, Peter I, et al. The prenatal gut microbiome: are we colonized with bacteria in utero? *Pediatr Obes.* 2017;12(Suppl 1):3–17.
145. Agrawal V, Hirsch E. Intrauterine infection and preterm labor. *Seminars in Fetal & Neonatal Medicine*. 2012;17(1):12–19.
146. Schoenmakers S, Steegers-Theunissen R, Faas M. The matter of the reproductive microbiome. *Obstet Med*. 2018;12(3):107–115.
147. Ralph SG, Rutherford AJ, Wilson JD. Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study. *BMJ*. 1999;319(7204):220–223.
148. Akintunde ME, Rose M, Krakowiak P, et al. Increased production of IL-17 in children with autism spectrum disorders and co-morbid asthma. *Journal of Neuroimmunology*. 2015;286:33–41.
149. Choi GB, Yim YS, Wong H, et al. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science*. 2016;351(6276):933–939.
150. Wang W, Sung N, Gilman-Sachs A, et al. T helper (Th) cell profiles in pregnancy and recurrent pregnancy losses: Th1/Th2/Th9/Th17/Th22/Tfh cells. *Front Immunol.* 2020;11:2025.
151. Wang WJ, Hao CF, Yi L, et al. Increased prevalence of T helper 17 (Th17) cells in peripheral blood and decidua in unexplained recurrent spontaneous abortion patients. *Journal of Reproductive Immunology*. 2010;84(2):164–170.
152. Nakashima A, Ito M, Shima T, et al. Accumulation of IL-17-positive cells in decidua of inevitable abortion cases. *American Journal of Reproductive Immunology*. 2010;64(1):4–11.
153. Lee SK, Kim JY, Lee M, et al. Th17 and regulatory T cells in women with recurrent pregnancy loss. *American Journal of Reproductive Immunology*. 2012;67(4):311–318.
154. Liu YS, Wu L, Tong XH, et al. Study on the relationship between Th17 cells and unexplained recurrent spontaneous abortion. *American Journal of Reproductive Immunology*. 2011;65(5):503–511.
155. Mazor M, Hershkovitz R, Ghezzi F, et al. Intraamniotic infection in patients with preterm labor and twin pregnancies. *Acta Obstetricia Et Gynecologica Scandinavica*. 1996;75(7):624–627.
156. Megli CJ, Coyne CB. Infections at the maternal-fetal interface: an overview of pathogenesis and defence. *Nature Reviews Microbiology*. 2022;20(2):67–82.
157. Ganguli S, Chavali PL. Intrauterine viral infections: impact of inflammation on fetal neurodevelopment. *Front Neurosci*. 2021;15:771557.
158. Hutton J, Rowan PJ. Vertical transmission and discordance of cytomegalovirus in twin pregnancies. *Front Cell Infect Microbiol*. 2021;11:676988.
159. Sobhani NC, Avvad-Portari E, Nascimento ACM, et al. Discordant Zika virus findings in twin pregnancies complicated by antenatal Zika virus exposure: a prospective cohort. *Journal of Infectious Diseases*. 2020;221(11):1838–1845.
160. Lorenz JM. Neurodevelopmental outcomes of twins. *Seminars in Perinatology*. 2012;36(3):201–212.
161. Bonellie SR, Currie D, Chalmers J. Comparison of risk factors for cerebral palsy in twins and singletons. *Developmental Medicine and Child Neurology*. 2005;47(9):587–591.

CHAPTER

Microbiome studies and twin research

36

Joohon Sung[a,b], **Hang A. Park**[a,c], **Soo Ji Lee**[a,b]

[a]*Genome and Health Big Data Lab, Graduate School of Public Health, Seoul National University, Seoul, Korea*

[b]*Institute of Health and Environment, Seoul National University, Seoul, Korea*

[c]*Department of Emergency Medicine, Hallym University Dongtan Sacred Heart Hospital, Hwaseong-si, Korea*

36.1 Basic concepts of the microbiome and microbial analysis: what are the differences between classical microbiology and microbiome studies?

It is estimated that about 30 trillion microbes (similar to the total number of human cells) inhabit an adult with 70 kg body weight. Thus, the number of microbial genes living in a human is hundreds of times more than that of human genes. Due to the enormous functional diversity of microbes, host, and microbes form an ecosystem, resembling a "rain forest." Each individual constitutes various shapes of microbial communities. The differences in the composition of this microbial community and their metabolites are thought to be one of the factors of disease susceptibility of the host and response to the treatment. Therefore, the study has increased dramatically to elucidate the biological role of microorganisms in human health over the past decade.

Microbes were found to contribute to the maintenance of homeostasis in the body by regulating host metabolism, by connecting in host immune or hormonal system through their metabolites.[1] Humans and microorganisms mainly have a symbiotic relationship exchanging complex chemicals or through metabolic interactions.[2] Digestion of dietary fibers into sugars is one of the representative examples of human-microbial symbiosis.[3] Although the human body does not have enzymes to digest complex carbohydrates such as fibers, it is an important energy source for the gut microbiota. Some anaerobic bacteria can metabolize fibers into short-chain fatty acids (SCFA) under specific conditions, which are then reabsorbed by the gut. The vital role of the short-chain fatty acid as anti-inflammation mediators in inflammatory bowel diseases (IBD) is now well understood.[4]

The maintenance of a healthy balance between the microorganism and the host is called eubiosis. For example, the gut commensal microbes are known to be involved in the formation of a physical barrier in the gut, either by making them resistant to

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00010-6

invasion by pathogenic bacteria, or by regulating the digestion and absorption of nutrients that provide energy to epithelial cells.[5–8] The disruption of the balance due to a change in the composition of the microbial community can lead to proliferation of pathogenic bacteria which is called dysbiosis. Many studies have suggested that dysbiosis may be directly related to the development of inflammatory or metabolic diseases such as obesity and cancer.[9,10] However, it is still difficult to clearly explain how changes in microbial composition and abundance lead to the development of common complex diseases.

Although the structure, function, and interactions of microorganisms play an important role in human metabolism, the identification, quantification, and characterization of microorganisms to identify them is quite difficult. The classical microbiology approach was based on cultivation-dependent techniques. This approach was often time-consuming and has been limited to the discovery of aerobic bacteria such as Escherichia coli, readily proliferating organisms in the laboratory, and majority of microbial species that have been difficult to culture. Moreover, the microbes that affect human health were thought to be pathogenic organisms, so the focus of classical microbiology was mainly on pathogens that occasionally caused devastating pandemics such as Plaque, cholera, or smallpox.[11–13] The presence of a wide variety of nonpathogenic micro-organisms, a.k.a "normal flora," was also well understood, but these were not the primary target of medical or clinical research.

In the 1980s, great progress was in microbiology research, with the development of culture-independent techniques, primarily PCR-based methods. The rapid development of DNA sequencing technology enabled researchers to identify numerous microbes at once, leading to a new field of "microbiome" (meaning a study of the? whole microbiota). The basic principle of this methodology is not to harvest bacterial DNA from isolated cultures in vitro, but rather to analyze DNA extracted directly from the samples. This allows researchers to investigate not only various aspects of the microbial communities but also the biological function of the whole microbiome. Notably, the Human Microbiome Project (HMP) was a landmark study that successfully characterized the taxonomic diversity and functional significance of the human microbiome with the support of the National Institute of Health (NIH).[13] However, the classical method of identifying microorganisms depended on visual (microscopic) identification is still useful when characterizing an important microorganism of interest. Table 36.1 compares the fundamental differences between the microbiome approach with the classical microbiology.

To summarize, microbiome is a research field studying microorganism as a whole, not limited to specific pathogens. Within relatively short period time, the microbiome studies have contributed to broaden our understanding of human health in view of microbial diversity and symbiosis-dysbiosis (=impaired symbiosis) between humans and microbes. Microbiome studies are still evolving to add new types of therapeutics for more and more human diseases and health conditions.

TABLE 36.1 Conceptual comparisons between classical microbiology and microbiome approach with an analogy to the genomics (*Mendelian disease** versus *common complex disease*** genomics).

	Conventional microbiology	**Microbiome**	**Human genome analogy**
Target microbes	Single of limited number of pathogens 	Numerous microbes including normal flora (Aiming at "total microbes") 	"Disease Genes" for Mendelian Disease are like "pathogens," whereas numerous "susceptibility loci" underlying common diseases are like "microbiome"
Methods for identification	Microscope and culture	DNA Sequencing and culturomics	Linkage studies (high penetrance) versus Association studies (low penetrance)
Ideas about the relationship between host and microbes	Microbes causes diseases and illness	Host and microbes constitute an ecosystem	Deterministic (high penetrance) versus probabilistic (low penetrance)
goals	Removing pathogens	Successful Symbiosis (disease susceptibility), restoring a healthy ecosystem	Diagnostic and therapeutic targets (both)
Methods for control	Antibiotics and vaccines	Pro- and prebiotics/Preserving diversity	Genotypic intervention (birth consult or gene therapy) versus phenotypic intervention (life-style modification) for the carriers

**Mendelian diseases in genomics is "a single-gene disorder that is heritable and has a very high probability of developing illness even at early stage of life." Polycystic kidney disease, hemophilia, and Huntington's disease are the typical examples of Mendelian diseases.*

***Common complex diseases are caused by multiple factors, including genes, environments, and lifestyles, together with the interactions within and between them. Heart diseases, cancers, dementia are the typical examples.*

36.2 Analytic approaches in microbiome studies

Starting with an early DNA-based method of extracting DNA of a microbial community of interest using fluorescence in situ hybridization (FISH), followed by the development of a polymerase chain reaction (PCR) method, and 16S ribosomal RNA (rRNA) gene-based microbial profiles became possible.[14] 16S rRNA is a bacteria-specific ribosome, essential for all known bacterial species. The 16S rRNA gene encodes 16S rRNA and known to have about 9 regions (hundreds of base pairs each) which are highly diverse, thus good targets to classify bacterial species. Taking advantage of this scientifically proven characteristics of 16S rRNA genes, 16S rRNA genes have been the "marker gene" to identify and classify bacteria in the first generation of microbiome studies.[15] The concept of identifying bacterial taxa using this region was proposed by Carl Woese in the 1970s,[16] and during 2001–2007, 215 novel bacterial species were identified using 16S rRNA gene sequencing, of which 29 were classified as new genera.[17] Oral/dental and gastrointestinal specimens were the most important reservoirs of new species and most anaerobes were found in the oral cavity and/or gastrointestinal tract.

The 16S rRNA gene has nine hypervariable regions (V1–V9) that can be used to distinguish taxa, including conserved regions. Rather than sequencing the entire 16S rRNA gene, deep sequencing of shorter sub-regions using massively parallel sequencing techniques of short read length was preferred.[18] Targeting only variable regions in the 16S rRNA genes is a "quick and dirty" method, compared with the whole DNA sequencing approach (called "whole metagenome sequencing (WMS)"). Except for the fact that WMS requires more analytic costs, the taxonomic results from WMS are generally more accurate than that from the 16S rRNA markers. In addition, there are multiple protocols by the choice of variable regions (out of 9 variable regions in the 16S rRNA genes) and sometimes inconsistency exists between different protocols of 16S rRNA marker analysis.[19–24]

Since the HMP project launched the "Phase 2, or iHMP (integrated HMP)" project in 2014,[25] WMS is rapidly replacing the above-mentioned 16S rRNA analysis. WMS utilizes fragments of the genomic DNA sequence obtained after breaking the whole genomic DNA of a sample. The sequence reads are compared against available databases either directly or after being assembled into continuous DNA fragments (= "contigs"). This allows the identification of *de novo* microbial genes not previous identified, and also has the advantage of predicting biological processes and pathways by comparing the genes to databases such as KEGG (the Kyoto Encyclopedia of Genes and Genomes).[26]

36.3 Assessing taxonomic composition, function, and diversity of microbial community

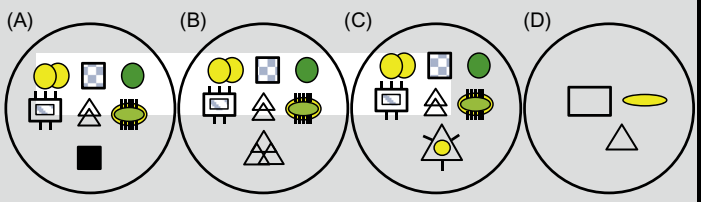
Frequently, the primary purpose of research lies in comparing the relative abundance of taxa between the host groups of interest. For instance, if certain bacterial species show

significant differences in relative abundance between IBD patients' stools and healthy controls, we can hypothesize that the microbes may be associated with the disease, IBD. The diversity of microbes is also an essential measure of the microbial ecosystem.

