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Literature review of genes responsible for intramuscular fat content and its methodology in swine

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ABSTRACT - The organoleptic value of pork, e.g. its taste and tenderness, as well as overall acceptability is positively influenced by fat content, including both inter- and intramuscular fat (IMF) content up to a certain threshold. Recently, a number of research dealt with studying the genetic background of IMF incorporation. Many genes have been identified that are involved in fat metabolism and in development of marbling in muscle tissue. The aim of this work is to review the current literaure written about the most important genes and gene families that play role in IMF metabolism. The most studied genes are FABP3 and FABP4, which are part of the FABP family. They have a key role in the transport and intermediate metabolism of lipids. Number of studies have recently been published discussing the role of SCD (stearoyl-CoA desaturase) encoding gene in IMF content. Since multiple genes have been already identified playing a key role in fat metabolism and in fat deposition in muscle tissue, its gene expression studies are crucial in genetic programmes as well as in nutrigenomical research.

Keywords: Swine, IMF, gene expression, SNP, GWAS

INTRODUCTION

The intensive genetic selection for higher growth rate and better feed efficiency resulted in high lean meat % in some pig hybrids. High lean meat % is a consequence of high protein and low fat deposition, thus the fat content of those pork is usually extremely low, such as 1% fat in loin chops. In general, most of the meat quality traits (fat content, level of marbling, drip loss, taste, tenderness, etc.) are in correlation with one another and these together shape the rejection and acceptibility for the consumer, or they provide a high value of pleasure (*Hocquette et al., 2010*). It is widely accepted that intramuscular fat (IMF) content together with tenderness explain much of the variability in eating quality and acceptability of fresh pork (*Fernandez et al., 1999b; Hamill et al., 2012*). However, the consumers have ambivalent demands regarding the appearance, marbling and organoleptic traits of pork. Studies confirmed that consumers less likely purchased marbled than leaner chops due to appeared lighter color, less lean, and overall less acceptable appearance, while according to the taste panel scores of the same people marbled chops were juicier, more

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tender, oily and flavorful than leaner chops (*Brever et al., 2001; Schwab et al., 2009; Font-i-Furnos et al., 2012*). *Font-i-Furnos et al. (2012)* revealed that the consumers preferred loins with higher IMF level despite the fact that they identified themself such as 'lean loin lovers' or 'marbled loin lovers'. It is repeatedly confirmed that the minimum IMF content recommended to ensure a good taste is between 2.2% and 3.4% (*Fernandez et al., 1999; Font-i-Furnos et al., 2012*).

Regarding the above mentioned trends, sufficient level of IMF is needed to enhance consumer acceptance of pork products, and as a consequence the IMF is receiving greater attention within swine genetic improvement programmes (*Schwab et al., 2009*). It is well documented that different genotypes have different capacity to deposit fat and to develop the inter- and intramuscular fat. The underlying molecular genetic mechanism of fat deposition in fatty and lean pigs has been at least partly elucidated in number of recent studies. Many genes are involved in fat metabolism of the body and in the appearance of fat in muscle tissue. The aim of the present paper is to review the current literaure and give a summary of the most important genes and gene families that play role in IMF metabolism.

GENETICAL BACKGROUND OF FAT DEPOSITION IN MEAT

IMF (intramuscular fat) includes all lipids found in muscles, including functional lipids, such as phospholipids which build up cell membranes, cholesterol, reserve lipids, triglicerides which serve as energy storage for the organism. Triglicerides can be found in the muscles of mammals, primarily in intramuscular adipocytes and in the cytoplasm of miofibrillums (*Hocquette et al., 2010; Guo et al., 2014*).

In muscle cells, intracellular long-chain fatty acids come primarily from material transported by heart-type fatty acid binding protein (H-FABP) *(Glatz et al., 2003*). The expression of H-FABP is realted to the modulation of proteins and enzymes, which participate in the handling and utilisation of the fatty acid. This explains the expressional abundance of H-FABP mRNS and the simultaneous increase of IMF-content (*Chen et al., 2012*). Adipocyta-type fatty acid binding protein (A-FABP) plays a critical role in the intracellular circulation of the fatty acid. It was identified as a candidate gene responsible for fat deposition in swine (Gerbens et al.,1998; Chmurzyńska, 2006). *Gerbens et al. (2001)* could not find a connection between the IMF content and the expression of A-FABP in large white × dutch landrace swine hybrids. However, *Damon et al. (2006)* showed that the protein content of A-FABP was doubled in the *longissimus dorsi* muscle of swine with high IMF content, contrary to swine with low IMF-

content and positive correlation coefficients were found between the protein level of A-FABP, adipocyte count and the lipid content.

