



Effect of genetic and environmental influences on hepatic steatosis: A classical twin study based on computed tomography

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ORIGINAL ARTICLE



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ABSTRACT

Background and aims: Non-alcoholic fatty liver disease (NAFLD) increases cardiovascular morbidity and mortality, and carries poor long-term hepatic prognosis. Data about the role of genetic and environmental factors in the hepatic lipid accumulation are limited. The aim of the study was to evaluate the genetic and environmental impact on the hepatic lipid accumulation within a cohort of adult twin pairs.

Patients and methods: We investigated 182 twin subjects [monozygotic (MZ, $n = 114$) and dizygotic (DZ, $n = 68$) same-gender twins (age 56.0 ± 9.6 years; BMI 27.5 ± 5.0 kg/m²; females 65.9%)] who underwent computed tomography (CT) with a 256-slice scanner. Using non-enhanced CT-images, we calculated the average value of hepatic attenuation [expressed in Hounsfield unit (HU)] suggesting hepatic lipid content. Crude data were adjusted to age, sex, BMI and HbA1c values. Intra-pair correlations were established, and structural equation models were used for quantifying the contribution of additive genetic (A), common environmental (C) and unique environmental (E) components to the investigated phenotype.

Results: The study cohort represented a moderately overweight, middle-aged Caucasian population. There was no significant difference between MZ and DZ twin subjects regarding hepatic CT-attenuation (57.9 ± 12.6 HU and 59.3 ± 11.7 HU, respectively; $p = 0.747$). Age, sex, BMI and HbA1c adjusted co-twin correlations between the siblings showed that MZ twins have stronger correlations of HU values than DZ twins ($r_{MZ} = 0.592$, $p < 0.001$; $r_{DZ} = 0.047$, $p = 0.690$, respectively). Using the structural equation model, a moderate additive genetic dependence (A: 38%, 95% CI 15–58%) and a greater unique environmental influence (E: 62%, 95% CI 42–85%) was found. Common environmental influence was not identified (C: 0%).

Conclusion: The results of our classical CT-based twin study revealed moderate genetic and greater environmental influences on the phenotypic appearance of hepatic steatosis, commonly referred to as NAFLD. Favorable changes of modifiable environmental factors are of great importance in preventing or treating NAFLD.

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Introduction

Hepatic lipid accumulation in the absence of regular alcohol intake is commonly referred to as non-alcoholic fatty liver disease (NAFLD). In recent years, NAFLD has become increasingly more prevalent, affecting about 25% of adults worldwide, carrying severe vascular and hepatic outcomes [1–3]. It has been demonstrated previously that NAFLD might increase cardiovascular risk. In the Framingham Heart Study fatty liver was associated with several cardiovascular risk factors even after adjustment for other fat compartments [4]. Clinical studies have documented independent associations between NAFLD and increased incidence of cardiovascular events [5, 6]. Furthermore, NAFLD may progress to NASH (nonalcoholic steatohepatitis) and ultimately to cirrhosis, hepatocellular carcinoma, and liver failure [7]. NAFLD is often associated with obesity, type 2 diabetes, hyperlipidemia, hypertension, insulin resistance and, therefore, some authors have suggested that NAFLD should be considered as a component of the metabolic syndrome [8, 9].

In the clinical setting, various imaging modalities including ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI) have been used to investigate and quantify hepatic steatosis [10–16]. In daily clinical practice, ultrasonography is routinely used but it has high intra- and interobserver variability. Nevertheless, improvements in methodology resulted in more reliable results [17, 18]. Notably, European guidelines for the management of NAFLD recommend using ultrasonography as a first choice for imaging in adults who are at risk for NAFLD [19]. Recently, CT-imaging for the measurement of hepatic steatosis has become increasingly popular in different research and clinical projects [20, 21]. Importantly, hepatic steatosis is quantifiable on non-contrast-enhanced CT-images and presents with decreased attenuation values of the parenchyma due to the inverse relationship between hepatic fat content and hepatic attenuation [10]. Although MRI proved to be more accurate measurement for evaluating hepatic lipid content, the availability of this method is limited, and its cost is relatively high. Therefore, MRI is limited and reserved mainly for research and clinical trials [22]. Undoubtedly, the exact diagnosis of NAFLD needs liver biopsy and should be based on histopathological investigations. Nevertheless, this invasive method is very rarely justified in practice [23]. Clinicians should base their diagnosis on laboratory findings, biomarkers and results of imaging procedures. Comparative studies with histopathological investigations validated the reliability and usefulness of ultrasonography, unenhanced CT or MRI [13, 21, 22].