As shown in Box 36.1, there are two basic types of diversity: α-diversity is an index estimating how many "different" microbial taxa could be detected in a sample,

Box 36.1 Key terms and concepts of microbiome

- *Microbiology* usually studies specific (often one) microorganism(s) of interest, while *microbiome* studies *total microorganisms*, and tries to understand overall microbes. (e.g., gut microbiome) This "totality" is not limited to the realm of bacteria, and often includes virus, archaea, and fungi.
- *Diversity matters*: given the totality of the microbiome research, diversity is usually evaluated first prior to focusing on specific microbes. There are two types of measures for estimating diversity (Fig. 36.1). (1) α-*diversity (=within sample diversity)* measures how many different species exist within certain local area (in ecology), or host system (in health). (2) β-*diversity (=between sample diversity)* measures how many nonoverlapping (unique) taxa exists in a certain area, host, or system. For example, As shown in Fig. 36.1, each species of microbes contributes to α-diversity regardless whether the species are shared by other samples. For β-diversity, unique species are counted (in the formal measure of β-diversity, number of unique species are divided by the overall diversity). Two measures have their own usages: α-diversity is often used to show the biodiversity of the individual sample, and often interpreted as ecological healthiness. On the other hand, β-diversity is used to identify specific taxa which is associated with the individuals (e.g., disease association).
- *Understanding of microbiome relies on analytic technologies*: Estimating the presence and abundance of total microbial species is a big deal. Instead of identifying one-by-one by culture or laboratory tests, microbiome studies rely on "high-throughput" methods of analyzing DNA sequences at once. The analysis largely consists of (1) laboratory works of generating DNA sequence information (all microbes mixed up), (2) bioinformatics works (=computational work) to discriminate, identify, and quantify taxa. Theoretically microbiome analysis can capture "all microbes" in the sample, but in reality, this complexity often leads to a certain degree of uncertainty.



samples	A	B	C	D
α-diversity count	7	7	7	3
β-diversity contributors	1 ■	1 ▲	1 ✳	3 ■▲

FIG. 36 1 A graphical explanation of diversity measurements: α- and β-diversity. In the imaginary samples A–D, the figure shows how α-diversity and β-diversity are measured. (see the text for more explanations)

- *Taxa and function*: Microbial analyses start with taxonomy (=a study of identifying and classifying species). Functions of the species are often guessed from the taxa rather than direct assays. Of course, recent microbial studies are evolving to more advanced analyses where functional genes are simultaneously identified by full-range sequencing (a.k.a whole metagenomics sequencing) or by adding RNA (gene expression) analysis.
- *Microbiome and human health:* How microbiome affects human health and diseases is the key question in the microbiome studies. Recently, microbiome studies are trying to apply the knowledge into therapeutic measures to modify the course of diseases too. For example, it was discovered that long-term use of antibiotics depletes microbial community in the gut (low diversity), resulting in a favorable environment for pathogen *Clostrigium difficile* and *C. difficile* colitis a.k.a pseudomembranous enterocolitis (PE). From this knowledge "fecal transplantation"[a] very effectively cured often fatal PE. See the last part for more detail.

[a] Fecal transmission is a clinical practice to inoculates stools to the CDC patients (or extracts of stools) which had been taken from healthy individuals.

(=within sample diversity), whereas β-diversity is an index estimating how different is the microbial composition in one sample compared to another. (=between-host diversity). If the α-diversity of the sample is higher, it is generally regarded as a healthier ecosystem than lower diversity. Sometimes not only the number of species (species richness) but the abundance of species is considered (species evenness) in estimating the α-diversity. Formal β-diversity metrics measure the degree of variation between samples. Metrics of β-diversity also consist of two types, whether 1) only the number of species are counted (qualitative β-diversity, e.g., "Jaccard" or "unweighted UniFrac") or 2) abundance of species are also considered (quantitative β-diversity e.g., "Bray-Curtis" or "weighted UniFrac").[27]

Fig. 36.2 shows the overall process of microbiome analysis.[28–30]

36.4 Microbiome associations with human diseases and the application of the knowledge to the treatment

Our understanding of the microbiome is increasing rapidly thanks to the research efforts such as the HMP.[31,32] Recently the "integrative Human Microbiome Project (iHMP)," a new round of HMP was launched to aim for a deeper understanding of microbiomes' role in the pathogenesis of human health and diseases in 2014. The primary difference between the HMP and iHMP is the addition of multiomics data in the iHMP. In addition to existing 16S rRNA gene data and WMS, the iHMP has focused on analyzing meta-transcriptomics (whole microbial RNA sequence analysis), meta-proteomics (whole microbial protein analysis), and meta–metabolomics (whole microbial metabolites analysis) data of the microbiomes. The introduction of these "multiomics" data enabled researchers to understand how the microbiome interacts with human health and diseases in greater detail. Moreover, the mechanistic insight into the pathogenesis reveals potential targets of intervention.

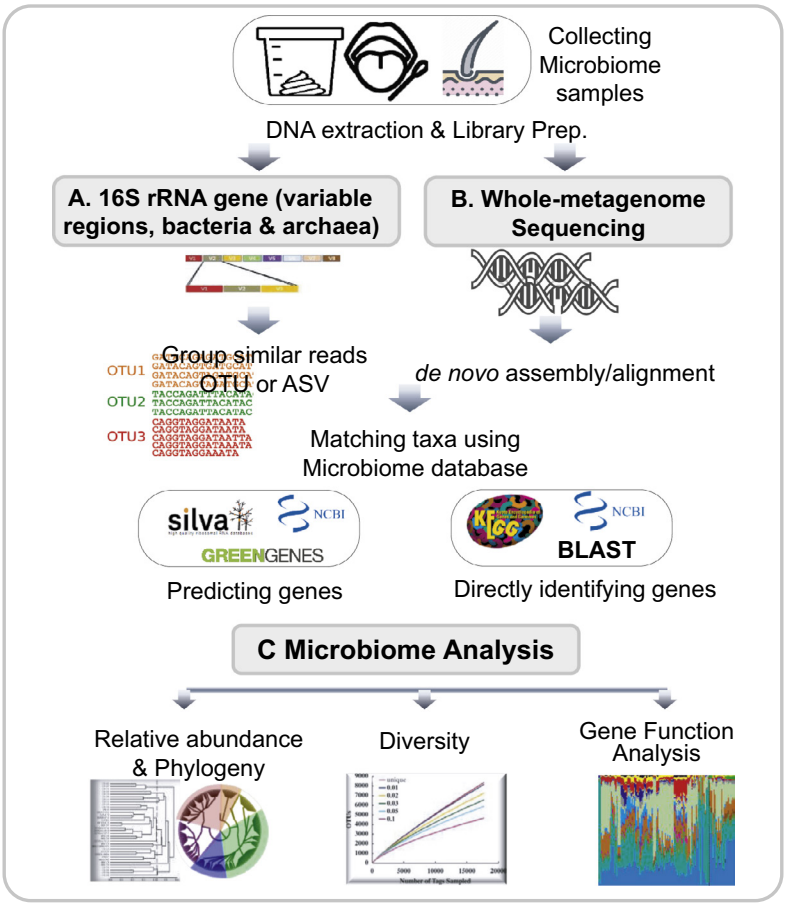


FIG. 36.2 A diagrammatic explanation about the process of microbiome analysis: after acquiring microbial biospecimens and DNA extraction, DNA sequences are analyzed either by targeting variable regions of 16S rRNA genes or by sequencing the whole genome (=whole metagenome analysis). (A) Because the 16S rRNA genes are specific to bacteria and archaea and nine variable regions bear information to differentiate taxa, 16S rRNA gene-based analysis provides a quicker and economical way of estimating taxa. Taxa are assessed based on limited genetic information, and it is called the "operational taxa unit (OTU)." Recently, amplicon sequence variant (ASV) clustering is simultaneously used with the OTU. (B) A whole metagenome sequencing (*WMS*) analyzes all genes of microbiota, including viruses and fungi. The process and informatics works are more challenging because the WMS data are much larger with more noises than the 16S rRNA gene analysis. (C) The relative abundance, particularly between the host groups of interest (e.g., between the obese and normal weight), provides a microbial association with diseases. Alpha- and beta-diversities are other vital indexes of the host-microbiome ecosystem. Functional analysis based on bacterial genes is frequently the aim of the whole analysis, revealing the mechanisms of host-microbiome interaction and potential targets of intervention. WMS analysis is costly but provides more reliable taxa and functional information. Note that quality controls are not mentioned.

Numerous microbiome studies have reported discoveries of the microbiome's roles and its importance in maintaining human health. One of the first examples is the microbiome and "pseudomembranous enterocolitis" caused by Clostridium difficile. Pseudomembranous enterocolitis is a severe and fatal infectious disease, which occurs in patients who take long-term antibiotic treatment. In the past, the application of higher doses and new types of antibiotics was the only possible treatment. However, an understanding of the etiology, that is, depletion of healthy microbes by using extensive long-term antibiotics, has led to an entirely new treatment method—the fecal microbiome therapy (FMT), previously a.k.a fecal transplantation. In 1989, Tvede et al. in Denmark successfully treated six C. difficile infection cases by FMT. In the same year, the first successful FMT treatment for Crohn's disease was also reported.[33] The list of "good" and "bad" bugs, as well as microbiome-related health conditions, are ever-increasing. To name a few examples: skin microbes are responsible for the development and severity of acne;[34] gut microbes modulate the gut serotonin secretion,[35] thus it may influence psychological symptoms mediated by neurotransmitter metabolism such as anxiety or depression;[36–38] gut microbes may modify the susceptibility of both type I and type II diabetes.[36,39–41] Armed with new evidence of the microbial roles in the pathogenesis of human health conditions, a novel class of therapeutic measures based on microbiome is also emerging. From the perspective of the microbiome, these intervention measures can be classified into pro-, pre-, and post-biotics. Definitions and examples of these are summarized in Table 36.2. Recently in the United States and EU, a new regulation called live biotherapeutic products (LBP) was introduced to regulate new therapeutic modalities involving live microorganisms.[42]

36.5 Twin research for microbiome studies

Twin research is generally a powerful study design in both identifying the degree of genetic contribution, as well as in identifying pure nongenetic contribution. It is particularly so in the area of microbiome studies (1) microbiome may have weaker effects on human diseases compared with lifestyle factors or genes;[43] (2) microbiome profiles are strongly influenced by many factors, particularly diet patterns or physical activities;[44–46] (3) microbiome profiles are known to have a weak direct association with genetics, but numerous genetic variations are strongly associated with lifestyle habits which again affects microbial profiles;[47,48] (4) age differences in the microbial profiles are well known;[49,50] (5) maternal microbiome is reported to be transferred to the newborns, of which influence is maintained until early adulthood.[49]

The degree to which microbiome profiles are influenced by genetic and environmental factors is an initial research question in the microbiome area. Here, twin studies are contributing to our understanding of what kind of microbial profiles, including viruses, might be heritable.[51] Interestingly, few microbes such as *genus Turucibacter, Intestinibacter*, or *Collinsella* show replicated evidence, at genus level, that their relative abundance is heritable across studies.[52] A genome-wide study about the beta

TABLE 36.2 Types of intervention measures to control human microbiome and examples of applications to health conditions.

Classification	Probiotics	Prebiotics	Postbiotics (metabiotics)	Others
Definition	Live microorganisms that when administered in adequate amounts confer a health benefit on the host	Compounds in food or supplements that induce the growth or activity of beneficial microbes	Substances released by microbes or by the lysis of microbes, which have bioactive function for the host	Direct or indirect control of microbes through other mechanisms
Examples	- Traditional food (kimchi, soy bean paste) - supplements: *Lactobacillus (L)* or *Bifidobacillus (B*) - FMT (fecal microbiota transplantation) - Ova of *Trichuris suis* (swine whipworm)	- Food components: fibers, oligosaccharides (fructans and galactans) - Various supplements intended to nourish beneficial microbes	- Various active metabolites (many are undisclosed for its chemical composition) - Supernatant of microbes - Lysis products of microbes	- Bacteriophage (phages are specific to bacteria, still conceptual)
Target conditions	- *(L)* or *(B):* various diseases, including irritable bowel syndrome, vaginitis, acne, autoimmune disease or general health promotion - FMT: PE, IBD	not specific	- Cardiovascular diseases: bacterial TMA inhibitor - Microbial metabolites for IBD - Bacteriocins (avidocin) for E coli-related urinary track infection	Atypical Mycobacterial infection of cystic fibrosis patients

FMT, fecal microbiome transplantation; *PE,* pseudomembranous enterocolitis; *IBD, i*nflammatory bowel disease.

diversity or relative abundance of microbes also failed to show reliable evidence that particular human genetic variants are linked to microbes, except for the Bifidobacterium and lactase gene variants.[48] The interpretation of twin studies indicates that the genetic control over microbiome profile may be weak. However, the "lack of strong heritability for microbiome profile" might not be conclusive because the current genome-microbiome studies have been limited in size, mainly due to the cost. A recent report from a large consortium reported more suggestive evidence of genetic controls over the microbial profile, in addition to the human lactase-Bifidobacterium

Link such as chromosome 3 short arm 25.1 region and genus *Gastranaerophilales*; chromosome 3 short arm 24.3 region and *genus Peptococcus*; and chromosome 4 short arm 15.33 region and *genus Intestinibacter*.[52]

Notably, differences, rather than the resemblance, within MZ pairs are often of utmost interest when nongenetic factors are studied. This study design is called a "cotwin-control study," stemming from the term of case-control study,[b] that is, a cotwin-control study replaces the control with the cotwin of the case. This study design is only plausible when the cases are twins and discordant (i.e., one cotwin has the disease, and the other cotwin is healthy). Studies comparing siblings[c] have been conducted more frequently due to their relative abundance and accessibility compared to twins. The cotwin-control study of MZs, albeit scarce, has a unique power in contrasting nonoverlapping environments. The cotwin-control study can be planned and conducted at several levels: (1) when the risk factors are different (analogous to a cohort study design in the epidemiologic study), exposed twin versus nonexposed cotwin. (2) when the health outcome of interest is different (analogous to a case-control study design), patients are compared with their cotwins. (3) when a differential intervention is tested to compare the therapeutic effects. Fig. 36.3. Illustrates the strength of cotwin-control study design compared with conventional study designs such as case-control studies or cohort studies.