There are numerous reports about the role of FABP gene family regarding IMF, especially about their role in lipid transport. FABP family means fatty acid binding proteins. These are transport proteins, transporting fatty acids and other lipophilic substances. The FABP family helps fatty acids in getting through extra- and intracellular membranes (*Chmurzyńska, 2006*). H-FABP genes and A-FABP genes are candidates concerning IMF content. The H-FABP gene is present in many tissues, with the highest expression in heart- and skeletal muscle. The genetical variations of the H-FABP gene locus are related to IMF content. *Tyra et al. (2010)* studied H-FABP and LEPR (leptin receptor) to find out if there is any correlation between IMF concentration and gene expression. It is obvious from their report, that H-FABP is an important regulating element of fat transport and it plays roles in other processes, such as transcription processes, cell proliferation, cell differentiation. The LEPR gene plays an important role in the incorporation of IMF because it encodes leptin, an important hormone of fat metabolism. The leptin hormone is a protein produced by fat cells, that contributes to fat metabolism (*Tyra et al., 2011a*). An other research also reported about the role of FABP3 and LEPR genes regarding IMF concentration. The LEPR and FABP3 genes are nestled on the 6. chromosome in swines (*Tyra et al., 2011b*). *Zhao et al. (2012)* studied the expression of LEPR and FABP genes in two groups of swine with different fat content. According to their results, the expression of the LEPR gene was smaller, while that of the FABP gene was higher in the stock containing less fat and more meat. The FABP gene family is summarized in the following table (*Table 1.*) (*Chmurzyńska, 2006; Smathers, 2011*).

STUDIES CONCERNING THE GENETICAL BACKGROUND OF IMF INCORPORATION

Genotyping

In general, there is a moderate, positive fenotypical correlation between the intramuscular fat content and backfat thickness. That means the higher the backfat thickness in a certain genotype the higher is the IMF. Since the lean meat % is indirectly measured with consideration of the backfat thickness and the loin diameter, therefore the backfat thickess is negatively correlated with the lean %. Regarding the causality, the higher lean meat % is associated generally with a lower IMF content. Because of this, it is useful to find single-nu-

cleotide polymorphisms (SNPs) that on one hand can increase the intramuscular fat content, but in the same time they do not increase the backfat thickness. An SNP is usually a biallelic locus at a specific position in the genome. At a specific base position, an allele may appear in most individuals, but in a minority of individuals, the position is occupied by an other allele (*Wang et al., 2019*).

Table 1
The FABP gene family

Gene name with number	Gene name	Protein name	Tissue distribution
FABP1	FABPL	FABP 1	liver
FABP2	FABPI	FABP 2	intestinal
FABP3	FABPH	FABP 3	muscle and heart
FABP4	FABPA	FABP 4	adipocyta
FABP5	FABPE	FABP 5	epidermal
FABP6	FABPIL	FABP 6	ileum
FABP7	FABPB	FABP 7	brain
PMP2	FABPM	FABP 8	peripherial nervous system, myelin
FABP9	-	FABP 9	-
fabp11	-	FABP 11	Fish
FABP12	-	FABP 12	human retinoblastoma
-	FABPT	-	Testis

In an experiment FABP3, LEPR, SCD, IGF1, IGF2, LIPE, LEP, MC4R, RETN, RYR1 was studied. Among these SNPs low polymorphisms of IGF1 alleles (rs322131043), LEP (rs45431504), LEPR (rs45435518), RETN (rs327132149) and RYR1 (rs344435545) were observed. The results of the study indicate that most of the SNPs correlated, except for FABP3 (rs1110770079). The strong selection pressure on growth rate, lean meat yield, and backfat thichess conservated the link between IMF and backfat thickness. However, the FABP3 SNP could be used as a marker to improve IMF content without changing backfat thickness in the Suhuai pig breeding system (*Wang et al., 2019*).