Despite the growing knowledge about the pathogenesis of NAFLD, the entire process is still not completely understood. Data about the contribution of genetic and environmental influences on the hepatic steatosis are limited [24, 25]. In an earlier study, our working group found only negligible role for heritability of NAFLD within healthy, middle-aged twin pairs, however, lipid accumulation was

visualized by using ultrasonography with its inherent limitations [26]. Currently, we intended to assess the effect of genetic and environmental influences on hepatic lipid accumulation within a cohort of adult twin pairs, undergoing CT-examination. Therefore, our objective was to evaluate the relative contribution of genetics versus environmental factors to hepatic steatosis, using CT-images available in participants of the BUDAPEST-GLOBAL clinical twin study.

Patients and methods

Study population

This study was a prospective, single-center, classical twin study involving monozygotic [MZ] and dizygotic [DZ] same-gender twins of self-reported Caucasian ethnicity. The investigation was conducted under the name of BUDAPEST-GLOBAL (Burden of atherosclerotic plaques study in twins – Genetic Loci and the Burden of Atherosclerotic Lesions) study; participants had been co-enrolled with the large, international, multicenter Genetic Loci and the Burden of Atherosclerotic Lesions (GLOBAL) clinical study (www.ClinicalTrials.gov: NCT01738828) [27]. Detailed study description and enrollment criteria were published previously [28]. The total study population consisted of 202 adult twin subjects (101 twin pairs) of whom 122 were MZ and 80 were same-gender DZ twin subjects. Participants were recruited from the Hungarian Twin Registry [29]. The study was approved by the National Scientific and Ethics Committee (institutional review board number: ETT TUKEB 58401/2012/EKU [828/PI/12], Amendment-1: 12292/2013/EKU [165/2013] and was carried out according to the principles stated in The Declaration of Helsinki. All subjects provided written informed consent.

The present analysis included 91 twin pairs (182 twin subjects; 120 women, 62 men); 10 twin pairs from the original cohort were excluded due to inadequate image quality. For the assessment of zygosity a self-reported questionnaire was used [30] and, based on this, 57 MZ and 34 DZ same-gender twin pairs were investigated. Main clinical and laboratory findings of twin pairs are summarized in Table 1.

Computed tomography (CT) scanning protocol

Each subject underwent a non-contrast enhanced CT-scan of the heart with a longer caudal coverage for visualizing the upper part of the abdomen (256-slice CT-scanner; Philips Brilliance iCT, Best, The Netherlands). Details of the study protocol were reported in our design paper [28]. Of note, the native CT-image acquisition resulted in a small (<1 mSv) radiation dose.

Hepatic parenchymal attenuation was measured to quantify hepatic lipid content (Fig. 1). We selected three circular regions of interest (ROI) with an area of at least 300 mm² on three cross-sectional images at different hepatic levels (one in the right hepatic lobe above the portal vein, one in the right hepatic lobe below the portal vein, and one in the left lobe). Special attention was taken to avoid hepatic larger vascular



Table 1. Demographic characteristics, medical history and clinical-laboratory data of twin subjects

