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Exploring the pig muscle transcriptome and candidate genes for lipid metabolism

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Exploring the pig muscle transcriptome and candidate genes for lipid metabolism

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fa constar

que Magí Passols Manzano ha realitzat sota la seva direcció el treball de recerca i la redacció de la memòria de la tesi doctoral titulada:

"Exploring the pig muscle transcriptome and candidate genes for lipid metabolism"

i certifica

que aquest treball s'ha dut a terme al Departament de Ciència Animal i dels Aliments de la Facultat de Veterinària de la Universitat Autònoma de Barcelona i al Grup de Recerca de Genòmica Animal del Centre de Recerca en Agrigenòmica considerant que la memòria resultant és apta per optar al grau de Doctor en Producció animal per la Universitat Autònoma de Barcelona,

I perquè quedi constància, signen aquest document a Bellaterra, a 28 de Setembre de 2023.

Dr. Josep Maria Folch Albareda Magí Passols Manzano

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“If A is success in life, then

A = X + Y + Z . Work is X, play is Y,

and Z is keeping your mouth shut."

Albert Einstein.

CONTENT

SUMMARY

Global meat consumption, with pork as a prominent choice, is on the rise. Enhancing meat quality is closely linked to consumer preferences for healthier and more flavourful meat products. Key factors influencing meat quality include fatty acid composition and intramuscular fat, although the underlying processes are intricate and multifaceted. This thesis is dedicated to uncovering the molecular mechanisms that affect lipid metabolism and fatty acid composition in pork. As pork stands as one of the primary source of human-consumed meat, the growing demand for high-quality meat highlight the importance of understanding the molecular processes controlling meat production and quality.

We studied the *longissimus dorsi* mRNA expression of 45 candidate genes related to lipid metabolism in a total of 354 animals. The eGWAS identified 301 eSNPs located in 27 eQTLs. Three out of 27 eQTLs corresponding to the *GPAT3, RXRA* and *UCP3* genes were classified as *cis*-acting eQTLs, whereas the remaining 24 eQTLs presented *trans*-acting effects. In addition, the eGWAS revealed two *trans*-eQTL hotspots, which regulate the expression of various candidate genes.

Moreover, RNA-Seq data from Backcross Iberian × Duroc pigs were used to identify 2,146 SNPs which presented allelic imbalance in a total of 1,621 genes through allelic-specific expression (ASE) analysis. Among the 2,146 SNPs, 69 were located in 52 genes involved in lipid metabolism and fatty acid composition pathways. The top ten ASE-SNPs located in *ACADM*, *ECHS1, UCP3, LPIN1, PRXL2B, FDFT1, PNPLA2, ACSL1* and *ETFA* genes were the most interesting based on the higher proportion of allele-specific expression.

Finally, a study on muscle transcriptome of 129 pigs by RNA-Seq was carried out with the aim to identify candidate genes related to lipid metabolism and muscle gene expression regulators. The eGWAS identified a total of 2,678 eQTLs located in 854 genes, of which 620 were classified as *cis*-eQTL and 2,058 as *trans*-eQTLs. Among the 854 genes, 101 were associated with lipid metabolism and fatty acid composition pathways. The main pathways identified for the 854 genes with significant eQTLs were metabolic processes, oxoacid metabolic process, and carboxylic acid metabolic process. At last,

ACAA1, CLN, CYP2B22, GBA and *LDHD* genes were proposed as candidate genes in modulating the levels of eight different fatty acids.

RESUMEN

El consumo global de carne, con el cerdo como una elección destacada, está en aumento. Mejorar la calidad de la carne está estrechamente relacionado con las preferencias del consumidor por productos cárnicos más saludables y sabrosos. Factores clave que influyen en la calidad de la carne incluyen la composición de ácidos grasos y la grasa intramuscular, aunque los procesos subyacentes son complejos y diversos. El objetivo de la tesis es descifrar los mecanismos moleculares que afectan al metabolismo de lípidos y la composición de ácidos grasos en la carne de cerdo. Dado que el cerdo es una de las fuentes principales de carne consumida por los humanos, la creciente demanda de carne de alta calidad destaca la importancia de comprender los procesos moleculares que controlan la producción y calidad de la carne.

Estudiamos la expresión de ARNm del músculo *longissimus dorsi* de 45 genes candidatos relacionados con el metabolismo de lípidos en un total de 354 animales. El eGWAS identificó 301 eSNPs ubicados en 27 eQTLs. Tres de los 27 eQTLs correspondientes a los genes *GPAT3*, *RXRA* y *UCP3* se clasificaron como regiones *cis*-eQTLs, mientras que los 24 restantes se identificaron como *trans*-eQTLs. Además, el eGWAS reveló dos *trans*-eQTL *hotspots*, que regulan la expresión de varios genes candidatos.

Además, los datos de RNA-Seq de 129 animales del retrocruce Ibérico × Duroc se usaron para identificar 2,146 SNPs que presentaban expresión alélica diferencial en un total de 1,621 genes mediante análisis de expresión alélica específica (ASE). De los 2,146 SNPs, 69 se encontraban en 52 genes relacionados con las vías del metabolismo de lípidos y la composición de ácidos grasos. Los 10 ASE-SNPs ubicados en los genes *ACADM*, *ECHS1*, *UCP3*, *LPIN1*, *PRXL2B*, *FDFT1*, *PNPLA2*, *ACSL1* y *ETFA* fueron los más interesantes debido a su mayor proporción de expresión alélica específica.

Finalmente, se llevó a cabo un estudio sobre el transcriptoma de músculo en 129 cerdos mediante los datos de RNA-Seq con el objetivo de identificar genes candidatos relacionados con el metabolismo de lípidos y reguladores de la expresión génica muscular. El eGWAS identificó un total de 2,678 eQTLs ubicados en 854 genes, de los cuales 620 se clasificaron como *cis*-eQTL y 2,058 como *trans*-eQTL. Entre los 854 genes, 101 estaban asociados con las vías del metabolismo de lípidos y la composición de ácidos

grasos. Los principales procesos identificados para los 854 genes con eQTLs significativos fueron procesos metabólicos, el proceso metabólico de oxoácidos y el proceso metabólico de ácidos carboxílicos. Por último, los genes *ACAA1*, *CLN*, *CYP2B22*, *GBA* y *LDHD* se propusieron como genes candidatos para modular los niveles relativos de ocho ácidos grasos diferentes.

LIST OF TABLES

GENERAL INTRODUCTION

PAPER I

PAPER III

GENERAL DISCUSSION

ANNEXES: Paper I

ANNEXES: Paper II

ANNEXES: Paper III

LIST OF FIGURES

GENERAL INTRODUCTION

PAPER I

PAPER II

PAPER III

LIST OF PUBLICATIONS

The present thesis is based on the work contained in the list of articles below:

- Paper I: **Passols, M**., Llobet-Cabau, F., Sebastià, C., R., Castelló, A., Valdés-Hernández, J., Sánchez, A., Folch, J.M (2023). "Expression genome-wide association study of lipid metabolism candidate genes in pig muscle". (In revision)

- Paper II: **Passols, M**., *et al.* "Expression quantitative trait loci (eQTL) identification and its relationship with lipid metabolism in pig muscle". (Preliminary manuscript)

- Paper III: **Passols, M.,** Vos de, J., Madsen, O., González-Prendes, R., Llobet-Cabau, F., Castelló, A., Sánchez, A., Folch, J.M (2023). "Deciphering allele-specific expression in muscle transcriptome of Duroc crossbreed pigs from RNA-seq data". (Manuscript in preparation)

RELATED PUBLICATIONS BY THE AUTHOR

Not included in the thesis

- Criado-Mesas L., Ballester M., Crespo-Piazuelo D., **Passols** M., Castelló A., Sánchez A., Folch JM. (2021). "Expression analysis of porcine miR-33a/b in liver, adipose tissue and muscle and its potential role in fatty acid metabolism". *PLoS One* 26;16(1):e0245858. https//doi: 10.1371/journal.pone.0245858.

- Valdés-Hernández J., Ramayo-Caldas Y., **Passols M.,** Sebastià C., Criado-Mesas L., Crespo-Piazuelo D., Esteve-Codina A., Castelló A., Sánchez A., Folch JM. (2023). "Global analysis of the association between pig muscle fatty acid composition and gene expression using RNA-Seq". *Scientific Reports* 11;13(1):535. http://doi: 10.1038/s41598-022-27016-x.

ABBREVIATIONS

3BCs	The three backcrosses together
ACACA	Acetyl-CoA carboxylase
ACSL4	Acyl-CoA Synthetase Long-Chain Family Member 4
ACSM5	Acyl-CoA synthetase medium-chain family member 5
ASE	Allelic Specific Expression
BC1_DU	25% Iberian x 75% Duroc backcross
BC1_LD	25% Iberian x 75% Landrace backcross
BC1_PI	25% Iberian x 75% Pietrain backcross
cDNA	Complementary DNA
ELOVL	ELOVL fatty acid *elongase*
eQTL	Expression quantitative trait
FA	Fatty Acid
FABP	Fatty Acid-Binding Protein
FASN	FA synthase
FADS	FA desaturase
GWAS	Genome-wide association study
IMF	Intramuscular Fat
MUFA	Monounsaturated fatty acid
NGS	Next generation sequencing
PCR	Polymerase-chain reaction
PPAR	Peroxisome proliferator activated receptor
PUFA	Polyunsaturated fatty acid
QTL	Quantitative trait *loci*
RNA-Seq	RNA sequencing
RT-qPCR	High-throughput real-time quantitative PCR
RXR	Retinoid X Receptor
SCD	Stearoyl-CoA Desaturase
SFA	Saturated fatty acid
SNP	Single nucleotide polymorphism
SSC	*Sus scrofa* chromosome

GENERAL INTRODUCTION

Chapter 1

1.1. Current situation of pork production

The economic significance of pig (*Sus scrofa*) is undeniable, as they are one of the most widely consumed sources of meat worldwide, alongside with chicken and beef. Pigs were domesticated around 10,000 years ago, and it was during the 1960s and 1970s that the establishment of selection programs and the crossbreeding of different breeds led to significant improvements in their production. Nowadays, the pig industry is one of the most important sectors in meat production. The development of an intensive and highly technified productive system has made possible the access of consumers to affordable and safe pig meat and other processed products.

In the upcoming years, China is expected to lead in contributing to the global increase in meat production, followed by Brazil and the United States. The increase in pork production is expected to remain restricted in the next three years due to the slow recovery from the outbreaks of Asian Swine fever (ASF) in China, the Philippines and Vietnam. It is hypothesized that the recovery from the above-mentioned diseases will culminate by the year 2023, predominantly in China, supported by the rapid development of large-scale production facilities that can ensure biosecurity. Based on current trends, it is projected that the global consumption of meat proteins will experience a 14% increase by 2030 relative to the average consumption levels observed between 2018 and 2020. Hence, pig meat production is estimated to experience an increase of approximately 44 Million tonnes during the next decade. This growth is primarily attributed to the expansion of both population and income levels (OECD-FAO Agricultural Outlook 2021-2030, agri-outlook.org).

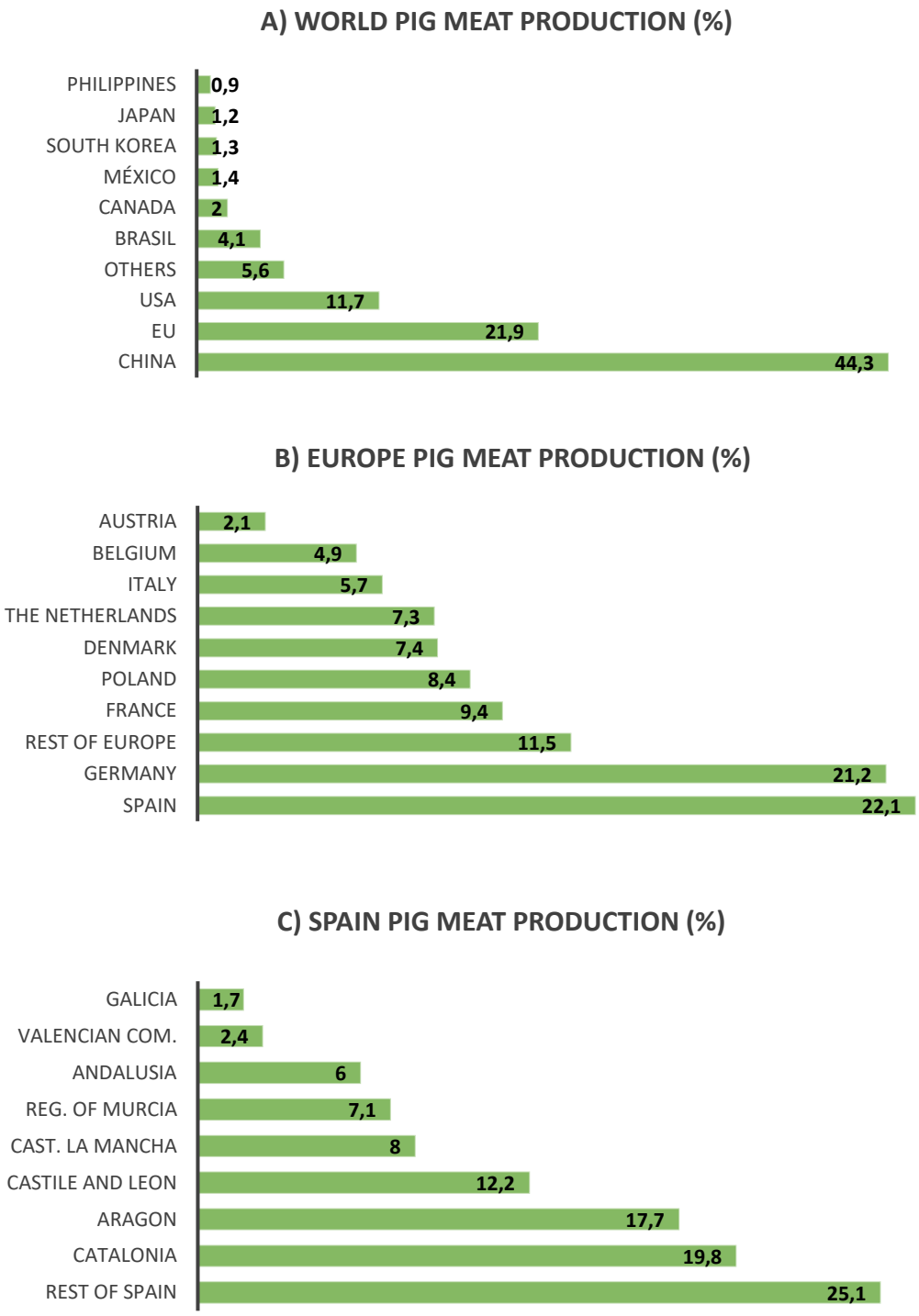

Figure 1.1. Percentage of pig meat production in A) world, B) in Europe and C) in Spain (MAPA, 2022).

In Europe, the two main pig meat producers are Spain (22.1 %) and Germany (21.2 %), followed by France (9.4 %) (Figure 1.1.A). Finally, Catalonia is leading the Spanish pig

meat production with 19.8 % in 2022 (MAPA: Ministerio de Agricultura, Pesca y Alimentación, Gobierno de España, Junio 2022) followed by Aragon and Castile and León with 17.7 % and 12.2 %, respectively.

The increase in global meat consumption is a multifaceted phenomenon that varies across societies and it is primarily driven by income and population expansion. This trend carries significant economic, sanitary, and environmental implications. Recent shifts in meat consumption patterns, including the increased popularity of chicken and pork and the consumption of processed meat products, have resulted in a high impact on public health, with increasing evidence associating high meat consumption and meat-derived products with a wide range of diseases (Webb & O'Neill, 2008; Wood et al., 1999).

Furthermore, the increase in livestock production has a negative impact on the environment because it is an important source of greenhouse gases (Godfray et al., 2018). On the other hand, it is expected that the increase of emissions by the meat sector of 5% by 2030 will be considerably less than the increase in meat production, due primarily to the increased contribution of poultry production and to projected higher meat output from a given stock of animals. The adoption of new technologies to reduce methane emissions, for example feed supplements that are not widely available today, could further reduce future per-unit emissions (OECD-FAO Agricultural Outlook 2021-2030, agri-outlook.org).

1.2. Main traits of interest in porcine meat production

Breeding programs for pigs adopt a strategic approach that involves setting measurable traits and achievable goals to improve the genetic characteristics of pig populations. These objectives are aligned with the needs of stakeholders who are involved in the production, processing, and consumption of pork products.

Classical genetic evaluation methodologies have significantly influenced the enhancement of pork production efficiency and the quality of carcasses. Improvement

in genetics can be achieved by measuring the inheritable trait of interest in selection candidates, through rigorous assessment and selection procedures.

In the past, swine breeding programs have primarily concentrated on the genetic improvement of crucial production traits that have a significant impact on profitability, such as growth rate, meat yield, feed efficiency, and piglet production. However, the intense selective pressure aimed at increasing the proportion of lean muscle tissue in livestock carcasses has resulted in a significant diminution of intramuscular fat (IMF) in certain breeds which has had an adverse impact on organoleptic properties of meat and consequent alterations in sensory attributes such as flavour and tenderness (Wood et al., 2004). Nevertheless, recently there has been a shift in consumer demands, with taste and nutritional composition emerging as relevant quality traits desired in meat products. As a result, over the past two decades, pig selection programs have incorporated the genetics of meat quality in order to meet the growing consumer demand for premium meat products (Wood & Whittemore, 2007)

1.3. Pig meat quality traits

Studies pertaining to the genetic basis of meat quality traits have concentrated on fundamental phenotypic characteristics that influence the technological and sensory properties of meat. These attributes include post-mortem pH, electrical conductivity, water-binding capacity, exudative loss, chromaticity, as well as the quantity and quality of IMF content. In addition, measuring meat quality traits proves challenging due to their complex nature, being influenced by a variety of stakeholders such as producers, slaughterers, processors, distributors, and consumers, each with unique quality requirements that may vary depending on the intended usage of the meat product.

Multiple factors contribute to the determination of meat quality, as illustrated in Figure 1.2. Encompassing animal welfare considerations aligned with ethical production practices, food safety concerns related to microbiological hazards, technological determinants such as pH, firmness, water-holding capacity, and cooking characteristics; sensorial attributes including aroma, texture, flavour, taste, juiciness, colour, and

marbling; and healthfulness and nutritional value as reflected by IMF content, lipid composition, and digestibility (Listrat et al., 2016; Webb & O'Neill, 2008).

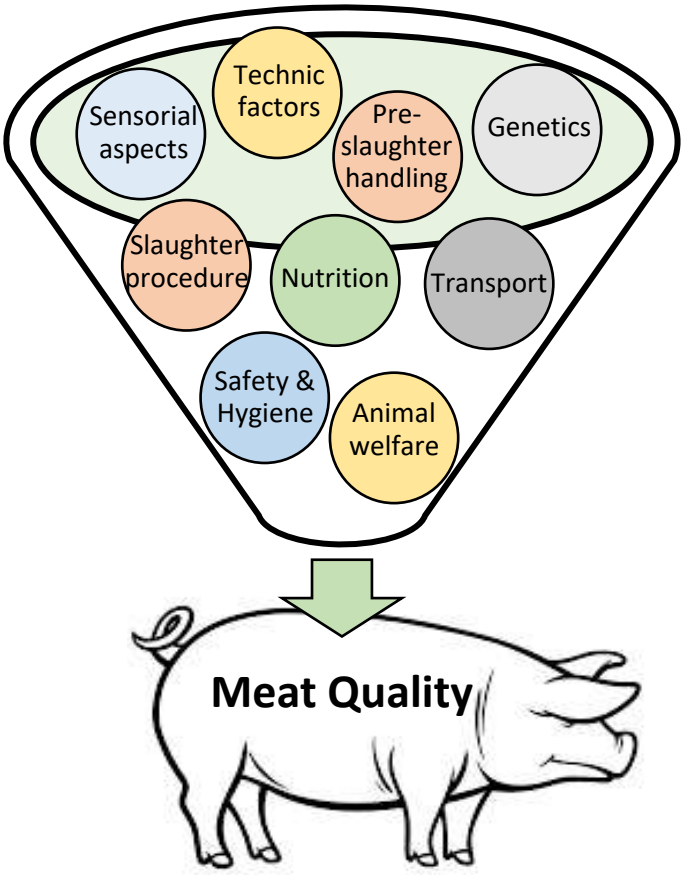

Figure 1.2. Main factors that affect pig meat quality.

1.3.1 Intramuscular fat content

IMF content, also known as marbling, is a crucial component of pork quality, affecting both sensory and technological properties of meat. IMF is found within the muscle fibers of pigs and is influenced by genetic and environmental factors, such as age, nutrition, and management practices. Higher levels of IMF in pork have been associated with improved juiciness, tenderness, and flavour, leading to increased consumer

acceptability (Wood et al., 2004). Additionally, IMF content has been linked to the oxidative stability and freshness of pork products. Therefore, understanding the mechanisms that regulate IMF deposition in pigs and its association with meat quality traits is essential for optimizing pig meat production and enhancing the overall quality of pork products.

1.3.2 Fatty acid composition

Fatty acids (FAs) are organic molecules composed of a hydrocarbon chain and a carboxylic acid group. They are essential components of many biological structures and play important roles in energy metabolism, signaling, and membrane structure. There are several categories of FAs, including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). These categories refer to the number of double bonds in the hydrocarbon chain and determine the physical properties and biological functions of the FA. Other important types of FAs include omega-3 and omega-6 FA, which are PUFAs with specific structural features that are important for health These FAs play a key role in cell structure, brain function and inflammation regulation as well as supporting cardiovascular health and cognitive function (Djuricic & Calder, 2021).

The FA composition of meat is an important factor that affects its quality, particularly the degree of unsaturation of the FAs present. MUFAs improve meat flavour and contribute to a better taste and lower oxidation rate of meat. Conversely, PUFAs are more susceptible to be oxidized, which produces rancidity and a consequent reduction of meat quality (Webb & O'Neill, 2008). On the other hand, the ratio of omega-6 to omega-3 FAs can also affect the nutritional value of the meat. Therefore, the FA profile of meat is an important consideration for both human health and meat quality (Wood et al., 2008).

1.4. Fatty acid metabolism

Lipids are a diverse group of organic compounds that are insoluble in water and are often considered membrane components whose function is to embed proteins into cell membranes. In the last two decades, studies on brain lipids have unequivocally demonstrated that many lipids have critical cell signaling functions (Bieberich, 2013). Some of the key functions of lipids include energy storage, insulation, protection of organs, cell membrane structure, and signaling. Lipids can be further classified into several subcategories, including FAs, phospholipids, and cholesterol, each with its own unique properties and functions. Overall, lipids are essential components of cells and tissues and are critical for maintaining proper cellular function and overall health. In addition, the main tissues for fat synthesis in animals are liver, adipose tissue and muscle (Duran-Montgé et al., 2009). As a key site of fat storage and release, adipose tissue is an important metabolic and endocrine organ that plays a critical role in regulating lipid metabolism and circulating free fatty acids (FFAs) throughout the body (Xing et al., 2016).

Furthermore, the metabolic pathways involved in FA metabolism, including lipolysis or FA β-oxidation and lipogenesis or *de novo* FA synthesis, are influenced by an individual's nutritional status and may be altered accordingly to Frühbeck et al. (2014). In the fed state, lipogenesis takes place where carbohydrates are converted into FAs and stored as triglycerides, which serve as an important energy reserve. This process is in contrast to the breakdown of FAs during the fasted state, and can occur via the uptake of exogenous FAs or through *de novo* lipogenesis, which is the endogenous synthesis of FAs (Ameer et al., 2014).

1.4.1 Fatty acid β-oxidation

Oxidation of FAs occurs in multiple regions of the cell within the human body; the mitochondria, in which only β-oxidation occurs; the peroxisome, where α- and β-oxidation occur; and ω-oxidation, which occurs in the endoplasmic reticulum (Talley &

Mohiuddin, 2023). β-oxidation is a significant source of metabolic energy during inter-prandial periods and high-energy demand states, such as exercise (Houten et al., 2016). Mitochondrial β-oxidation of FAs requires four steps, all of which occur in the mitochondrial matrix, to produce three energy storage molecules per round of oxidation, including one $NADH^{+}$, one $FADH^{2}$, and one acetyl-CoA molecule (Talley & Mohiuddin, 2023).

The β-oxidation or lipolysis is a metabolic pathway (Figure 1.3) that breaks down FAs into acetyl-CoA, which can be used in the citric acid cycle to generate energy. The process involves a series of four enzymatic reactions that sequentially remove two-carbon units from the FA chain. The first step is catalyzed by acyl-CoA dehydrogenase, which converts the FA to a trans-enoyl-CoA. This is followed by hydration of the double bond by enoyl-CoA hydratase, which converts the molecule to a hydroxy acyl-CoA. The third step is catalyzed by hydroxy acyl-CoA dehydrogenase, which converts the hydroxy acyl-CoA to a ketoacyl-CoA. Finally, thiolase cleaves the ketoacyl-CoA to produce acetyl-CoA and a FA chain two carbons shorter than the original molecule (Talley & Mohiuddin, 2023). This process is regulated by several factors, including the availability of FAs and their transport into the mitochondria, as well as the activity of the enzymes involved in the pathway. Defects in β-oxidation enzymes or transporters can lead to a variety of metabolic disorders, such as FA oxidation disorders, which can result in hypoglycaemia, muscle weakness, and other symptoms (Thangavelu, 2010). Therefore, the process of FA oxidation presents an alternate means of generating high-efficiency energy, which concurrently prevent muscle catabolic breakdown.

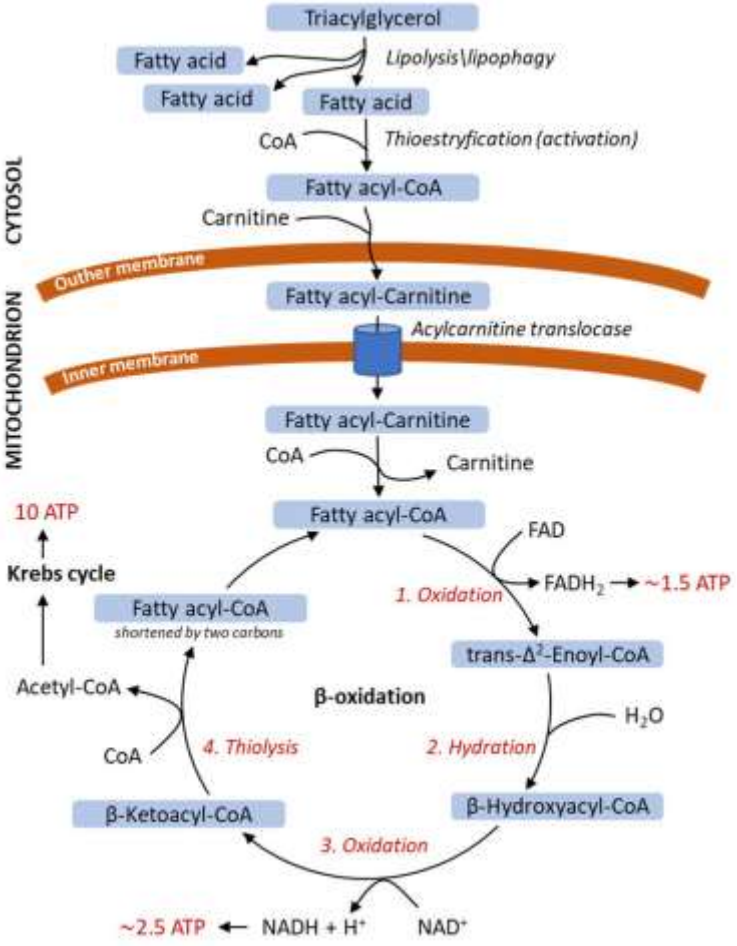

Figure 1.3. Schematic representation of mitochondrial FA β-oxidation pathway (reprinted from Kloska et al., 2020).