The genome-wide association studies (GWAS) make it possible to study over one-hundred thousand individual nucleotides (SNP) (*Pearson et al., 2008*). As a result of GWAS, several illnesses associated genes, genes associated with economically important properties and genes associated with various metabolic and biological functions have been identified (*Viterbo et al., 2018*). The fatty acid composition of Duroc swine showed that saturated fatty acids (SFA) and mono- unsaturated fatty acids (MUFA) are highly heritable, according to genetic estimations they are at 0.50-0.57, while polyunsaturated fatty

acids (PUFA) are moderately at between 0.25 and 0.46. These heritabilitiy values imply large potential for improvement of pork quality in terms of its fatty acid profile (*Gjerlaug-Enger et al., 2011*). In a study 470 genotyped animal and 39919 SNP subsets were used in an association test. In this study the genome sequence of the same region (SSC14) of the chromosome 14 (SSC14) of swine showed significant association with stearic acid (18:0), oleic acid (18:1) and SFAs. Top SNP ALGA008191 was located at 5 kb near the stearoyl-CoA desaturase (SCD) gene. This gene is directly involved in desaturation of stearic acid into oleic acid. In the results an important QTL was identified on SSC14 (120–124 Mb) associated with stearic acid, oleic acid and SFA in Duroc swine. The candidate gene in the chromosomal segment is the SCD gene that has direct effect on fatty acid composition (*Viterbo et al*., 2018). Other research also corroborates that the SSC14 region with 120–124 Mb has tight connections with SFA, MUFA, C18:1 and with the C18:1/C18:0 desaturation index. The most associated window was located either at 121–122 Mb, for the muscles, or at 122–123 Mb, for SF. This region; that according to estimations; can incorporate the 44.8% of the genetic variant of C18:1/C18:0, corresponds to the SCD gene site and thus confirms that the haplotype in the SCD gene promoter has an association with the desaturation of C18:0 to C18:1 (*Ros-Freixedes et al., 2016*).

Gene expression studies

RT-qPCR-based gene expression studies

Numerous studies have investigated the genetic background of IMF incorporation. Multiple genes have been identified, that play a key role in the fat metabolism of the organism and in the appearance of fat in muscle tissue. During fat incorporation different mechanisms are governed by the genes, i.a. they take part in lipid metabolism, lipid transport, de novo fatty acid synthesis, fatty acid oxidation, fat deposition, elongation and desaturation (*Lim et al., 2016; Chmurzynska, 2006; Kulkarni et al., 2007; Meadus et al., 2011; Xing et al., 2016*). *Zhang et al. (2002)* reported, that in the initial phase of fat deposition, ATN1 gene appears as a co-expressor during the transcription process. EEF1A2 has a relation with mRNA translation, it bounds to aminoacylated tRNA and it helps the detachment of the ribosome during the elongation process (*Kulkarni et al., 2007*).

Several researches in recent years have dealt with the genetic background of IMF content and gene expression. *Serao et al. (2011)* studied the IMF content of 72 swines through a candidate gene expression approach. The swines originated from three different genetical groups: an indigenous Piau swine with