The cotwin-control study is particularly compelling for the studies of "omics" studies, including the microbiome, which typically has thousands of measures with strong genetic influences. For example, epigenomic profiles of DNA-methylation are under strong genetic influence, so that more than 30,000 CpG sites are regulated by genetic variants.[53] For example, when a researcher is interested in detecting epigenetic changes due to smoking habits, small differences in the average genetic constitution between two groups would result in unwanted differences in the epigenetics level.

The above-mentioned cotwin-control study designs have been adopted for the microbiome research area. The first study widely accepted as the human evidence of microbial contribution involved twins and adopted the cotwin-control design: suggesting that microbial profile may be associated with human obesity.[54] These findings about "obesogenic germ" was further proved by transplanting the stools of lean and obese cotwins into mice respectively and proved that transplanted mouse got leaner or fatter.[55] Somewhat mystified[d] but still well-replicated findings about "good (Bacteriodetes) and bad (Firmicutes) bugs" for weight control are originated from this cotwin-control study. It is not surprising that reliable evidence about the

[b] A typical case-control study recruit patients (=cases) and normal references (= controls) to understand the etiology of the disease by a comparison between the two.

[c] "Sibling" means a genetic relationship regardless of their sex, including sisters and brothers.

[d] The *Bacteriodetes (*"good bugs") or *Firmicutes* ("bad bugs") were measured at phylum level, which should include striking diversity within them at species or even at family level (note that its analogy can be "all vertebrate animals are good to human while all invertebrate animals are harmful"). The so-called B/Fratio (the ratio of *Bacteriodetes to Firmicutes*) has not been substantiated as a measure of obesity or predicting obesity.

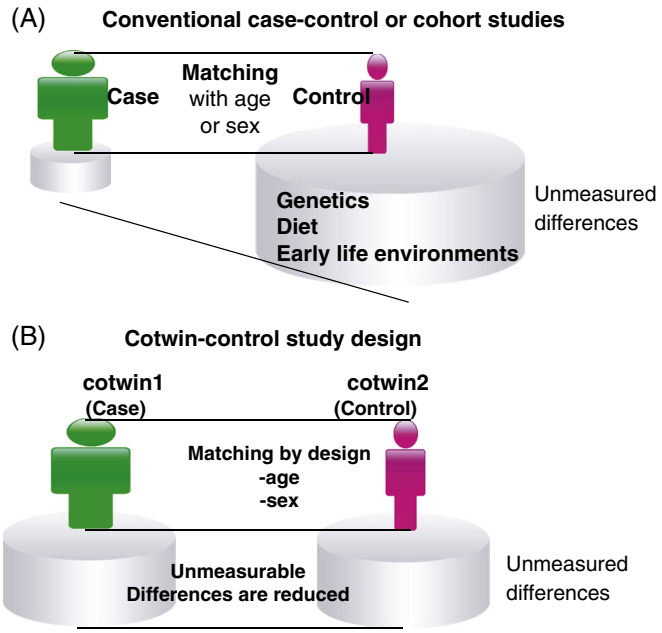


FIG. 36.3 Illustrated example of explaining conventional study designs (case-control or cohort) versus cotwin-control study design. Note that even after matching with "measurable" variables such as age, sex, and other important factors, there may still exist numerous unmeasurable factors which have associations with the disease of interest in the conventional approach. The cotwin-control study, by design, cancels off the majority of even unmeasured differences.

role of the microbiome and human-microbe ecosystem has been studied applying the cotwin-control design, expanding its use to more vaguely understood area of virus.[56,57] Findings from microbiome twin research provided true so that the initial observations were further verified by functional and mechanistic studies how microbiota induces ulcerative colitis.[58] For more complex interactions between host genetics microbiome and metabolic traits, cotwin-control design was also applied to prove that *Prevotella species* modifies the nonalcoholic fatty liver diseases for the obese.[59,60] In addition, classical twin study design estimating heritability provided valuable information of the host genetic influences on the microbiome profile.[48] Box 36.2 summarized the key discoveries of the microbiome are according to the type of twin study designs.

Box 36.2 Key findings of the microbiome through twin research

- Microbiome profiles are more similar between MZT than DZT (classical twin study design).
- Existence or relative abundance of specific taxa, however, does not show reliable genetic influences, i.e., heritability (classical twin study design).
- Human genetic variants of lactase genes are linked to the abundance of Bifidobacterium spp, but very few species show genetic associations (genome-wide association study, twin adaptation).
- Microbes play vital roles in the development and sequel of obesity and metabolic syndrome, including NAFLD and type I/type II diabetes (cotwin-control study design).
- A range of diseases show an association between microbes and diseases (cotwin-control study design).
- Diet, physical activity, and lifestyle environments also participate in the human-microbiome ecosystem (cotwin-control study design).

Abbreviation: *MZT, monozygotic twins; DZT, dizygotic twins; NAFLD, nonalcoholic fatty liver disease.*

36.6 Summary and conclusion

A growing body of evidence indicates that the microbiome and its interaction with humans are essential in maintaining health. Thanks to the recent development of genetic analysis and bioinformatics, our understanding of this essential ecosystem is advancing. Twin research and its study design have played an essential role in estimating the genetic influences (e.g., heritability or genome-wide association studies) and high-quality evidence of microbes associated with the susceptibility of diseases. The knowledge from the microbiome research is now used for the development of new therapeutic measures, which may provide a new hope to conquer intractable diseases.

References

1. Schroeder BO, Backhed F. Signals from the gut microbiota to distant organs in physiology and disease. *Nature Medicine*. 2016;22:1079–1089.
2. Dorrestein PC, Mazmanian SK, Knight R. Finding the missing links among metabolites, microbes, and the host. Immunity. 2014;40(6):824–832.
3. Makki K, Deehan EC, Walter J, et al. The impact of dietary fiber on gut microbiota in host health and disease. *Cell Host Microbe*. 2018;23:705–715.
4. Maslowski KM, Vieira AT, Ng A, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature*. 2009;461:1282–1286.
5. Sekirov I, Russell SL, Antunes LC, et al. Gut microbiota in health and disease. *Physiological Reviews*. 2010;90:859–904.
6. Bäumler AJ, Sperandio V. Interactions between the microbiota and pathogenic bacteria in the gut. Nature. 2016;535(7610):85–93.

7. Ming L, Zhang Q, Yang L, et al. Comparison of antibacterial effects between antimicrobial peptide and bacteriocins isolated from Lactobacillus plantarum on three common pathogenic bacteria. *International journal of clinical and experimental medicine*. 2015;8:5806–5811.
8. Ramakrishna BS. Role of the gut microbiota in human nutrition and metabolism. *Journal of Gastroenterology and Hepatology*. 2013;28(Suppl 4):9.–17.
9. Clemente JC, Ursell LK, Parfrey LW, et al. The impact of the gut microbiota on human health: an integrative view. *Cell*. 2012;148:1258–1270.
10. Brown CT, Sharon I, Thomas BC, et al. Genome resolved analysis of a premature infant gut microbial community reveals a Varibaculum cambriense genome and a shift towards fermentation-based metabolism during the third week of life. *Microbiome*. 2013;1:30.
11. Piret J, Boivin G. Pandemics Throughout History. Front Microbiol. 2021;11:631736.
12. Opal SM. A Brief History of Microbiology and Immunology. In: Artenstein, A. (eds) *Vaccines: A Biography*. Springer, New York, NY. 2010:31–56. https://doi.org/10.1007/978-1-4419-1108-7_3.
13. Malla MA, Dubey A, Kumar A, et al. Exploring the human microbiome: the potential future role of next-generation sequencing in disease diagnosis and treatment. *Frontiers in immunology*. 2018;9:2868.
14. Olsen GJ, Lane DJ, Giovannoni SJ, et al. Microbial ecology and evolution: a ribosomal RNA approach. *Annual Review of Microbiology*. 1986;40:337–365.
15. Bergey's Manual® of Systematic Bacteriology. 2 ed: Springer, New York, NY. XXII, 722 p.
16 Woese CR, Fox GE. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *PNAS*. 1977;74:5088–5090.
17. Woo PC, Lau SK, Teng JL, et al. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. *Clinical Microbiology and Infection*. 2008;14:908–934.
18. Claesson MJ, Wang Q, O'Sullivan O, et al. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. *Nucleic Acids Res*. 2010;38:e200.
19. Claesson MJ, O'Sullivan O, Wang Q, et al. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. *PLoS One*. 2009;4:e6669.
20. Hamady M, Knight R. Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. *Genome Research*. 2009;19:1141–1152.
21. Huse SM, Dethlefsen L, Huber JA, et al. Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. *Plos Genetics*. 2008;4:e1000255.
22. Liu Z, DeSantis TZ, Andersen GL, et al. Accurate taxonomy assignments from 16S rRNA sequences produced by highly parallel pyrosequencers. *Nucleic Acids Res*. 2008;36:e120.
23. Nossa CW, Oberdorf WE, Yang L, et al. Design of 16S rRNA gene primers for 454 pyrosequencing of the human foregut microbiome. *World Journal of Gastroenterology*. 2010;16:4135–4144.
24. Soergel DA, Dey N, Knight R, et al. Selection of primers for optimal taxonomic classification of environmental 16S rRNA gene sequences. *The ISME journal*. 2012;6:1440–1444.
25. Integrative H. The integrative human microbiome project. *Nature*. 2019;569:641–648.
26. Kanehisa M. *Post-Genome Informatics*. Oxford: OUP; 2000.

27. Goodrich JK, Di Rienzi SC, Poole AC, et al. Conducting a microbiome study. *Cell*. 2014;158:250–262.
28. Venter JC, Remington K, Heidelberg JF, et al. Environmental genome shotgun sequencing of the Sargasso Sea. *Science*. 2004;304:66–74.
29. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010;464:59–65.
30. Truong DT, Franzosa EA, Tickle TL, et al. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. *Nature Methods*. 2015;12:902–903.
31. Huttenhower C, Gevers D, Knight R, et al. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486:207–214.
32. Lloyd-Price J, Mahurkar A, Rahnavard G, et al. Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature*. 2017;550:61–66.
33. Bennet JD, Brinkman M. Treatment of ulcerative colitis by implantation of normal colonic flora. Lancet. 1989;1(8630):164.
34. Belkaid Y, Segre JA. Dialogue between skin microbiota and immunity. Science. 2014;346(6212):954–959.
35. Yano JM, Yu K, Donaldson GP, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*. 2015;161:264–276.
36. Wen L, Ley RE, Volchkov PY, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature*. 2008;455:1109–1113.
37. Zheng P, Zeng B, Zhou C, et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Molecular Psychiatry*. 2016;21:786–796.
38. Simpson CA, Diaz-Arteche C, Eliby D, et al. The gut microbiota in anxiety and depression—a systematic review. *Clinical Psychology Review*. 2021;83:101943.
39. Murri M, Leiva I, Gomez-Zumaquero JM, et al. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. *Bmc Medicine [Electronic Resource]*. 2013;11:46.
40. Larsen N, Vogensen FK, van den Berg FW, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One*. 2010;5:e9085.
41. Vatanen T, Franzosa EA, Schwager R, et al. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. *Nature*. 2018;562:589–594.
42. Cordaillat-Simmons M, Rouanet A, Pot B. Live biotherapeutic products: the importance of a defined regulatory framework. *Experimental & Molecular Medicine*. 2020;52:1397–1406.
43. Manor O, Dai CL, Kornilov SA, et al. Health and disease markers correlate with gut microbiome composition across thousands of people. *Nature Communication*. 2020;11:5206.
44. Louis P, Scott KP, Duncan SH, et al. Understanding the effects of diet on bacterial metabolism in the large intestine. *Journal of Applied Microbiology*. 2007;102:1197–1208.
45. Sonnenburg ED, Zheng H, Joglekar P, et al. Specificity of polysaccharide use in intestinal bacteroides species determines diet-induced microbiota alterations. Cell. 2010;141(7):1241–1252.
46. Santacruz A, Marcos A Fau - Wärnberg J, Wärnberg J Fau - Martí A, et al. Interplay between weight loss and gut microbiota composition in overweight adolescents. (1930-7381 (Print)).
47. Spor A, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews Microbiology*. 2011;9:279–290.

48. Goodrich JK, Davenport ER, Beaumont M, et al. Genetic determinants of the gut microbiome in UK twins. *Cell Host Microbe*. 2016;19:731–743.
49. Nicholson JK, Holmes E, Kinross J, et al. Host-gut microbiota metabolic interactions. *Science*. 2012;336:1262–1267.
50. Mariat D, Firmesse O, Levenez F, et al. The firmicutes/bacteroidetes ratio of the human microbiota changes with age. *Bmc Microbiology*. 2009;9:123.
51. Goodrich JK, Waters JL, Poole AC, et al. Human genetics shape the gut microbiome. Cell. 2014;159(4):789–799.
52. Kurilshikov A, Medina-Gomez C, Bacigalupe R et. al. Large-scale association analyses identify host factors influencing human gut microbiome composition. Nat Genet. 2021. 53(2):156–165.
53. Gaunt TR, Shihab HA, Hemani G, et al. Systematic identification of genetic influences on methylation across the human life course. *Genome biology*. 2016;17:61.
54. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. *Nature*. 2009;457:480–484.
55. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013;341(6150):1241214.
56. Reyes A, Haynes M, Hanson N, et al. Viruses in the faecal microbiota of monozygotic twins and their mothers. *Nature*. 2010;466:334–338.
57. Moreno-Gallego JL, Chou SP, Di Rienzi SC, et al. Virome Diversity Correlates with Intestinal Microbiome Diversity in Adult Monozygotic Twins. *Cell Host Microbe*. 2019;25:261–272 e5.
58. Lepage P, Hasler R, Spehlmann ME, et al. Twin study indicates loss of interaction between microbiota and mucosa of patients with ulcerative colitis. *Gastroenterology*. 2011;141:227–236.
59. Lim MY, You HJ, Yoon HS, et al. The effect of heritability and host genetics on the gut microbiota and metabolic syndrome. *Gut*. 2017;66:1031–1038.
60. Si J, You HJ, Yu J, Sung J, Ko G. Prevotella as a Hub for Vaginal Microbiota under the Influence of Host Genetics and Their Association with Obesity. Cell Host Microbe. 2017;21(1):97–105.