Variables	Twin subjects (total, $n = 182$)	Monozygotic (MZ) twin subjects ($n = 114$)	Dizygotic (DZ) twin subjects ($n = 68$)	p Value (MZ vs. DZ twins)
<i>Demographic characteristics, medical history and clinical data</i>				
Female n (%)	120 (65.9%)	70 (61.4%)	50 (73.5%)	0.095
Age (years)	56.0 \pm 9.6	54.6 \pm 9.7	58.5 \pm 8.9	0.006
BMI (kg/m ²)	27.5 \pm 5.0	27.6 \pm 4.8	27.4 \pm 5.3	0.848
Waist circumference (cm)	96.1 \pm 13.2	96.0 \pm 13.6	96.6 \pm 12.8	0.758
Hypertension n (%)	75 (41.2%)	46 (40.4%)	29 (42.6%)	0.760
Diabetes mellitus n (%)	16 (8.8%)	11 (9.6%)	5 (7.4%)	0.600
Current smoker n (%)	31 (17.0%)	19 (16.6%)	12 (17.6%)	0.864
<i>Laboratory parameters</i>				
Fasting glucose (mmol/L)	5.31 \pm 1.27	5.34 \pm 1.47	5.26 \pm 0.85	0.629
HbA1c (%)	5.5 \pm 0.9	5.6 \pm 1.0	5.2 \pm 0.8	0.008
Total cholesterol (mmol/L)	5.54 \pm 1.08	5.65 \pm 1.13	5.38 \pm 0.99	0.095
LDL-cholesterol (mmol/L)	3.47 \pm 0.99	3.55 \pm 1.06	3.35 \pm 0.85	0.180
HDL-cholesterol (mmol/L)	1.60 \pm 0.38	1.59 \pm 0.40	1.64 \pm 0.34	0.357
Triglycerides (mmol/L)	1.57 \pm 1.06	1.67 \pm 1.23	1.39 \pm 0.65	0.043
Serum creatinine (μ mol/L)	75.9 \pm 8.8	79.6 \pm 8.8	70.7 \pm 8.8	0.161
ALT (U/l)	20.2 \pm 11.6	21.4 \pm 12.7	18.1 \pm 9.0	0.043
AST (U/l)	22.2 \pm 10.8	23.0 \pm 12.1	20.7 \pm 7.9	0.129
GGT (U/l)	34.8 \pm 39.1	37.4 \pm 28.9	30.6 \pm 39.4	0.260
Insulin (μ U/mL)	7.9 \pm 7.5	8.4 \pm 8.2	7.1 \pm 6.0	0.250
C-peptide (ng/mL)	2.3 \pm 1.4	2.2 \pm 1.3	2.3 \pm 1.5	0.635
hsCRP (ng/mL)	2.9 \pm 4.5	2.7 \pm 2.9	3.1 \pm 6.4	0.658

Continuous variables are presented as mean \pm SD, while categorical as n (%). P values represent two-sided p values for independent t -tests done between the monozygotic (MZ) and dizygotic (DZ) twin groups. BMI: body mass index, hsCRP: high sensitive C-reactive protein, HbA1c: hemoglobinA1c, HDL: high-density lipoprotein, LDL: low-density lipoprotein, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: γ -glutamyl transpeptidase

structures [21]. For further analysis we used the mean values of CT-attenuation measured in three ROIs of the liver. Hepatic attenuation was measured by two radiologists (ZD, ÁLJ both with 5 years of experience with CT) in consensus. CT-attenuation was expressed in Hounsfield unit (HU). Readers were blinded for the zygosity of subjects investigated.

Anthropometric data, medical history and laboratory analysis

We recorded basic anthropometric parameters (weight, height, waist circumference) in each subject. Brachial blood

pressure was measured prior to the CT-exam. We used questionnaires for evaluating past medical history and current lifestyle (smoking, dietary habits, physical activity). Fasting peripheral blood draw was performed before the CT-examination and we used standard methods in certified laboratory for assessing laboratory parameters.

Statistical analysis

For evaluating genetic and environmental influences on NAFLD, first we assessed co-twin correlations of HU in MZ and DZ pairs separately, and then we evaluated the