1.4.2 *De novo* fatty acid synthesis

Lipogenesis is a metabolic pathway involved in the synthesis of FAs from excess carbohydrates and then can be incorporated into triglycerides for energy storage. The process of storing energy from carbohydrate-derived carbon precursors occurs in the cytosol of cells and is performed by a series of enzymes beginning with the production of acetyl-CoA by ATP citrate lyase. Acetyl-CoA is then metabolized by the rate-limiting enzyme of FA synthesis pathway, acetyl-CoA carboxylase 1 (*ACACA*) to produce the

limiting reagent, malonyl-CoA (Figure 1.4). The polypeptide, fatty acid synthase (*FAS*), which serves multiple enzymatic functions, catalyzes the stepwise elongation of acyl chains by iterative addition of malonyl-CoA molecules via a series of biochemical reactions, ultimately producing saturated, short (C14:0) to medium (C18:0) chain FAs, with palmitic acid (C16:0) constituting the predominant (80-90%) end-product (Jayakumar et al., 1995). Various elongation and desaturase enzymes can further modify FAs. Mammalian organisms are equipped with a repertoire of seven different elongase enzymes (*ELOVL1-7*) that exhibit varying substrate specificities, acting as key mediators in the elongation of FAs through the catalysis of malonyl-CoA addition (Guillou et al., 2010).

Lipogenesis or synthesis *de novo* pathway in adipocytes is affected by several dietary and hormonal factors such as insulin, which is enhanced with the expression of *GLUT4* receptor. In the presence of sufficient glucose, insulin upregulates acetyl-CoA carboxylase and the other enzymes involved in elongation of the carbon chain, such as *FASN* and *ELOVLs* gene family (Ojha et al., 2014). A diet rich in carbohydrates stimulates lipogenesis in adipocytes, while fasting reduces it. Glucose enhances lipogenesis by stimulating insulin secretion and by upregulating several lipogenic genes. In contrast to the effects of insulin, growth hormone inhibits lipogenesis in adipocytes both directly by downregulating fatty acid synthase and indirectly by reducing the sensitivity of adipocytes to insulin action. Leptin (*LEP*) also affects lipogenesis in adipocytes, decreasing adiposity. The enzymes *FAS*, *ELOVL1*, *ELOVL3*, and *ELOVL6* are recognized as strictly responsible for *de novo* FA metabolism; whereas *ELOVL2* and *ELOVL5* are enzymes that exclusively metabolize dietary FAs. On the other hand, desaturase genes are a family of genes that encode desaturase enzymes, such as *FADS1*, *FADS2*, *FADS3* and *SCD* genes. Desaturases are involved in the synthesis of unsaturated FAs from the essential FAs provided by the diet (Nakamura & Nara, 2004). These enzymes play a key role in introducing double bonds into the essential FAs, such as linoleic and α-linoleic, to synthesize other PUFAs. This process is particularly important because the human body cannot produce essential FAs which must be obtained through the diet.

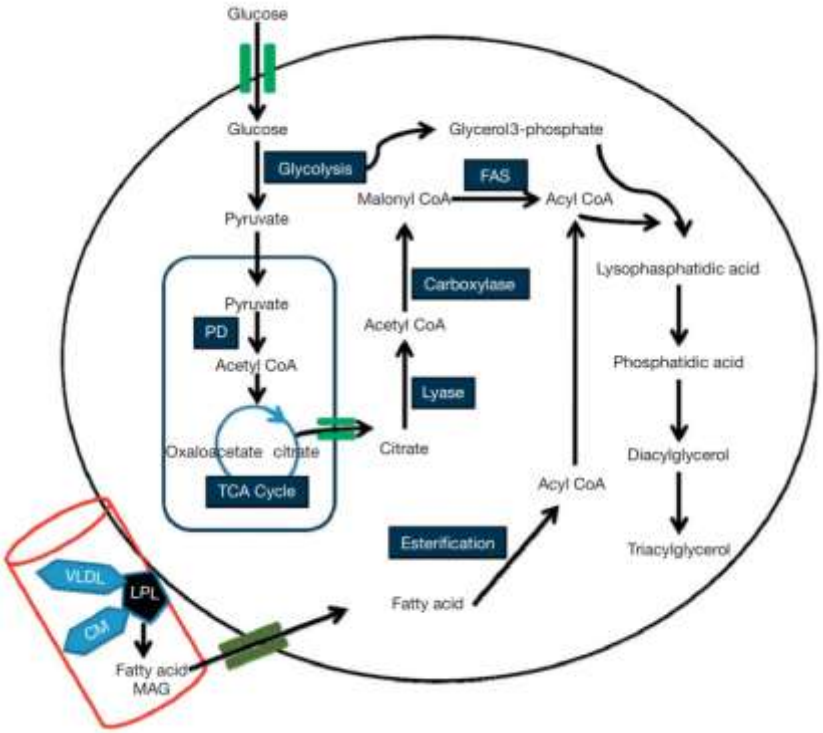

Figure 1.4. Schematic representation of *de novo* lipogenic pathway (reprinted from (Ojha et al., 2014).

In addition, FA metabolism is tightly regulated by transcription factors that bind to specific DNA sequences in the promoter regions of genes involved in this process (Fatehi-Hassanabad & Chan, 2005). These transcription factors act as either activators or repressors of gene expression, depending on the specific regulatory context. For example, the peroxisome proliferator-activated receptor (*PPAR*) family of transcription factors plays a central role in regulating the expression of genes involved in FA oxidation and storage (Christofides et al., 2022). PPARs form heterodimers with retinoid X receptor (*RXR*) and bind to *PPAR* response elements (*PPREs*) in the promoter regions of target genes. Other transcription factors, such as sterol regulatory element-binding proteins (*SREBPs*) and carbohydrate response element-binding protein (*ChREBP*), also play important roles in regulating FA metabolism. Overall, transcription factors serve as critical regulators of gene expression in the context of FA metabolism.

With the aim of modify the FA composition in meat, it is crucial to comprehend the genetic mechanisms underlying their metabolic processes.

1.5. Pig Genomics

The emergence of genomics has revolutionized swine genetics, allowing the utilization of genetic markers to selectively breed for traits that enhance feed efficiency, growth, meat quality, and litter size. Genomic selection relies on genotyping genetic markers covering the whole genome to predict the breeding values of the animals, considering that all quantitative trait *loci* (QTL) are in linkage disequilibrium with at least one maker (Goddard & Hayes, 2007). In this context, the sequencing of the swine genome has facilitated the genotyping of animals for thousands of single nucleotide polymorphisms (SNPs), enabling the identification of markers linked to relevant traits (Sukanta & Ram, 2020). Furthermore, the implementation of genomic selection has provided a powerful tool to accurately predict the genomic breeding value. As a result, the integration of genomic information enables the efficient selection of desirable traits, significantly enhancing the precision of breeding value estimation and expediting early evaluations.

In 2003, the Swine Genome Sequencing Consortium (SGSC) started the sequencing of the pig genome (Schook et al., 2005) and in 2012 the *Sscrofa 10.2* assembly was published (Groenen et al., 2012). Different DNA sequencing methodologies were employed. First, automatic DNA sequencing, based on Sanger sequencing method was used. This method is accurate and reliable, but it is limited by its low throughput and high cost. It is still used today for sequencing small fragments of DNA, such as individual genes, and for validating results obtained from other methods. Subsequently, Next-Generation Sequencing (NGS) method was used to finish the sequence and complete the gaps. NGS has some advantages:

1. High throughput: NGS can sequence millions of DNA fragments simultaneously, allowing for the analysis of large amounts of data in a short period of time.
2. Cost-effective: The cost of sequencing has decreased significantly due to NGS, making it more accessible for researchers.

3. High accuracy: NGS can produce highly accurate and reliable results due to the redundancy of sequencing multiple copies of the same DNA fragment.
4. Detection of novel variants: NGS can detect rare or novel variants that may be missed by traditional sequencing methods.
5. Detection of structural variants: NGS can detect large structural variants such as deletions, insertions, inversions, and translocations.
6. Simultaneous analysis of multiple genes: NGS can sequence multiple genes at once, which is useful for analyzing complex diseases with multiple genetic factors.
7. Data analysis: NGS produces vast amounts of data, but advances in bioinformatics have made it easier to analyze and interpret the data.

From these two methodologies, the 2.70 Gigabases (Gb) sequence of the *Sscrofa* 10.2 assembly was obtained from a single female Duroc animal. In 2017, an improvement of the previous assembly was made and *Sscrofa* 11.1 assembly was available. This assembly was constructed with data obtained through third-generation sequencing (TGS) technologies (PacBio RSII long reads), generating a 65x genome coverage over a total sequence length of 2.5 Gb. Nowadays, the genomes of several pigs from different breeds have been re-sequenced, and are available in open-access databases like NCBI and FAANG data repositories. The most recent version of the *Sscrofa* 11.1 reference genome available in the Ensembl database is release 110 (September 2023). This version encompasses a comprehensive annotation of 22,063 protein-coding genes and 13,154 non-coding genes. Furthermore, a total of 60,273 transcripts have been detected to date.

In addition, there is information in Ensembl database about more than 70 million short variants, including SNPs, insertions and deletions (Indels). NGS methodologies have facilitated the extensive identification of SNPs in the porcine genome, as demonstrated by Ramos et al. (2009), thereby enabling the creation of high-throughput genotyping arrays comprising a comprehensive set of SNPs that are uniformly dispersed throughout the entirety of the *Sscrofa* genome. The first whole genome genotyping array, the *PorcineSNP60 BeadChip* (*Illumina*), was commercialized in 2008, before the completion

of the pig genome sequence. This array contains 62,163 polymorphisms spread out along the chromosomes. A few years later, the *Axiom Porcine Genotyping Array*, manufactured by *Affymetrix*, was commercialized, featuring a comprehensive marker panel of 658,692 *loci*, of which 56,000 were SNPs sourced from *Illumina*'s chip technology, thereby ensuring compatibility with prior investigations.

Over the past few decades, significant advances have been made in pig breeding. The genomic selection of important traits can be achieved by improving the accuracy of breeding value predictions and obtaining early evaluations. For instance, sow prolificacy traits, with low heritability and expression only in mature females, present a challenge for traditional selection methods. In addition, genomic selection offers valuable opportunities for traits that cannot be measured in live animals, such as meat quality traits.

Advancements in genome mapping and sequencing have enabled extensive molecular measurements within cells and tissues. These technologies can be applied to study a biological system, providing a comprehensive view of its intricate biology at an unprecedented level of detail. Collectively, the scientific disciplines focused on high-throughput measurement of biological molecules are referred to as "*omics*" (Micheel et al., 2012). Various *'omics'* methodologies have been employed in pigs. These new '*omics'* technologies outline the system genetics approach, which integrates different levels of information (Table 1.1.).

Table 1.1. Description of the main '*omics*' and their technologies. Table adapted from (Singh et al., 2022).

Type of Omics	Definition	Technology
Genomics	Analysis of the structure and function of a genome	Whole-genome sequencing
		Whole-exome sequencing
		High-density genotyping
Epigenomics	Analysis of chemical modifications, chromatin structure, conformation, and its interaction with proteins	Bi-sulfite sequencing
		ChIP-Seq
		DNase-Seq
		3C and 4C
Transcriptomics	Study of the expression levels of all gene transcripts in a particular cell, at a particular time, and in a particular state.	Microarrays
		RNA-Seq
		High throughput RT-qPCR
		Single-cell transcriptome analysis
Proteomics	Detection of quantitative and/or qualitative variation on proteins	Tandem mass spectrophotometry
Metabolomics	Detection of quantitative and/or qualitative variation on metabolites	Gas chromatography
		Mass spectrophotometry
		Nuclear magnetic resonance
Microbiomics	Study of the microbiota, their genomes and the surrounding environmental conditions from an entire habitat	16S rRNA sequencing
		Whole-metagenome shotgun sequencing
Phenomics	Collection of a high number of phenotypic data	Image or video analysis-based

The integration of functional genomics into the study of traits of interest in pigs is made possible by the development of high-throughput techniques, which allow for the investigation of a wide range of biological systems (Figure 1.5).

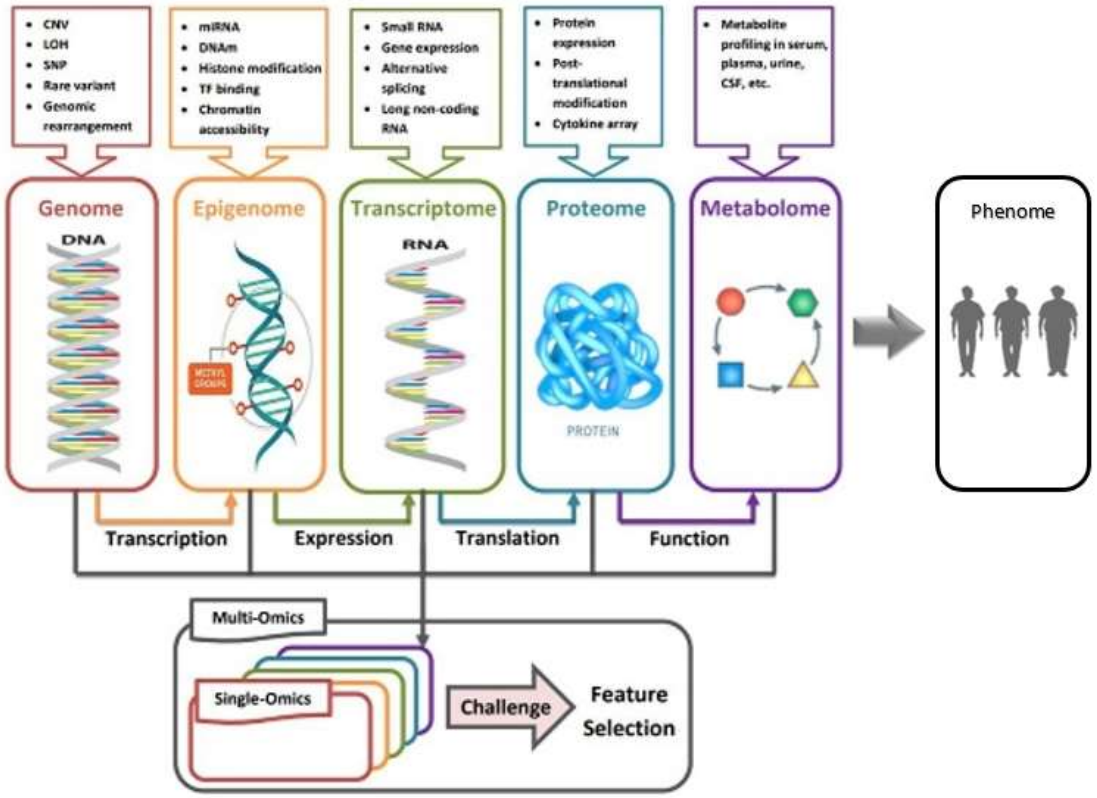

Figure 1.5. Different biological multi-omic systems and their relation with the phenotype (adapted from Momeni et al. (2020)).

The application of these new genomic tools has the advantage of generating information in parallel on multiple genes and gene products, which in turn provides the opportunity to identify pathways and interacting genes (Andersson & Georges, 2004). Thus, the aforementioned methodology is facilitating the elucidation of the interrelationships among genes, thereby enhancing our comprehension of the genetic underpinnings of complex traits.

1.5.1. Gene expression studies

Gene expression studies involve the analysis of the activity of genes in cells, tissues, or organisms, and are used to investigate a wide range of biological processes. There are many different techniques used to study gene expression, including microarray analysis, RNA sequencing (RNA-Seq), and quantitative real-time PCR (qPCR). These methods were used to measure the abundance of different RNA molecules in a sample, and to identify which genes are being expressed, and at what level. Gene expression studies can also be used to investigate the regulation of gene expression, by identifying the transcription factors and other regulatory elements that control the activity of specific genes.

During the 1990s, reverse transcription quantitative polymerase chain reaction (RT-qPCR) was the preferred approach for assessing gene expression levels, whether for individual genes or multiple targets. The fundamental concept underlying RT-qPCR involves real-time monitoring of the DNA polymerase chain reaction process, allowing for the detection of PCR amplicon amplification along each amplification cycle, via employment of a fluorescent dye system and a thermocycler with fluorescence-detection capabilities. In the 2010s, array platforms appeared to study gene expression by multiplex RT-qPCR with customized designs. Among them, are Fluidigm Dynamic Array (*Fluidigm*) (Spurgeon et al., 2008) or the TaqMan Open Array platforms (*Life Technologies*) which allow to study of numerous genes in several animals per array in a cost-effective way. Different studies in our group were carried out selecting different candidate genes for lipid metabolism in three different pig tissues and were quantified in a Fluidigm Dynamic array (Ballester et al., 2017b; Criado-Mesas et al., 2020; Puig-Oliveras et al., 2016; Revilla et al., 2018).

On the hand, we have the Microarrays, which consist in DNA molecules deposited or synthesized onto the surface of a microscope slide, which allows the expression analysis of thousands of genes simultaneously by DNA hybridization. In pigs, the first array commercialized was the Porcine AROS v1.0, *Operon* Gene-Chip Porcine microarray (*Affymetrix*) in 2003 and consisted of a set of 10,665 oligos. Latterly, these arrays were improved and customized and become a powerful tool for detecting differential gene expression. For instance, the GeneChip® Porcine Genome Array from *Affymetrix*

contains 23,937 probe sets that interrogate approximately 20,201 *Sus scrofa* genes was the most widely used, but other arrays were commercialized such as the *PigOligoArray* from *Illumina*, which contains 20,400 70-mer oligonucleotides and the Snowball array from *Affymetrix* comprises 1,091,987 probes (47,845 probe sets) with a mean coverage of 22 probes/transcript (Freeman et al., 2012; Steibel et al., 2009). Utilizing microarray technology, comparative analyse of muscle transcriptomes were conducted in pigs exhibiting differences in IMF content and composition, revealing the identification of differentially expressed genes (Damon et al., 2012; Hamill et al., 2013; Pena et al., 2013; Sun et al., 2013; Yu et al., 2013). After that, microarray technology was progressively replaced by sequencing methods.

NGS can be applied to whole genome sequence or to the sequencing of transcriptomes, which is called RNA-Seq. Some RNA-Seq studies have reported differentially expressed genes in pigs associated with sex, breed, growth and meat quality traits (Cardoso et al., 2018; Corominas et al., 2013b; Esteve-Codina et al., 2011; Ghosh et al., 2015; Jiang et al., 2013; Puig-Oliveras, et al., 2014; Zhao et al., 2011). While both microarray and RNA-Seq methodologies are viable for quantifying gene expression, the application of microarrays is constrained by their lower sensitivity and higher background noise. In contrast, RNA-Seq offers the advantage of enabling the determination of transcript abundance across a broader spectrum of expression levels with increased dynamic range. The advantages and disadvantages of three transcriptomic methodologies are summarized in Table 1.2.

Table 1.2. Main advantages and disadvantages about Microarray, RNA-Seq and RT-qPCR methodologies.

Technique	Advantages	Limitations
Microarray	• Low cost • Large number of samples • High throughput	• Limited number of genes • Low sensitivity • High background
RNA-Seq	• High accuracy and specificity • Low background • High dynamic range • Identification of novel transcripts, splice junctions, SNPs and non-coding RNAs	• High cost • Requires a NGS platform and high bioinformatics tools for data analysis
RT-qPCR	• Low cost • Speed and efficiency • High sensitivity and specificity	• Limited dynamic range • Risk of contamination • Requirement of careful optimization

Regarding to RT-qPCR, this method confers a faster and more cost-effective alternative to other RNA quantification techniques, such as RNA-Seq, facilitating accurate and specific high-throughput mRNA quantification across a broad dynamic range (Kuang et al., 2018). Although the methodology is perceived to be relatively simple, there are a number of steps and reagents that require optimization and validation to ensure reproducible data that accurately reflect the biological questions being posed (Taylor et al., 2019).

Several studies have utilized RNA-Seq methodology to identify differentially expressed genes in skeletal muscle and adipose tissue of pigs, which are associated with meat quality traits and these findings were subsequently validated using RT-q-PCR (Ayuso et al., 2015; Gao et al., 2019; Gorni et al., 2011; Óvilo et al., 2014; Xing et al., 2019; Zhao et al., 2019). In addition, a few gene expression studies have been reported to study candidate genes in relation to lipid metabolism traits: *ACSL4* (Corominas et al., 2012), *APOA2* (Ballester et al., 2016), *DGAT1* and *DGAT2* (Cui et al., 2011), *ELOVL6* (Corominas

et al., 2015), *FABP4* and *FABP5* (Ballester et al., 2017a), *FADS2* (Gol et al., 2018), and *IGF2* (Criado-Mesas et al., 2019) among others.

1.5.2. Regulation of gene expression

The gene expression regulation is a complex process that allows cells to control which genes are turned on or off, and to what extent. This regulation involves a series of molecular events that act at the transcriptional, post-transcriptional, translational, and post-translational levels. Key players in this process include transcription factors, epigenetic modifiers, RNA processing enzymes, ribosomes, and protein kinases/phosphatases. The overall outcome of gene expression regulation is the production of proteins with specific functions that are required for proper cellular function and development. Hence, gene expression disruption can result in alterations of protein functions.

Within the context of gene expression, transcriptional regulation has been recognized as the foremost critical process, and its investigation has been facilitated by well established methodologies. At the transcriptional level, the control of gene expression is modulated by proteins that can be classified into two categories: sequence-specific DNA binding proteins, including transcription factors, and other key regulatory factors like TATA-binding proteins. Despite the extensive research on transcriptional regulation, post-transcriptional regulation has gained significance in a multitude of biological processes due to its capacity to obtain a quick response to various cellular and environmental signals.

1.6. Genetic studies into meat quality traits in pig

1.6.1. QTLs, GWAS and candidate genes

Quantitative Trait *Loci* (QTL) is a specific region on a chromosome that is associated with the variation of a quantitative trait (Turner et al., 2013). In other words, a QTL is a genetic

locus that influences a complex trait that is controlled by multiple genes and environmental factors. QTL mapping is a statistical method used to identify the location and effect of QTLs on a trait of interest. This method involves analyzing the genetic polymorphism of molecular markers in a population and associating it with the variations of the trait. This approach can be used to identify genes that underlie complex traits, which can provide insights into the genetic basis of important biological processes (Zargar et al., 2015). QTLs are important in understanding the genetic basis of complex traits, and can be used in various fields of study such as genetics, genomics, and plant breeding.

The pursuit of QTLs in pigs has persisted for approximately twenty years, starting with the initial revelation of a QTL associated with growth rate and fatness on SSC4 (Andersson et al., 1994). Subsequent to the initial discovery, numerous scientific publications have documented the existence of thousands of QTLs associated with diverse traits in pigs. The Pig QTLdb, established by Hu et al. (2005), currently archives a comprehensive collection of 23,273 QTLs/associations derived from more than 700 publications, encompassing 681 distinct traits distributed along the *Sus scrofa* genome, as illustrated in Figure 1.6.

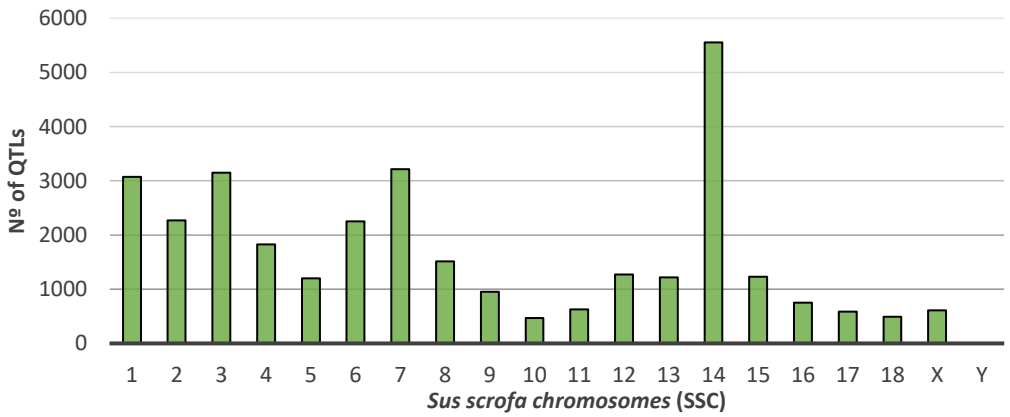

Figure 1.6. Distribution of *Sus scrofa* QTLs throughout its genome as reported by the Pig QTLdb (https://www.animalgenome.org/cgi-bin/QTLdb/SS/index; accessed June 2023).

Recent advancements in the field of pig genotyping have led to significant improvements in the identification of QTLs, primarily due to the development of high-density SNP panels. These SNP panels have been used in conducting GWAS aimed at identifying genomic regions associated with various traits, allowing for the selection of pigs with desirable genetic profiles (Zargar et al., 2015). GWAS analysis is an approach that analyse the whole genome to identify genetic variations, typically SNPs, which are associated with specific traits. This methodology has provided multitude associations for a wide range of traits and diseases, improving our understanding of the genetic basis of complex traits in pigs (Uffelmann et al., 2021).

GWAS and QTL mapping are two common approaches used in genetic research to identify genetic variants associated with complex traits. While both methods aim to identify genetic *loci* that influence trait variation, they differ in their scale and resolution (Zargar et al., 2015). GWAS examines the association between hundreds of thousands to millions of genetic markers across the genome and a trait of interest in a large sample of individuals. On the other hand, QTL mapping focuses on identifying regions of the genome that have a significant effect on a trait using linkage analysis with mapped markers, typically microsatellites. In swine, GWAS have identified several genomic regions associated with various traits in different populations, encompassing a comprehensive catalogue of noteworthy *loci* regulating FA composition in pork (Crespo-Piazuelo et al., 2020; M. Muñoz et al., 2013; Ramayo-Caldas et al., 2012b; Yang et al., 2013; Zhang et al., 2016).

Candidate genes to explain traits of interest have been identified through a combination of their physiological function and their proximity to QTLs associated with the trait. Although a large number of QTLs have been identified in pigs, only a limited number of candidate genes have been evaluated functionally to identify causal polymorphisms of the QTL. Some of these candidate genes are summarized in Table 1.3.

Table 1.3. Candidate genes identified in QTL or GWAS studies associated with traits of interest in swine production (adapted from Ernst & Steibel, 2013).