CHAPTER

Chromosomal anomalies, monogenetic diseases, and leukaemia in twins

37

Irén Haltrich, Anna Lengyel
2nd Department of Pediatrics, Semmelweis University, Budapest, Hungary

37.1 Genetic background

Chromosomal abnormalities (CA) are microscopically visible DNA changes affecting either the number and/or the structure of chromosomes. Human somatic cells are diploid, which means that they each carry 23 pairs of chromosomes: 22 pairs of autosomes and 1 pair of sex chromosomes (XX in females and XY in males). We inherit one set of 23 chromosomes from our mother and the other set from our father. This numerically and structurally characteristic diploid set of chromosomes constitutes an individual's karyotype (Fig. 37.1). We carry two copies of every autosomal gene on each pair of the chromosomes (the copy inherited from our mother is called the "maternal allele" of the gene, whereas the one from our father is the "paternal allele"; Fig. 37.2).

Chromosomal disorders comprise two main groups: numerical (i.e., the presence of an abnormal number of chromosomes in cells) and structural aberrations (translocations, deletions, duplications, inversions, and complex rearrangements). Amongst the numerical abnormalities, a group termed as "aneuploidies" are relevant to this chapter: aneuploidies are defined as the gain or loss of one or more chromosomes. These CAs arise from faults in cell division (chromosomal nondisjunction: when the homologous chromosomes or sister chromatids fail to separate during cell division; Fig. 37.2); meanwhile, structural CAs are generated by the misrepair of DNA double-strand breaks.

Various pathogenetic mechanisms can lead to disease, as CAs can affect one or more genes in a complex manner:

a. Gene dosage effects: aneuploidies, deletions, and duplications alter the dosage of the genes localized within the CA.
b. The breakpoint of the CA may directly disrupt a gene or various regulatory elements.

Twin Research for Everyone. DOI: https://doi.org/10.1016/B978-0-12-821514-2.00008-8

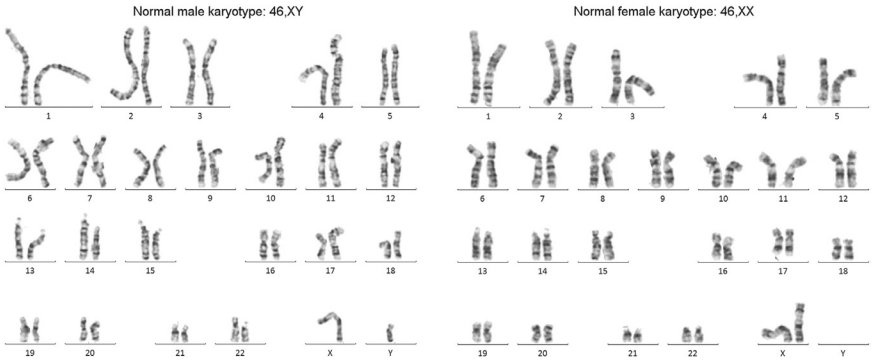


FIG. 37.1 Normal male and female Giemsa-banded karyotypes.

c. Positional effects: rearrangements can relocalize/truncate/fuse entire genes, or disrupt the normal genomic architecture; which can significantly change the regulation of gene expression and/or gene-gene interactions.
d. Epigenetic mechanisms, for example, genomic imprinting. *(See Chapter 34).*

CAs are discovered in approximately 15% of patients with multiple congenital abnormalities and/or intellectual disability; and in up to 30%–50% of fetal loss and still births. CAs do not occur more frequently in twins compared to singletons (overall prevalence in twins: 0.16%–0.63%).[1–4] Individual CAs are too infrequent for reliable statistical calculations; with the possible exception of Down syndrome (DS; estimated prevalence: 0.1%–0.2%).[1–7] Data regarding the risk of DS specifically are somewhat contradictory: multiple studies have reported that MZ pregnancies have a lower risk for DS per twin[7,8]; however, a third study observed only a tendency for a lower risk, but no statistically significant difference compared to singletons.[4]

Monogenic disorders result from genetic modifications of a single gene and are classified according to their inheritance pattern:

a. Autosomal dominant: a genetic change in one allele of the gene is sufficient for the manifestation of the disease.
b. Autosomal recessive: both alleles of the gene must be altered; individuals with one pathologic allele are referred to as "disease carriers."
c. X-linked disorders: caused by mutations in genes found on the X chromosome, inheritance can be either dominant or recessive. We will discuss this in more detail later.

The global prevalence of monogenic disorders is approximately 1%, and some have been reported more frequently in twins.[9]

Genetic mosaicism and chimerism: we must define and differentiate between two further genetic phenomena relevant to the topic of twin discordance. *Mosaicism* occurs when one individual carries two or more genetically distinct cell lines *that originated from a single zygote.* Mosaicism is reported as the percentage of the different cell lines, and the levels may differ between tissues. *Chimerism* occurs when

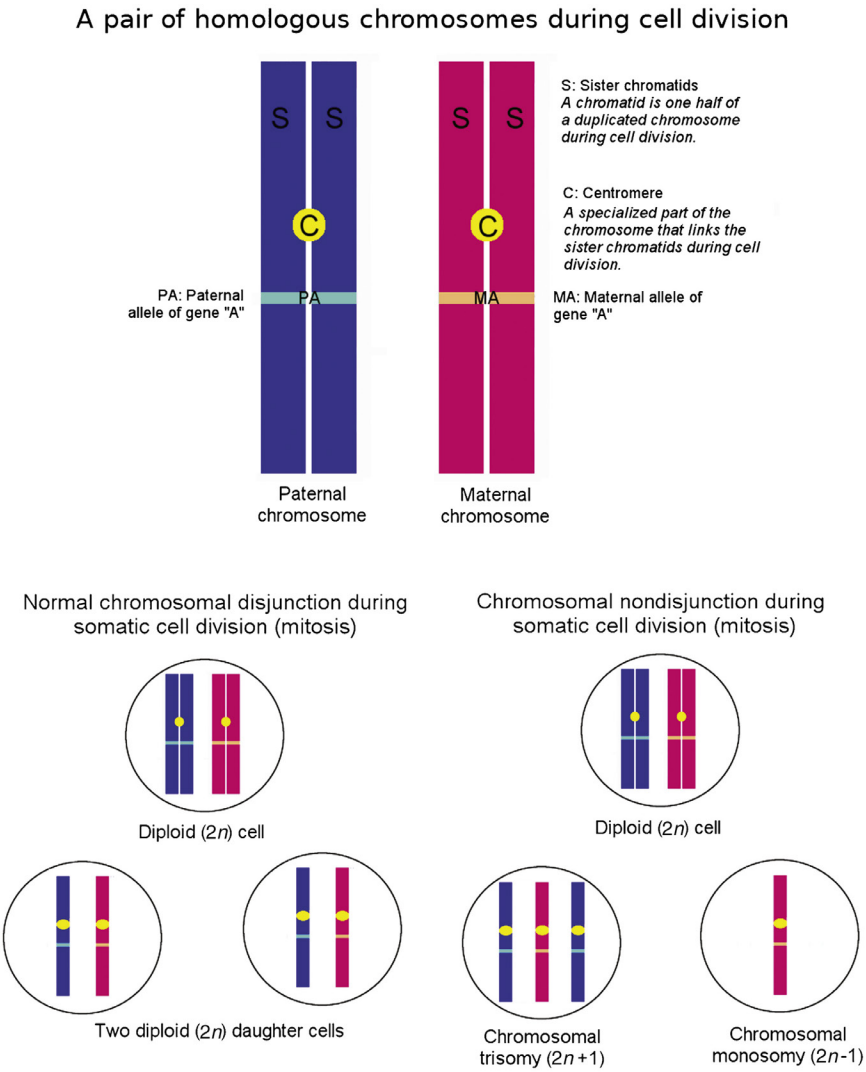


FIG. 37.2 Schematic figure of a pair of homologous chromosomes during cell division, normal chromosomal disjunction, and chromosomal nondisjunction.

one individual carries multiple genetically distinct cell lines *that originated from multiple zygotes.*

Genetic testing methods utilized in clinical practice include traditional Giemsa (G-) banding and fluorescent in situ hybridization (FISH). During G-banding dividing cells (routinely peripheral blood lymphocytes) are stained with a special dye that results in banded chromosomes, each recognizable by its size and band pattern (Fig 37.1). The entire karyotype of a cell is visualized in a microscope, and allows

for the identification of aneuploidies and larger structural abnormalities (resolution: 5–10 million base pairs). The FISH technique uses fluorescent DNA probes that bind to specific regions of chromosomes. This allows better sensitivity and resolution (depending on the length of the specific probes, but at least an order of a magnitude higher than G-banding), and is very useful in cases of mosaicism; however, it is not a genome-wide method (in practice this means that FISH is indicated only if the patient's clinical presentation is highly suggestive of a specific disorder). Chromosomal microarrays (CMA) represent the first-line modern technique recommended in cases suspicious for a CA, but without a recognizable "syndromic" phenotype. These methods investigate the entire genome in a potentially very high resolution, and can identify copy number changes as small as 1000 base pairs, but are unable to detect balanced structural anomalies (where there is no change in DNA copy number), and their usefulness in mosaicism is limited. Nucleotide level changes, gene mutations are identified by sequencing methods, which can be targeted (one specific gene or a gene panel in genetically related disorders) or genome-wide (whole genome/exome sequencing).

37.2 Mechanisms of twin discordance

As mentioned above, the study of discordant MZ twins has been and continues to be, extremely valuable in genetic research. Twins can be discordant phenotypically, in which case they each have the genetic disorder in question; however, they present with different types/severity of symptoms. There is an abundance of reports of genetically discordant twins as well.[10] We will discuss the underlying mechanisms through examples of relatively common genetic conditions; relevant genotypic and phenotypic information of the mentioned disorders are listed in Table 37.1.

37.3 Postzygotic chromosomal nondisjunction and chromosomal mosaicism

a. Early postzygotic nondisjunction can lead to heterokaryotic monozygosity, which means that the developing twins will have different karyotypes.[10,11] This mechanism has often been described in regards to twins discordant for common aneuploidies: Down syndrome (Fig. 37.3) and Turner syndrome (TS; Fig. 37.4). An early report from 1982 was of twin boys, one of whom had typical DS, whereas his monochorionic (MC) twin brother was phenotypically unaffected. Both children showed 47,XY,+21/46,XY mosaicism in peripheral blood, however, the skin fibroblasts of the DS boy were 100% trisomic for chromosome 21, while the unaffected brother's fibroblasts were 100% normal. A similar genetic background was described in a pair of twin girls discordant for TS: analysis of blood lymphocytes showed similar levels of 45,X/46,XX mosaicism in both children, however the affected girl had 99% 45,X cells

TABLE 37.1 Main clinical characteristics of the discussed genetic disorders.

Disorder	Genetic background	Main features
Down syndrome	Trisomy 21: an extra (full or partial) copy of chromosome 21 Non-mendelian inheritance	• Developmental delay (DD) and short stature • Intellectual disability (ID; mild to moderate) • Behavioral disorders • Characteristic minor anomalies: *flattened face and occipital region, up-slanted almond-shaped eyes, short neck, small ears, small hands and feet, single palmar crease, loose joints, sandal gap between I.-II. toes* • Weak muscle tone (infants) • Variable structural birth defects: *heart defects (~50% of individuals), defects of the digestive tract* • Increased risk for diseases: *vision problems, hearing loss, hypothyroidism, gastro-esophageal reflux, celiac disease, leukemia, testicular germ cell tumors, Alzheimer's disease*
Turner syndrome	Monosomy X: (full or partial) absence of the second sex chromosome Non-mendelian inheritance	• Short stature • Intelligence is mostly normal (*learning difficulties, behavioral problems may be present*) • Early loss of ovarian function, infertility • Absent puberty • Characteristic minor anomalies: *webbed neck, puffiness of hands and feet, low posterior hairline, cubitus valgus/genu valgum (forearm/lower leg angled away from the body)* • Variable structural birth defects: *skeletal abnormalities, kidney defects, heart defects*
Fragile X syndrome	Triplet repeat mutation in *FMR1* gene XD inheritance	• Males are more severely affected • DD, ID/learning difficulties • Behavioral disorders: *autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), anxiety, impulsivity* • Seizures • Characteristic minor anomalies: *long, narrow face, large ears, prominent forehead and jaw, flat feet* • Males often have enlarged testicles

(*Continued*)

TABLE 37.1 Main clinical characteristics of the discussed genetic disorders. *Continued*

Disorder	Genetic background	Main features
Duchenne muscular dystrophy	Mutations in the *DMD* gene XR inheritance	• Progressive muscle disorder • Early signs: motor and speech DD, worsening muscle wasting and weakness, enlarged calves, clumsiness, waddling gait, Gower's sign: *due to lower limb weakness, children need to push themselves up with their hands from a squatting position* • Complications: scoliosis, joint contractures, enlarged heart, inability to walk, breathing difficulties
Beckwith–Wiedemann syndrome	Variable genetic/epigenetic alterations relating to chromosome 11p15.5 Inheritance pattern depends on the genetic alteration	• Pre- and post-natal overgrowth, which slows down by adolescence (adult height is usually normal) • Lateralized overgrowth: one side of the body is larger • Abnormally large organs • Additional signs: *abnormally large tongue, facial naevus, ear creases or pits, neonatal hyperinsulinism or transient hypoglycaemia, omphalocele, kidney abnormalities* • Increased risk for certain childhood tumors (*predominantly Wilms tumor and hepatoblastoma*)
22q11.2 deletion syndrome (*DiGeorge syndrome, velocardiofacial syndrome and other related syndromes are grouped under this term*)	Microdeletion of chromosome region 22q11.2 AD inheritance	• Great phenotypic variability • Heart defects • Immunodeficiency, recurrent infections • Submucosal cleft palate • Hearing problems • Kidney abnormalities • Hypercalcaemia • Feeding difficulties • Breathing problems • DD, ID/learning difficulties • Behavioral disorders: *ASD, ADHD* • Short stature

AD, autosomal dominant; XD, X-linked dominant; XR, X-linked recessive; FMR1, FMRP Translational Regulator 1; DMD, Dystrophin.