high fat content, a meat-type crossbred stock (Landrace x Large White x Pietrain) and crossbreeds of the two genotypes in both sexes. The animals were slaughtered in different body weights (30, 60, 90 and 120 kg). IMF content was measured in the *M. longissimus dorsi* and correlated with the mRNA expression of the probed genes. The genes involved in different mechanisms such as lipid transport (FABP3, LDLR), transcription and translation process (ATN1, EEF1A2) and energy utilisation (MGP) were studied. According to their results FABP-3 (Fatty-acidbinding protein 3) and LDLR (Low density lipoprotein receptor) genes support the deposition of fat in muscle. The first gene is responsible for lipid transport process in cells, while the latter is responsible for the internal transport mechanism in the connective tissue. These genes show positive correlation with the intramuscular fat content. The ATN1 (that plays role as a transcription and translation factor) and the MGP genes (which takes part in cell differentiation) did not show significant correlation with IMF content *(Kulkarni et al., 2007*). It has been shown that higher degree of gene expression can result in a higher degree of protein synthesis, however, this may not result in a higher rate of muscle deposition (*Serao et al., 2011*). With advancements of RNA sequencing technologies, differences founded in transcriptomes can be correlated with the features of the animals. *Lim et al. (2016)* obtained differently expressed genes (DEG) with RNA sequencing, that are in tight relationship with the IMF content *of longissimus dorsi*. Two groups of Berkshire swine with different IMF content (extremely high and low IMF content group) were analyzed with multidimensional scale analysis and 134 genes were identified. The functional analysis has shown that the studied, differently expressed genes and lipid metabolism (SCD, FASN) have evident biological association and the degree of expression of DEG is defining in regard of the IMF content (*Lim et al., 2016*). *Wang et al. (2017)* explored molecular mechanisms in the *M. longissimus dorsi* of Laiwu swine with transcriptome analysis. The swine breed, widespread in Northern China, is explicitly fatty and has an exceptionally high IMF content (9-12%). Samples were taken from animals of different ages (60, 120, 240 and 400 days old). The most intensive period of fat incorporation into muscle was between 120-240 days. Genes with connections to lipid biosynthesis (FOSL1, FAM213B and G0S2), transcription factors (TF) (EGR1, KLF5, SREBF2, TP53 and TWIST1) and some of the steroid and lipid biosynthesis pathways were identified, respectively. During the 120-240-day period of intensive fat incorporation 10 up- and down regulated DEG were found that take part in numerous biological process, such as in the metabolism of energy (ATP5J2, DNAJB1), transcription (EGR1, KLF11), metabolism of fat

(G0S2, CYP1A1, FAM213B), or development of muscle (CHRNG, TNNT2). In lipid biosynthesis five up regulated (CYP1A1, SERPINA1, LDLR, EGR1 and FOSL) and five down regulated (DIAPH1, SORBS1, PDK4, ACSL1 and ASPA) genes were reported (*Wang et al., 2017*).

SCD (stearoyl-CoA desaturase) and FASN are genes contributing to lipid metabolism. *Lim et al. (2016)* had studied their role and found significant association between IMF content and the expression of genes. Further reports also confirm the relationship of these two genes with the amount of IMF *(Renaville et al., 2012; Zappatera et al., 2016*).

RNA sequencing studies

One of the genomics methods is RNA-seq which serves to analyze transcriptome profiles. Recently, this method has been used extensively, because results obtained by RNA-seq are more informative than the ones obtained by gene expression microarray technology. Contrary to RNA-seq, microarray technology can not identify new transcripts, inversions and alternative splice variants *(Hurd and Nelson, 2009*). The main application of RNA sequencing is to evaluate DEG among studied groups. In case of swine this method has been utilised to ascertain the transcriptome profile depending on breed, type of tissue and phenotype *(Ropka-Molik et al., 2014; Esteve-Codina et al., 2011; Corominas et al., 2013*). The RNA-seq method provides information on transcripted sequences, hence it can be used to identify genetical mutations (*Martínez-Montes et al., 2016*). Individuals with different phenotypes of a given trait can be compared by transcriptome studies, providing a powerful approach to identify genetic pathways and networks with different expression among livestock species such as swine or cattle. These studies contributed to the recognition of procedures associated with IMF deposition. Transcriptome studies utilising RNA-seq in breed and tissue of different age brought highly relevant results concerning genetic expression patterns and networks upon which IMF is based on (*Munoz et al., 2018*).

With RNA-seq the complete RNA complement or transcriptome of a single cell or cell population can be analysed and highly expands the possibility of transcriptome studies to analyze a gene (isoforms, translocational events, nocleotid variants and transcription post-transformations) (*Ketkar and Kulkarni, 2015*).

THE ROLE OF GENES INVOLVED IN IMF INCORPORATION

The role of the MAPK cascade in the incorporation of IMF

Based on the results of a novel research *(Won et al., 2018*), it is possible that elements of the Mitogen-Activated Protein Kinase (MAPK) cascade also play role in the process of IMF incorporation. MAPK pathway is a name for the cascade mechanism that has a strong realtionship with information flow between cells. The participating proteins convey a signal from the receptors on the surface of the cell to the DNA inside the nucleus. Hence, MAPK plays an important regulating role in many different cellular processes during proliferation, differentiation and mitosis among others. Furthermore, it has an unarguable key role in the development of preadipocytes to adipocytes. MAPK phosphatase 1 (MKP1) is a part of this pathway, that is an important regulator in the development of adipocytes. The BMPER, FOXO 1, SOX9, PTN1, CD40 and EGF genes are regulating elements of the MAPK cascade. Of these, the SOX9 gene inhibits the development of adipocytes. The FOXO 1 gene induces the development of preadipocytes to adipocytes during the early stages of fat cell differentiation. The BMPER gene activates BMP4 which catalyses the accumulation of lipids. Because the amount of IMF is determined by the amount of adipocytes, genes having correlation to the MAPK pathway can influence IMF content by regulating the level of adipocyte differentiation (*Won et al., 2018*).