Gene name	Gene	Trait(s)
Calpastatin	*CAST*	Meat quality
Carbonic anhydrase 3	*CA3*	Meat quality
ELOVL fatty acid elongase 6	*ELOVL6*	Meat quality
Fatty acid binding protein 4	*FABP4*	Meat quality
Fatty acid binding protein 5	*FABP5*	Meat quality
Insulin like growth factor 2	*IGF2*	Growth and carcass composition
Leptin	*LEP*	Growth and carcass composition
Leptin receptor	*LEPR*	Growth and carcass composition
Melanocortin 4 receptor	*MC4R*	Growth and carcass composition
Myopalladin	*MYPN*	Carcass composition
Phosphoenolpyruvate carboxykinase 1	*PCK1*	Meat quality
POU class 1 homeobox 1	*POU1F1*	Growth and carcass composition
Protein kinase AMP-activated non- catalytic subunit gamma 3	*PRKAG3*	Meat quality
Prolactin receptor	*PRLR*	Litter size
Retinal binding protein 4	*RBP4*	Litter size
Ryanodine receptor 1	*RYR1*	Stress susceptibility and meat quality
Stearoyl-CoA desaturase	*SCD*	Meat quality
Acetyl-CoA carboxylase alpha	*ACACA*	Meat quality
Acyl-CoA synthetase long-chain 4	*ACSL4*	Meat quality
Cytochrome P450 2 subfamily E1	*CYP2E1*	Meat quality
Peroxisome proliferator-activated receptor gamma coactivator 1-alpha	*PPARGC1 A*	Meat quality
Fatty acid desaturase 1	*FADS1*	Meat quality
Fatty acid desaturase 2	*FADS2*	Meat quality
Fatty acid desaturase 3	*FADS3*	Meat quality
Patatin-like phospholipase domain-containing protein 2	*PNPLA2*	Meat quality
Hydroxyacyl-CoA dehydrogenase	*HADH*	Meat quality
ELOVL fatty acid elongase 7	*ELOVL7*	Meat quality
Phosphoinositide-3-kinase subunit 1	*PIK3R1*	Meat quality
Lipin 1	*LIPIN*	Meat quality
Acyl-CoA synthetase short-chain 1	*ACSS1*	Meat quality
Carnitine palmitoyltransferase 1A	*CPT1A*	Meat quality
Estrogen-related receptor alpha	*ESRRA*	Meat quality
Fatty acid synthase	*FASN*	Growth and carcass composition

1.6.2 eQTL mapping

Expression Genome-Wide Association Study (eGWAS), also known as expression GWAS, is a genetic analysis method that investigates the association between genetic polymorphisms, such as SNPs, and gene expression levels. The goal of eGWAS is to identify genetic variants that influence gene expression, elucidating the molecular mechanisms underlying different traits. This analysis can identify expression quantitative trait loci (eQTLs), which are genomic regions associated with gene expression variation. eQTL identification can provide insights into the genetic basis of complex traits and may lead to the identification of potential candidate genes, which can modify these traits. Through the utilization of mRNA transcript abundance as a phenotypic representation of interest, it is possible to quantitatively measure the impact of regulatory variations on a continuous scale (Xinghua, 2020) (Figure 1.7).

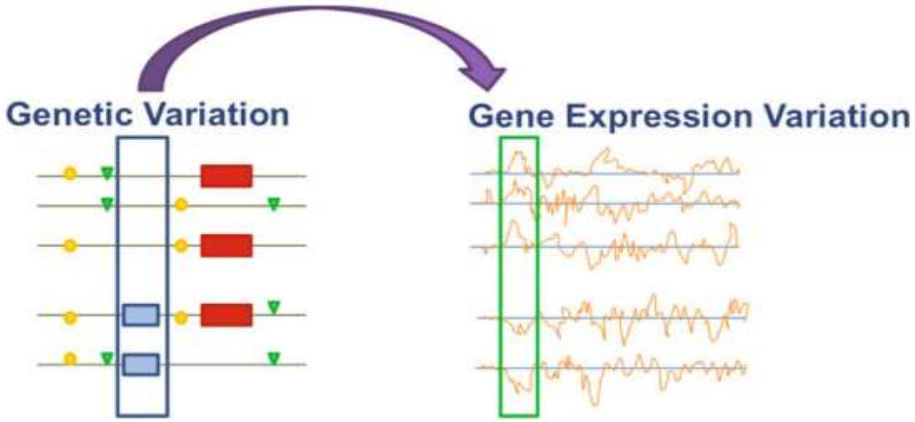

Figure 1.7. Pairwise associations are made by linking genetic variants in a given population, as represented by the blue box on the left, to measured gene expression levels in the green box on the right. . This process is followed in an eQTL analysis, which focuses on studying the effects of genetic variants on gene expression variation (Figure adapted from Xinghua, 2020).

In addition, in eQTL analysis, it becomes possible to distinguish between the *cis* and *trans*-acting modes of action, which can lead to the identification of hotspot *loci* and regulators. Essentially, a *cis*-eQTL refers to a genetic variant that is located near or within the gene being studied, and directly influences its expression levels. On the other hand, a *trans*-acting eQTL refers to a genetic variant that is located in a different genomic location than the studied gene and may indirectly affect the target gene expression (Figure 1.8)

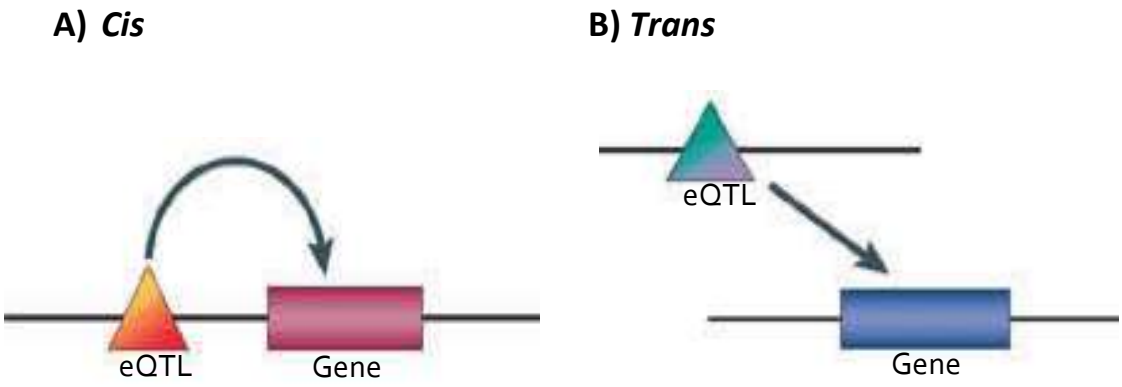

Figure 1.8. Illustration of A) *Cis*- and B) *Trans*-acting eQTL regions. In the *cis*-eQTL the expression of a gene located close to the SNP varies according to the presence of one allele. In contrast, a *trans*-eQTL occurs when the SNP that influences the expression level of the target gene is located at a considerable distance, even in a different chromosome.

Cis-acting eQTLs, which are typically characterized by their proximity to the target gene, are known to explain a significant proportion of variance in gene expression and are of considerable interest. In contrast, *trans*-eQTLs, which often regulate multiple genes, have been identified as regulatory hotspots in previous studies (Schadt et al., 2003).

In recent years, there has been a significant increase in the eQTL mapping studies, due to advances in genomic technology and computational methods. Although the first eQTL mapping studies were conducted in the early 2000s (Jansen & Nap, 2001; Schadt et al., 2003), researchers have been able to perform large-scale eQTL mapping studies in a

wide range of organisms. Consequently, there is now an abundance of data on the genetic control of gene expression available across multiple species. Up to the present time in pigs, the predominant approach has been to employ transcriptomic data derived from skeletal muscle, with a focus on eQTL mapping studies related to production traits. Some of them are summarized in Table 1.4. Moreover, a few studies of our group analyzed muscle (Criado-Mesas et al., 2020; Puig-Oliveras et al., 2016), liver (Ballester, et al., 2017b), and adipose tissue (Revilla et al., 2018) eQTLs.

Table 1.4. Summary of eQTL studies for genes associated with growth, fatness and meat quality production traits in pigs.

Related trait	References
Growth	(Heidt et al., 2013; Ponsuksili et al., 2011; Steibel et al., 2011)
Fatness and FA composition	(Cánovas et al., 2012; Criado-Mesas et al., 2020; González-Prendes et al., 2019; Heidt et al., 2013; M. Muñoz et al., 2013; Ponsuksili et al., 2011; Revilla et al., 2018; Steibel et al., 2011).
Meat quality	(Ballester, et al., 2017a; Criado-Mesas et al., 2020; González-Prendes et al., 2019; Heidt et al., 2013; M. Muñoz et al., 2013; Pena et al., 2013; Ponsuksili et al., 2011; Puig-Oliveras et al., 2016; Steibel et al., 2011).

1.6.3 Allelic Specific Expression Analysis

Allelic-specific expression (ASE) analysis is a molecular biology technique used to study gene expression patterns, specifically looking at the differential expression of alleles inherited from each parent. It is based on the fact the expression of the alleles inherited from the mother and the father can be measured separately. The grade of expression varied from complete monoallelic expression (MAE) to preferential overexpression of an allele from a single parent. Thus, when one of the two alleles is completely silenced is known as MAE (Figure 1.9).

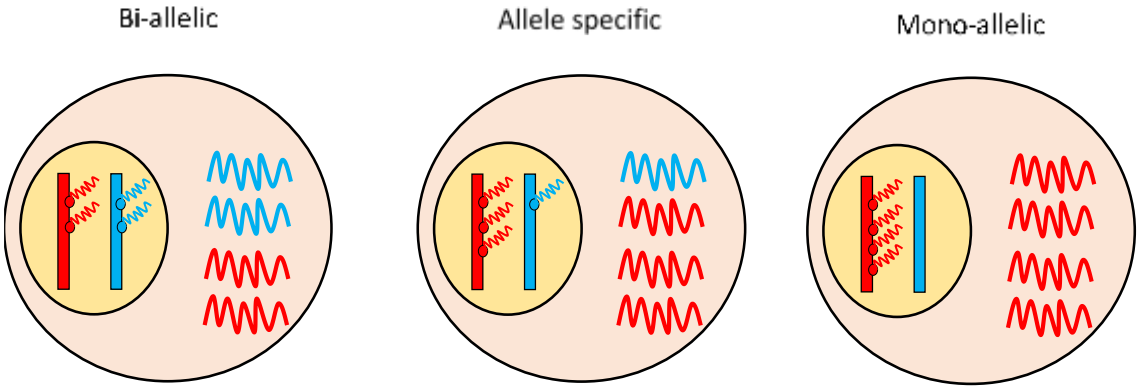

Figure 1.9. Schematic representation of different types of gene expression on allele level. Red and blue colour represent two alleles of a gene.

ASE analysis has been used in various forms since the 1980s, but it has become increasingly popular in the field of genetics and genomics in recent years due to advances in sequencing technologies. Today, ASE analysis is commonly used in a variety of research fields and has been used to identify genetic variants that are associated with different traits and to study the impact of epigenetic modifications on gene expression.

Before the arrival of RNA-Seq, differential allelic expression analysis was conducted using techniques such as *in situ* hybridization, cloning and sequencing of RNA, and amplified fragment length polymorphism (AFLP). *In situ* hybridization involved the use of specific probes for maternal and paternal alleles to detect differential allele expression in tissue samples (Ohlsson et al., 2001). This allowed for visualization of the spatial distribution and relative abundance of transcribed RNA from each allele. Cloning and sequencing of RNA required cloning RNA transcripts into vectors and sequencing them individually. This enabled determination of the RNA sequence of each allele and quantification of their relative expression. AFLP was a technique that selectively amplified DNA fragments using specific primers for different alleles. Quantification of the amplified fragments allowed estimation of the relative expression of each allele. While these techniques provided insights into differential allelic expression, they had

limitations in terms of scalability and the ability to analyze a large number of genes and samples simultaneously. The emergence of RNA-Seq revolutionized the field by enabling comprehensive and high-throughput analysis of gene expression, including differential allelic expression, at a genomic level.

In order to conduct ASE analysis using RNA-Seq, the data are derived from individuals who exhibit heterozygosity in one or multiple SNPs. The RNA-Seq reads are mapped to the reference genome, and the number of reads that correspond to each allele is counted. The utility of ASE analysis lies in its ability to identify genetic factors that contribute to phenotypic variation. For example, if a certain allele is consistently overexpressed compared to the other allele in a population, it may be associated with a particular trait. Additionally, ASE analysis can be used to study epigenetic modifications, such as DNA methylation and histone modifications, which can also affect allele-specific expression. Hence, ASE analysis is a powerful tool for studying gene expression patterns and understanding the genetic and epigenetic factors that contribute to phenotypic variation.

So far, there have been few ASE studies detecting candidate genes associated with production traits in livestock animals, such as in cattle (Bruscadin et al., 2021; de Souza et al., 2020), broilers (Zampiga et al., 2018) and only a few in pigs (Liu et al., 2020; Stachowiak et al., 2018; Stachowiak & Flisikowski, 2019). In a 2020 study, Liu et al. identified candidate genes (*PHKG1*, *NUDT7*, *FADS2*, and *DGAT2*) associated with pig production traits through ASE analysis. Stachowiak et al. (2018) suggested that *PPARGC1A* is subjected to *cis*-regulation in pig backfat, while Stachowiak & Flisikowski (2019) reported allelic imbalance for *ACACA, LEP, SCD* and *TNF* genes involved in lipid metabolism in pig skeletal muscle.

The ASE approach offers a valuable tool for understanding the genetic mechanisms underlying complex traits in pigs. The discovery of key genes through ASE analysis may eventually lead to the development of more effective breeding strategies for improving pig production.

1.7. The IBMAP cross

The IBMAP consortium was created in 1996 with the collaboration among the *Universitat Autònoma de Barcelona* (UAB), the *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria* (INIA), and the *Institut de Recerca i Tecnologia Agroalimentàries* (IRTA). First, a F2 cross between Guadyerbas Iberian boards and Landrace sows was generated for the identification of growth, carcass, and fatty acid composition QTLs. Later, backcrosses of Iberian boars with Landrace, Duroc, and Pietrain sows were obtained (Figure 1.10.) and samples for gene expression analyses were collected from hypothalamus, liver, adipose tissue and *longissimus dorsi* muscle.

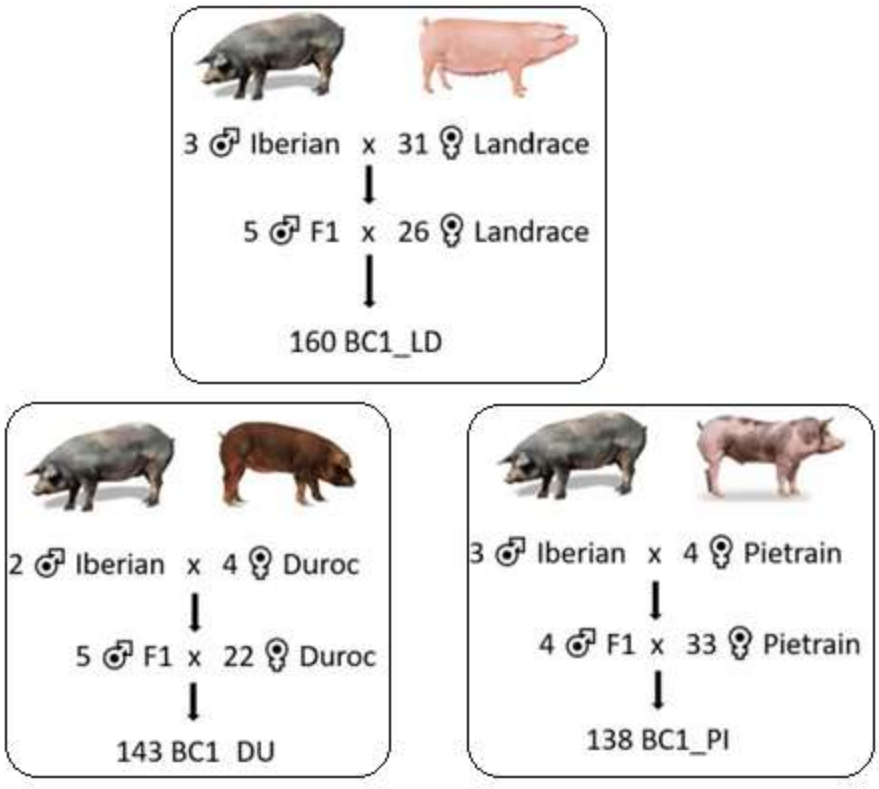

Figure 1.10.: Schematic representation of the three IBMAP backcrosses (BC1_LD, BC1_DU and BC1_PI).

Different swine breeds were selected based on their divergent phenotypic characteristics pertaining to meat quality, growth rate, fatness, productivity, and feed efficiency. The Iberian pig breed, local and rustic, is raised in Spain and is distinguished for its superior meat quality and cured products, attributed to its high levels of SFAs and MUFAs, primarily oleic acid. However, its productivity is comparatively lower than that of conventional pig breeds (Serra et al., 1998).

In contrast, Landrace pig is a commercial breed characterized by its long, lean body type with a straight and narrow back, ideal for efficient meat production. Landrace sows are known for their exceptional reproductive performance and can produce large litters with high growth rates. Additionally, Landrace pigs have a high feed conversion ratio, indicating good feed efficiency and are well-suited for intensive commercial production systems. On the other hand, they are also characterized by lower IMF levels and an elevated proportion of PUFAs. It has undergone intense selective breeding for production attributes, exhibiting enhanced growth and high prolificacy.

Concerning to the Duroc pig, it is characterized by a medium-sized and muscular body type. This breed is renowned for its high-quality meat, with well-marbled and its juiciness. Furthermore, they exhibit good growth rates, feed conversion efficiency, and tend to produce carcasses with high meat quality due to their increased IMF deposition, which makes them a highly desirable choice for commercial swine production systems seeking to maximize productivity and profitability. They also present excellent reproductive performance and can produce large litters of piglets. Additionally, Duroc pigs are known for their docile temperament and adaptability to a wide range of climatic conditions (Yoder et al., 2011).

In relation to Pietrain commercial pig breed, is characterized by its short and compact body type. This breed is well-known for its high lean meat yield and with low levels of IMF. Pietrain pigs are typically fast-growing and have good feed efficiency, but less prolific than other commercial breeds such as Landrace (Kouba & Sellier, 2011). Moreover, they are known to have a higher risk of developing stress-related disorders and require careful management.

1.7.1. QTLs identified in the IBMAP population

The primary aim of the IBMAP consortium was to identify QTLs that are linked to both pork meat quality and growth traits. The initial investigations, which were carried out on the F2-cross between Iberian and Landrace pigs, employed microsatellite markers to pinpoint significant regions that are associated with carcass quality, growth, fatness, and the FA composition present in backfat on chromosomes SSC2, SSC3, SSC4, SSC6, SSC7, SSC8, SSC10, SSC12, and SSCX (Clop et al., 2002, 2003; Mercadé et al., 2005; Mercadé, et al., 2006b; G. Muñoz et al., 2007; Óvilo et al., 2000; Pérez-Enciso et al., 2000, 2005). Clop et al. (2003) carried out the first report of a genome scan for QTLs affecting FA composition in pigs of the IBMAP cross.

Furthermore, novel technological advancements such as the *PorcineSNP60 BeadChip* (*Illumina*) were employed to genotype the animals and enhance the precision of the localization of the previous QTLs, as well as to identify novel genomic *loci* linked to the analysed phenotypic traits (Corominas et al., 2013a; Fernández et al., 2012; M. Muñoz et al., 2013; Ramayo-Caldas, et al., 2012b; Revilla et al., 2014).

Rencently, Crespo-Piazuelo et al. (2020) have identified a multitude of potential genes in both backfat and skeletal muscle tissue (*longissimus dorsi*) among the three backcrosses from the IBMAP population. In this study, nine and six significant associated regions with different phenotypic traits were identified in backfat and *longissimus dorsi* muscle, respectively. Moreover, a comprehensive set of 52 genes has been proposed to account for the variability in FA composition traits within both the backfat and skeletal muscle tissues.

1.7.2. Candidate genes identified in the IBMAP population

The main objective of examining complex traits is to identify the genes implicated and unravel their molecular mechanisms and roles. Within the mentioned QTLs, the IBMAP consortium has identified numerous potential candidate genes linked to growth, fatness, and meat quality traits as illustrated in Table 1.5.

Table 1.5. Main positional candidate genes analyzed in the IBMAP population.

Chr	QTL associated traits	Candidate genes	Reference
SSC2	Growth and fatness	*IGF2*	Estellé et al., 2005
SSC4	Growth and FA composition	*APOA2*	Ballester et al., 2016
		DECR	Clop et al., 2002
		DGAT1; FABP4	Mercadé et al., 2005, 2006b
		FABP5	Estellé et al., 2006
		PRRX1	Crespo-Piazuelo et al., 2020
SSC6	Fatness and IMF	*ACADM*	Kim et al., 2006
		FABP3; LEPR	Ovilo et al., 2002; 2005
SSC8	FA composition	*CDS1*	Mercadé et al., 2007
		ELOVL6	Corominas et al., 2013b
		FABP2; MTTP	Estellé et al., 2005, 2009
		SETD7	Revilla et al., 2014
		HADH	Crespo-Piazuelo et al., 2020
SSC12	FA composition	*ACACA; FASN; GIP*	Muñoz et al., 2007
SSC13	FA composition	*LIPI; NRIP1*	Crespo-Piazuelo et al., 2020
SSC14	FA composition	*ELOVL3; SCD*	Crespo-Piazuelo et al., 2020
SSC16	FA composition	*ELOVL7; PIK3R1*	Crespo-Piazuelo et al., 2020
SSC17	FA composition	*ABHD12; ACSS1; PANK2*	Crespo-Piazuelo et al., 2020
SSCX	FA composition, growth, fatness and IMF	*ACSL4*	Corominas et al., 2012; Mercadé et al., 2006a

Among the genes on this list, *ELOVL6* has been among the genes that have been subject to more detailed investigation. The *ELOVL6* gene plays a crucial role in elongating SFAs and MUFAs containing 12-16 carbons to C18, significantly impacting the levels of palmitic (C16:0) and palmitoleic (C16:1n7) FAs (Jakobsson et al., 2006). In previous studies conducted by Corominas et al. (2013b), the *ELOVL6* gene was extensively investigated as the main positional candidate gene in a QTL on SSC8 for FA composition in both IMF and backfat. The FAs affected by this QTL were palmitic and palmitoleic , as well as the elongation rations of C18:0/C16:0 and C18:1n-7/C16:1n-7. Notably, the *ELOVL6:c.-533C>T* polymorphism was found to be associated with the content of palmitic and palmitoleic FAs, along with the elongation ratios, in muscle and backfat of BC1_LD animals. Furthermore, this polymorphism demonstrated an association with

ELOVL6 mRNA levels in adipose tissue. Therefore, the concurrence of the biological role of *ELOVL6* with the detected QTL impact on FA composition on SSC8 reinforces the significance of *ELOVL6* as the candidate positional gene responsible for this QTL. Through the characterization of both the coding and the proximal promoter regions of the porcine *ELOVL6* gene, numerous mutations were identified (Corominas et al., 2013b). Moreover, the SNP *ELOVL6*:c.-394G>A, which is in a complete linkage disequilibrium with *ELOVL6*:c533C>T SNP, was associated with differences in the methylation levels of the *ELOVL6* gene promoter and its expression (Corominas et al., 2015).

1.7.3. NGS tools in the IBMAP population

NGS technologies enable the high-throughput sequencing of genomes and transcriptomes, resulting in a significant abundance of genomic data being generated. The IBMAP consortium used these new techniques like RNA-Seq to analyse the transcriptome and identify differentially expressed genes in liver (Ramayo-Caldas, et al., 2012a), adipose tissue (Corominas, et al., 2013b), muscle (Fernández et al., 2014; Puig-Oliveras, et al., 2014) and hypothalamus (Pérez-Montarelo et al., 2014) in BC1_LD animals. For liver, adipose tissue and muscle, differential expression analysis were conducted between two divergent groups of animals characterized by their differential composition of SFAs and MUFAs in one group, and a higher concentration of PUFAs in the other. The results obtained showed that in the group of animals with a higher proportion of SFAs and MUFAs there was a reduction of liver FA oxidation (Ramayo-Caldas, et al., 2012a), an increase of *de novo* lipogenesis in adipose tissue (Corominas, et al., 2013b), and a higher FA and glucose uptake and increased lipogenesis in muscle tissue (Puig-Oliveras, et al., 2014). Remarkably, common pathways related to LXR/RXR activation, PPARs and β-oxidation were identified in the three RNA-Seq studies. Moreover, Puig-Oliveras, et al. (2014) also utilized this methodology to identify gene interactions and pathways affecting pig traits, such as growth and fatness. Three transcription factors were identified: the peroxisome proliferator activated receptor gamma (PPARG), E74 like ETS transcription factor 1 (ELF1) and PR/SET domain 16

(PRDM16), as key transcription factors regulating growth traits. In another study, Muñoz et al. (2013) also combined QTL and eQTL mapping to identify candidate genes with potential effect on backfat thickness and intramuscular FA composition. Accordingly, utilization of the RNA-Seq technique has enabled the identification of potential candidate genes and pathways associated with lipid metabolism pathwats and FA composition.

OBJECTIVES

Chapter 2

This PhD thesis was done under the framework of the AGL2017-82641-R and PID2020-112677RB-C22 projects funded by the *Ministerio de Economía y Competitividad* (MINECO). The present research has been performed using the animal material generated by the IBMAP Consortium involving INIA, IRTA and UAB groups.

The main objective was to investigate the genetic and molecular factors that influence the fatty acid composition in pigs.

The specific objectives of this thesis were:

1. To study the expression and regulation of a set of lipid-related genes in *longissimus dorsi* muscle of pigs from three different genetic backgrounds, to better understand the expression and regulation of these genes.

2. To identify variants from porcine *longissimus dorsi* muscle through allele-specific expression analysis with RNA-Seq data to decipher relevant genetic variants in candidate genes for lipid metabolism and fatty acid composition.

3. To characterize the transcriptome architecture of the porcine *longissimus dorsi* muscle by RNA-Seq and to identify potential lipid metabolism candidate genes and regulators of muscle gene expression.

PAPERS AND STUDIES

Chapter 3

PAPER I

Identification of genomic regions, genetic variants and gene networks regulating candidate genes for lipid metabolism in pig muscle

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Abstract

The intramuscular fat content and fatty acid composition of porcine meat have a significant impact on its quality and nutritional value. This research aimed to investigate the expression of 45 genes involved in lipid metabolism in the *longissimus dorsi* muscle of three experimental pig backcrosses, with a 25% of Iberian background. To achieve this objective, we conducted an expression Genome-Wide Association Study (eGWAS) using gene expression levels in muscle measured by high-throughput real-time qPCR for 45 target genes and genotypes from the PorcineSNP60 *BeadChip* or Axiom Porcine Genotyping Array and 65 SNPs located in 20 genes genotyped by a custom-designed *Taqman* OpenArray in a cohort of 354 animals. The eGWAS analysis identified 301 eSNPs associated with 18 candidate genes (*ANK2, APOE, ARNT, CIITA, CPT1A, EGF, ELOVL6, ELOVL7, FADS3, FASN, GPAT3, NR1D2, NR1H2, PLIN1, PPAP2A, RORA, RXRA* and *UCP3*). Three *cis*-eQTLs (expression quantitative trait *loci*) were identified for *GPAT3*, *RXRA*, and *UCP3* genes, indicating that a genetic polymorphism proximal to the same gene is affecting its expression. Furthermore, 24 *trans*-eQTLs were detected and 8 candidate regulatory genes were located in these genomic regions. Additionally, two *trans*-regulatory hotspots in SSC13 and SSC15 were identified. Moreover, a co-expression analysis performed on 89 candidate genes and the fatty acid composition revealed the regulatory role of four genes (*FABP5*, *PPARG*, *SCD*, and *SREBF1*) in modulating the levels of α-linolenic, arachidonic, and oleic acids, as well as regulating the expression of other candidate genes associated with lipid metabolism. The findings of this study offer novel insights into the functional regulatory mechanism of genes involved in lipid metabolism, thereby enhancing our understanding of this complex biological process.