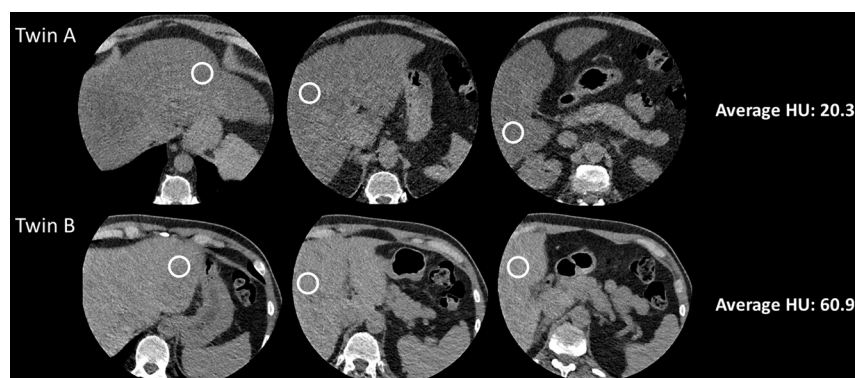


Figure 1. Evaluation of hepatic attenuation in a dizygotic twin pair. Measurements were performed in three regions of interest with at least 300 mm² (white circles), and average values of the three measurements were used for analysis. HU: Hounsfield unit

heritability using structural equation models often called ACE models. In the ACE model, the best fit model substantiates the quantification of genetic or environmental effects on phenotype investigated (in our study: hepatic lipid accumulation assessed by CT-attenuation, expressed in HU). All models were corrected for age, sex, BMI and HbA1c values. Log likelihood-based 95% confidence intervals (CI) were calculated for all estimated parameters. The most parsimonious model best describing our data was found by eliminating, C (AE model), A (CE model) or AC (E model). Deterioration of fit was assessed using $-2 \times \log$ -likelihood values. All calculations were performed using R version 3.6.0. [31]. Twin modelling was performed using OpenMx version 2.12.2. [32]. Using the structural equation model, the effect of genetic and environmental influences on a given phenotype can be partitioned into additive genetic effects (A), common (or shared) environmental (C) and unshared (or unique) environmental (E) factors, which drive the variance in the phenotype for each twin. Additive genetic effect (A) is perfectly ($r = 1.0$) correlated across MZ twins and correlated less ($r = 0.5$) across DZ twins. Environmental components are grouped as common factors (C), which equally effect the siblings, and unique factors (E), which cause differences within families. Both MZ and DZ twins shared 100% of their C factors and none of their E factors. Since measurement error in the phenotype is also uncorrelated across measurements, it appears as part of the unique environmental component.

Continuous variables are expressed as mean \pm standard deviation (SD) whereas categorical variables are expressed as numbers and percentages. MZ and DZ twins were compared using Student's t-tests and Chi-square tests. Correlations were calculated using Pearson correlation coefficients. Descriptive statistics and correlations were calculated using IBM SPSS Statistics version 23 (IBM, Armonk, NY, USA). The level of significance was considered as $p < 0.05$.

Results

Our study cohort (91 twin pairs, 182 twin subjects) represented a moderately overweight, middle-aged Caucasian population with a slight female predominance (Table 1). Mean values of laboratory data (including hepatic, renal and metabolic parameters) were within the normal range.

There was no significant difference between MZ and DZ twin subjects regarding hepatic CT-attenuation (57.9 ± 12.6 HU and 59.3 ± 11.7 HU, $p = 0.747$, respectively).

Age-, sex-, BMI- and HbA1c-adjusted co-twin correlations between the siblings showed that MZ twins have stronger correlations of HU values than DZ twins ($r_{MZ} = 0.592$, $p < 0.001$; $r_{DZ} = 0.047$, $p = 0.690$, respectively).

Using the structural equation model, the role of environmental influences was greater (E: 62%, 95% CI 42–85%) compared to a moderate, additive genetic dependence (A: 38%, 95% CI 15–58%). Common environmental influence was not identified (C: 0%). Detailed model information is given in Table 2.