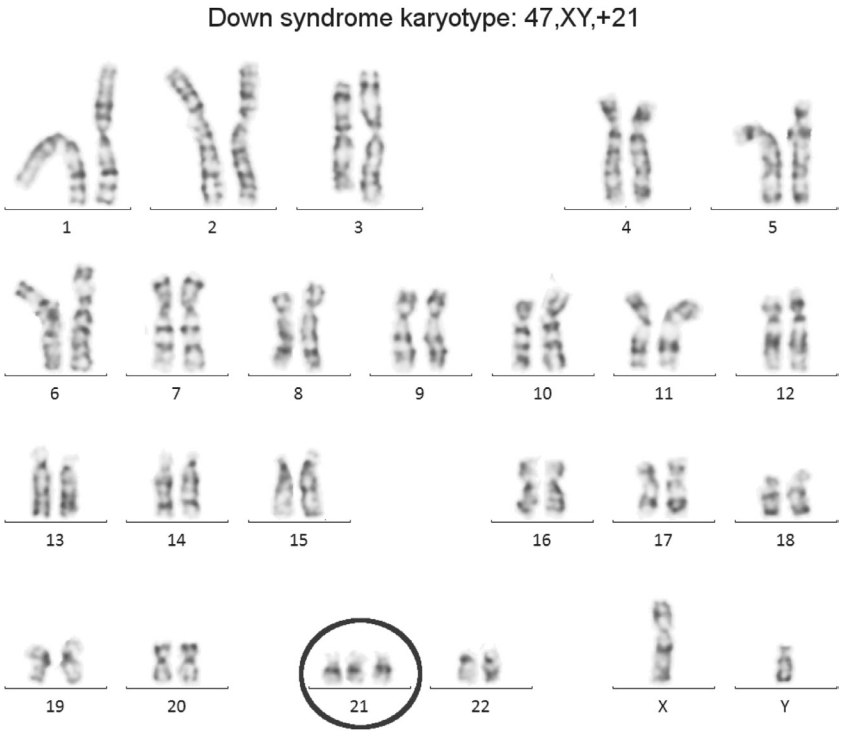


FIG. 37.3 Down syndrome Giemsa-banded karyotype.

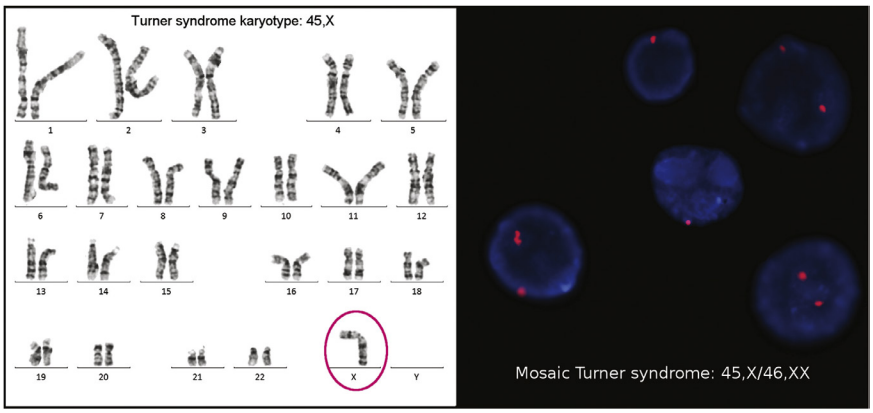


FIG. 37.4 Turner syndrome Giemsa-banded karyotype (left), and fluorescent in situ hyridization (FISH) pattern of monosomy X mosaicism (right).

On the right image, the X chromosome centromeres are fluorescently marked with specific probes. The cells with two red signals are the normal 46,XX cells, and the cells with one red signal are the monosomic (TS) cells.

in buccal smear, in contrast to the unaffected twin with 98% normal 46,XX cells. These cases are attributed to chromosomal nondisjunction leading to two separate cell lines, which then developed into genetically discordant twins. The blood "mosaicism" seen in the healthy twins is due to intrauterine mixing of blood via placental vascular anastomoses, and actually represent cases of chimerism.[12,13] Several similar cases have been described in the literature.[10,14–17]

Here we must highlight an important aspect of MC twin pregnancies complicated by prenatally detected discordance: aneuploidies confer an elevated risk of fetal loss, and the demise of one twin can, in turn, lead to the death of the unaffected twin due to acute transplacental exsanguination, which requires heightened care in pregnancy management.[17,18] The best method for reliable prenatal detection of CAs is dual amniocentesis.[11]

b. Chromosomal mosaicism can cause phenotypic discordance: in these cases, both twins carry the same genetic alteration, albeit with different levels of mosaicism; the dissimilar percentage of abnormal cells then leads to dissimilar disease presentation. Such has been reported for TS (the twin girls showed varying levels of 45,X/46,XX mosaicism in lymphocytes and fibroblasts, but the sister with lower rates of X monosomy had a milder phenotype than her typical TS twin[19]).

c. MZ twins discordant for phenotypic sex are a rare but fascinating occurrence. This phenomenon is usually explained by a single zygote undergoing early postzygotic nondisjunction that results in an embryo with two distinct cell lines. The twinning process could then lead to two genetically distinct MZ embryos (i.e., heterokaryotic twins); or alternatively, each embryo can carry both cell lines with disparate tissue distribution (i.e., levels of mosaicism). Different distribution in the gonads can subsequently lead to opposite gonadal differentiation despite monozygosity, or to various disorders of sexual development in one or both twins.[20] Amongst these cases the most frequent situation involves 45,X/46,XY mosaicism, which often results in a healthy male and a TS female twin.[21] 46,XX/46,XY mosaicism can lead to the birth of a normal female and a normal male.[22] Several other chromosomal constitutions are feasible,[23] and have been reported (e.g., 47,XXY/46,XX[20]; 45,X/47,XYY).[24]

37.4 Different levels of triplet repeat expansion

Trinucleotide (or triplet) repeat disorders are a group of genetic disorders caused by a so-called triplet repeat mutation, wherein sets of three nucleotides expand in copy number. After reaching a certain threshold, the expansion becomes unstable and leads to disease presentation. An example of a triplet repeat disorder is Fragile X syndrome, caused by the expansion of CGG repeats in the *FMR1* gene [normal copy number of the triplet is between 5 and 40, Fragile X syndrome manifests over 200

copies, and the intermediate 50–200 copies represent a premutation (predisposing to premature ovarian failure)]. Analogous to chromosomal mosaicism, Fragile X syndrome discordance between MZ twins may arise due discordant triplet repeat expansion length.[25]

37.5 Postzygotic point mutations

Twins genetically discordant for pathogenic point mutations are extremely rare but are tremendously valuable in understanding disease etiology. Generally speaking, the search for disease-causing mutations is complicated by the presence of thousands of normal variations (SNVs) in the DNA sequence of each individual. As MZ twins are assumed to carry identical SNVs, any potential difference in sequence between the affected and unaffected twin may be incriminated in the discordant syndrome/phenotype. The study of a MZ twin pair has thus led to the identification of the disease-causing gene for van der Woude syndrome, the most common syndromic orofacial clefting syndrome.[10,26]

On the other hand, phenotypic discordance between twins carrying the same point mutation causing a monogenic disorder is relatively common,[10,23,27] which may be attributed to various environmental, epigenetic or genetic (e.g., undiscovered difference in mosaicism) factors.

37.6 Skewed X-inactivation

X-inactivation is an epigenetic process by which one of the X chromosomes is silenced in 46,XX cells to ensure dosage compensation between males and females. Normally the process is random, which means that there is no parent-of-origin preference; and healthy women have an approximately equal amount of active maternally inherited and paternally inherited X chromosomes. Skewed X-inactivation, therefore, means that the silencing process was nonrandom, and this mechanism underlies the manifestation of certain X-linked disorders in females. For example, Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene and is inherited in an X-linked recessive manner. This means that females should only be carriers of the mutation because they have two X chromosomes; and under normal circumstances ~50% of their active X's are healthy, which provides sufficient compensation. However, nonrandom X-inactivation can lead to the healthy copy being silenced in a majority of cells, resulting in the manifestation of DMD.

In the context of twins, there have been many reports of twin girls discordant for DMD, or other X-linked recessive disorders, due to a difference in the randomization of X-inactivation. Cases can show either random inactivation for the normal twin and nonrandom for the affected twin; or mirror inactivation, when the mutated copy is preferentially active in the affected sibling and the normal copy is preferentially active in the healthy twin.[10,23,28]

37.7 Other epigenetic mechanisms

Beckwith–Wiedemann syndrome (BWS) is a genetic imprinting disorder, which has an increased prevalence among (mostly female) MZ twins, who are usually discordant for the disorder. BWS may result from multiple genetic/epigenetic changes, for example, uniparental disomy (this occurs when an individual inherits both copies of a chromosome/a part of a chromosome from the same parent and no copy from the other parent) of the disease-causing region on chromosome 11p, which can occur in only one twin; or abnormal methylation of key genes within the region, which can likewise differ between MZ twins.[29] Dissimilarity of epigenetic phenomena has also been implicated in phenotypically discordant twins for many chromosomal and monogenic disorders. MZ twins with 22q11.2 deletion syndrome (which includes DiGeorge syndrome and genetically related syndromes) for instance are frequently reported to have discordant congenital heart defects.[30]

37.8 Copy number variations (CNVs)

CNVs are submicroscopic deletions and duplications of chromosome segments, typically between one thousand to five million base pairs in size. In relation to the aforementioned 22q11.2 deletion syndrome, a pair of MZ twins with discordant phenotypes has been reported with different-sized deletions.[31] CNVs play an important role in human disease, susceptibility to disease, and phenotypic variability within a specific disorder; however, as CNVs also represent an extensive portion of normal human variation, their interpretation in individual patients is often confounding. Whether or not differential CNV patterns in MZ twins contribute to discordance is not yet fully understood, but the study of such twin pairs has led to the identification of possibly pathogenic CNVs for various congenital malformations and neurodevelopmental disorders.[32]

37.9 The value of twin studies in leukaemia research

Leukaemia is a malignant progressive disease in which the bone marrow and other blood-forming tissues produce an increased number of immature or abnormal white blood cells called blasts or leukaemia cells (human mature blood cells originate from haematopoietic stem cells in a process referred to as haematopoiesis, meanwhile the aberrant process is called leukaemogenesis). The abnormal cells suppress the production of normal blood cells, leading to infections caused by neutropenia, bleeding due to thrombocytopenia, and fatigue from anaemia. The different types of leukaemia are classified according to the course of the disease (acute or chronic) and the predominant type of affected white blood cell (lymphoid or myeloid). Acute lymphocytic leukaemia (ALL) is the most common type in childhood, acute and chronic myelogenous leukaemias (CML, AML) are more common in adults, meanwhile chronic lymphocytic leukaemia occurs primarily in elderly people. Half

of all leukaemia cases occur in children and teenagers; the cumulative risk of the most common ALL is ~1 in 2000 up to the age of 15 years.[33,34]

The causal factors of leukaemia are acquired genetic alterations, especially CAs [translocations, deletions, inversions, duplications, amplifications, changes of the normal diploid 46 chromosome number (less than 46 chromosomes—hypodiploidy, more than 46 chromosomes–hyperdiploidy, near haploidy with ~30 chromosomes)], which occur in hematopoietic stem cells or in their committed progenitors. The breakpoints of CAs usually alter key regulatory genes (transcriptional factors, tyrosine kinases) that are necessary for normal haematopoietic cell self-renewal, proliferation, differentiation (an abbreviation list of all mentioned genes can be found at the end of the chapter). The genetic changes may therefore alter the normal cellular function of these key genes, leading to the initiation of leukaemogenesis. Balanced CAs often result in gene fusions, which are a hallmark of the disease. These gene fusions facilitate leukaemogenesis by two main mechanisms:

a. Overexpression of a hematopoietic proto-oncogene: in this scenario one of the genes involved in the fusion is a constitutively (in other words always, continuously) expressed gene, which comes in proximity with the other, not continuously expressed proto-oncogene, leading to the latter's abnormal upregulation. This upregulation is due to the influence of the constitutive gene's regulatory elements. The proto-oncogene turns into an oncogene, which is essentially a malfunctioning gene that alters normal cellular processes, leading to abnormal cell growth, and consequently cancer (in this case, leukaemia). A well-known example of this is seen in T-cell ALL where regulatory elements of T-cell receptor (TCR) genes deregulate the expression of various partner genes.

b. The creation of a hybrid gene that consequently results in an abnormal fusion protein. Leukaemia-associated fusion proteins share several structural and functional similarities, suggesting that they impart a leukaemic phenotype by way of transcriptional dysregulation. A classic example of a fusion protein is the BCR-ABL1 hybrid protein generated by the t(9;22)(q34;q11) translocation in CML, ALL, and AML. The fusion protein is encoded by sequences from both BCR and ABL and has an increased tyrosine kinase activity contributing to the immortality of leukaemic cells.