Keywords: eGWAS, Lipid metabolism, Muscle, Pig, Regulatory genes

Implications

The relationships between the genes that regulate lipid metabolism and the fatty acids are complex. Deepening the knowledge in this field is key to have a better understanding of how fatty acids are produced and, therefore, how can they be modulated.

Introduction

The percentage of intramuscular fat (IMF) and its fatty acid (FA) composition are recognized as key factors that influence meat quality and play a significant role in determining the nutritional value of meat (Wood et al., 2008). Moreover, IMF is directly related to meat flavour, juiciness, tenderness and firmness, which are relevant traits for consumers. Hence, in accordance with consumers, a high quantity of backfat has less acceptance, although meat with high IMF is considered a desirable trait. Some FAs are essential for humans, such as ω-3 and ω-6 polyunsaturated FAs (PUFAs), as they are not produced by *de novo* biosynthesis in the organism and must be provided through the diet. Therefore, the FA profile of pork is a key factor determining its quality and overall healthiness (Simopoulos, 2002) can also affect meat nutritional values and its sensory quality parameters (Chernukha et al., 2023). However, excessive fat can result in a less desirable texture and taste. To ensure a high-quality product, pork producers carefully balance genetics, nutrition, and management practices to optimize the level of IMF in the meat. With proper techniques, consumers can appreciate pork that is both flavourful and healthy, as the right amount of IMF can provide essential nutrients such as ω-3 and ω-6 FAs (Chernukha et al., 2023). Commercial pig breeds, such as Pietrain and Landrace exhibit superior efficiency in growth and leaner carcasses compared to Duroc or Iberian pigs. However, these carcasses have less IMF, which affects its meat quality. On the contrary, meat from Iberian breed is characterized by high IMF deposition and higher mono-unsaturated FA (MUFA) percentage, which provide more oxidative stability and improve meat taste and colour. Furthermore, this breed is widely used for dry-cured products, such as loin and ham (Lopez-Bote, 1998). A common practice involves crossing the Iberian pig with Duroc breed to enhance growth and feed efficiency.

Three different backcrosses, between Iberian × Duroc (BC1_DU), Iberian × Landrace (BC1_LD), and Iberian × Pietrain (BC1_PI) pigs were generated (Á. M. Martínez-Montes et al., 2018) producing animals with large phenotypic differences in growth, carcass and meat quality traits, such as the IMF content and FA composition. Different studies based on these animals used several analytical techniques such as quantitative trait *loci* (QTL) mapping and genome-wide association studies (GWAS) to identify genes associated with

growth, IMF content, and FA composition (Crespo-Piazuelo et al., 2020; Puig-Oliveras, et al., 2014; Ramayo-Caldas et al., 2012).

In previous studies of our group, the expression of candidate genes for lipid metabolism was analysed in *longissimus dorsi* muscle, adipose tissue, and liver of an Iberian × Landrace backcross (Puig-Oliveras et al., 2016; Revilla et al., 2018; Ballester et al., 2017). Moreover, Criado-Mesas et al. (2020) conducted an eGWAS with the same list of candidate genes of Puig-Oliveras et al. (2016) but including *longissimus dorsi* muscle expression data of 355 animals of the three backcrosses. These studies were specifically centred on the expression of candidate genes implicated in FA metabolism, aiming to identify eQTLs regulating gene expression. Detecting eQTLs is a valuable strategy to study complex trait genetics, revealing genetic variants linked to gene transcription levels that may contribute to phenotypic variation.

The main goal of this research is to study the expression and regulation of a selected set of 45 candidate genes for lipid metabolism in the porcine *longissimus dorsi* (LD) muscle in a total of 354 animals belonging to three different backgrounds. Additionally, we aimed to investigate the interrelationship between the 45 candidate genes utilized in this study, the 44 genes employed in the study conducted by Criado-Mesas et al. (2020) and the FA composition derived from the same population.

Material and methods

Pig population

The IBMAP population was obtained by crossing Iberian boars with Duroc, Landrace and Pietrain sows, and then F1 boars were crossed again with the respective Duroc, Landrace and Pietrain sows. In the present study, 354 animals were used, of which 122 belong to the BC1_DU (25% Iberian and 75% Duroc), 114 to the BC1_LD (25% Iberian and 75% Landrace) and 118 to the BC1_PI (25% Iberian and 75% Pietrain). All animals were maintained under the same intensive conditions and fed *ad libitum* with cereal-based commercial diet on NOVA GENÈTICA S.A. experimental farm (Lleida, Spain). Detailed information of generation schemes, diet, growth, and housing condition of the three

backcrosses is described in (Á. M. Martínez-Montes et al., 2018). Slaughtering procedures were conducted in a certified abattoir according to the institutional and national guidelines for the Good Experimental Practices and approved by the Ethical Committee of the Institution (IRTA – Institut de Recerca i Tecnologia Agroalimentàries). The *longissimus dorsi* muscle samples were collected, snap-frozen in liquid nitrogen, and stored at -80º C until further RNA isolation. Diaphragm samples were collected for DNA extraction.

Genotyping

Genomic DNA was isolated from diaphragm tissue by the standard method of phenol-chloroform extraction and was quantified with a NanoDrop-2000 spectrophotometer (Thermo Scientific). Animals from BC1_LD and BC1_PI were genotyped using the PorcineSNP60 Beadchip (*Illumina* Inc.; San Diego, USA) (Ramos et al., 2009) and BC1_DU animals were genotyped using Axiom Porcine Genotyping Array (*Affymetrix*). PLINK v1.90b4.3 software (Purcell et al., 2007) was used to remove markers that showed a minor allele frequency (MAF) of less than 5% and SNPs with more than 5% of missing genotypes. For eGWAS analysis, we used 38,423 SNPs that were common between the two SNP-genotyping arrays and were mapped in the *Sscrofa11.1* assembly. Moreover, 65 SNPs located in positional candidate genes were genotyped in the 354 pigs using custom-designed *Taqman* OpenArray genotyping plates in a QuantStudio™ 12K flex Real-Time PCR System (ThermoFisher Scientific) and were also included in the eGWAS analysis. Of these 65 SNPs, 11 SNPs were located within the fatty acid elongase 6 (*ELOVL6*) gene. Eight, nine and three were SNPs located on the fatty acid desaturase 1, 2 and 3 (*FADS1*, *FADS2* and *FADS3*) genes, respectively. Five of them were located in fatty acid synthase (*FASN*) gene, four in fatty acid elongase 1 (*ELOVL1*) gene, three in fatty acid elongase 7 (*ELOVL7*) gene, three in fatty acid binding protein 4 (*FABP4*) gene and three in thrombospondin 1 (*THBS1*) gene. The remaining 18 were located with one or two SNPs in the *ACACA*, *ACSL4*, *ANK2*, *CPT1A*, *GPAT3*, *LPL*, *NR1D2*, *PLIN1*, *SLC27A1*, *SREBF2* and *USF1* genes. The SNPs and their positions are detailed in Supplementary Table S1. A total of 38,488 SNPs were used for further analysis.

RNA isolation and gene expression

Total RNA extraction from *longissimus dorsi* tissue was performed with the Ribopure kit (Ambion), following the manufacturer's protocol. RNA integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies) and purity and quantification using a NanoDrop-2000 spectrophotometer (Thermo Scientific). Only the RNAs with integrity above seven (RIN >7) were used for the analysis. One µg of RNA was converted to cDNA using *High-Capacity cDNA Reverse Transcription Kit* (Applied Biosystems) in 20 µl total volume, following the manufacturer's instructions. The cDNA samples were loaded into a *Dynamic Array* 48.48 chip in a BioMark system (Fluidigm: San Francisco, CA, USA) through an integrated fluidic circuit controller following the manufacturer's instructions.

The expression of 45 target genes was analysed. After checking their stability, *ACTB* and *TBP* were used as reference genes. Primers used for the analyses were designed using PrimerExpress 2.0 software (Applied Biosystems) and are detailed in Supplementary Table S2. Data were collected using the Fluidigm Real-Time PCR analysis software 3.0.2 (Fluidigm) and analysed using the DAG expression software 1.0.4.11 (Ballester et al., 2013) applying the relative standard curve method. Samples targeted in this study were analysed in duplicate. The normalized quantity (NQ) values of each sample and assay were used to compare the gene expression among animals. Data normality was checked by applying the Shapiro-Wilk test in R and $\log_2$ transformation of the NQ value was applied when required. The sex and breed effects were also tested by using a linear model with the *lm* function in R.

Furthermore, the gene expression data of 44 candidate genes generated by Criado-Mesas et al. (2020) in the same 3BCs population, were used to analyse the gene co-expression patterns and its association with FA composition.

Expression Genome-wide association study

Genomic association studies between each gene expression measure and SNPs genotypes (eGWAS) were performed through a linear model using GEMMA software (Zhou & Stephens, 2012).

$y = W\alpha + x\beta + u + \varepsilon$; $u \sim MVNn(0, \lambda\tau^{-1}K)$, $\varepsilon \sim MVNn(0, \tau^{-1}In)$,

in which: **y** is the vector of phenotypes for **n** individuals; **W** is a matrix nxc of covariables (fixed effects) that includes sex (2 levels), backcross (3 levels) and slaughtering batch (9 levels); **α** is a c vector with corresponding coefficients, including the intercept; **x** is an n vector with the marker genotypes; **β** is the size of the marker effect, **u** is an n vector of random effects (additive genetic effects), **ε** is an n vector of errors. The random effects vector is assumed to follow a normal multivariate n-dimensional distribution (MVNn) where τ^{-1} is the variance of residual errors; **λ** is the quotient between the two components of variance; **K** is an nxn Kinship matrix calculated from the SNPs. The vector of errors is assumed to follow a distribution MVNn, where **In** is an nxn identity matrix.

GEMMA software calculates the *p*-value from the Wald statistical test for each SNP comparing the null hypothesis that the SNP has no effect versus the alternative hypothesis that the SNP effect is different from zero.

An eGWAS using GEMMA software was conducted between 38,488 SNPs distributed along the genome of the animals and 45 lipid-related genes in *longissimus dorsi* muscle. The False Discovery Rate (FDR) multiple testing correction method of Benjamini and Hochberg (Benjamini & Hochberg, 1995) was using the *p.adjust* function of R. For each gene, SNPs were considered significant at a threshold of FDR ≤ 0.05.

Identification of cis and trans eQTLs

The *cis*-eQTL mapping window was defined from 1 Mb upstream of the start of the gene to 1 Mb downstream of the gene end and all other regions were considered as *trans*-eQTLs. Significant SNPs separated less than 10 Mb apart were considered as belonging to the same genomic interval or eQTL. In this study, only eQTL intervals containing two or more SNPs were considered for further analysis.

Gene annotation

The annotation of the genes contained in the eQTLs was performed with Biomart (Smedley et al., 2015) tool from the Ensembl project (www.ensembl.org; release 108) using the *Sscrofa 11.1* reference assembly. Additionally, a 1 Mb extension was included at both ends of the genomic region. Functional predictions of the significant SNPs comprised in the eQTL regions were carried out using Variant Effect Predictor (VEP) (McLaren et al., 2010) and the Ensembl Genes 108 Database. With these tools, the location of eSNPs regarding a gene can be classified as outside of the gene, in untranslated regions (UTR) or in the coding sequence.

Co-expression and functional analysis

For this analysis, we used the gene expression data of the 45 candidate genes mentioned earlier, besides to the 44 candidate genes employed by Criado-Mesas et al. (2020) measured in the same animals. Furthermore, this analysis incorporated the relative quantification data of 14 different FAs in the *longissimus dorsi* muscle, as previously obtained by Crespo-Piazuelo et al. (2020).

Weighted gene expression networks were calculated using the PCIT algorithm (Watson-Haigh et al., 2009), which employ first-order partial correlation coefficients and an information theory approach to detect primary gene interactions. Only significant interactions between genes were considered for further analysis. Networks were represented with the Cytoscape v3.9.1 (Shannon et al., 2003) program.

Gene functional classification

The ShinyGO v0.77 (Ge et al., 2020) program was used to identify the main biological functions and the gene ontology association from the most important pathways of the genes mapped within the eQTLs. Moreover, STRING v11.5 (Jensen et al., 2009) was used to perform the functional enrichment analysis of genes found significantly associated in

the eGWAS analysis, and also to integrate and cluster the genes regarding their Gene Ontology.

Results

Sex and Genetic background effect on gene expression

In this study, including the three backcrosses (3BCs), 24 out of the 45 genes presented significant sex effect (*p*-value ≤ 0.05) on pig muscle gene expression: *ADIPOQ, ADIPOR1, ADIPOR2, AGPAT2, ANK2, APOE, ARNT, CD36, CYP2U1, EGF, ELOVL5, ELOVL6, ESRRA, FADS2, FASN, GPAT3, HADH, LPL, ME1, NR1D2, NR1H2, PDK4, PLIN1,* and *USF1* (Figure 1). We have identified more genes over-expressed in females (20), than in males (4).

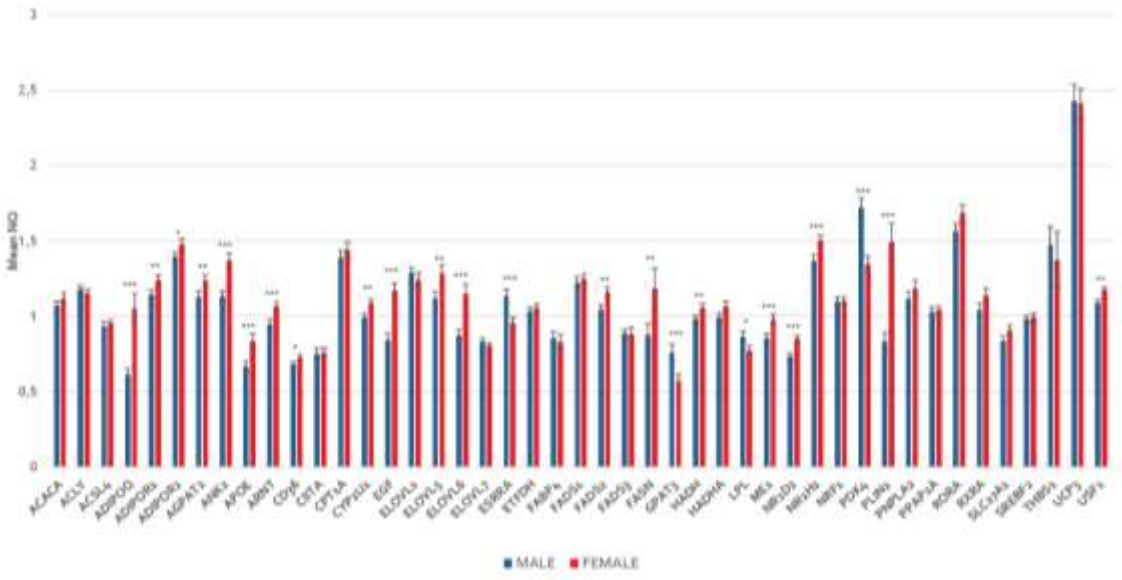

Figure 1. Comparison between females (red bars) and males (blue bars) of the mRNA levels (Mean NQ) of 45 lipid-related genes in animals from the 3BCs. Data are represented as mean ± standard error of the mean (SEM). Significant differences are labelled as: * p-value ≤ 0.05, ** p-value ≤ 0.01, *** p-value ≤ 0.001.

Furthermore, a significant backcross effect (*p-value* < 0.05) on gene expression levels was detected in 35 out of 45 analysed genes: *ACACA, ACSL4, ADIPOQ, ADIPOR1, ADIPOR2, AGPAT2, ANK2, APOE, ARNT, CD36, CPT1A, EGF, ELOVL1, ELOVL5, ELOVL6, ELOVL7, ESRRA, ETFDH, FADS1, FADS2, FADS3, FASN, GPAT3, HADH, HADHA, ME1, NR1H2, NRF1, PDK4, PLIN1, PNPLA2, RORA, RXRA, SLC27A1, SREBF2* (Figure 2). Overall, 24, 7 and 4 genes were over-expressed in BC1_DU, BC1_LD and BC1_PI backcrosses, respectively.

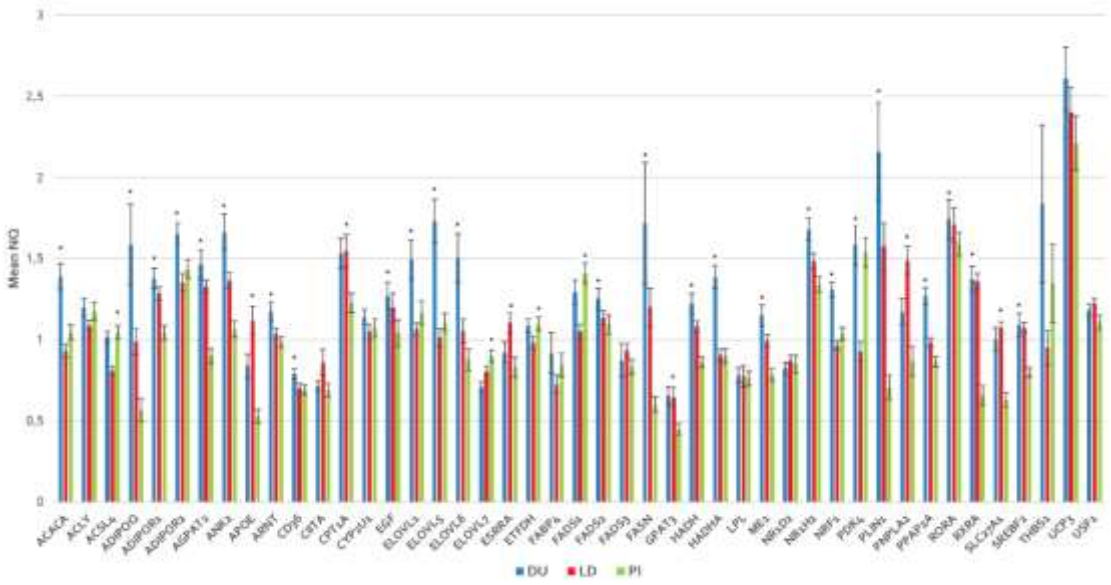

Figure 2. Comparison between the three experimental backcrosses in the mRNA levels of 45 lipid-related genes. Data presents mean ± standard error of the mean (SEM). Significant differences are labelled as: * p-value ≤ 0.05. DU, LD and PI represent the BC1_DU, BC1_LD and BC1_PI populations, respectively.

Genome-wide association studies for gene expression and eQTL identification

An eGWAS was performed between the muscle gene expression values and the genotypes of 38,488 SNPs distributed along the *Sus Scrofa* chromosomes in 354 animals. The eGWAS identified 301 eSNPs located in 27 genomic *Sus Scrofa* chromosome regions associated with the expression of *ANK2, APOE, ARNT, CIITA, CPT1A, EGF, ELOVL6,*

ELOVL7, FADS3, FASN, GPAT3, NR1D2, NR1H2, PLIN1, PPAP2A, RORA, RXRA and *UCP3* (FDR<0.05) genes. Unfortunately, none of the 65 SNPs located in candidate genes and genotyped by *Taqman* OpenArray were found significant in the eGWAS analysis.

A total of 24 eQTLs showed *trans*-regulatory effects on gene expression of 12 genes (Table 1). In addition, three were classified as *cis*-acting eQTLs for *GPAT3, RXRA* and *UCP3* genes, which suggests that there is a mutation in the same gene or in a proximal genomic region affecting its expression (Table 1). Both *cis* and *trans*-eQTLs were represented in Figure 3.

Table 1: Significant eQTLs for the 45-muscle gene expression study in 3BCs animals. Start and end positions refer to the eQTL interval and are based on Sus scrofa 11.1 assembly. Lengths are given in base-pairs. Gene annotation was performed considering one additional Mb at the start and at the end of the eQTL interval. The SNPs column indicates the number of SNPs within the eQTL.

Inter-val	Gene	Chr eQTL	Start pos (bp)	End pos (bp)	Size (Mbp)	SNPs	Top SNP	*q-value*	MAF	eQTL	Candidate genes
1	*ANK2*	6	129477727	132846185	3.36	3	rs81391604	0.0431	0.2	Trans	
2	*ANK2*	7	90911258	92399480	1.4	3	rs340169919	0.0096	0.22	Trans	
3	*ANK2*	7	105856850	105892246	0.04	2	rs81223355	0.0065	0.24	Trans	
4	*ANK2*	14	55291076	82159674	26.9	118	rs80792689	0.0065	0.18	Trans	*NRBF2*
5	*APOE*	13	77105634	82197802	5.1	14	rs80831731	0.0035	0.11	Trans	
6	*APOE*	15	93185092	94923471	1.74	6	rs333806503	0.0135	0.11	Trans	*STAT1, STAT4*
7	*ARNT*	14	56474441	58410680	1.99	5	rs80792689	0.0033	0.18	Trans	
8	*EGF*	8	131373563	131452301	0.08	2	rs81211121	0.0032	0.38	Trans	
9	*ELOVL6*	13	24228663	24253641	0.02	2	rs80853212	0.0429	0.05	Trans	
10	*ELOVL6*	13	77105634	82197802	5.1	14	rs80831731	0.0002	0.11	Trans	
11	*FASN*	5	27631915	28327581	0.7	2	rs81334652	0.0269	0.06	Trans	
12	*FASN*	8	79839602	79853747	0.02	2	rs81401770	0.0426	0.08	Trans	*NR3C2*
13	*FASN*	13	52057695	61140536	9.1	3	rs80909668	0.0374	0.41	Trans	
14	*FASN*	13	77105634	82197802	5.1	14	rs80831731	0.0065	0.11	Trans	
15	*FASN*	15	74207778	74235458	0.03	3	rs80850172	0.0040	0.11	Trans	
16	*FASN*	15	93185092	94923471	1.74	6	rs81301298	0.0183	0.09	Trans	*STAT1, STAT4*
17	*GPAT3*	8	134733478	135607348	0.88	8	rs81336088	0.0003	0.25	Cis	

18	*NR1D2*	6	99307882	99580947	0.27	3	rs81347503	0.0246	0.29	Trans	
19	*PLIN1*	13	77105634	82197802	5.1	14	rs81447187	0.0078	0.11	Trans	
20	*PPAP2A*	6	45156998	46387903	1.23	8	rs81395741	0.0089	0.11	Trans	*USF2*
21	*RORA*	14	2722953	2758196	0.36	2	rs80785221	0.0291	0.21	Trans	
22	*RXRA*	1	29427957	31162256	1.74	2	rs80801544	0.0086	0.12	Trans	
23	*RXRA*	1	270278445	274019182	0.37	11	rs81352834	3.8E-08	0.13	Cis	
24	*RXRA*	4	111702541	119798655	8.09	2	rs80985433	0.0014	0.09	Trans	
25	*RXRA*	12	20659791	20767619	0.1	5	rs81214864	0.0383	0.07	Trans	*MLX, STAT3, STAT5*
26	*UCP3*	9	8362141	8406364	0.44	2	rs81413811	0.0228	0.3	Cis	*UCP2*
27	*UCP3*	X	96608820	96624858	0.02	2	rs81473579	0.0228	0.09	Trans	

Abbreviations: Chr = Chromosome; bp = base pairs; Mbp = Megabase pairs;

MAF = Minor Allele Frequency

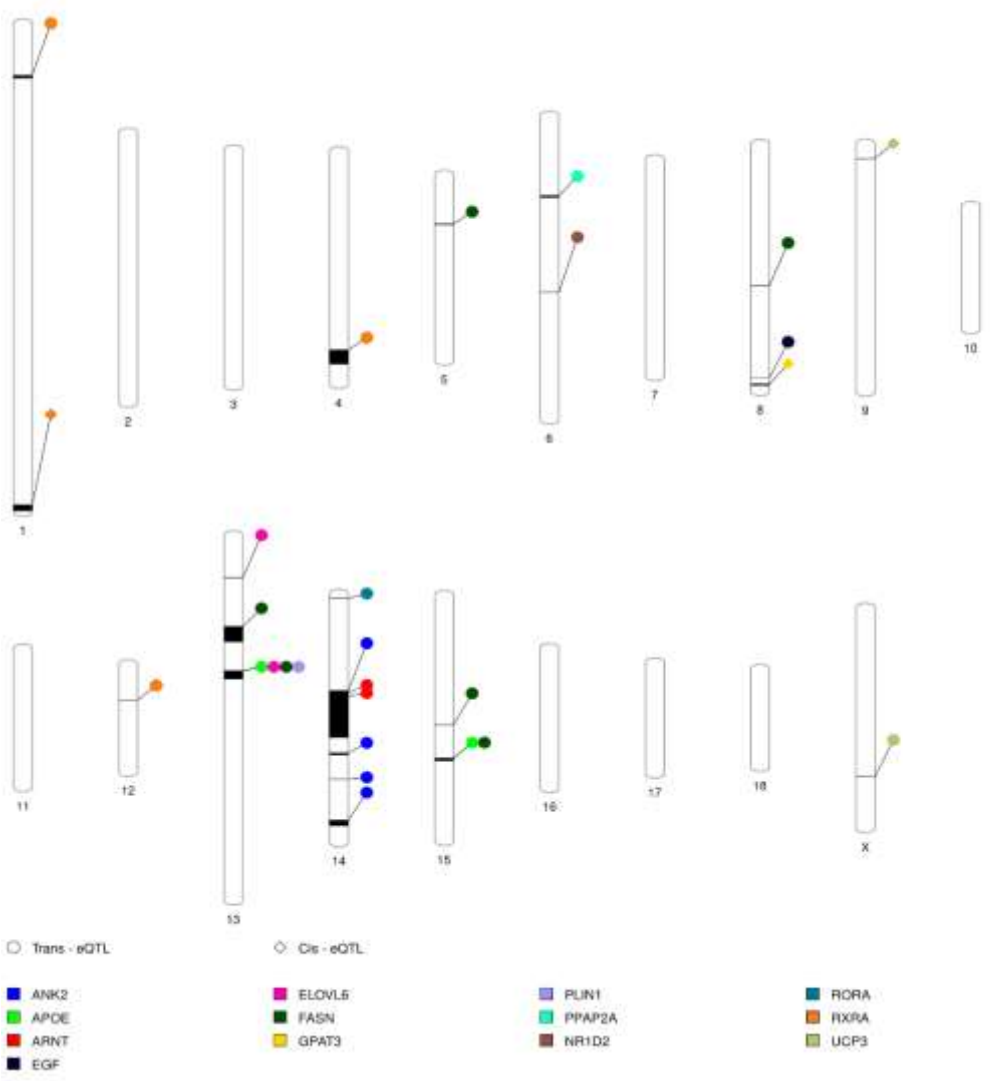

Figure 3. PhenoGram plot representing regions associated with gene expression of 45 lipid-related genes along pig chromosomes in the 3BCs study. The shape indicates the type of eQTL and the colour indicates the gene name as indicated in the legend.