BMPER (BMP-binding endothelial cell precursor-drived regulator) is a key gene contributing to angiogenesis and vascularisation. *Liu et al. (2014)* came to the conclusion that paralellism can be drawn between the IMF concentration and the level of BMPER mRNA.

Meng et al. (2018) studied HSL (Hormone-sensitive lipase), FASN (Fatty acid synthase), and FABP4 genes. By adding lecitine and/or L-carnitine to the feed the mRNA expression of these genes showed increase and fatty acid deposition also occured *(Meng et al., 2018).*

The Forkhead transcription factor O1(FoxO1) gene plays an important role in the hormone activated signal pathway integration of the complex transcription cascade, which helps the differentiation of the clonal cell line. The expression of the FoxO1 gene was studied by *Pang et al*. in *2009*. During their research, they studied this gene in 1 and 180 days old swine. The expression of FoxO1gene was determined by qRT-PCR. The studied tissues were the following: visceral adiposa, subcutaneous adipose, liver and muscle.

Their results show that the expression profile of the 1 day and 180 days old animals were different. In case of the 1 day old animals the order of expression was the following: visceral adipose>subcutaneous adipose>liver>muscle. In

case of the 180 days old animals: subcutaneous adipose>visceral adipose>liver>muscle. The expression of FoxO1 was the highest in subcutaneous fat and the difference in between the various tissues is not as high as in case of the 180 days old animals. They were significantly higher in the subcutaneous adipose, visceral adipose and liver tissue of 1 day old animals, than in the 180 days old animals. *(Pang et al., 2009*).

Table 2
Genes, gene families and their metobolic roles in IMF metabolism and incorporation

Gene or gene family name	Abbreviation	Effect of genes on metabolism	Methodology of investigation	Reference
Stearoyl-CoA desaturase	SCD	directly involved in desaturation of stearic acid into oleic acid	GWAS	*Viterbo et al,.* 2018
Fatty acid synthase	FASN	lipid metabolism	DEG	*Lim et al.,* 2016
BMP-binding endothelial cell precursor-drived regulator	BMPER	key gene contributing to angiogenesis and vascularisation	Real-Time Reverse Transcription-PCR	*Liu et al.,* 2014
Hormone-sensitive lipase	HSL	rate-limiting enzyme for triacylglycerol hydrolysis	Real-Time Reverse Transcription-PCR	*Meng et al.,* 2018
Leptin receptor gene	LEPR	encodes leptin, an important hormone of fat metabolism	Real-Time Reverse Transcription-PCR	*Tyra et al.,* 2011
Heart -type fatty-acid-binding protein	FABP3 (FABP-H)	lipid transport	Real-Time Reverse Transcription-PCR	*Tyra et al.,* 2011
Adipocyte-type fatty acid binding protein	FABP4 (FABP-A)	plays a critical role in the intracellular circulation of the fatty acid	Real-Time Reverse Transcription-PCR	*Damon et al.,* 2006

CONCLUSIONS

There are various methods to explore the genetic background and the underlying mechanisms of IMF incorporation. From different methods of the molecular biological techniques, particularly gene expression studies are the most informative ones. The most studied genes are FABP3 and FABP4, which are part of the FABP family. They have a key role in the transport and intermediate metabolism of lipids. Number of studies have recently been published discussing the role of SCD (stearoyl-CoA desaturase) encoding gene in IMF content.

Since multiple genes have been already identified that play a key role in fat metabolism and in fat deposition in muscle tissue and the pork quality traits such as taste and tenderness are positively correlated with its fat content, gene expression studies are crucial in genetic programmes as well as in nutri-genomical research. Such explorations are paving the way to understand the pathways and to modify metabolism to achieve higher pork quality.

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