Recurrent CAs are used to define distinct disease entities and are included in the World Health Organization classification of haematological malignancies. They are independent prognostic indicators, play a crucial role in risk stratification, and facilitate clinical decision making (e.g., selecting the appropriate type and intensity of treatment), which increases survival rates and reduces long-term side effects. Recent profiling of subtle copy number alterations and mutational analyses have allowed further refinement of cytogenetic risk stratification groups by including submicroscopic alterations that coexist and cooperate with CAs and have been recognized to have prognostic and therapeutic relevance. Patients with the most common B-cell precursor ALL (BCP-ALL) are thus classified into three risk groups with distinct event-free survival (EFS) rates according to their CAs: (1) low risk (EFS 81~91%, representing more than half of the cases;) intermediate (EFS ~73%) and (3) high risk (EFS ~54%)[35,36] (these

entities will be discussed in further detail later). In childhood, the less frequent T-cell progenitor phenotype ALL (T-ALL) generally has worse outcomes than BCP-ALL.[37]

Leukaemia of twins is no different biologically, clinically, or in its age incidence from leukaemia in singletons. Although only 0.6% of childhood BCP-ALL occurs in twins, a relatively high number of associated studies are at the focus of scientific interest. Concordant twin leukaemia studies have provided a multistep leukaemogenesis model—similar to Knudson's famous "two hit" retinoblastoma model—applicable generally to the origin, pathogenesis, latency, and prognosis of childhood leukaemia.[38,39]

Twins with concordant leukaemia were first described in 1882[40] followed by several further reports of concordant MZ twin pairs in the 1900s, which raised the hypothesis regarding the prenatal (or *in utero*) origin of childhood leukaemia.[41,42] Scientific evidence emerged later with the development of molecular genetic techniques and accumulation of knowledge related to twin embryogenesis. Sixty percent of MZ twin embryos split 3–7 days after fertilization and develop a single MC placenta resulting in vascular anastomoses and blood cell chimerism. Asymmetry of the vascular anastomoses can lead to uneven distribution of blood, causing twin-twin transfusion syndrome and reciprocal anemia/polycythemia. Very rarely blood chimerism can also occur in DZ twins by fusing of the two placentae. Cytogenetic and molecular genetic studies of MZ twins demonstrated that the high level of leukaemia concordance might be attributable to *in utero* origin of the disease and subsequent spread to the cotwin via shared placental circulation as an "intraplacental metastasis" (Fig. 37.5).[38,43]

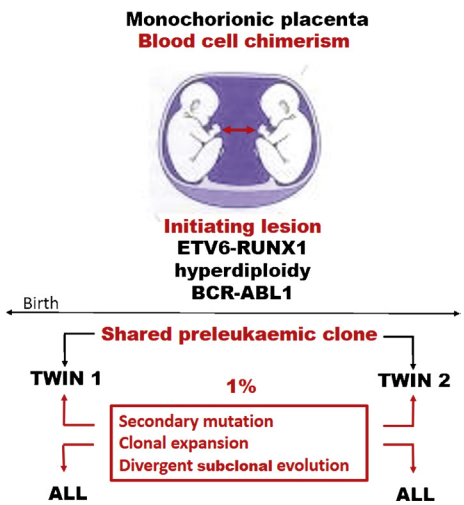


FIG. 37.5 Schematic representation of concordant leukaemia in identical twins.

The initiating lesions occur *in utero* in one twin, which then spread to the cotwin via intraplacental anastomosis. Majority of children with this preleukaemic first hit mutation remain healthy. Approximately 1% develop overt leukaemia upon acquisition, usually postnatal, of secondary mutations contributing to clonal diversification. If a monozygotic, monochorionic twin has ALL, the probability of the cotwin also developing ALL is 10%–15%.

The evidence supporting the prenatal origin of leukaemia comes from studies that demonstrated that twin pairs have the same recurrent translocation, with identical unique translocation breakpoints and immunoglobulin heavy chain (IgH) or TCR rearrangements.[44–46] Additionally, retrospective scrutiny of archived neonatal blood spots (Guthrie cards) identified the presence of leukaemia specific translocations in 1% of all neonates immediately after birth, and the translocation breakpoints were again identical in MZ twins. Leukaemia-associated CAs are detectable in cord blood from newborn infants at rates 100-fold higher than the incidence of ALL.[47,48] These prenatal CAs were interpreted as critical initiating lesions, but represent "silent" preleukaemic mutations. The development and clinical manifestation of leukaemia require additional secondary (usually postnatal) mutational events targeting genes that are important for immune cells in general, B-cell lineage differentiation, and cell-cycle control. These additional genetic alterations drive the expansion of the initial clone leading to divergent clonal evolution, which contributes to overt leukaemia. In some instances, the initial mutation itself has the potential to generate secondary mutations, but in the majority of cases, other factors (infectious and/or environmental exposure, constitutive CAs) trigger the acquisition of secondary alterations.[38,49–52] The "intraplacental metastasis" process is visualized on Fig. 37.5. Further examples of primary and secondary genetic alterations, arranged according to the aforementioned BCP-ALL cytogenetic risk groups, can be found in Fig. 37.6.

An important aspect of concordant twin leukaemia studies is that they provide the opportunity to measure the latency period, that is, the time from the initial preleukaemic event to clinically evident disease, reflecting the accumulation of

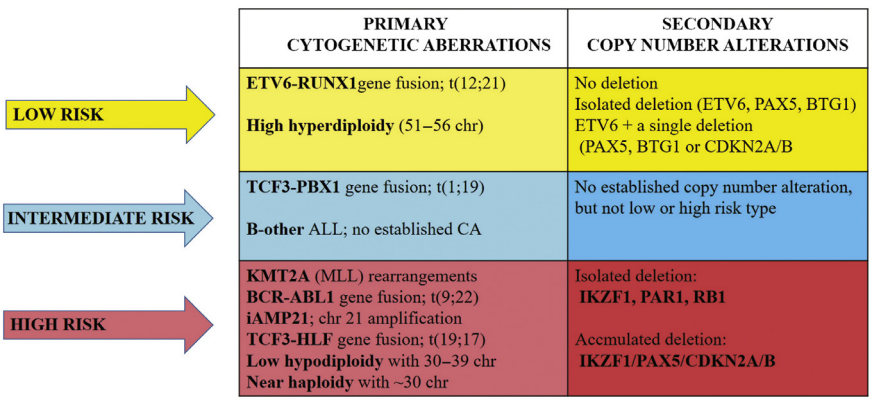


FIG. 37.6 Genetic risk groups of childhood BCP-ALL.

Majority of the recurrent chromosomal aberration are primary initiating lesions originated "in utero," but require secondary postnatal mutations and possibly a dysregulated immune system. The prenatal origin for several fusion genes (ETV6/RUNX1, BCR/ABL1, TCF3/PBX1, KMT2A/different partner genes), as well as hyperdiploidy, has been proven by concordant leukaemia twin studies in which both twins had identical molecular rearrangements. The secondary abnormalities can significantly affect the prognosis and could determine a highly variable postnatal latency. Abbreviation: chr: chromosome.

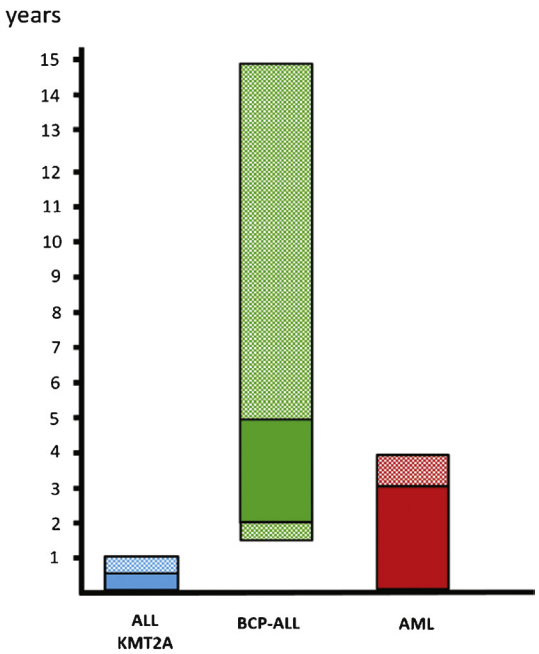


FIG. 37.7 Age distribution of childhood twin leukaemia.

The presented age distribution of infant leukaemia with KMT2A rearrangements, BCP-ALL and AML, based on published concordant twin leukaemia data (see references) correspond to the childhood leukaemia age specific incidence in the general population. The columns indicate the general age incidence for each subtype, and the shaded part of each column shows the peak age incidence.

harmful environmental/infectious effects triggering secondary genetic aberrations. The latency period can be the same in cotwins, but is different in the majority of cases; the published highest latency period was 14 years,[43] meanwhile the greatest difference between twins was 9 years.[38] ALL specific initial, *in utero* preleukaemic cytogenetic entities are associated with distinctive age-incidence distribution patterns both in twins and singletons: ALL associated with KMT2A mutations occurs in infants, most frequently under 6 months of age; BCP-ALL is most common in 2–5-year-old children, but is found in older children and in teenagers as well; childhood AML occurs predominantly from birth up to 3 years of age (Fig. 37.7). The concordance rate in twin pairs approaches 100% for infants, is 10%–15% in older children and less than 1% in adults.[38,51,52]

The high rate of concordance in infant leukaemia reflects that KMT2A rearrangements (with a peak incidence at 6 months of age) have a very short latency period and could be sufficient alone to cause leukaemia. Besides the majority of neonatal and infant ALL, therapy-related AML (t-AML) is also frequently associated with chromosome 11q23 rearrangements involving KMT2A gene, which provides clues

about disease etiology. Most reported t-AML cases occur in patients previously treated with DNA topoisomerase II (Topo II) inhibitors. Topo II is a ubiquitous ligase enzyme with roles in DNA replication and transcription. Topo II inhibitors block the ligase function of Topo II, resulting in double-strand breaks near the enzyme's consensus DNA binding sites. KMT2A rearrangement breakpoints in both infant leukaemia and t-AML are related to this consensus binding site, suggesting that prenatal exposure to natural and synthetic compounds that can inhibit Topo II (flavonoids present in a number of fruits and vegetables, compounds found in soybeans, tea, cocoa and wine, quinolone antibiotics, benzene metabolites, etc.) may play a role in disease etiology.[53–56]

The most frequent recurrent founder mutation in childhood ALL is the genomic fusion of transcription factors ETV6 and RUNX1 produced by the t(12; 21) translocation. Single cell, *in utero* origin of this leukaemia-initiating mutation was first demonstrated by Ford et al.[44] in a pair of MZ twins diagnosed with concordant ALL. The ETV6-RUNX1 gene fusion arises at a much higher (100-500 fold) frequency than the corresponding leukaemia, indicating an obligatory requirement for additional mutations in disease development. Epidemiological data reveal that the associated ALL is very common in young children, but extremely rare after 15 years of age. These observations suggest that for the leukaemic transformation of ETV6-RUNX1 positive cells there should be critical pre- and/or post-natal time windows and a specific progenitor target cell. Recent studies have demonstrated that a candidate target cell for childhood BCP-ALL is an early B-cell progenitor cell type restricted to fetal lymphopoiesis.[46,57] In mouse models, fetal haemopoietic stem cells expressing ETV6-RUNX1 have a limited lifespan and do not contribute to the early B cell progenitor pool of adults. This can explain the low penetrance of preleukaemia in children and the declining incidence of ETV6-RUNX1 positive ALL with age due to natural loss of the fetal preleukaemic clone.[39,58,59]

MZ twin studies with concordant ALL were pivotal in proving that hyperdiploid BCP-ALL can also arise *in utero* from a single cell clone in early B lineage progenitors. Compared to the other cytogenetic subtypes, hyperdiploid ALL is associated with relatively few secondary CAs, which are furthermore not consistently retained at relapse. Taken together, these observations indicate that the high hyperdiploid pattern is the main driver event in this common pediatric malignancy.[48,60]

The twin study of Cazzaniga et al. has shed light on the critical role of IKZF1 deletion, which is a frequent secondary genetic abnormality in BCR-ABL1 positive childhood ALL. IKZF1 gene encodes the B-cell transcription factor IKAROS, and its aberrations have a significant effect on the development of overt leukaemia, its clinical course, and response to therapy. The presented twin pair shared the same prenatally generated BCR-ABL1 fusion gene with identical breakpoints, but were discordant for the secondary IKZF1 deletion. Similar treatment led to different outcomes: the twin with the deletion relapsed and could not achieve remission, while the cotwin without the deletion has been in asymptomatic remission for years. This demonstrates that IKZF1 loss greatly accelerates BCR-ABL1-driven leukaemia development and is associated with an adverse prognosis.[61]

Contrary to the example above, a pair of MZ MC twins with concordant DS were described with synchronous progression of acute megakaryoblastic leukaemia (AMKL). They developed AMKL simultaneously at the age of 11 months. The twins were found to have three, *in utero* originated identical CAs: (1) GATA1 rearrangement, a known first hit mutation predisposing DS patients to AMKL; (2) a t(3;7)(q27;q32) translocation with a breakpoint involving the tumor suppressor CUX1 gene and (3) tetrasomy 21, an extra, nonconstitutional chromosome 21. Both twins were treated according to the standard DS myeloid leukaemia protocol and had a parallel treatment response with full remission.[62] This twin study allowed for the identification of CUX1 as a new driver gene that could promote a GATA1-mutated clone into AMKL in DS patients. However, large cohort studies suggest that the prenatal origin of AML is less frequent compared to ALL cases.