In addition, in SSC13 we found an eQTL that affects the expression of four genes (*APOE, ELOVL6, FASN* and *PLIN1*). Moreover, in SSC15 there is another eQTL that regulates two other genes (*APOE* and *FASN*).

From the 258 eQTL-associated SNPs, 230 were successfully annotated with VEP of Ensembl (*Sscrofa* 11.1 annotation release 108) of which 34.8% (80 SNPs) were located in intergenic regions. The remaining 65.2% (150) of SNPs were mapped within 93 genes: 121 (52.6%) in intronic regions, 10 in upstream regions, five in downstream regions, four in non-coding transcript regions, three in 3'UTR regions and six in the coding regions of genes, five determining synonymous mutations and one being a missense mutation (Supplementary Table S3).

Cis-eQTLs

Concerning the *GPAT3* gene in the eGWAS results, one of the annotated *cis*-SNPs (*GPAT3* g.134933342T>C) was mapped within of the *GPAT3* gene. However, this SNP was not the most significant associated SNP (*p*-value = 9.00×10^{-8}). The most significant *cis*-SNP for *GPAT3* (g.135550523A>C; *p*-value = 1.70×10^{-8}) was located in the *SCD5* gene (Figure 4).

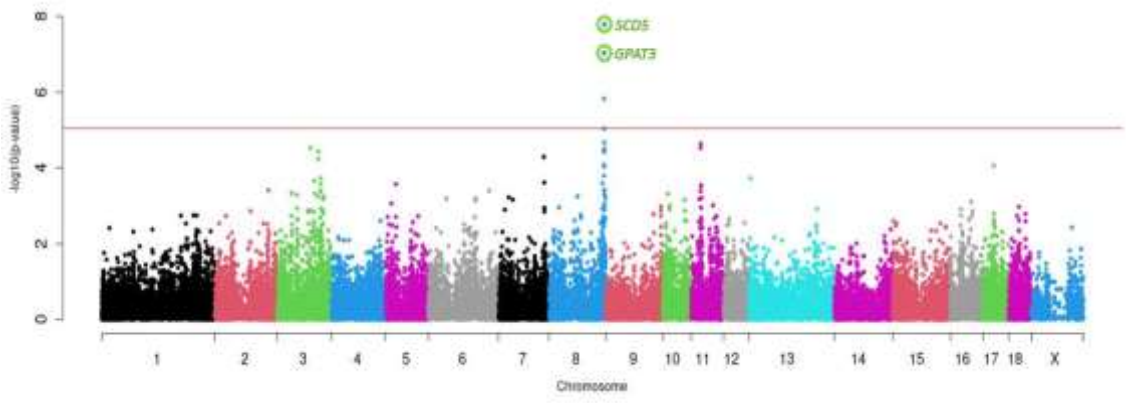

Figure 4. GWAS plot of *GPAT3* gene expression in muscle. Chromosome positions in Mb based on Sscrofa 11.1 assembly of the pig genome are represented in the X-axis and the –log10 (p-value) is on the Y-axis. The horizontal line represents the p-value for a genome-wide FDR < 0.05. The *SCD5* g.135550523A>C (rs81344869) and GPAT3 g.134933342T>C (rs81269758) SNPs are circled and labelled in colour green.

Regarding the *RXRA cis*-eQTL, the variant *NCS1* g.270313674C>T (rs81352834) was the most significantly associated polymorphism (*p*-value = 9.84×10^{-13}), and the second most significantly associated variant was *RXRA* g.273242436A>G SNP (rs80827620) (*p*-value = 9.73×10^{-11}) (Figure 5).

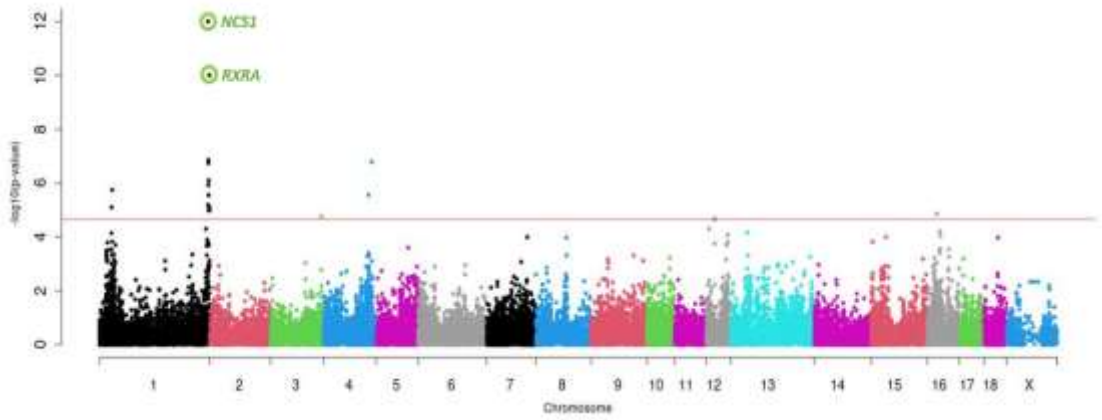

Figure 5. GWAS plot of *RXRA* gene expression in muscle. Chromosome positions in Mb based on Sscrofa 11.1 assembly of the pig genome are represented in the X-axis and the –log10 (p-value) is on the Y-axis. The horizontal line represents the p-value for a genome-wide FDR < 0.05. The *NCS1* g.270313674C>T (rs81352834) and RXRA g.273242436A>G (rs80827620) SNPs are circled and labelled in colour green.

In relation to the Uncoupling Protein 3 (*UCP3*) gene in the eGWAS results, the SNP g.8362141G>A (rs81413811), located within the *UCP2* gene, was the most significantly associated SNP with the *UCP3* gene expression in muscle (*p*-value = 6.54×10^{-7}) (Figure 6).

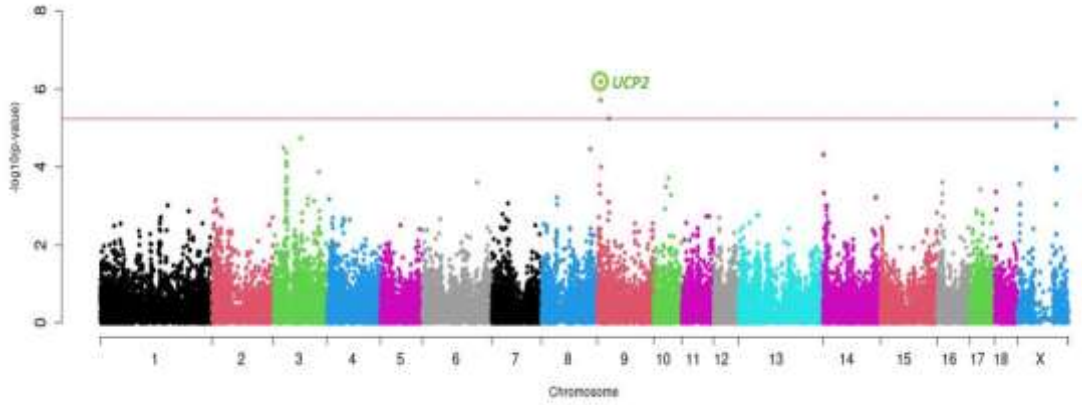

Figure 6. GWAS plot of *UCP3* gene expression in muscle. Chromosome positions in Mb based on Sscrofa 11.1 assembly of the pig genome are represented in the X-axis and the –log10 (p-value) is on the Y-axis. The horizontal line represents the p-value for a genome-wide FDR < 0.05. The *UCP2* g.8362141G>A SNP is circled and labelled in colour green.

Trans-eQTLs

A total of 786 genes located within 24 *trans*-eQTL genomic regions were identified in our study. Among them, we detected potential lipid metabolism regulatory genes in six genomic regions (Table 1).

Gene expression correlations

In order to identify co-expression patterns in the candidate genes, a co-expression correlation using PCIT algorithm (Watson-Haigh et al., 2009) was performed. The analysis included the muscle expression data of the 45 candidate genes used in this study, the 44 candidate genes utilized in the study of Criado-Mesas et al. (2020), and the composition data of 14 different FAs in the 3BCs pigs. Hence, a total of 89 candidate genes and 14 FAs were analyzed and a network graph was generated by Cytoscape software (Shannon et al., 2003) (Figure 7).

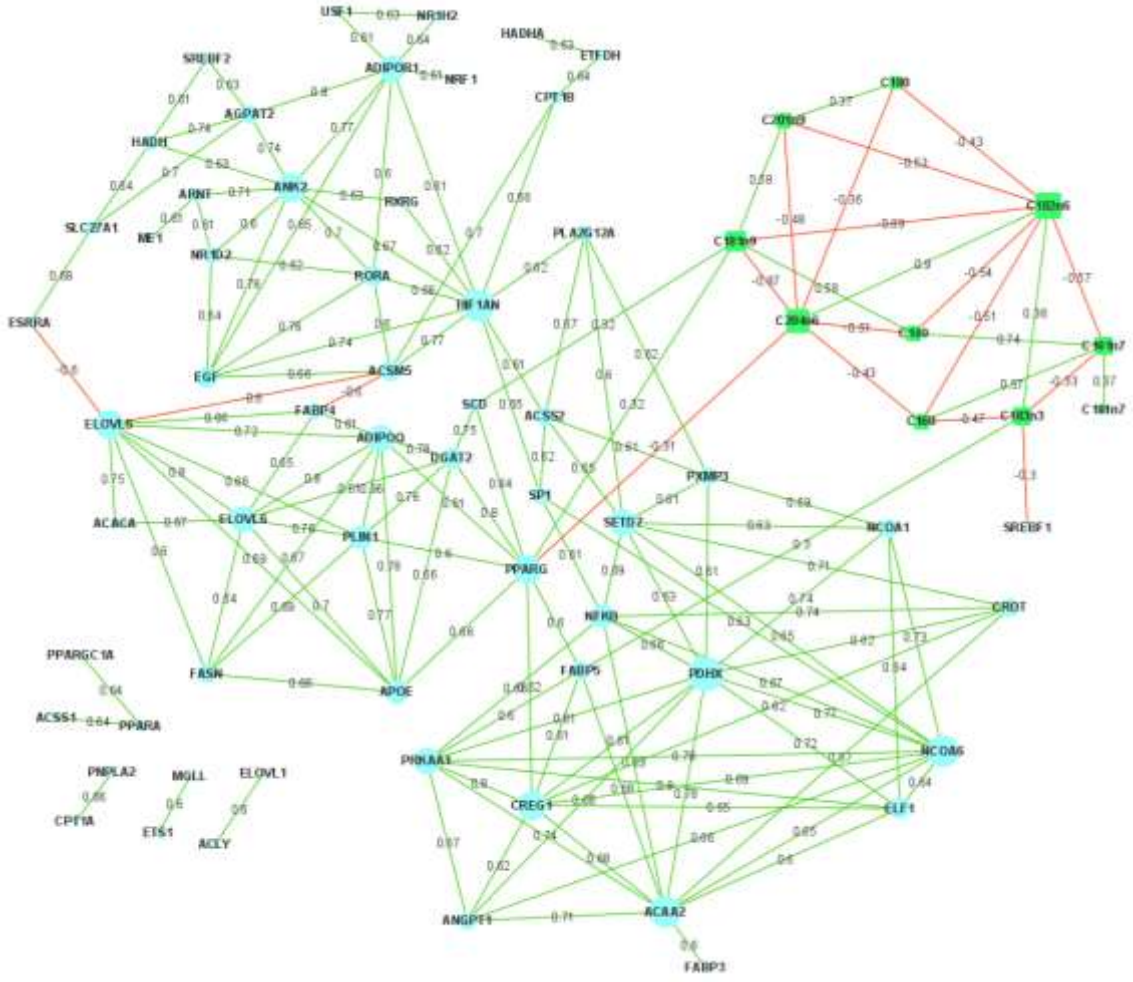

Figure 7. Gene co-expression network in 3BCs using the PCIT algorithm (Watson-Haigh et al., 2009). After filtering by significance and r ≥ |0.6| for genes and r ≥ |0.3| for FAs, 58 of the 90 initial genes and 10 different FAs are shown in this network. Node size represents the degree of a node. Green and red lines indicate the positive and negative correlations, respectively.

Notably, the genes *PDHX*, *HIF1AN*, *ACAA2*, and *NCOA6* exhibited the highest number of correlated connections with other genes. Regarding the 14 analysed FAs, only oleic acid (C18:1n-9), α-linolenic acid (C18:3n-3) and arachidonic acid (C20:4n-6) displayed significant correlations with genes. Specifically, C18:1n-9 exhibited positive correlations with the expression of the genes *SCD* (r = 0.32) and *PPARG* (r = 0.32). On the other hand, C18:3n-3 exhibited a negative correlation with the *SREBF1* gene expression (r = –0.3)

and a positive correlation with the *FABP5* gene (r = 0.3). Conversely, C20:4n-6 displayed a negative correlation with the *PPARG* gene expression. Finally, the strongest correlations within the entire network were observed between the genes *ADIPOQ1* and *PLIN1*, with a correlation value of 0.96, between the genes *SCD* and *PPARG*, with a correlation value of 0.84, between the linoleic acid (C18:2n-6) and C20:4n-6, with a correlation value of 0.9 and between C18:2n-6 and C18:1n-9, with a correlation value of –0.89.

Discussion

Sex and genetic background effects

In general terms, our results show that there are differences in muscle gene expression between males and females, particularly in genes associated with fat accumulation and storage. In females, the expression of genes related to lipid metabolism is generally higher, which may contribute to differences in fat distribution between the sexes (Varlamov et al., 2014). Hormonal differences between males and females are thought to play a role in the regulation of gene expression. For instance, estrogen and progesterone are more abundant in females and can stimulate the expression of genes associated with fat storage (Varlamov et al., 2014). Previously, a sexual dimorphism in the transcriptional regulation of genes related to lipid metabolism has been reported in various tissues, such as muscle, liver, and backfat (Ballester, Ramayo-Caldas, et al., 2017; Puig-Oliveras et al., 2016; Revilla et al., 2018). Therefore, it is relevant to comprehend the mechanisms underlying sexual dimorphism in gene expression.

The genes over-expressed in females are involved in FA metabolism (*APOE, CYP2U1, EGF, ELOVL5, ELOVL6, FADS2, FASN, HADH, PLIN1*), transcriptional regulation and control (*ANK2, ARNT, NR1D2, NR1H2, USF1*), energy metabolism (*ADIPOQ, ADIPOR1, ADIPOR2*) and lipid metabolic process (*AGPAT2, CD36, ME1*). Conversely, some of the four genes showing higher expression in males (*ESRRA, GPAT3, LPL* and *PDK4*), are relevant regulators of lipolytic pathways (Cunningham et al., 2022). In humans, men exhibit higher activity in lipolytic pathways, while women tend to have higher rates of lipogenesis and triglyceride accumulation, putting them at a greater risk for weight gain

and obesity development (Varlamov et al., 2014). In a similar way, female pigs seem to develop obesity more readily than male pigs (Zhang & Lerman, 2016). On the other hand, a comparison between Iberian and Duroc pigs reported a breed effect on the expression of genes involved in energy balance and lipogenesis (Bahelka et al., 2007; Benítez et al., 2018; Font-I-Furnols et al., 2019). In summary, genes more related to lipogenic pathways and biosynthesis pathways were more expressed in BC1_DU whereas genes related to lipolytic pathways were higher expressed in BC1_LD. Finally, 4 out of 45 genes were over-expressed in BC1_PI and were mainly related to lipogenic pathways and biosynthesis of unsaturated FAs.

Altogether, these results indicated a strong effect of sex and breed on gene expression levels. Therefore, they were considered in association studies and included as co-factors in our model.

Cis-eQTLs

The Glycerol-3-Phosphate Acyltransferase 3 (*GPAT3*) gene is involved in pathways such as the triglyceride biosynthetic process through the conversion of glycerol-3-phosphate to lysophosphatidic acid in the synthesis of triacylglycerol, and pathways related with gluconeogenesis (Cao et al., 2006). In the *GPTA3* eGWAS results, the most significant polymorphism was located on an intronic region of Stearoyl-CoA Desaturase 5 (*SCD5*) gene, at 0.57 Mb upstream from the *GPAT3* gene. The associated pathways of *SCD5* gene include lipid metabolism through stearoyl-CoA 9-desaturase and acyl-CoA desaturase activity, as well as biosynthesis of FAs and unsaturated FAs (Stelzer et al., 2016). On the other hand, the second most significant SNP was located on an intronic region of *GPTA3* gene. These results suggest the presence of another polymorphism within or near of this gene as a causative mutation affecting *GPTA3* gene expression levels.

Regarding Retinoid X Receptor Alpha (*RXRA*) in the eGWAS findings, this gene is involved in pathways such as hormone-mediated signalling pathway and regulation of RNA transcription (Stelzer et al., 2016). Furthermore, the *RXRA* gene forms the complex *PPARA-RXRA*, which increases lipid catabolism and FA β-oxidation. (Tontonoz et al., 1994; Vitali et al., 2018). In the *RXRA* eGWAS results, the most significant SNP was

located on an intronic region of *NCS1* gene, at 3.4 Mb downstream from the *RXRA* gene, and the second most significantly associated variant was located within an intronic region of *RXRA* gene. These findings indicate the presence of an additional polymorphism located within or in close proximity to this gene, which may act as a causal mutation influencing *RXRA* gene expression levels.

The Uncoupling Protein 3 (*UCP3*) gene, a target of another *cis*-eQTL region identified, is implicated in pathways including β-oxidation of FAs and it has been involved in chemical reactions and pathways such as FA metabolism process and adaptative thermogenesis (Han et al., 2012; Lin et al., 2017). This *cis*-eQTL, located in SSC9 contains a mutation located in an intronic position of *UCP2* gene, at 0.15 Mb downstream from the *UCP3* gene. *UCP2* gene, a paralog of *UCP3* gene, plays a role in non-shivering thermogenesis, obesity and diabetes *mellitus*, while its pathways include respiratory electron transport, ATP synthesis and heat production (Lin et al., 2017).

In this study, the associations between gene expression and genetic markers may be influenced by linkage disequilibrium with the causal mutation. Nevertheless, additional investigations are necessary to validate the findings derived from these analyses.

Trans-eQTLs

The Ankyrin 2 (*ANK2*) eGWAS results unravelled a total of four *trans*-eQTLs, but only one located at 55.2 Mb – 82.1 Mb in SSC14 (spanning 26.9 Mb and with 118 significant SNPs) contained a candidate gene (*NRBF2*) that could regulate the expression of *ANK2*. The *ANK2* gene belongs to the ankyrin family and has been suggested as a susceptibility gene for obesity, based on studies in mice with a human variant linked to type 2 diabetes (Lorenzo et al., 2015). Furthermore, *AnkB*-deficient adipocytes displayed increased levels of *GLUT4*, plasma membrane, glucose uptake, and lipid accumulation (Lorenzo & Bennett, 2017). Nuclear Receptor Binding Factor 2 (*NRBF2*) was mapped in this region and was associated as a lipid metabolism regulatory gene. Polymorphisms in *NRBF2* gene has been associated with specific PUFA levels in plasma in humans (Hu et al., 2015). Furthermore, *NRBF2* has also demonstrated its ability to interact with several other receptors, including peroxisome proliferator-activated receptor alpha (*PPARA*), thyroid

hormone receptor beta (*THRB*), retinoic acid receptor alpha (*RARA*), and retinoid X receptor alpha (*RXRA*), which is also related to different candidate genes for lipid metabolism, such as *FABP4, FASN, LPL* and *PLIN1* (Ouyang et al., 2020).

The Fatty Acid Synthase (*FASN*) eGWAS results identified *trans*-eQTL covering the genomic region of 79.8 Mb to 79.85 Mb, wherein a transcription factor associated with lipid metabolism, the Nuclear Receptor Subfamily 3 Group C Member 2 (*NR3C2*), was identified. Polymorphisms in the *NR3C2* gene have been associated with neuroendocrine parameters, carcass composition and meat quality traits in pigs, suggesting its important role in the regulation of lipidic genes (Terenina et al., 2013). Unfortunately, no evidence has been found linking the gene *NR3C2* to the regulation of *FASN*. However, further analysis would be necessary to determine if there is any relationship between these genes.

The further *trans*-eQTL region identified in the eGWAS results of the candidate gene *PPAP2A* was located in SSC6 at 45.1 Mb – 46.3 Mb, where Upstream Transcription Factor 2 (*USF2*) was found. The *USF2* gene has been identified as a transcriptional regulator of the human *APOC3* gene which is recognized as an inhibitor of lipoprotein lipase, and as such, its overexpression in mice has been observed to result in a substantial increase in plasma triglyceride concentrations, consistent with its inhibitory function (Lai et al., 2005). With this information, it suggests that the *USF2* gene could play a regulatory role in genes related to lipid metabolism.

The Retinoid X Receptor Alpha (*RXRA*) eGWAS unravelled a *trans*-eQTL, positioned at 20.6 Mb – 20.7 Mb in SSC12. Through a comprehensive analysis of this genomic *locus*, which spans 0.1 Mb, a total of five SNPs have been identified that show significant associations with *RXRA* gene expression levels, with three candidate regulatory genes being mapped (*MLX*, *STAT3, STAT5*).

The MAX Dimerization Protein (*MLX*) gene has been found to be correlated with the *ChREBP* transcription factor, and it has been observed that this complex is capable of modulating the transcriptional activity of genes involved in lipid metabolism, including *ACC1* and *FASN* (Donald, 2012). According to a model proposed by Ma et al. (2005), two

ChREBP-Mlx heterodimers would bind to the two E boxes of the ChoRE to provide a transcriptional complex necessary for glucose regulation.

The Signal Transducer and Activator of Transcription 3 (*STAT3*) gene has also been identified in the *RXRA* eQTL. According to Wu et al. (2018), *STAT3* gene plays a crucial role in regulating the *FAS* and *CPT1α1b* lipid metabolism genes, as their promoter regions are associated with the binding locus of *STAT3* in yellow catfishes. Unfortunately, we were unable to locate any relevant literature concerning this transcription factor's association with the *RXRA* gene or its involvement with lipid metabolism genes in pigs.

On the other hand, the STAT5A/B family was also mapped at *RXRA trans*-eQTL. According to Kliewer et al. (1999), the *PPARγ* is a key member of the nuclear hormone receptor superfamily. It collaborates with *RXRA* to effectively regulate specific genes associated with adipocyte differentiation and insulin sensitization. In relation with this, Meirhaeghe et al. (2003) indicated that *PPARγ3* could play a role in maintaining lipid balance in humans by influencing the Growth Hormone/*STAT5B* pathway. Hence, it suggests that the STAT proteins not only control the expression of genes specific to fat tissue but also serve as targets of regulation by transcriptional factors such as *PPARγ*. In addition, an study conducted by Si & Collins (2002) on the regulation of haematopoiesis in humans and its association with transcription factors, it was discovered that the JAK2/STAT pathway, specifically the activation of *STAT5*, plays a crucial role in enhancing RAR transcriptional activity in cultured hematopoietic cells.

Trans-eQTL hotspot regions

Furthermore, we have detected two hotspot regions that modulate the expression of several genes. A *trans*-eQTL hotspot located on SSC13 and spanning 5.1 Mb (77.1 – 82.2 Mb) was associated with the expression of four genes: *APOE, ELOVL6, FASN* and *PLIN1.* The Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Beta (*PIK3CB*) gene was mapped in this region. *PIK3CB* participates in the PI3K-Akt signalling pathway and mTOR signalling pathway, and the regulation of the PI3K-Akt-mTOR signalling pathway on lipid metabolism has been mentioned by different studies (Dibble, 2013; D.

Liu et al., 2016). Furthermore, inhibition of the PI3K-Akt-mTOR pathway leads to a decrease in intracellular lipid accumulation along with a reduction in mRNA expression and protein content of genes involved in *de novo* FA synthesis (Y. Zhao et al., 2023). Hence, we can suggest that *PIK3CB* is involved in muscle lipid metabolism, being an interesting candidate gene to explain the differences in the expression of four genes associated with the SSC13 hotspot. Other potential genes related to lipid metabolism (*ESYT3*, *RBP1* and *RBP2*) were detected, but no evidence was found for their possible regulation of the four genes in the mentioned hotspot.

The second *trans*-hotspot region covering 1.74 Mb on SSC15 (93.1 – 94.9 Mb) was associated with the expression of *APOE* and *FASN* genes. In this region, we detected two candidate regulatory elements, *STAT1* and *STAT4*, which have been associated with lipid metabolism pathways. Zhang et al. (2019), suggested that *STAT1* may regulate the expression of genes associated with lipid metabolism and FA synthases (FAS), such as *FASN*, the key enzymes in *de novo* lipogenesis, which promotes the synthesis of long-chain FAs. A previous study has indicated that *STAT1* regulates adipogenesis and adipolysis (Stephens et al., 1996). In addition, the Myostatin (*MSTN*) gene was also mapped in this hotspot region. The *MSTN* gene has been described by Xin et al. (2020) as being involved as a regulator of AMP kinase activity in cattle. The down-regulation of *MSTN* triggers the activation of AMPK signalling pathways to regulate glucose and lipid metabolism, which highlights its possible role in lipid metabolism. Moreover, based on their findings, Pan et al. (2021) propose that *MSTN* exerts an inhibitory effect on adipogenesis and promotes lipolysis in the subcutaneous adipose tissue of pigs, primarily through the activation of ERK1/2 and PKA signalling pathways. These results indicate that *MSTN* may act as a powerful regulator of genes involved in lipid metabolism pathways.

The two *trans* hotspots detected in this study are associated with *APOE* and *FASN* gene expression, suggesting that different genetic variants are regulating the muscle expression of these genes, but further studies are required to identiy these variants.

Muscle gene expression and FA composition correlation networks

Our study investigates the relationship between specific gene expression and the quantities of different FAs in the porcine *longissimus dorsi* muscle. *SCD* and *PPARG* genes are found to play a crucial role in regulating C18:1n-9 levels, with a positive correlation observed between their expression and the abundance of this FA. Similarly, the genes *FABP5* and *SREBF1* are implicated in controlling C18:3n-3 levels, with a positive correlation for *FABP5* and a negative correlation for *SREBF1*. Furthermore, the study explores the association between C20:4n-6 and the *PPARG* gene, revealing a negative correlation and suggesting a regulatory role of *PPARG* in C20:4n-6 metabolism. Moreover, we want to highlight the importance of the *PPARG* gene due to its substantial number of connections with other genes and its association with FAs, suggesting a significant role of this gene in FA metabolism and with potential implications in pork quality. In addition, the genes *PDHX*, *HIF1AN*, *ACAA2*, and *NCOA6* display significant correlations with multiple genes, indicating their role as central regulators. The positive and negative correlations between the genes and the FAs are shown in Figure 7.