Table 2. Detailed model information regarding single trait classical twin model of CT-based liver attenuation

Variable	Full model	Estimated parameters	A	95% CI	C	95% CI	E	95% CI	Model-2LL	AIC	BIC	Difference to model -2LL	Difference to full model <i>p</i>
Liver attenuation (HU)	ACE	ACE	0.38	[0.05–0.58]	0.00	[0.00–0.24]	0.62	[0.42–0.85]	471.03	119.03	-320.93		
	AE	AE	0.38	[0.15–0.58]			0.62	[0.42–0.85]	471.03	117.03	-325.43	0.00	1.00
	CE	CE			0.23	[0.03–0.42]	0.77	[0.58–0.97]	475.71	121.71	-320.75	4.68	0.03
	E	E					1.00	[1.00–1.00]	480.73	124.73	-320.23	9.70	0.008

A: additive genetic factor, C: common environmental factor, E: unique environmental factor, HU: Hounsfield unit



Discussion

Based on the results of our CT-based classical twin study, the contribution of unique environmental factors appears to have a greater role than genetic determinants in the development of hepatic steatosis.

We were uniquely positioned to address this question by performing a classical twin study which has value even in the current omics era of molecular genetic studies [33]. Our cohort consisted of middle-aged (56.0 ± 9.6 years) adult twin subjects ($n = 182$), with a female predominance (65.9%) and a higher number of MZ vs. DZ twin subjects ($n = 114$ vs. $n = 68$, respectively). These numbers are in line with former investigations as females and MZ twin pairs are more willing to participate in different research projects [34]. The sample size of our current investigation is also comparable to that of former classical twin studies [34].

We used non-enhanced CT-images for evaluating hepatic lipid accumulation, similarly to prior studies [10]. There is no consensus for CT-based criteria for NAFLD; both attenuation absolute values (expressed in HUs) and derived numbers (spleen-to-liver attenuation ratio or difference in attenuation values between liver and spleen) have been used [10, 21]. In our twin study, we preferred to use absolute numbers of HUs instead of derived ratios or differences. We considered more appropriate for assessing hepatic lipid content by measuring absolute attenuation values.

We found that environmental influences outweighed genetic influences in the development of hepatic lipid accumulation. Our current CT-based findings corroborate our previous investigation using ultrasonography [26], although the results of ACE models are numerically slightly different. Nevertheless, we expected a slight numerical difference as we used different methodology in a different twin population.

Our results have profound clinical implications. Since our study suggests that the development of NAFLD is predominantly influenced by environmental factors, the importance of lifestyle changes (including healthy diet, medical nutrition therapy, nutraceutical/dietary supplements, regular physical activity or behavioral intervention) is substantial in the primary prevention of NAFLD and related conditions [35, 36]. In addition, bariatric surgery in obese people may reduce NAFLD [37]. Although several different drugs have shown some potential promising results in early clinical Phase-2 and Phase-3 trials, there are no currently approved drugs for the treatment and prevention of NAFLD and NASH [38, 39].

Our study has several limitations. Hepatic steatosis was estimated based on CT-attenuation, without any histological validation. Nevertheless, unenhanced CT-based evaluation of NAFLD is accepted, and histopathological correlations have been already published by others [21]. Furthermore, while biopsy-based steatosis measurements are severely hindered by “geographic miss” [40] our CT-based approach allows for the quantification of hepatic steatosis in a large, substantial portion of the liver. In our study, zygosity was classified using validated questionnaires; a method widely

used in clinical twin studies [30]. Our study was performed in adult twin subjects of Caucasian population; therefore, the generalizability of our findings is limited.

In conclusion, our classical CT-based twin study documented moderate genetic and greater environmental influences on the phenotypic appearance of hepatic steatosis commonly referred to as NAFLD. Therefore, favorable changes of modifiable environmental factors are of great importance in preventing or treating NAFLD.

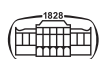
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Authors' contribution: GJ, PMH conceived the study, GJ, ZDD drafted the manuscript, MK performed the statistical analysis, contributed to interpretation, ALJ, ZDD, AP, IFS, JK, ADT, DLT performed the clinical study, collected data, PMH provided continuous supervision of the study, SV, BM, PMH critically reviewed the manuscript with intellectual contribution to interpretation. GJ wrote the final version of manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read the final version of the manuscript and agreed to submit it to IMAGING for Open Access publication.

Conflict of interest: Szilard Voros, MD, is a shareholder in Global Genomics Group, LLC, and receives salary from Global Genomics Group, LLC. The authors have no other financial relationships, or conflicts of interest to disclose.

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