Data regarding the prenatal origin of childhood T-ALL are less comprehensive. Convincing evidence was presented by Ford et al.[45] in a study of MZ twins concordant for T-cell leukaemia/lymphoma, with identical rearrangements of TCR genes, but with an extraordinarily long latency period (9 and 11 years respectively). A preclinical phase of clonal expansion of more than 10 years has been described in one further patient with ataxia telangiectasia and T-ALL.[63] In a 13 infant T-ALL cohort study, the *in utero* origin of leukaemia associated somatic mutations could be demonstrated in three patients only.[64] This and other follow-up pediatric T-ALL studies suggest that T-ALL associated CAs are most probably postnatal events and concordant T-ALL leukaemias may be extremely rare in MZ twins.

On the basis of deep targeted sequencing on blood DNA from 52 MZ and 27 DZ twin pairs aged 70 to 99 years, concordance for clonal hematopoiesis is limited in elderly twins.[65] Mutational landscape analysis revealed very similar results in age-matched samples from MZ and DZ twin pairs. In addition, a disparity in clonal size and trajectory over time was observed even between MZ twins harboring the same driver gene mutations. Therefore, nongenetic factors and events may play major roles in the acquisition of somatic mutations and in possible malignant transformation in elderly twins and singletons.[66] However, a number of germline variants predispose to hematological malignancies both in twins and in the general population.

Gene abbreviation list

ABL1 ABL Proto-Oncogene 1, Non-Receptor Tyrosine Kinase
BCR Activator Of RhoGEF And GTPase
BTG1 Anti-Proliferation Factor 1
CDKN2A Cyclin Dependent Kinase Inhibitor 2A
CDKN2B Cyclin Dependent Kinase Inhibitor 2B
CUX Cut Like Homeobox 1
ETV6 ETS Variant Transcription Factor 6
GATA1 GATA Binding Protein 1
IKZF1 IKAROS Family Zinc Finger 1

KMT2A	Lysine Methyltransferase 2A (formerly: MLL)
PAR1	pseudoautosomal regions, PAR1
PAX5	Paired Box 5
PBX1	PBX homeobox 1
RB1	RB Transcriptional Corepressor 1
RUNX1	RUNX Family Transcription Factor 1
TCF3	Transcription Factor 3

References

1. Li S-J, Ford N, Meister K, et al. Increased risk of birth defects among children from multiple births. *Birth Defects Research. Part A, Clinical and Molecular Teratology*. 2003;67:879–885. https://doi.org/10.1002/bdra.10093.
2. Tang Y, Ma C-X, Cui W, et al. The risk of birth defects in multiple births: a population-based study. *Maternal and Child Health Journal*. 2006;10:75–81. https://doi.org/10.1007/s10995-005-0031-5.
3. Glinianaia SV, Rankin J, Wright C. Congenital anomalies in twins: a register-based study. *Human Reproduction*. 2008;23:1306–1311. https://doi.org/10.1093/humrep/den104.
4. Kroeldrup L, Larsen LA, Fagerberg C, et al. Chromosomal aberrations in monozygotic and dizygotic twins versus singletons in Denmark during 1968–2009. *Twin Res Hum Genet*. 2017;20:216–225. https://doi.org/10.1017/thg.2017.17.
5. Sperling L, Kiil C, Larsen LU, et al. Detection of chromosomal abnormalities, congenital abnormalities and transfusion syndrome in twins. *Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol*. 2007;29:517–526. https://doi.org/10.1002/uog.3918.
6. Boyle B, McConkey R, Garne E, et al. Trends in the prevalence, risk and pregnancy outcome of multiple births with congenital anomaly: a registry-based study in 14 European countries 1984-2007. *Bjog*. 2013;120:707–716. https://doi.org/10.1111/1471-0528.12146.
7. Sparks TN, Norton ME, Flessel M, et al. Observed rate of down syndrome in twin pregnancies. *Obstetrics and Gynecology*. 2016;128:1127–1133. https://doi.org/10.1097/AOG.0000000000001690.
8. Boyle B, Morris JK, McConkey R, et al. Prevalence and risk of Down syndrome in monozygotic and dizygotic multiple pregnancies in Europe: implications for prenatal screening. *Bjog*. 2014;121:809–819. discussion 820. https://doi.org/10.1111/1471-0528.12574.
9. Shur N. The genetics of twinning: from splitting eggs to breaking paradigms. *American journal of medical genetics Part C, Seminars in medical genetics*. 2009;151C:105–109. https://doi.org/10.1002/ajmg.c.30204.
10. Zwijnenburg PJG, Meijers-Heijboer H, Boomsma DI. Identical but not the same: the value of discordant monozygotic twins in genetic research. *Am J Med Genet Part B, Neuropsychiatr Genet Off Publ Int Soc Psychiatr Genet*. 2010;153B:1134–1149. https://doi.org/10.1002/ajmg.b.31091.
11. Machin G. Non-identical monozygotic twins, intermediate twin types, zygosity testing, and the non-random nature of monozygotic twinning: a review. *American journal of medical genetics Part C, Seminars in medical genetics*. 2009;151C:110–127. https://doi.org/10.1002/ajmg.c.30212.

12. Rogers JG, Voullaire L, Gold H. Monozygotic twins discordant for trisomy 21. *American Journal of Medical Genetics*. 1982;11:143–146. https://doi.org/10.1002/ajmg.1320110204.
13. Rohrer TR, Gassmann KF, Rauch A, et al. Growth of heterokaryotic monozygotic twins discordant for Ullrich-Turner syndrome during the first years of life. *American Journal of Medical Genetics. Part A*. 2004;126A:78–83. https://doi.org/10.1002/ajmg.a.20446.
14. Gilbert B, Yardin C, Briault S, et al. Prenatal diagnosis of female monozygotic twins discordant for Turner syndrome: implications for prenatal genetic counselling. *Prenatal Diagnosis*. 2002;22:697–702. https://doi.org/10.1002/pd.383.
15. Su P-H, Chen J-Y, Chen S-J, et al. 45,X/46,XX mosaicism in a mother and one of her discordant monozygotic twin daughters: report of one case. *Acta Paediatrica Taiwanica = Taiwan Er Ke Yi Xue Hui Za Zhi*. 2006;47:252–254.
16. Choi SA, Ko JM, Shin CH, et al. Monozygotic twins discordant for Down syndrome: mos 47,XX,+21/46,XX and 46,XX. *European Journal of Pediatrics*. 2013;172:1117–1120. https://doi.org/10.1007/s00431-013-1998-3.
17. Chang Y-L, Yi W-P, Chao A-S, et al. Monozygotic twins discordant for trisomy 21: discussion of etiological events involved. *Taiwan J Obstet Gynecol*. 2017;56:681–685. https://doi.org/10.1016/j.tjog.2017.08.019.
18. Lewi L, Blickstein I, Van Schoubroeck D, et al. Diagnosis and management of heterokaryotypic monochorionic twins. *Am J Med Genet Part A*. 2006;140A:272–275. https://doi.org/10.1002/ajmg.a.31052.
19. Lebl J, Zahradníková M, Vlasak I, et al. Discordant growth pattern and ovarian function in monozygotic twins with 45,X/46,XX mosaicism. *Hormone Research*. 2001;55:102–105. https://doi.org/10.1159/000049978.
20. Tachon G, Lefort G, Puechberty J, et al. Discordant sex in monozygotic XXY/XX twins: a case report. *Human Reproduction*. 2014;29:2814–2820. https://doi.org/10.1093/humrep/deu275.
21. Tho SP, Jackson R, Kulharya AS, et al. Long-term follow-up and analysis of monozygotic twins concordant for 45,X/46,XY peripheral blood karyotype but discordant for phenotypic sex. *American Journal of Medical Genetics. Part A*. 2007;143A:2616–2622. https://doi.org/10.1002/ajmg.a.31992.
22. Zech NH, Wisser J, Natalucci G, et al. Monochorionic-diamniotic twins discordant in gender from a naturally conceived pregnancy through postzygotic sex chromosome loss in a 47,XXY zygote. *Prenatal Diagnosis*. 2008;28:759–763. https://doi.org/10.1002/pd.2031.
23. Machin GA. Some causes of genotypic and phenotypic discordance in monozygotic twin pairs. *American Journal of Medical Genetics*. 1996;61:216–228. http://doi.org/10.1002/(SICI)1096-8628(19960122)61:3<216::AID-AJMG5>3.0.CO;2-S.
24. Bohec C, Douet-Guilbert N, Basinko A, et al. Difficult diagnosis and management of an heterokaryotypic monochorionic twin pregnancy with discordant fetal sex and 45,X/47,XYY karyotypes. *Fetal Pediatr Pathol*. 2010;29:424–430. https://doi.org/10.3109/15513815.2010.505630.
25. Helderman-van den Enden AT, Maaswinkel-Mooij PD, Hoogendoorn E, et al. Monozygotic twin brothers with the fragile X syndrome: different CGG repeats and different mental capacities. *Journal of Medical Genetics*. 1999;36:253–257.
26. Kondo S, Schutte BC, Richardson RJ, et al. Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. *Nature Genetics*. 2002;32:285–289. https://doi.org/10.1038/ng985.

27. Han JY, Lee IG, Jang W, et al. Identification of a novel de novo nonsense mutation of the NSD1 gene in monozygotic twins discordant for Sotos syndrome. *Clinica Chimica Acta*. 2017;470:31–35. https://doi.org/10.1016/j.cca.2017.04.025.
28. Abbadi N, Philippe C, Chery M, et al. Additional case of female monozygotic twins discordant for the clinical manifestations of Duchenne muscular dystrophy due to opposite X-chromosome inactivation. *American Journal of Medical Genetics*. 1994;52:198–206. https://doi.org/10.1002/ajmg.1320520215.
29. Weksberg R, Shuman C, Caluseriu O, et al. Discordant KCNQ1OT1 imprinting in sets of monozygotic twins discordant for Beckwith-Wiedemann syndrome. *Human Molecular Genetics*. 2002;11:1317–1325. https://doi.org/10.1093/hmg/11.11.1317.
30. Singh SM, Murphy B, O'Reilly R. Monozygotic twins with chromosome 22q11 deletion and discordant phenotypes: updates with an epigenetic hypothesis. *Journal of Medical Genetics*. 2002;39:e71. https://doi.org/10.1136/jmg.39.11.e71.
31. Halder A, Jain M, Chaudhary I, et al. Chromosome 22q11.2 microdeletion in monozygotic twins with discordant phenotype and deletion size. *Mol Cytogenet*. 2012;5:13. https://doi.org/10.1186/1755-8166-5-13.
32. Peng R, Zhou Y, Xie H-N, et al. MCDA twins with discordant malformations: submicroscopic chromosomal anomalies detected by chromosomal microarray analysis and clinical outcomes. *Prenatal Diagnosis*. 2016;36:766–774. https://doi.org/10.1002/pd.4859.
33. Parkin DM, Stiller CA, Draper GJ, et al. The international incidence of childhood cancer Znt. *J. Cancer*. 1988;42:511–520.
34. Howlader N, Noone AM, Krapcho M, et al. *SEER cancer statistics review, 1975–2016*. Bethesda, MD: National Cancer Institute; 2019.
35. Moorman AV. New and emerging prognostic and predictive genetic biomarkers in B-cell precursor acute lymphoblastic leukaemia. *Haematologica*. 2016;101:407–416. http://doi.org/10.3324/haematol.2015.141101.
36. Hamadeh L, Enshaei A, Schwab C, et al. Validation of the United Kingdom copy-number alteration classifier in 3239 children with B-cell precursor ALL. *Blood Adv*. 2019;3:148–157. http://doi.org/10.1182/bloodadvances.2018025718.
37. Zhang J, Ding L, Holmfeldt L, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature*. 2012;481:157–163. Published 2012 Jan 11. http://doi.org/10.1038/nature10725.
38. Greaves MF, Maia AT, Wiemels JL, et al. leukaemia in twins: lessons in natural history. *Blood*. 2003;102:2321–2333. http://doi.org/10.1182/blood-2002-12-3817.
39. Greaves M. A causal mechanism for childhood acute lymphoblastic leukaemia. *Rev Cancer*. 2018;18:471–484. http://doi.org/10.1038/s41568-018-0015-6.
40. Senator H. Zur Kenntniss der Leuka¨mie und Pseudoleuka¨mie im Kindesalter. *Berliner Klinische Wochenschrift*. 1882;35:533–536.
41. Hrubec Z, Robinette CD. The study of human twins in medical research. *New England Journal of Medicine*. 1984;310:435–441.
42. MacMahon B, Levy MA. Prenatal origin of childhood leukaemia. evidence from twins. *New England Journal of Medicine*. 1964;270:1082–1085. http://doi.org/10.1056/NEJM196405212702102.
43. Greaves M. In utero origins of childhood leukaemia. *Early Human Development*. 2005;81:123–129. http://doi.org/10.1016/j.earlhumdev.2004.10.004.
44. Ford AM, Bennett CA, Price CM, et al. Fetal origins of the TEL-AML1 fusion gene in identical twins with leukaemia. *PNAS*. 1998;95:4584–4588. http://doi.org/10.1073/pnas.95.8.4584.