Conclusions

In the present work, we identified genetic variants associated with the expression of lipid-related genes in muscle. These genetic variants were grouped in 27 eQTLs from which three were described as *cis*-acting major regulators of *GPAT3*, *RXRA* and *UCP3* gene expression levels. The other 24 regions were *trans*-eQTLs, which affect different lipid-related genes along the chromosomes. In addition, two *trans*-regulatory hotspots regulating the expression of several genes were identified in SSC13 and SSC15. Furthermore, the co-expression analysis identified four regulatory genes modulating the levels of different FAs in the 3BCs, as well as the expression of other lipid metabolism genes. Our results increase the knowledge of the genetic basis of gene expression regulation in muscle lipid metabolism. Overall, the expression of genes related to lipid metabolism is regulated in a complex way and further validations are needed to corroborate our findings.

Ethics approval

Animal care and procedures were carried out following the Spanish Policy for Animal Protection RD1201/05 and the European Union Directive 86/609 about the protection of animals used in experimentation.

Data and model availability statement

All relevant data produced or evaluated in this research are disclosed in the paper as well as its supplementary information files. Additional materials can be requested to JMF.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author used the ChatGPT tool in order to correct potential grammatical errors, search for synonyms, enhance the author's written sentences, as well as to translate different words related to this field.

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Author contributions

JMF conceived the study and was the principal investigator of the project with the participation of AS. JMF collected the animal samples. MP, FLC and LCM performed the total RNA isolation. Real-time qPCR expression analyis was performed by MP, FLC, AC and LCM. MP, FLC and LCM performed the bioinformatic analysis to generate the gene expression data. MP, FLC, CS and JVH participated in data processing and bioinformatic methods. JMF, CS and MP proposed the statistical methodology and performed the global association study and gene functional analysis. JMF reviewed the statistical methodology and conceived the structure of the paper. MP and JMF wrote the paper. All authors critically revised and approved the final manuscript.

Declaration of interest

The authors declare no conflict of interest

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PAPER II

Expression quantitative trait *loci* (eQTL) identification and its relationship with lipid metabolism in pig muscle

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Abstract

Pig is one of the main sources of meat in the world, so the quality of its meat and its nutritional values are gaining more interest. Genetic dissection of gene expression could help us to understand the genetic architecture of complex phenotypes such as meat quality in pigs. This work aimed to identify expression quantitative trait loci (eQTLs) within the transcriptome of the *longissimus dorsi* muscle from a crossbreed of 129 Iberian x Duroc pigs using RNA-Seq data and analyse its relationship with lipid metabolism and FA composition. The muscle gene expression data and the SNP genotypes obtained from the Axiom Porcine Genotyping Array (*Affymetrix*) were used to carry out the expression genome-wide association studies (eGWAS) and the eQTL mapping. A total of 2,678 eQTLs were identified, of which 620 were *cis*-eQTLs corresponding to 598 *cis*-genes and 2,058 *trans*-eQTLs corresponding to 604 *trans*-genes. A total of 854 genes presented significant associations with the expression-SNPs (eSNPs), among them 101 genes were associated with lipid metabolism pathways. In addition, a co-expression analysis performed on 75 *cis*-genes and 71 *trans*-genes related to lipid metabolism and fatty acid (FA) composition revealed the regulatory role of five genes (*ACAA1, CLN, CYP2B22, GBA* and *LDHD*) in modulating the levels of eight different FAs, as well as regulating the expression of other candidate genes associated with lipid metabolism. Our results increase the knowledge of the genomic basis of gene expression regulation in pig skeletal muscle.

Introduction

The pig is an important livestock animal because it is one of the main sources of meat in the world. Meat is considered an important source of nutrients, although a high consumption can increase the risk of some types of chronic diseases (Godfray et al., 2018). In the last few years, consumers are more concerned about healthy and high-quality food. The relationship between muscle growth, fat deposition, and the content of intramuscular fat (IMF) and fatty acid (FA) composition are significant determinants of porcine meat quality characteristics, including its sensory attributes and nutritional value (Wood et al., 2004, 2008b). According to Wood et al. (2008a), a high backfat content is considered a less desirable trait, whereas meat with high IMF is known for its enhanced taste and juiciness. Furthermore, the FA composition of IMF plays a crucial role in meat quality parameters. A previous study conducted by Wood et al. (2008b) reported that monounsaturated FAs (MUFAs) provide greater oxidative stability compared to polyunsaturated FAs (PUFAs), resulting in improved meat taste and colour. On the other hand, PUFA consumption decreases the risk of suffering cardiovascular diseases, being healthier than saturated FAs (SFA) (Michas et al., 2014). In pork, the SFA and MUFA are positively correlated to tenderness, whereas PUFA are negatively correlated (Cameron & Enser, 1991).

Investigating the gene expression patterns in metabolic tissues offers a potential pathway to elucidate the molecular mechanisms influencing the phenotypic variation observed in IMF content and FA composition in animals. Next-generation sequencing (NGS) technologies have provided new tools for both gene-expression profiling and transcriptome characterization. The RNA sequencing (RNA-Seq) method is based on the sequencing of RNA molecules present in a given sample. The obtained counts corresponding to each transcript can be used for quantification and the sequences can be mapped to the genome for their annotation. The transcriptome analyses allow not only to study the gene expression variation, but also the identification of new isoforms, splicing events, and different promoter signals. Previous studies on the porcine skeletal muscle identified genes affecting IMF content and FA composition (Cardoso et al., 2017; Crespo-Piazuelo et al., 2020; Criado-Mesas et al., 2020; González-Prendes et al., 2019; Muñoz et al., 2018; Puig-Oliveras et al., 2016), drip loss (Heidt et al., 2013), and lipid

metabolism (Steibel et al., 2011a) among other traits. Moreover, several studies identified candidate genes for FA composition in muscle through RNA-Seq analysis (Crespo-Piazuelo et al., 2020; Huang et al., 2018; Jin et al., 2021; Y. Liu et al., 2020; Puig-Oliveras et al., 2014).

The detection of expression Quantitative Trait *Loci* (eQTL) has been proposed as a good strategy to deepen the study of the genetic architecture of complex traits (Gilad et al., 2008). This technique allows for the identification of candidate genes, causal variants, and molecular pathways associated with phenotypic differences in complex traits. Several studies have been published using the eGWAS-based eQTL detection methodology to identify various traits, some of which are related to productive characteristics and meat quality traits. Furthermore, different analyses have been performed using eQTL detection methodology and meat quality traits, such as meat colour, muscle size, IMF deposition, drip loss, FA composition and pH (Leal-Gutiérrez et al., 2020; Y. Liu et al., 2020; Ponsuksili et al., 2010; Steibel et al., 2011b). In addition, several studies have been carried out in our group to identify eQTLs for lipid metabolism candidate genes across different pig tissues and crosses (Ballester et al., 2017; Criado-Mesas et al., 2020; Puig-Oliveras et al., 2016; Revilla et al., 2018) and using real-time qPCR expression data.

The aim of this work was to study the porcine *longissimus dorsi* muscle transcriptome profile by RNA-Seq, to identify eQTL associated with lipid metabolism and FA composition pathways.

Material and methods

Pig population

In the present study, 129 pigs were used (59 females and 70 males) belonging to an experimental backcross (25% Iberian and 75% Duroc, BC1_DU). Animal care and procedures were carried out following the Spanish Policy for Animal Protection RD1201/05 and the European Union Directive 86/609 about the protection of animals used in experimentation. All animals were maintained under the same intensive

conditions and fed *ad libitum* with a cereal-based commercial diet on NOVA GENÈTICA S.A. experimental farm (Lleida, Spain). Detailed information of generation schemes, diet, growth, and housing conditions was described in (Martínez-Montes et al., 2018). Slaughtering procedures were conducted in a certified abattoir according to the institutional and national guidelines for the Good Experimental Practices and approved by the Ethical Committee of the Institution (IRTA – Institut de Recerca i Tecnologia Agroalimentàries). The *longissimus dorsi* muscle samples were collected, snap-frozen in liquid nitrogen, and stored at -80º C until further RNA isolation. Diaphragm samples were collected for DNA extraction.

Genotyping

Genomic DNA was isolated from diaphragm tissue by the standard method of phenol-chloroform extraction and was quantified with a NanoDrop-2000 spectrophotometer (Thermo Scientific). The 129 animals were then genotyped using the Axiom Porcine Genotyping Array 660K (*Affymetrix*) according to the manufacturer's protocol and only SNPs mapping against the *Sscrofa 11.1* assembly were used. Quality Control (QC) was done using PLINK v1.90b4.3 software (Purcell et al., 2007) filtering out SNPs with a minor allele frequency (MAF) of ≤ 5%, missing genotype call rate per SNP ≥ 1% and linkage disequilibrium (R^2) ≥ 0.7. Then, the missing genotypes were imputed using Beagle v5.1 software (Browning et al., 2018). Finally, 76,318 SNPs distributed along all autosomes and X chromosomes passed the criteria and were used for further analysis.

RNA isolation and sequencing

Total RNA was isolated from the *longissimus dorsi* tissue of 129 animals using the RiboPure kit (Ambion, Austin, TX) following the manufacturer's protocol. RNA integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies) and purity and quantification using a NanoDrop-2000 spectrophotometer (Thermo Scientific). Only the RNAs with integrity above seven (RIN >7) were used for the RNA-Seq experiment. Library preparation and sequencing were performed at CNAG Institute (*Centro Nacional de Análisis Genómico*, Barcelona, Spain). For each sample, one paired-end library was

prepared using TruSeq Stranded mRNA kit (Illumina, Inc.; San Diego CA, USA). To discriminate among samples, libraries were labelled by barcoding and pooled to be run in Illumina HiSeq 3000/4000 instruments (Illumina, San Diego CA). In brief, in this study 2 × 75 bp reads, a mean of 45.09 million of paired-reads per sample, and an average of 90.06% (ranging from 80.51 to 96.09%) of uniquely mapped reads were generated.

Bioinformatic analyses

We ran MultiQC v0.7 (Ewels et al., 2016) and FastQC v0.11.9 (Andrews, 2010) for the quality control and basic statistics of reads. RNA-Seq reads were mapped by using the STAR software v2.7.9a (Dobin et al., 2013) with default parameters to the pig reference genome assembly *Sscrofa 11.1* and to the annotation from Ensembl Genes 107 Database. Transcripts were assembled and quantified by HTSeq v0.10.0 (Anders et al., 2015). Data pre-processing and quality control were performed with the EdgeR v3.10.5 (Robinson et al., 2009) and Limma v3.26.0 (Ritchie et al., 2015) R packages. First, unexpressed genes were filtered out retaining genes having more than one read per million in at least 25% of the samples. Finally, a total of 11,054 genes were considered to be expressed in the muscle samples and further analysed. Expressed gene counts were normalized using the *log* counts per million (logCPM) with the Limma-trend approach (Law et al., 2014).

Expression Genome-wide association studies

Genomic association studies between each gene expression measure and SNPs genotypes (eGWAS) were performed through a linear model using GEMMA v0.98.1 software (X. Zhou & Stephens, 2012).

$y = W\alpha + x\beta + u + \varepsilon$; $u \sim MVNn(0, \lambda\tau^{-1}K)$, $\varepsilon \sim MVNn(0, \tau^{-1}In)$,

in which: y was the vector of phenotypes for n individuals; W is a matrix nxc of covariables (fixed effects) that includes a column of ones, sex (2 levels) and slaughtering batch (5 levels); α is a c vector with corresponding coefficients, including the intercept; x is an n vector with the marker genotypes; β is the size of the marker effect, u is an n

vector of random effects (additive genetic effects), ε is an n vector of errors. The random effects vector is assumed to follow a normal multivariate n-dimensional distribution (MVNn) where τ-1 is the variance of residual errors; λ is the quotient between the two components of variance; K is an nxn Kinship matrix calculated from the SNPs. The vector of errors is assumed to follow a distribution MVNn, where ln is an nxn identity matrix.

GEMMA software calculates the p-value from the Wald statistical test for each SNP comparing the null hypothesis that the SNP has no effect versus the alternative hypothesis that the SNP effect is different from zero.

An eGWAS using GEMMA software were conducted between 76,318 SNPs distributed along the genome of the animals and 11,054 genes expressed in muscle. An FDR (False Discovery Rate) filtering method of Benjamini and Hochberg (Benjamini & Hochberg, 1995) with the function *p.adjust* of R was applied for each gene with a cut-off threshold of FDR ≤ 0.01. Only associations with a *p-value* lower than this established threshold were considered significant.

Identification of *cis*- and *trans*-eQTLs

In order to define the regions, chromosomes were divided into windows of 10 Mb long. Within these windows, the first and the last SNPs were taken as the start and the end of the eQTL. Subsequently, we categorized the eQTLs as either *cis* or *trans*, depending on their distance from the target gene. The *cis*-eQTL mapping window was defined from 0.5 Mb upstream of the start of the gene to 0.5 Mb downstream of the gene end; all other regions were defined as *trans*-eQTLs. Additionally, we filtered-out the eQTLs with less than three significant SNPs.

Gene annotation

The extraction of the genes contained in the eQTLs was performed with Biomart (Smedley et al., 2015) tool from the Ensembl project (www.ensembl.org; release 107) using the *Sscrofa 11.1* reference assembly. Additionally, a 1 Mb extension was included at both ends of the genomic region. Functional predictions of the significant SNPs

comprised in the eQTLs were carried out using Variant Effect Predictor tool (McLaren et al., 2016) and the Ensembl Genes 107 Database. With these tools, the location of expression SNPs (eSNPs) in relation to a gene can be defined as outside of the gene, in untranslated regions (UTR) or in the coding sequence.

Gene functional classification

The ShinyGO (v0.77) (Ge et al., 2020) program was used to identify the main biological functions and the gene ontology association from the most important pathways of the genes mapped within the eQTLs. Moreover, the STRING (v11.5) (Jensen et al., 2009) program was used to perform the functional enrichment analysis of genes found significantly associated in the eGWAS studies and also to integrate and cluster the genes regarding their Gene Ontology.

Co-expression and functional analysis

In order to prioritize eQTLs influencing the expression of genes associated with lipid metabolism and FA metabolism, we employed a filtering approach based on gene ontology utilizing the Biomart tool (Smedley et al., 2015) from the Ensembl project (www.ensembl.org; release 107).

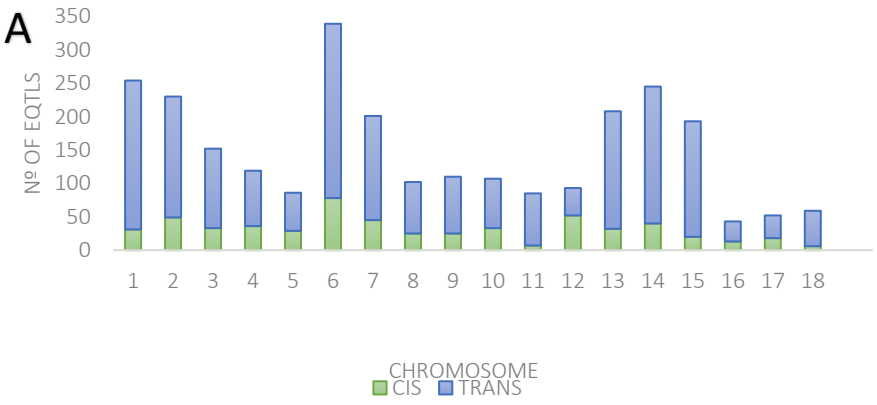
Furthermore, we performed a co-expression analysis with the gene expression data of lipid metabolism-related genes associated with eQTLs and the relative quantification data of 14 different FAs in the *longissimus dorsi* muscle, as previously obtained by Crespo-Piazuelo et al. (2020). Weighted gene expression networks were calculated using the PCIT algorithm (Watson-Haigh et al., 2009), which employs first-order partial correlation coefficients and an information theory approach to detect primary gene interactions. Only significant interactions between genes were considered for further analysis. Networks were represented with the Cytoscape v3.9.1 (Shannon et al., 2003) program.

Results

Identification of eQTL regions

An eGWAS was performed by combining the pig muscle gene expression values measured by RNA-Seq, and the genotypes of 76,318 distributed along the *Sus Scrofa* chromosomes in 129 BC1_DU animals. The eGWAS identified a total of 34,003 significantly associated eSNPs located in 2,678 *Sus scrofa* chromosome regions associated with the expression of 854 genes (FDR<0.01). Chromosomes SSC6, SSC14, SSC2, SSC7 and SSC13 presented a higher number of significantly associated eSNPs, while SSC18, SSC11, and SSC5 showed a lower number of significantly associated eSNPs.

A total of 2,058 eQTLs showed *trans*-regulatory effects on gene expression of 604 genes. On the other hand, 620 eQTLs were classified as *cis*-acting eQTLs for 598 genes. The analysis of eQTL genomic regions revealed that *cis*- and *trans*-eQTLs were widely distributed on all autosomes. Both *cis*- and *trans*-eQTLs are represented in Figure 1A. Furthermore, from the 854 significantly associated genes, we identified 250 with only *cis*-eQTLs, 256 only with *trans*-eQTLs and 348 genes that presented both *cis*- and *trans*-regions (Figure 1B).



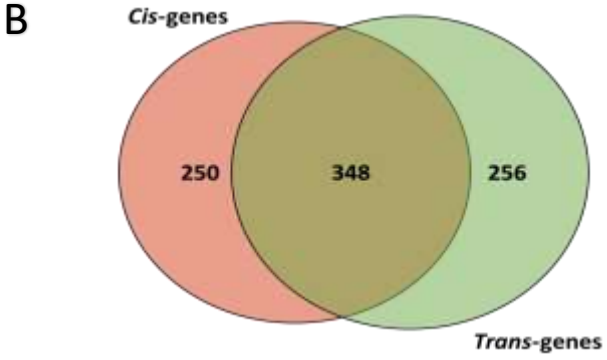


Figure 1. A. Distribution of *cis*- and *trans*-eQTLs along the autosomes. The green group represents the *cis*-eQTLs (CIS). The blue group represents the *trans*-eQTLs (TRANS). B. Venn diagram of *cis*-genes and *trans*-genes significantly associated in the eGWAS analysis.

Furthermore, we analysed the eSNPs associated to the eQTLs, and the predictions of their impact were performed using Variant Effect Predictor from Ensembl tools. We observed that a higher proportion of eSNPs were located in intronic (50.25%), intergenic (27.5%) or upstream (9.8%) regions. The less-represented SNP positions were in downstream (6.1%), coding regions (3.2%) and 3' UTR (2.1%) regions. A 0.71% of eSNPs are missense, thus producing an amino-acid change in the corresponding protein. In addition, we performed an eQTL mapping with the significantly associated eSNPs. Among the 34,003 eSNPs (Figure 2), 3,846 corresponded to eSNPs located in *cis*-eQTLs (blue line on the diagonal in Figure 3) and the other 30,083 eSNPs were associated to *trans*-eQTLs.

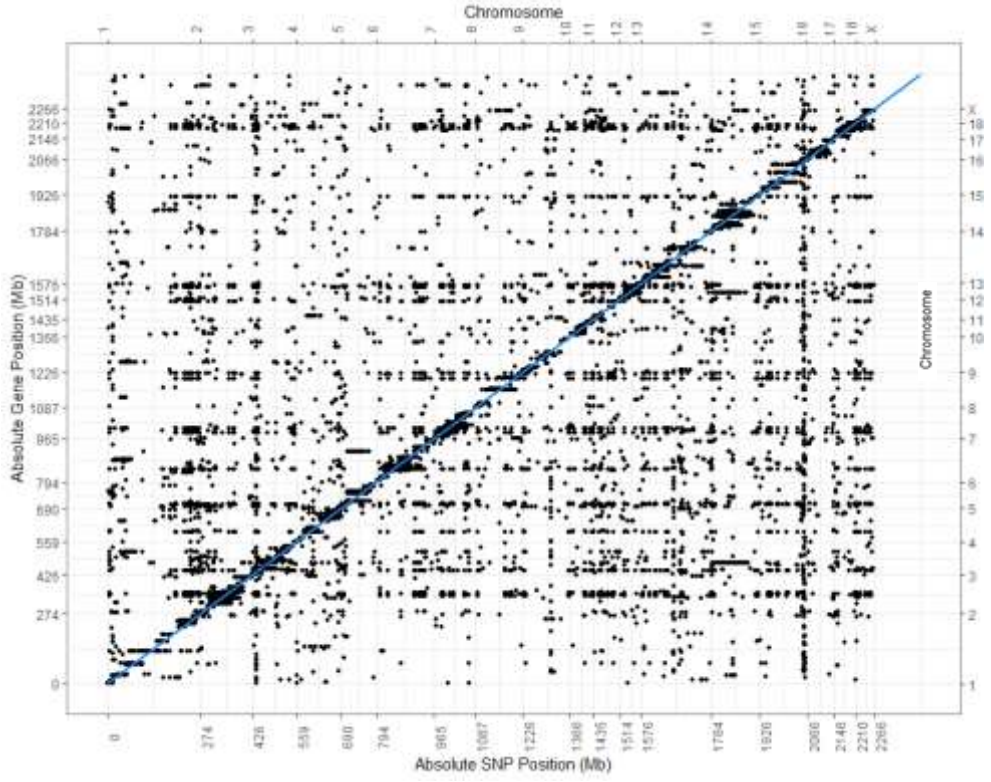


Figure 2. Scatter plot of all characterized eQTL. Each dot represents an eSNP-gene pair, with the vertical direction linking to the eSNP and the horizontal direction linking to the gene. The blue line represents the *cis*-acting eSNPs.

Functional analysis of the eQTL-associated genes

To understand biological processes that could be affected by significantly associated genes in the eGWAS studies, a total of 854 genes were analysed with ShinyGO software. A total of 76 clusters of overrepresented GO biological processes were identified, being the top three related to metabolic processes: small molecule metabolic process, oxoacid metabolic process and carboxylic acid metabolic process. All the GO biological terms and genes are described in Supplementary Table S1.

Selection and functional analysis of lipid and FA metabolism genes

Out of the 2,678 eQTLs associated with the 854 genes significantly associated in the eGWAS study, we filtered the genes based on their ontology related to lipid and FA metabolism pathways. A total of 275 eQTLs identified in 101 candidate genes were selected for further analysis (Supplementary Table S2). Among these, 78 eQTLs located in 75 genes were identified in *cis*-regions, while 197 eQTLs located in 71 genes were found in *trans*-regions. In addition, we observed 30 genes with only *cis*-regulatory regions, 26 genes with *trans*-regions, and 45 genes with both *cis*- and *trans*-eQTLs.

In addition, we performed a functional analysis with ShinyGO software for the 101 candidate genes for lipid and FA metabolism. These genes were divided into 10 different sub-processes based on their function (Supplementary Table S3). We want to highlight six genes (*ACOX3, BDH2, HACL1, PEX7, PEX13* and *SLC25A17*) involved in at least seven lipid metabolism processes (FA metabolism, carboxylic acid metabolism, oxoacid metabolic, lipid oxidation, FA oxidation, FA catabolism and FA β-oxidation). All the sub-processes for the 101 lipid metabolism genes are described in Supplementary Table S3.

Gene expression and fatty acid composition correlations

In order to identify co-expression patterns in the lipid-related genes and muscle FA composition, a co-expression correlation using the PCIT algorithm (Watson-Haigh et al., 2009) was performed. The analysis included the muscle gene expression data of the 75-lipid metabolism candidate genes containing 78 *cis*-eQTLs and the relative abundance of 14 different FAs present in *longissimus dorsi* muscle in the 129 BC1_DU pigs.

On the other hand, a second correlation analysis was performed between the gene expression data of 71 candidate genes involved in lipid metabolism containing 197 *trans*-eQTLs and the relative abundance of 14 FAs present in the *longissimus dorsi* muscle of the 129 animals in the BC1_DU population. A network graph was constructed using the Cytoscape software (Shannon et al., 2003) (Figure 3 and 4).

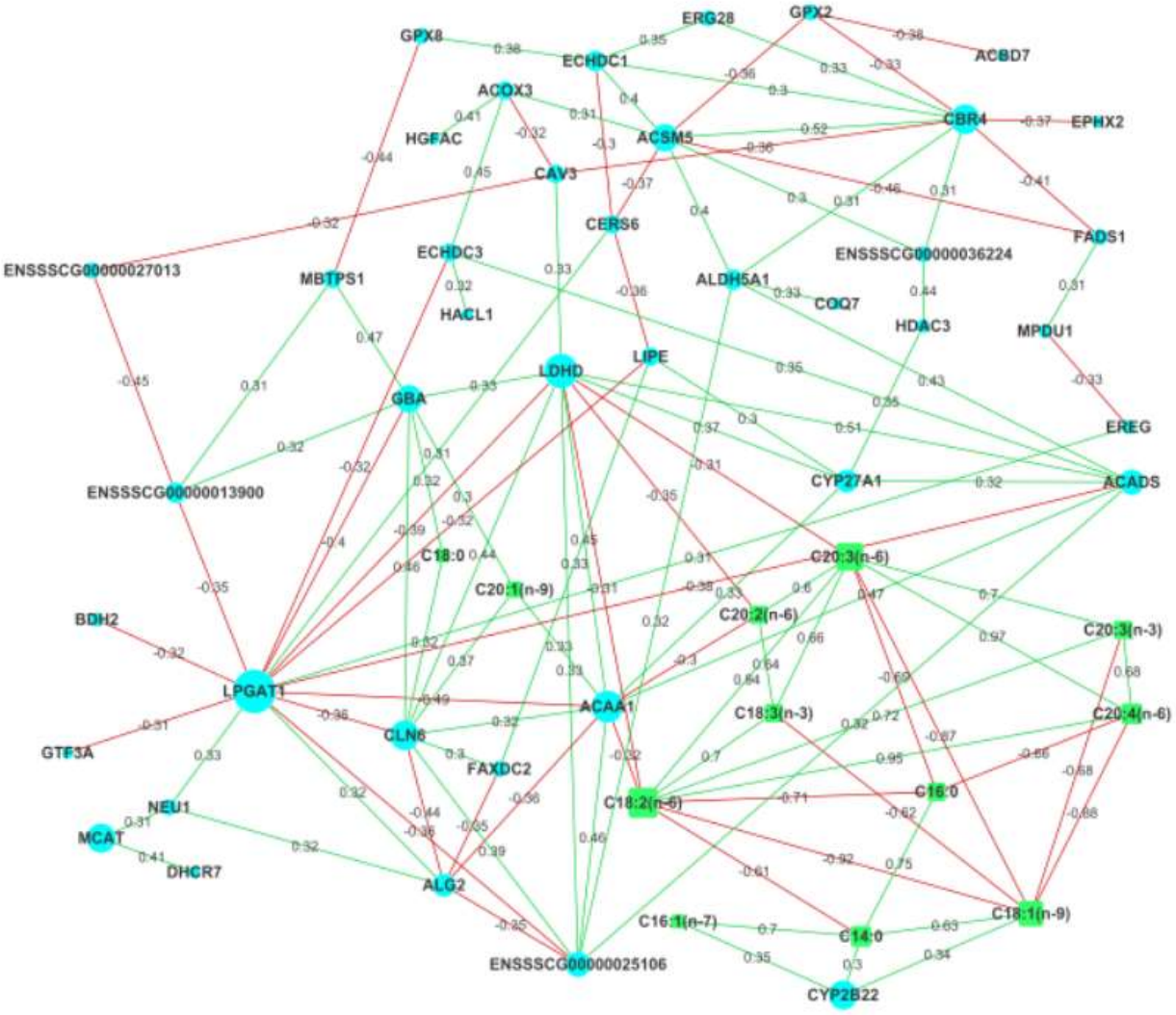


Figure 3: Gene co-expression network in 129 animals using the PCIT algorithm (Watson-Haigh et al., 2009). After filtering by significant r ≥ |0.3| for genes and r ≥ |0.6| for FAs, 39 of the 75 *cis*-lipid initial genes and 12 different FAs are shown in this network. The node size represents the number of connections with other nodes. Green and red lines indicate positive and negative correlations, respectively.

Notably, the genes *LPGAT3*, *ACAA1*, *LDHD*, and *ACSM5* exhibited the highest number of correlated connections with other genes. Regarding the analysed FAs, 8 out of 14 FAs displayed significant correlations with five genes (*ACAA1, CLN, CYP2B22, GBA* and *LDHD*). *ACAA1* gene expression exhibited negative correlations with the linoleic (C18:2(n-6)) (r = –0.32) and eicosadienoic (C20:2(n-6)) (r = –0.3) acids and positive correlations with the eicosanoid (C20:1(n-9)) (r = 0.33) acid. *CLN6* gene expression presented positive correlations with stearic acid (C18:0) (r = 0.32) and C20:1(n-9)) (r = 0.32). *CYP2B22* gene expression showed positive correlations with palmitoleic (C16:1(n-7) (r = 0.35), oleic (C18:1(n-9) (r = 0.34) and myristic (C14:0) (r = 0.3) acids. Moreover, *GBA* gene expression exhibited positive correlations with C18:0 (r = 0.32) and C20:1(n-9) (r = 0.3). Conversely, *LDHD* gene expression showed negative correlations with the relative amount of C20:1(n-9) (r = –0.31), C20:2(n-6) (r = –0.35) and C20:3(n-6) (r = –0.31). Finally, the strongest correlations within the entire network were observed between the genes *CBR4* and *ACSM5*, with a correlation value of 0.52 and between the C20:4(n-6) and C20:3(n-6), with a correlation value of 0.97.

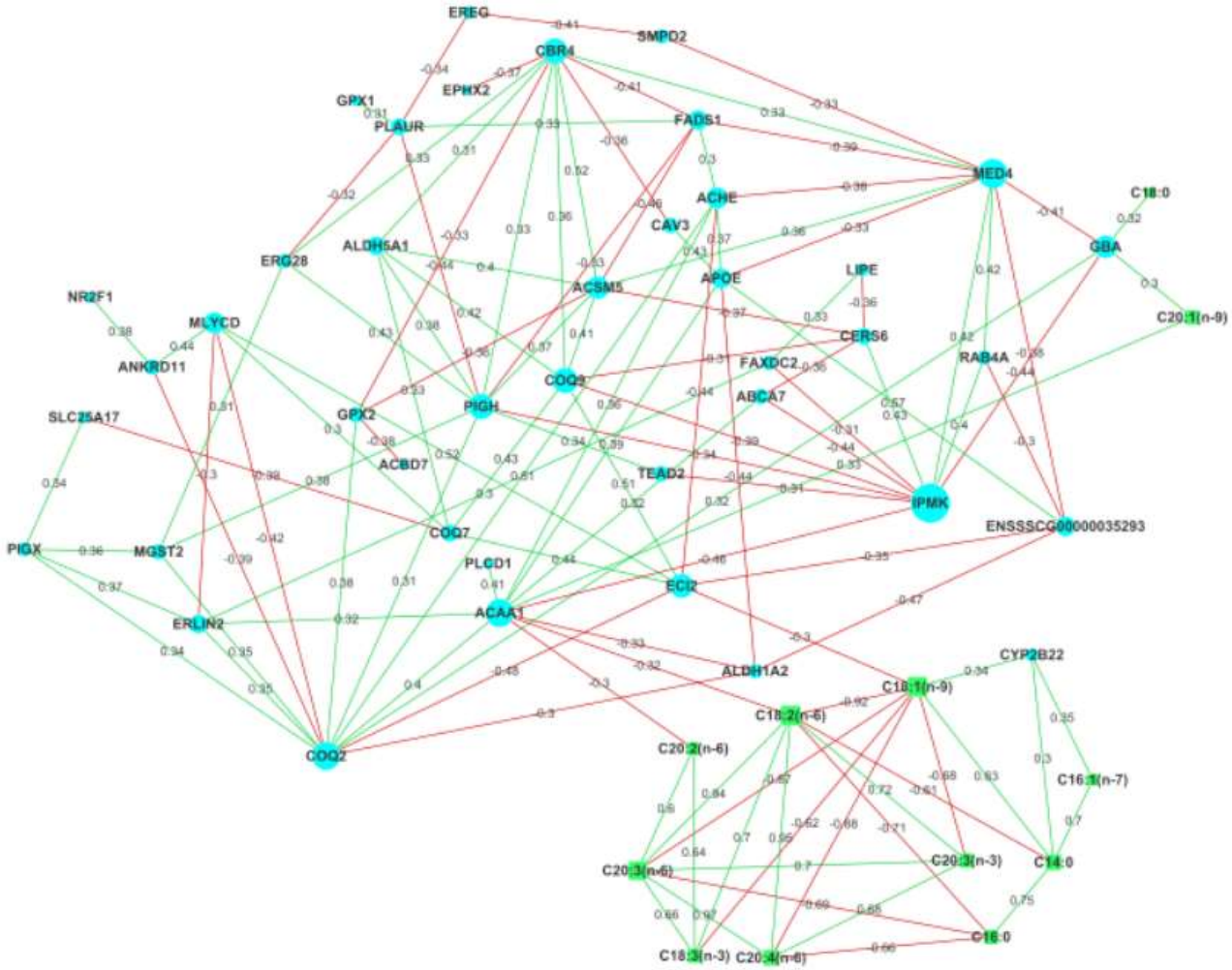


Figure 4: Gene co-expression network in 129 animals using the PCIT algorithm (Watson-Haigh et al., 2009). After filtering by significant r ≥ |0.3| for genes and r ≥ |0.6| for FAs, 40 of the 71 *trans*-lipid initial genes and 12 different FAs are shown in this network. The node size represents the number of connections with other nodes. Green and red lines indicate positive and negative correlations, respectively.

Regarding the genes associated to the *trans*-eQTLs, the *IPMK, ACAA1, COQ2* and *CBR4* genes showed the highest number of connections within the entire network. In relation to the FAs, a total of 7 out of 14 presented significant correlations with a set of four genes. *ACAA1* gene expressions values showed two negative correlations with C18:2(n-6) (r = –0.32) and C20:2(n-6) (r = –0.3) and positive correlations with C20:1(n-9) (r = 0.33).

Moreover, *CYP2B22* gene expression presented three positive correlations with C14:0 (r = 0.3), C16:1(n-7) (r = 0.35) and C18:1(n-9) (r = 0.34). On the other hand, *ECI* gene expression exhibited a negative correlation (r = –0.3) with the C18:1(n-9). Conversely presented positive correlations with the C18:0 (r = 0.32) and C20:1(n-9) levels. Lastly, the strongest correlations within the network were between the genes *CBR4* and *ACSM5* (r = 0.52) and between C20:4(n-6) and C20:3(n-6), exhibiting a correlation coefficient of 0.96.

Discussion

eQTL analysis

A *cis*-eQTL region, which modulates the expression of the analyzed gene in the eGWAS, is characterized by its proximity to the analyzed gene. We observed that 23.16% of the detected eQTLs (620) were located in *cis*-regions, associated with a total of 598 genes. These findings imply the presence of genetic variants regulating its expression either within the same gene or in its proximal genomic region. On the other hand, *trans*-eQTLs are associated with the expression of distant genes and frequently are located on different chromosomes (Cheung & Spielman, 2009). *Trans*-acting effects are often weaker than *cis* effects, hence *trans*-eQTLs are indeed more difficult to detect than *cis*-eQTLs. However, we observed that 76.84% of the eQTLs were located in *trans*-regions and were associated with the expression of 604 genes. It should be noted that the classification of *cis*- and *trans*-eQTL depends on the chosen window *cis*-regions (Ponsuksili et al., 2010), defined as 0.5 Mb on either side of the associated gene in our study, which influences the number of regions categorized as *cis* or *trans*.

Furthermore, in 348 genes both *cis*- and *trans*-eQTLs were detected, suggesting that various factors are regulating gene expression.

Functional analysis of the eQTL-associated genes

In order to explore the pathways associated with the genes significantly associated in the eGWAS analysis, we performed a gene ontology analysis using ShinyGO software. A

total of 76 clusters of overrepresented gene ontology biological processes were identified, where the top three overrepresented pathways were related to metabolic processes, in accordance with the analyzed tissue, which is *longissimus dorsi*. Skeletal muscle is a metabolic tissue due to its role in energy production, glucose regulation, and FA utilization (Merz & Thurmond, 2021).

Out of the 76 biological processes identified in the functional analysis, seven were found to be associated with lipid and FA metabolism pathways, while eight were related to both positive and negative regulatory processes. Interestingly, the metabolic processes of oxoacids and carboxylic acid, which are among the top three identified metabolic processes in the functional analysis, are connected to important metabolic pathways. These processes are closely linked the Krebs cycle, lipid and FA metabolism, among others, demonstrating their importance in cellular activities and homeostasis (Smedley et al., 2015).

Candidate genes for lipid metabolism

This study focused on investigating the genetic underpinnings of lipid and FA metabolism pathways by employing an extensive approach. From the 854 genes that showed significant associations in the eGWAS study, a total of 101 genes with 275 eQTLs were linked to lipid and FA metabolism based on gene ontology annotations.

Within this subset, a comprehensive analysis identified 78 *cis*-eQTLs, affecting 75 genes, eight of which were categorized as novel genes. On the other hand, 197 eQTLs located in *trans*-regions regulate the expression of 71 genes, including nine novel genes. Furthermore, we compared both results to identify common genes. We identified a total of 30 genes with *cis*-eQTLs, 26 genes with *trans*-eQTLs, and 45 genes with both *cis*- and *trans*-eQTLs. In relation to the 45 common genes, the modulation of gene expression is influenced by genetic factors located in both proximal and distal regions relative to the target gene, suggesting a complex genetic regulation landscape involving several mechanisms and regulatory factors.

In addition, a detailed investigation of the 101 lipid-metabolism genes was performed using ShinyGO software. Through this analysis, these genes were divided into ten

distinct sub-processes, each aligning with a specific functional aspect. Particularly noteworthy were six genes (*ACOX3*, *BDH2*, *HACL1*, *PEX7*, *PEX13*, and *SLC25A17*) that exhibited involvement in seven lipid and FA metabolism pathways. Interestingly, the genes *ACOX3*, *BDH2* and *SLC25A17* have been identified as candidate genes related to meat quality traits. The Acyl-CoA oxidase 3 (*ACOX3*) gene is involved in peroxisomal lipid and FA metabolism (Stelzer et al., 2016). In a recent study published by Yu et al. (2023), it is proposed that the gene *ACOX3* plays a role in regulating IMF deposition in the skeletal muscle of Qinchuan beef cattle. Furthermore, Ropka-Molik et al. (2020) examined molecular mechanisms linked to fat accumulation in Large White and Pietrain pigs through transcriptome analysis of skeletal muscle, identifying the involvement of the *ACOX3* gene in fat deposition. Other investigations have demonstrated a clear correlation between *ACOX3* gene expression and the regulation of IMF in broilers (Liu et al., 2017).

Regarding the 3-Hydroxybutyrate dehydrogenase 2 (*BDH2*) gene, it functions differently from mitochondrial type-BDH1, as it participates in cytosolic ketone body utilization and contributes to auxiliary energy supply mechanisms during periods of starvation (Guo et al., 2006). In terms of lipid deposition, *BDH2* gene expression has been noted to exhibit a positive correlation with adiposity, contributing to the production of precursors essential for lipid and sterol synthesis (Bonnet et al., 2008; Drew et al., 2015). In addition, Wang et al. (2021) demonstrated significant downregulation in both mRNA and protein levels of *BDH2* within the high lipid deposition group in Nanyang black pigs, presenting it as a prospective candidate gene for meat quality traits.

The solute carrier family 25 member 17 (*SLC25A17*) gene encodes a peroxisomal transporter of coenzyme-A, FAD and NAD+ cofactors (Agrimi et al., 2012) and it could have a role in the α-oxidation of FAs (Van Veldhoven, 2010). Furthermore, in a study performed by González-Prendes et al. (2017), discovered both *cis*- and *trans*-eQTLs that regulate the expression of the lipid-metabolism gene *SLC25A17* in porcine skeletal muscle.

Gene expression and fatty acid composition correlations

In this study, we investigated the relationship between the genes containing *cis*-eQTLs and the FAs composition in the porcine *longissimus dorsi* muscle. The *ACAA1* gene expression appears to be linked to specific FAs (C20:1(n-9), C18:2(n-6) and C20:2(n-6)), indicating a potential role in FA metabolism and the processing or regulation of omega-6 PUFAs. The positive correlations displayed between the C18:0 and C20:1(n-9) and the *CLN6* gene expression, suggests that it is involved in lipid metabolic processes of this two FAs. On the other hand, the positive correlations between the *CYP2B22* gene expression and C16:1(n-7), C18:1(n-9), and C14:0 FA composition, may indicate a role of this gene in regulating these FAs. Interestingly, the *LDHD* gene expression showed a negative correlation with the relative levels of C20:1(n-9), C20:2(n-6), and C20:3(n-6), suggesting that it is involved in the processing or regulation of omega-6 and omega-9 PUFAs and its probable role in meat quality traits. Finally, the role of *LPGAT3*, *ACAA1*, *LDHD*, and *ACSM5* genes in this study is notable. These genes exhibit several connections with other genes and demonstrate associations with numerous FAs. These findings suggest that these genes could play a pivotal role in the metabolism of FAs, with potential implications for pork quality. Nevertheless, further investigations are needed to validate our findings and the mechanisms by which these genes influence FA composition. The positive and negative correlations between the genes and the FAs are shown in Figure 3.

Regarding the genes containing *trans*-eQTLs, the *IPMK*, *ACAA1*, *COQ2*, and *CBR4* genes displayed the highest number of connections within the entire network, indicating their role as central regulators. Concerning the FAs, 7 out of 14 FAs exhibit correlations with a specific set of four genes (*ACAA1, CYP2B22, ECI2* and *GBA*). Notably, *ACAA1* gene expression revealed dual negative correlations with C18:2(n-6) and C20:2(n-6), alongside a positive correlation with C20:1(n-9), suggesting an important role in FA regulation. Furthermore, *CYP2B22* gene expression displayed three positive correlations with C14:0, C16:1(n-7), and C18:1(n-9), potentially playing a significant role in the regulation of these FAs. In contrast, *ECI* gene expression showed a negative correlation with C18:1(n-9) and positive correlations with C18:0 and C20:1(n-9).

The aforementioned genes, which exhibit correlations with various FAs, may affect meat quality traits and further investigations are required to understand the regulation and function of these genes.

Conclusions

In the present work, we identified significant eQTLs associated with the expression of genes in muscle, including both *cis*- and *trans*-eQTLs, offering insights into the genetic control of gene expression. Among the 854 genes significantly associated in the eGWAS study, 101 genes were linked to lipid metabolism pathways. Furthermore, the co-expression analysis of genes related to lipid metabolism and FA composition highlighted the regulatory influence of specific genes (*ACAA1*, *CLN*, *CYP2B22*, *GBA*, and *LDHD*) in modulating FA levels and interacting with other genes involved in lipid metabolism. Our results increase the knowledge of the genetic basis of gene expression regulation in pig skeletal muscle.

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PAPER III

Deciphering allele-specific expression in muscle transcriptome of Duroc crossbreed pigs from RNA-Seq data

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Abstract

Analysis of gene expression is a promising method to increase our knowledge on candidate genes involved in economically relevant traits in livestock, such as meat quality. Allele-specific expression (ASE), referring to the differences observed between the expression of the two alleles of a *locus*, is a frequent occurrence in mammalian transcriptomes. The aim of this work was to study the porcine *longissimus dorsi* muscle transcriptome using the ASE analysis with RNA-Seq data to decipher relevant genetic variants in candidate genes for lipid metabolism and fatty acid composition. In our study, muscle RNA-Seq data from 129 pigs genotyped with the Axiom Porcine Genotyping Array 660K (*Affymetrix*) were analysed. We identified 13,113 SNP variants that were common between DNA chip genotyping positions and RNA-Seq data. After quality control, a total of 2,146 SNPs associated to 1,621 genes showed at least three samples with ASE. These SNPs were widespread along the chromosomes, being 35.6% located in coding regions and 24.6% in 3'UTR. Among these 1,621 genes, 52 have been detected to be involved in lipid and fatty acid metabolism pathways, suggesting their potential contribution to meat quality traits. Some of the potential regulatory variants described here were found to be associated with the expression patterns of genes that are related to meat quality traits in livestock. Consequently, these variants may contribute to our understanding of the genetic control underlying these phenotypes.

Introduction

In the last decade, NGS has been used in human, livestock and plant research (Gholami et al., 2014; L. Liu et al., 2018; Rochus et al., 2018; Wang et al., 2011). RNA-Seq, an NGS-derived method, is a powerful technique to study the transcriptome. Gene expression is the link between the genotype and the phenotype, and gene expression differences are one of the main causes of phenotypic variation among individuals. The RNA-Seq approach provides sequencing data for the detection of both transcript expression levels and single-nucleotide polymorphisms (SNPs). While a gene typically consists of two alleles within a diploid genome, regulatory factors can cause one allele to be preferentially expressed over the other, a phenomenon known as allele-specific expression (ASE) (Wu et al., 2015). By integrating genetic and expression data, ASE analyses provide detailed information on the relative expression levels of the two alleles of a gene from the same individual, allowing for the identification and quantification of each allele's contribution to gene expression (Pastinen, 2010). The procedure to detect ASE using RNA-Seq data includes aligning reads to a reference genome and counting the reads of each allele in the heterozygous variant *locus*. Is expected that the two alleles of a genein an individual, one from each parent, are expressed at similar levels, a phenomenon referred to as bi-allelic expression. Nonetheless, for a few genes, alleles can show variation in their expression due to genetic or epigenetic factors (Hasin-Brumshtein et al., 2014; Knight, 2004). The grade of expression varied from complete monoallelic expression (MAE) (Metsalu et al., 2014) to preferential overexpression of the allele inherited from on parent. Describing ASE for genes affecting complex traits of economic relevance in animal production may help to understand the molecular mechanisms underlying these traits and, hence can contribute to increasing the accuracy of animal breeding programs. There are many causes of ASE; however, one of the most common is the presence of polymorphisms at regulatory sites acting in *cis*, such as *cis*-expression quantitative trait *loci* (*cis*-eQTLs). These polymorphisms, for instance, may lie on target sites of transcription factors (TFs), compromising the affinity to its binding site, and leading to changes in the transcription rate for the allele (de Souza et al., 2020; Jaenisch & Bird, 2003). Additionally, the pattern of ASE can be parent-of-origin dependent, namely, genomic imprinting (Latham et al., 1995). Genomic

imprinting is considered a significant factor in determining the phenotypic effects of genetic selection, particularly with respect to economically important traits such as intramuscular fat (IMF) and lipid metabolism pathways (de Souza et al., 2020). Moreover, multiple studies with high throughput technologies for genome and transcriptome analyses were successfully employed to better understand ASE at the genome level (Gregg et al., 2010; Pinter et al., 2015) and several studies have employed RNA-Seq data for transcriptome analysis in pigs to enhance our comprehension of ASE and its association with candidate genes implicated with economically important traits (de Souza et al., 2020; Y. Liu et al., 2020; Stachowiak et al., 2018; Stachowiak & Flisikowski, 2019).

In this study, we conducted a genome-wide analysis of ASE in skeletal muscle samples from 129 backcross Duroc pigs (BC1_DU) with the aim to describe the variation among individuals and the presence of ASE patterns on genes associated with lipid metabolism and fatty acid (FA) composition. After detecting ASE, we performed *in silico* analysis to identify the main functions of the candidate genes.

Materials and methods

Pig population

In the present study, 129 pigs (59 females and 70 males) belonging to an experimental backcross (25% Iberian and 75% Duroc, BC1_DU) were used. Animal care and procedures were carried out following the Spanish Policy for Animal Protection RD1201/05 and the European Union Directive 86/609 about the protection of animals used in experimentation. All animals were maintained under the same intensive conditions and fed ad libitum with a cereal-based commercial diet on NOVA GENÈTICA S.A. experimental farm (Lleida, Spain). Detailed information of generation schemes, diet, growth, and housing condition of the three backcrosses is described in Martínez-Montes et al. (2018). Slaughtering procedures were conducted in a certified abattoir according to the institutional and national guidelines for the Good Experimental Practices and approved by the Ethical Committee of the Institution (IRTA – Institut de Recerca I Tecnologia Agroalimentàries). The *longissimus dorsi* muscle samples were collected,

snap-frozen in liquid nitrogen, and stored at -80ºC until further RNA isolation. Diaphragm samples were collected for DNA extraction.

DNA extraction, genotyping, and quality control (QC)

Genomic DNA was extracted from diaphragm tissue by the standard phenol-chloroform extraction method and quantified with a NanoDrop-2000 spectrophotometer (Thermo Scientific). The 129 animals were genotyped using the Axiom Porcine Genotyping Array 660K (*Affymetrix*) according to the manufacturer's protocol. Quality control (QC) was carried out with PLINK v1.90b4.3 software (Purcell et al., 2007) filtering out SNPs with minor allele frequency (MAF) < 5%, missing genotypes < 1% and Hardy-Weinberg equilibrium < $1x10^{-6}$. Missing genotypes were imputed with Beagle v5.1 software (Browning et al., 2018) by setting default parameters. Only the SNPs on the autosomes and the X chromosome were used. Finally, 405,194 SNPs passed all criteria and were kept for further analysis.

RNA isolation and sequencing

Total RNA extraction from *longissimus dorsi* tissue was performed with the Ribopure kit (Ambion), following the manufacturer's protocol. RNA integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies) and quantification using a NanoDrop-2000 spectrophotometer (Thermo Scientific). Only the RNAs with integrity above seven (RIN >7) were used for the analysis. Sequencing was performed at the CNAG institute (*Centro Nacional de Análisis Genómico, Barcelona, España*). Libraries for 129 samples were generated using the TruSeq Stranded mRNA kit (Illumina), following the manufacturer's recommendations, and sequenced on the Illumina HiSeq 3000/4000 instrument, generating an average of 44.2 million of 75 bp paired-end reads per sample.

Read mapping, annotation and variant calling

We ran MultiQC v1.14 (Ewels et al., 2016) for quality control. RNA-Seq reads were mapped by using the STAR v2.5.2a software (Dobin et al., 2013) with default parameters to the reference genome assembly *Sscrofa 11.1* and to the annotation database Ensembl Genes 105. Transcripts were assembled and quantified by HTSeq v2.0.3 (Anders et al., 2015). In order to prevent false positive ASE signals due to allelic mapping biases that could influence allele counts, Picard Toolkit 2019 v2.27.4 (Broad Institute) was used to mark the duplicates and to add unique read groups to each bam file. Finally, SAMtools Stats v1.9 (Danecek et al., 2021) was used to check the quality of the mapping results obtained.

Variant calling was carried out in the 129 animals using Freebayes software v1.3.2. Freebayes is a Bayesian genetic variant caller designed to find SNPs, Indels and multinucleotide polymorphisms (Garrison & Marth, 2012). Moreover, Freebayes uses short-read alignments for any number of individuals from a population and uses a reference genome to determine the most likely combination of genotypes at each position in the population (Garrison & Marth, 2012). The variant calling analysis identified a total of 3.49 million variants in the genome of 129 animals from the BC1_DU population. Finally, variant filtration and quality control were performed with SNPEff and SNPSift software v5.1 (Cingolani et al., 2012).

Allele-specific expression analysis

We carried out an overlapping between the variants obtained from DNA genotyping chip (*Affymetrix*) and the variants detected with Freebayes software in muscle RNA-Seq data. Therefore, we detected 13,113 SNPs that were common between them and were used for further analysis. In addition, we filtered out variants that did not pass the following criteria: genotype quality < 30 and SNP-*loci* with < 10 reads mapped. Additionally, SNP-*loci* with < 3 reads, or with < 1% of the total reads of the least frequent of the alleles were removed. Moreover, variants found in less than three animals were discarded, as well as Insertion/Deletion variants. Furthermore, an exact binomial test was used to assess the deviation from the expected 0.5 reference/alternate ratio. Finally, the False

Discovery Rate (FDR) filtering method of Benjamini and Hochberg (Benjamini & Hochberg, 1995) with the *p. adjust* function of R was applied in order to remove the false positives. Variants with *p.adjust* ≤ 0.05 were kept for further analysis, thus 2,146 SNP variants were considered allele-specific variants.

Variant and gene annotation

Functional predictions of the significant ASE-SNPs were carried out using Variant Effect Predictor (VEP) (McLaren et al., 2010) and the Ensembl Genes 108 Database. The candidate genes associated with ASE and the Gene Ontology (GO) terms related to biological processes and molecular functions were obtained using Biomart (Smedley et al., 2015) tool from the Ensembl project (www.ensembl.org; release 108) using the *Sscrofa 11.1* reference assembly.

Gene functional classification

The ShinyGO v0.77 program (Ge et al., 2020) was used to identify the main biological functions of the most important pathways of the genes associated with the ASE variants. Moreover, the STRING v11.5 (Jensen *et al*., 2009) program was used to carry out a functional enrichment analysis of genes found significantly associated in ASE analysis.

Lipid metabolism candidate genes

The Gene Ontology (GO) terms obtained using Biomart (Smedley, *et al*., 2015) tool from the Ensembl project (www.ensembl.org; release 108) were used to identify genes associated in ASE analysis related to lipid metabolism and fatty acid composition pathways.

Co-expression and functional analysis

Weighted gene expression networks were calculated using the PCIT algorithm (Watson-Haigh et al., 2009) which employed first-order partial correlation coefficients and an

information theory approach to detect primary gene and FA interactions. Only significant interactions between genes were considered for further analysis. Networks were represented with the Cytoscape v3.9.1 (Shannon et al., 2003) program. Furthermore, for this analysis, we used the relative quantification data of 14 different FAs in the *longissimus dorsi* muscle, as previously described by Crespo-Piazuelo et al. (2020).

Results

Filtered genotypes from *Affymetrix* SNP chip and muscle RNA-Seq SNPs from Freebayes were compared to identify 13,113 common variants. Among them, 8,092 SNPs passed the filtering for genotype quality and minimum number of reads and were used to perform the ASE analysis. It means that only 1.22 % of the 658,652 SNPs present in the genotyping chip from *Affymetrix* were tested for ASE analysis. Moreover, to identify ASE variants with reference/alternate alleles ratio different from 0.5, an exact binomial test was applied. Overall, 2,146 SNPs associated to 1,621 unique genes were expressed and overlapped with at least one heterozygous SNP in three individuals and were thus detectable as affected by ASE.