45. Ford AM, Pombo-de-Oliveira MS, McCarthy KP, et al. Monoclonal origin of concordant T-cell malignancy in identical twins. *Blood*. 1997;89:281–285.
46. Alpar D, Wren D, Ermini L, et al. Clonal origins of ETV6-RUNX1^{+} acute lymphoblastic leukaemia: studies in monozygotic twins. *Leukaemia*. 2015;29:839–846. http://doi.org/10.1038/leu.2014.322.
47. Gale KB, Ford AM, Repp R, et al. Backtracking leukaemia to birth: identification of clonotypic gene fusion sequences in neonatal blood spots. *PNAS*. 1997;94:13950–13954. http://doi.org/10.1073/pnas.94.25.13950.
48. Maia AT, Tussiwand R, Cazzaniga G, et al. Identification of pre-leukaemic precursors of hyperdiploid acute lymphoblastic leukaemia in cord blood. *Genes, chromosomes & cancer*. 2004;40:38–43. http://doi.org/10.1002/gcc.20010.
49. Wiemels JL, Ford AM, Van Wering ER, et al. Protracted and variable latency of acute lymphoblastic leukaemia after TEL-AML1 gene fusion in utero. *Blood*. 1999;94:1057–1062.
50. Bateman CM, Colman SM, Chaplin T, et al. Acquisition of genome-wide copy number alterations in monozygotic twins with acute lymphoblastic leukaemia. *Blood*. 2010;115:3553–3558. http://doi.org/10.1182/blood-2009-10-251413.
51. Hein D, Borkhardt A, Fischer U. Insights into the prenatal origin of childhood acute lymphoblastic leukaemia. *Cancer and Metastasis Reviews*. 2020;39:161–171. http://doi.org/10.1007/s10555-019-09841-1.
52. Ma Y, Dobbins SE, Sherborne AL, et al. Developmental timing of mutations revealed by whole-genome sequencing of twins with acute lymphoblastic leukaemia. *PNAS*. 2013;110:7429–7433. http://doi.org/10.1073/pnas.1221099110.
53. Greaves M, Hughes W. Cancer cell transmission via the placenta. *Evol Med Public Health*. 2018;2018:106–115. http://doi.org/10.1093/emph/eoy011.
54. Dimartino JF, Cleary ML. Mll rearrangements in haematological malignancies: lessons from clinical and biological studies. *British Journal of Haematology*. 1999;106:614–626. http://doi.org/10.1046/j.1365-2141.1999.01439.x.
55. Cimino G, Rapanotti MC, Biondi A, et al. Infant acute leukaemias show the same biased distribution of ALL1 gene breaks as topoisomerase II related secondary acute leukaemias. *Cancer Research*. 1997;57:2879–2883.
56. Ross JA, Potter JD, Reaman GH, et al. Maternal exposure to potential inhibitors of DNA topoisomerase II and infant leukaemia (United States): a report from the Children's Cancer Group. *Cancer Causes & Control*. 1996;7:581–590. http://doi.org/10.1007/BF00051700.
57. Teuffel O, Betts DR, Dettling M, et al. Prenatal origin of separate evolution of leukaemia in identical twins. Leukaemia. 2004;18:1624-1629. doi: http://doi.org/10.1038/sj.leu.2403462
58. Boiers C, Richardson SE, Laycock E, et al. A human IPS model implicates embryonic B-myeloid fate restriction as developmental susceptibility to B acute lymphoblastic leukaemia-associated ETV6-RUNX1. *Developmental Cell*. 2018;44:362–377. e367. http://doi.org/10.1016/j.devcel.2017.12.005.
59. Schindler JW, Van Buren D, Foudi A, et al. TEL-AML1 corrupts hematopoietic stem cells to persist in the bone marrow and initiate leukaemia. *Cell Stem Cell*. 2009;5:43–53. http://doi.org/10.1016/j.stem.2009.04.019.
60. Bateman CM, Alpar D, Ford AM, et al. Evolutionary trajectories of hyperdiploid ALL in monozygotic twins. *Leukaemia*. 2015;29:58–65. http://doi.org/10.1038/leu.2014.177.
61. Cazzaniga G, van Delft FW, Lo Nigro L, et al. Developmental origins and impact of BCR-ABL1 fusion and IKZF1 deletions in monozygotic twins with Ph+ acute lymphoblastic leukaemia. *Blood*. 2011;118:5559–5564. http://doi.org/10.1182/blood-2011-07-366542.

62. Bache I, Wadt K, Mehrjouy MM, et al. A shared somatic translocation involving CUX1 in monozygotic twins as an early driver of AMKL in Down syndrome. *Blood cancer journal*. 2020;10:27. Published 2020 Mar 3. http://doi.org/10.1038/s41408-020-0293-6.
63. Taylor AM, Metcalfe JA, Thick J. Mak YF. leukaemia and lymphoma in ataxia telangiectasia. *Blood*. 1996;87:423–438.
64. Mansur MB, van Delft FW, Colman SM, et al. Distinctive genotypes in infants with T-cell acute lymphoblastic leukaemia. *British Journal of Haematology*. 2015;171:574–584. http://doi.org/10.1111/bjh.13613.
65. Fabre MA, McKerrell T, Zwiebel M, et al. Concordance for clonal hematopoiesis is limited in elderly twins. *Blood*. 2020;135:269–273. http://doi.org/10.1182/blood.2019001807.
66. Hansen JW, Pedersen DA, Larsen LA, et al. Clonal hematopoiesis in elderly twins: concordance, discordance, and mortality. *Blood*. 2020;135:261–268. http://doi.org/10.1182/blood.2019001793.

SECTION

Conclusion 8

Summary and concluding statement

Adam D. Tarnoki[a], David L. Tarnoki[a], Jennifer R. Harris[b], Nancy L. Segal[c]

[a]*Medical Imaging Centre, Semmelweis University, Hungarian Twin Registry, Budapest, Hungary*

[b]*Centre for Fertility and Health, The Norwegian Institute of Public Health, Oslo, Norway*

[c]*Department of Psychology, California State University, Fullerton, CA, United States*

In the introduction to this book, we noted that this is a very exciting time to be involved in the study of twins. It is also an exciting time to look to the future of twin research.

Important findings and breakthroughs are happening all the time. You have read about some of them in the different chapters of this book, but some stories are worth retelling—and when the history of twins is written again, even more chapters will be added.

Throughout the years, twin registries have grown to occupy a special niche in science, particularly because new technologies and analytical approaches have often enhanced the known advantages of twin designs. For example, epigenetic analyses are informing us about factors affecting behavior and disease in identical twins who differ with regards to environmental exposures, life-style factors, and/or medical life histories. Comparing the microbiomes of MZ (identical) and DZ (fraternal) twins also promises to uncover new and important findings regarding the onset and progression of human disease. Moreover, twin registries are valuable resources for helping us find answers to significant public health challenges. This is illustrated, for example, by the number of twins studies that could rapidly investigate various aspects of the coronavirus (COVID-19) pandemic and were featured in the 19th *International Congress on Twin Studies* that took place in Budapest in 2021.

Twin researchers have further enriched their data through the addition of new types of genetic, omics, imaging, and environmental measures. This has laid a strong foundation for new knowledge generation. We foresee that today's twin-based research programs will continue to grow and provide new opportunities in which twin research will contribute to breakthroughs across a wide field of scientific inquiry.

Twin registries are also growing in size, number and specificity, owing to increased twinning rates. Although we have described factors that increase the chance of DZ twinning, research on th causes of MZ twinning is still underway with promising results. We still have a great deal to learn about many aspects of twins and twin-ning, so it is not surprising that new twin registries are emerging in the world, and more investigators are implementing twin research designs into their research programs. Physicians are also better able to detect and manage multiple birth pregnancies which are generally riskier than singleton pregnancies, for both mothers and infants. In our modern, changing world, psychologists are also continually adapting

and developing new suggestions for parenting twins. Areas of special interest concern placing twins in the same or separate classrooms and identifying the best interventions for improving twins' average language difficulties. Communication between researchers and parents is also critical, representing a subject of renewed focus within the *International Society of Twin Studies.*

Beyond the scientific promise and progress of twin studies, meeting and observing twins up close and personally provides a unique and fascinating experience. Each twin pair reflects a distinctive story about human developmental processes. Think about the twins you know, and this will become clear.

In the future, twin studies will continue to play an important role, along with emerging genome and molecular research methods, in shedding light on answers to big questions. Findings from these studies will continue to elucidate the reasons why people differ in the ways they develop and age, identify factors affecting health and well-being, and explain how environmental and genetic factors combine to affect human behaviors, physical traits, and diseases.

Thank you for reading our book in which we shared current information with you on the current state of twin-based science. In closing, we hope that you have gained appreciation for the valuable contributions that twin research have made to our understanding of individual differences in health, disease, and development. If so, then our mission as editors has been accomplished. We thank the authors of the chapters, the experts who reviewed them, and the publisher Elsevier, who allowed us to share this information with you. Finally, we thank twins worldwide for their participation in research and for sharing their stories with us.

Appendix—Resources

Books by the editor, Nancy L. Segal

Entwined Lives: Twins and What They Tell Us About Human Behavior. (2000).
Indivisible by Two: Lives of Extraordinary Twins. Cambridge, MA. (2007).
Someone Else's Twin: The True Story of Babies Switched at Birth. Books (2011).
Born Together—Reared Apart: The Landmark Minnesota Twin Study (2012).
Twin Mythconceptions: False Beliefs, Fables, and Facts About Twins. (2017).
Accidental Brothers: The Story of Twins Exchanged at Birth and the Power of Nature and Nurture. (2018, co-author: Y.S. Montoya).
Deliberately Divided: Inside the Controversial Study of Twins and Triplets Adopted Apart. (2021).
Uniting Psychology and Biology: Integrative Perspectives on Human Development. (1997; co-editors: G.E. Weisfeld and C.C. Weisfeld).

Journals and Magazines.

Twin Research and Human Genetics.
https://www.cambridge.org/core/journals/twin-research-and-human-genetics
Behavior Genetics.
http://bga.org/journal/
Twins Magazine.
https://twinsmagazine.com/

Professional Organizations.

International Society for Twin Studies.
https://twinstudies.org/
Behavior Genetics Association. bga.org
International Society for the Study of Individual Differences.
https://issidorg.com/

Parents and Twins Organizations.

Multiples of America.
https://multiplesofamerica.org/
International Council of Multiple Birth Organizations (ICOMBO).
Icombo.org
Twins Days Festival.
Twinsdays.org
International Twins Association (ITA).
http://www.intltwins.org/index.php/en/
Twiniversity.
https://www.twiniversity.com/
Twin Mom. twinmom.com
Twin Loss Support Groups.
Twinless Twins. twinlesstwins.org
The Twins Trust Bereavement Support Group (BSG). twinstrust.org/bereavement.html

Index

Page numbers followed by "*f*" and "*t*" indicate, figures and tables respectively.

D

E

F

G

H

I

L

M

N

U

V

X

Z

TWIN RESEARCH FOR EVERYONE

From Biology to Health, Epigenetics, and Psychology

Edited by Adam D. Tarnoki, David L. Tarnoki, Jennifer R. Harris, and Nancy L. Segal

Adam D. Tarnoki, Radiologist and Researcher, Medical Imaging Centre, Semmelweis University, Hungarian Twin Registry, Budapest, Hungary

David L. Tarnoki, Radiologist and Researcher, Medical Imaging Centre, Semmelweis University, Hungarian Twin Registry, Budapest, Hungary

Jennifer R. Harris, Director of Research, Center for Fertility and Health, Norwegian Institute of Public Health, Oslo, Norway

Nancy L. Segal, Professor of Psychology at California State University, Fullerton and Director of the Twin Studies Center, CA, USA

Twin Research for Everyone is a comprehensive, applied resource in twinning and twin studies that is grounded in the most impactful findings from twin research in recent years. While targeted to undergraduate and graduate students, this compendium will prove a valuable resource for scholars already familiar with twin studies, as well as those coming to the field for the first time. Here, more than 40 experts across an array of disciplines examine twinning and twin research methodologies from the perspectives of biology, medicine, genetics, epigenetics, neuroscience, and psychology. The different chapters provide clear instruction in both basic and advanced research methods, family and parenting aspects of twinning, twin studies as applied across various disease areas and medical specialties, genetic and epigenetic influences on differentiation, and academic, neurological, and cognitive development.

The presentation of existing studies and methods instruction empowers students and researchers to apply twin-based research and advance new studies across a range of biomedical and behavioral fields, highlighting current research trends and future directions.

Key features

- Offers unique insights into twinning rates, mechanisms, and factors surrounding twinship
- Provides clear instruction in both basic and advanced twin research methods and study design
- Features leading international experts in twin biology, genetics, health, and psychology
- Examines findings from past and recent twin studies across a broad array of health and behavioral studies

ELSEVIER

ACADEMIC PRESS

An imprint of Elsevier

elsevier.com/books-and-journals

ISBN 978-0-12-821514-2