Distribution of ASE variants

The ASE variants were observed to be spread throughout the genome (Figure 1). The percentage of tested SNPs showing ASE per chromosome ranged from 9.2 % (SSC6) to 1.16 (SSCX). The variants that exhibit allelic imbalance biased towards the alternate allele account for 70.65%, whereas the remaining 29.35% are predominantly comprised of the reference allele.

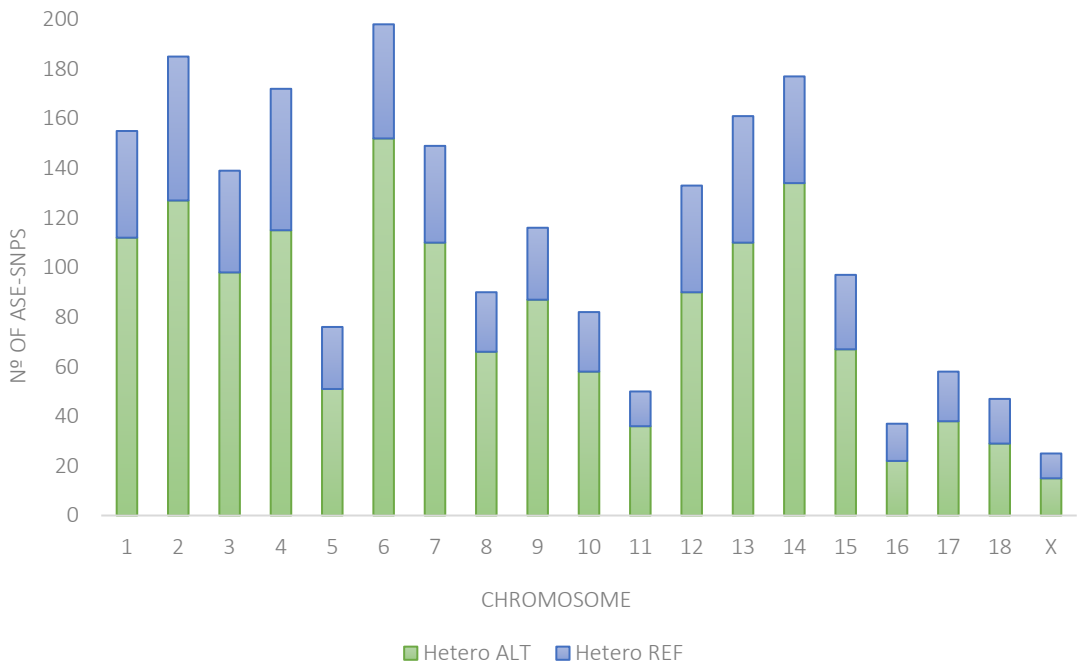

Figure 1: Distribution of ASE-SNPs along the chromosomes. The green group represents the ASE variants with a high proportion of alternative alleles (HeteroALT). The blue group represents the ASE variants with a high proportion of reference alleles (HeteroREF).

Annotation of SNPs and genes showing ASE

The effect of ASE-SNPs were predicted using VEP from Ensembl tools. We observed that a higher proportion of ASE-SNPs consisted of synonymous (28.8%), 3'UTR (24.6%), or Intron (17.1%) variants. The less-represented SNP positions were in intergenic regions (1.6%) and non-coding transcripts or within splicing regions (less than 1.3%) (Figure 2). A 6.8% of ASE-SNPs are missense, thus producing an amino-acid change in the corresponding protein. The number of ASE variants identified in each gene varied between 1 and 13.

The number of animals showing ASE for each ASE-SNP varied between 3 and 96. In 860 SNPs (40.01 %) ASE was identified in more than 50 individuals, while only 26 SNPs (1.21 %) showed ASE in 3 individuals.

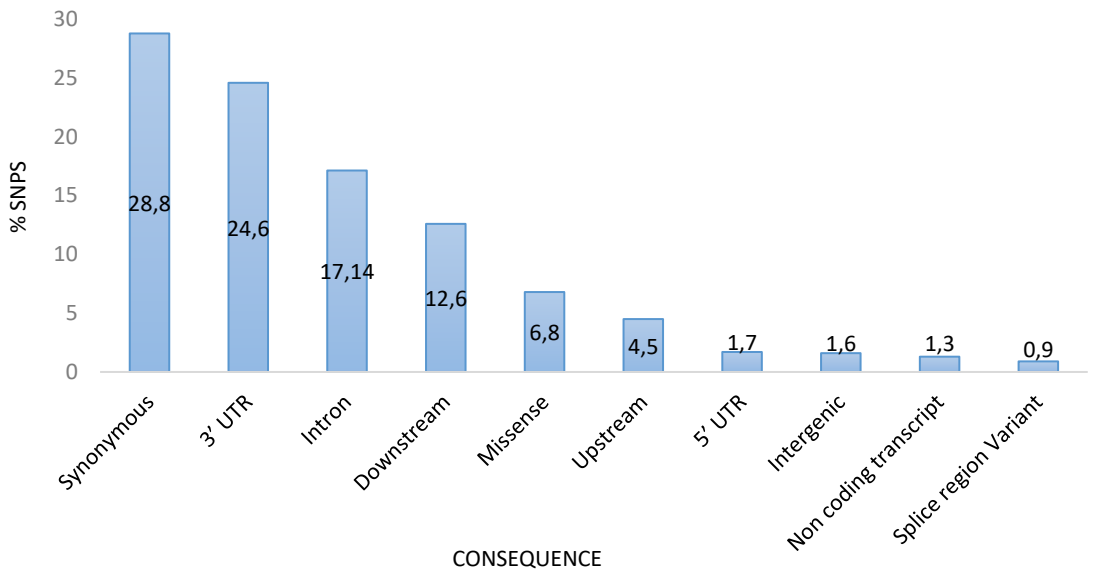

Figure 2: Distribution of ASE-SNPs according to their annotation.

Among the 1621 genes affected by 2146 ASE-SNPs, a total of 337 genes were identified to be affected by more than one ASE-SNP. Out of these 337 genes, 243 exhibited 2 ASE-SNPs, 65 showed 3 ASE-SNPs, 19 displayed 4 ASE-SNPs, and 9 genes were affected by 5 ASE-SNPs. Additionally, two genes showed 6 ASE-SNPs, while the genes *MYPN*, *NEXN*, and *NEB* were found to be affected by 7, 9, and 13 ASE-SNPs, respectively.

For the gene *MYPN*, located on chromosome 14, the SNPs rs321819726 and rs327360999 were found in a total of 78 and 77 animals, respectively. In both cases, all animals exhibited allelic imbalance (100%). Interestingly, both variants were located in the downstream region of the *MYPN* gene. On the other hand, for the gene *NEXN*, located on chromosome 6, the SNP rs81217583 was found in a total of 20 animals, of which 19 showed allelic imbalance (95%). This polymorphism is located in the 5' UTR region of the *NEXN* gene. Finally, for the *NEB* gene, located on chromosome 15, the SNP rs323475128 was identified in a total of 68 animals, all of which displayed allelic imbalance at this position (100%). This SNP is situated in the protein-coding region of the *NEB* gene, causing a synonymous mutation.

Functional analysis of ASE lipid metabolism genes

To understand biological processes underpinning production traits that could be affected by genes showing ASE, a selection of 1,621 genes showing ASE in at least three animals were analysed with ShinyGO software, which recognized 1,537 genes based on its pig gene database. Twenty clusters of overrepresented GO biological processes were identified, being the top three related to muscle biology: muscle system process, actin cytoskeleton organization, and actin filament process. All the GO biological terms and genes are described in Supplementary Table S1.

In addition, we conducted a gene ontology filtering aiming to select those genes affected by ASE that are associated with lipid and fatty acid metabolism. To achieve this, among the 2,146 ASE-SNPs, 69 SNPs (supplementary Table S2) were located in 52 potential lipid metabolism regulatory genes according to gene ontology descriptions. These genes can be divided into 8 different sub-processes based on their function (Table 1). We want to highlight eight genes (*ABCD3*, *ACAD11*, *ACADM7*, *CPT2*, *ECHS1*, *ETFA*, and *IRS*) involved in at least seven lipid metabolism pathways.

Table 1: Different sub-processes for the 52 lipid metabolism regulatory genes. The FDR column indicates the FDR (B&H) correction (Benjamini & Hochberg, 1995) of the enrichment analysis.

Pathway	FDR	Nº genes	Genes
Fatty acid metabolic process	$5{,}76\times10^{-27}$	21	*PPARA MAPK14 ETFA WDTC1 ACADM CPT2 QKI CRAT GPAT4 LPIN1 CBR4 ACAD11 UCP3 PDK4 ACSL1 PRKAG2 ACAD9 IRS1 PRXL2B ABCD3 ECHS1*
Oxoacid metabolic process	$1{,}31\times10^{-19}$	22	*PPARA MAPK14 ETFA WDTC1 ACADM CPT2 QKI CRAT GPAT4 LPIN1 CBR4 ACAD11 UCP3 PDK4 ACSL1 PRKAG3 PRKAG2 ACAD9 IRS1 PRXL2B ABCD3 ECHS1*
Lipid modification	$4{,}90\times10^{-18}$	14	*PPARA MAPK14 ETFA ACADM CPT2 CRAT EPHX2 AGT CYP2E1 ACAD11 PDK4 IRS1 ABCD3 ECHS1*
Fatty acid oxidation	$2{,}92\times10^{-16}$	11	*PPARA MAPK14 ETFA ACADM CPT2 CRAT ACAD11 PDK4 IRS1 ABCD3 ECHS1*
Lipid biosynthetic process	$1{,}47\times10^{-13}$	16	*PPARA MTOR WDTC1 QKI GPAT4 LPIN1 CBR4 ABHD5 PNPLA2 SLC27A1 PDK4 ACSL1 DEGS1 FDFT1 PRXL2B ABCD3*
Fatty acid catabolic process	$1{,}24\times10^{-12}$	9	*ETFA ACADM CPT2 CRAT LPIN1 ACAD11 IRS1 ABCD3 ECHS1*
Lipid catabolic process	$2{,}50\times10^{-12}$	12	*ETFA LIPE ACADM CPT2 CRAT LPIN1 ABHD5 ACAD11 PNPLA2 IRS1 ABCD3 ECHS1*
Fatty acid β-oxidation	$8{,}50\times10^{-12}$	8	*ETFA ACADM CPT2 CRAT ACAD11 IRS1 ABCD3 ECHS1*

Comparison of ASE-SNP location with QTL and eQTL studies

We compared our ASE-SNPs with a recently published study that used 189 Duroc x Luchuan to identify candidate genes for meat quality traits through ASE analysis (Liu et al., 2020). A total of 138 SNPs were common between both studies (Supplementary Table S3), which represented 6.33% of all ASE identified here. Furthermore, we observed 181 common genes, with at least one SNP showing ASE between both studies. Twelve of these were identified as novel genes.

In addition, we verified whether these ASE-SNP-containing genes were associated with meat quality traits in previous studies in the same population by GWAS (Crespo-Piazuelo

et al., 2020). We identified 19 ASE genes located in Quantitative Trait *Loci* (QTL) regions associated with FA composition in muscle: the amount of arachidic acid (C20:0) and eicosatrienoic acid (C20:3n-3) and the ratio of oleic/stearic acids (C18:1n-9/C18:0). Supplementary Table S4 describes all ASE genes associated with these traits.

Gene expression and fatty acids composition correlations

In order to identify co-expression patterns between we use the gene expression of the 52 genes affected by ASE-SNPs muscle FA compositions, a co-expression network using the PCIT algorithm (Watson-Haigh et al., 2009) was performed. The expression data of those 52 genes in muscle and the relative abundance of 14 different FAs present in *longissimus dorsi* muscle in the 129 BC1_DU pigs were analysed. Figure 3 shows the main relationships between genes related to lipid metabolism and the aforementioned FAs.

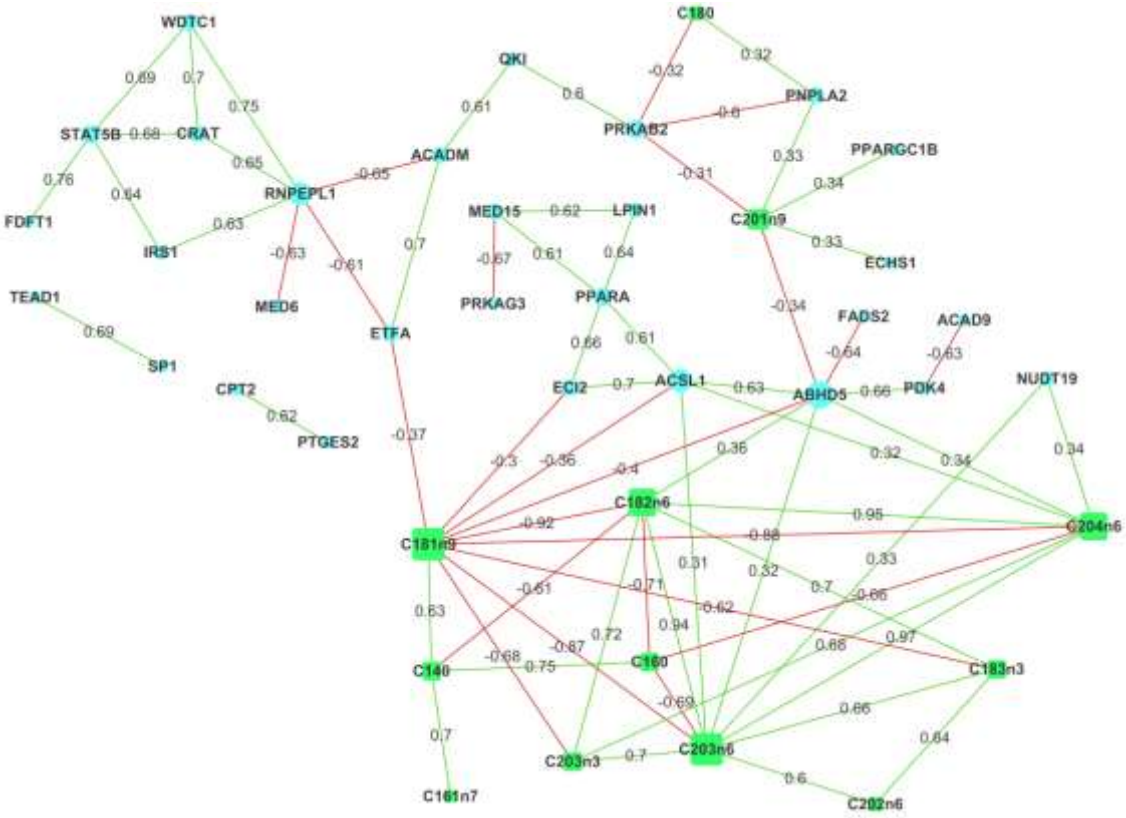

Figure 3: Gene co-expression network in 129 animals using the PCIT algorithm (Watson-Haigh et al., 2009). After filtering by significant r ≥ |0.6| for genes and r ≥ |0.3| for FAs,

29 of the 52 initial genes and 12 different FAs are shown in this network. The node size represents the number of connections with other nodes. Green and red lines indicate positive and negative correlations, respectively.

Notably, the genes *ABHD5*, *ACSL1*, and *RNPEPL1* exhibited the highest number of correlated connections with other genes and FAs. Regarding the FAs, 6 out of 12 displayed significant correlations with genes. Specifically, oleic acid (C18:1n-9) exhibited negative correlations with four genes (*ABHD5, ACSL1, ECI2* and *ETFA*) and five FAs (linoleic (C18:2n-6), α-linolenic (C18:3n-3), eicosatrienoic (C20:3n-3), dihomo-ɣ-linolenic (C20:3n-6) and arachidonic (C20:4n-6) acids). On the other hand, this FA presented a positive correlation with myristic acid (C14:0). Furthermore, C20:3n-6 exhibited positive correlations with three genes (*ACSL1, ABHD5* and *NUDT19*) and with five FAs (C18:3n-3, C18:2n-6, eicosadienoic (C20:2n-6), C20:3n-3 and C20:4n-6). Conversely, this FA presented negative correlations with two FAs (palmitic acid (C16:0) and C18:1n-9).

Interestingly, the strongest positive correlations within the entire network were observed between C20:4n-6 and C20:3n-6 FAs, with a correlation value of 0.97. For genes, the highest positive correlation was found for *STAT5B* and *FDFT1* with a value of 0.75. On the other hand, the strongest negative correlation was observed between C18:2n-6 and C18:1n-9 FAs, with a correlation value of –0.92. Similarly, the correlation between the genes *PRKAG3* and *MED15* was –0.67.

Top ASE-SNPs in lipid metabolism genes

From the 69 ASE-SNPs located in the 52 lipid metabolism genes, we selected those variants that exhibited a higher proportion of allele-specific expression. The top 10 ASE-SNPs are summarized in Table 2.

Table 2: Top 10 ASE-SNPs showing a higher proportion of ASE.

Gene Symbol	SNP	ASE Individuals ($p \leq 0,05$)	Total tested	ASE proportion (%)	Gene Location
ACADM	rs81211675	74	74	100,00	Synonymous
ECHS1	rs80915078	21	21	100,00	Synonymous
UCP3	rs326806805	18	18	100,00	Downstream
UCP3	rs321574540	3	3	100,00	Downstream
LPIN1	rs345575817	64	65	98,46	3' UTR
PRXL2B	rs322383150	64	65	98,46	3' UTR
FDFT1	rs81215802	57	58	98,28	Synonymous
PNPLA2	rs339524839	58	60	96,67	3' UTR
ACSL1	rs55618935	20	21	95,24	3' UTR
ETFA	rs80813406	30	33	90,91	Synonymous

Discussion

ASE-SNP location and effect prediction

After performing functional predictions using the VEP (McLaren et al., 2016) from the Ensembl Genes 108 Database, we observed that 35.6% of the variants were located in the protein-coding regions, some of which caused non-synonymous mutations that may result in amino acid change and potential alteration of protein function. Furthermore, we also observed that 26.2% of the ASE-SNPs were located in 3' and 5' UTR regions, with a higher prevalence in 3' UTR regions.

Furthermore, this study analyzed those genes that were affected by more than one ASE-SNP. Notably, the genes *MYPN*, *NEXN*, and *NEB* were significantly influenced by several ASE-SNPs. In the specific case of gene Myopalladin (*MYPN*) gene, we found seven ASE-SNPs, two of them (rs321819726 and rs327360999) in a high number of animals (78). Intriguingly, both SNPs caused an allelic imbalance in all animals. These SNPs, positioned downstream of the gene, could influence post-transcriptional regulation. The *MYPN* gene is involved in integrin-mediated cell adhesion, as well as actin binding and cytoskeletal protein binding (Stelzer et al., 2016).

On the other hand, Nexilin F-Actin Binding Protein (*NEXN*) gene showed a total of nine ASE-SNPs, with rs81217583 being the most prevalent SNP affecting a subset of 20 animals, with the majority of these animals exhibiting allelic imbalance (95 %). This SNP was located in the 5' UTR region of the *NEXN* gene, potentially affecting the transcriptional regulation. The related pathways related to *NEXN* gene include actin filament binding and calmodulin-dependent protein kinase activity (Stelzer et al., 2016).

Finally, the Nebulin (*NEB*) gene was influenced by 13 ASE-SNPs, including the SNP rs323475128, which is located in a protein-coding region, causing a synonymous mutation in 68 animals, all displaying allelic imbalance (100 %). This gene is associated with specific pathways, including the striated muscle contraction pathway. Moreover, the ontology for this gene is linked to actin binding and the structural constituent of muscle (Stelzer et al., 2016).

The analysis of the top 3 genes with the highest number of ASE-SNPs is directly related to the structure and function of the muscle.

Functional analysis

In order to explore if the ASE may affect FA composition traits recorded in the BC1_DU pigs, we performed a gene ontology analysis using ShinyGO software. Twenty clusters of overrepresented biological processes were identified, being the top three related to muscle biology, in accordance to the RNA-Seq analyzed tissue, which is the *longissimus dorsi*. Furthermore, a gene ontology filtering was conducted to focus on genes affected by ASE that are associated with lipid and fatty acid metabolism. Among the 2,146 ASE-SNPs, 69 were identified in 52 potential lipid metabolism genes and were categorized into eight distinct sub-processes according to their functions. Notably, the genes *ABCD3*, *ACAD11*, *ACADM7*, *CPT2*, *ECHS1*, *ETFA*, and *IRS* were found to be involved in at least seven lipid metabolism pathways, suggesting their crucial roles in lipid metabolism processes.

Moreover, the comparison of our ASE-SNPs with the findings of a recently published study by Y. Liu et al. (2020) revealed 181 common genes between both studies, where 12 of them were identified as novel genes, suggesting that these genes may have

implications for production traits, but further investigations are needed to validate these results. Notably, our study corroborated the findings of the previous research, as several genes affected by ASE in both studies were previously reported by our group. These genes, including *ACSL1* (Valdés-Hernández et al., 2023), *ACSM5* (Criado-Mesas et al., 2020; Puig-Oliveras et al., 2016), *ATF3* (Criado-Mesas et al., 2020), *EGF* (Ballester et al., 2017), *FABP3* (Puig-Oliveras et al., 2016), *FADS2* (Crespo-Piazuelo et al., 2020; Revilla et al., 2018), *MFN2* (Martínez-Montes et al., 2018) and *RETSAT* (Martínez-Montes et al., 2017), appear to be associated with meat quality traits. The shared genes and SNPs offer promising targets for further investigations to better understand the molecular mechanisms influencing lipid metabolism pathways and their potential influence on fatty acid composition in pigs.

Furthermore, we extended our investigation to assess whether the genes containing ASE-SNPs were linked to meat quality traits in previous studies within the same population through GWAS (Crespo-Piazuelo et al., 2020). Notably, we identified 19 ASE genes situated in QTL regions that were associated with FA composition in muscle. Specifically, these QTL regions were linked to the amount of different and important FAs (C20:0, C20:3n-3, ratio C18:1n-9/C18:0) that may influence meat quality traits.

This exploration of GWAS data reinforces the relevance of the identified ASE genes in relation to FA composition in muscle. The presence of ASE-SNPs within these QTL regions suggests a molecular mechanisms determining the observed FA composition variation.

Gene expression and FA composition correlations

PNPLA2 and *PRKAB2* genes were found to exhibit significant correlations with the levels of C18:0 and C20:1n-9, suggesting their potential involvement in the regulation of these fatty acids.. *PNPLA2* gene expression has a positive correlation with the abundance of both FAs in muscle. On the other hand, the expression level of the *PRKAB2* gene exhibits an inverse relationship with the abundance of C18:0 and C20:1n-9 FAs. *PNPLA2* gene is related to glycerophospholipid biosynthesis and regulation of Insulin-like Growth Factor (IGF). On the other hand, *PRKAB2* gene is a regulatory subunit of the AMP-activated

protein kinase (AMPK). AMPK control the activity of key enzymes involved in regulating *de novo* biosynthesis of FA and cholesterol (Stelzer et al., 2016). Furthermore, the abundance of C20:1n-9 shows a positive correlation with the expression of *PPARGC1B* and *ECHS1* genes, while exhibiting a negative correlation with the expression of the *ABHD5* gene. *PPARGC1B* gene is related to fat oxidation and non-oxidative glucose metabolism. Moreover, *ECHS1* gene is associated to mitochondrial FA β-oxidation and to leucine, isoleucine and valine metabolism. On the other hand, *ABDH5* gene is related to triglyceride metabolism pathway (Stelzer et al., 2016). The *NUDT19* gene expression has shown a positive correlation with C20:4n-6 and C20:3n-6. However, the allelic imbalance was observed in only 9 out of 34 heterozygous individuals at the SNP *locus* within the gene, suggesting that it is not the cause of the observed correlation. *NUDT19* gene participates in the peroxisomal lipid metabolism pathway mediating the hydrolysis of a wide range of CoA esters (Stelzer et al., 2016). Additionally, the expression of *ACSL1* gene has been found to be correlated with the levels of C20:4n-6 and C20:3n-6 (positively correlated) and C18:1n-9 (negatively correlated), suggesting its role in modulating the abundance of these FAs. Moreover, *ACSL1* expression has also been positively correlated with the expression of three genes: *ABHD5, ECI2* and *PPARA*. In our study, we found an SNP (rs55618935) located in the 3' UTR region of *ACSL1* gene that exhibited ASE in 20 out of 21 heterozygous individuals. This finding suggests a direct influence of the SNP on allelic imbalance at the *locus*. *ACSL1* encodes for an isozyme of the long-chain-fatty-acid-coenzyme A ligase family and is related to long-chain fatty acid-CoA ligase activity (Stelzer et al., 2016). Concerning to *PPARA* gene expression, it displays significant correlations, ranging from 0.61 to 0.66, with several genes, indicating its role as central regulating the expression of lipid metabolism genes. *PPARA* gene is associated with regulation of the gene expression and to the signalling receptor activity pathway (Stelzer et al., 2016). Another interesting gene is the *ETFA* gene, which expression has a negative correlation with C18:1n-9 content. Additionally, *ETFA* and *ACADM* gene expression are positively correlated, while *EFTA* and *RNPEPL1* gene expressions are negatively correlated. *ETFA* gene participates in catalyzing the initial step of FA β-oxidation (Stelzer et al., 2016). Therefore, *ETFA* gene is associated with electron transport in mitochondrial β-oxidation, which is involved in the metabolism of FAs found in meat, such as C18:1n-9. Its potential connection with FAs and their impact

on lipid metabolism is under investigation. On the other hand, *ACADM* gene is associated to acyl CoA dehydrogenase activity and catalyzes the first step of the mitochondrial FA β-oxidation (Stelzer et al., 2016).

Top ASE-SNPs in lipid metabolism genes

Among these 52 ASE-genes associated to 69 ASE-SNPs, we selected the top 10 ASE-SNPs located in *ACADM*, *ECHS1, UCP3, LPIN1, PRXL2B, FDFT1, PNPLA2, ACSL1* and *ETFA* as the most interesting based on the higher proportion of allele-specific expression.

Regarding the Acyl-CoA Dehydrogenase Medium Chain (*ACADM)* gene, it catalyses the first step of mitochondrial FA β-oxidation, allowing the production of energy from fats (Wipt & George, 2008). Bruun et al. (2013) reported a synonymous polymorphism in *ACADM* gene that have significant effects on pre-mRNA splicing and thus protein function and may affect the FA β-oxidation in humans. Interestingly, we identified a polymorphism in a total of 74 animals, and all of them exhibited allelic imbalance at this position. This SNP is located in a protein-coding region of this gene, resulting in a synonymous mutation. Hence, the genotyped SNP may be in strong linkage disequilibrium with the causal mutation producing ASE.

The Enoyl-CoA Hydratase, Short Chain 1 (*ECHS1*) gene encodes the protein responsible for carrying out the second step of β-oxidation of FAs in the mitochondria. Moreover, it plays a role specifically for short- and medium-chain fatty acids (Stelzer et al., 2016). In the study published by Chen et al. (2019), observed that the *ECHS1* gene was differentially expressed in finishing pigs, thereby improving meat quality traits. Additionally, Peng et al. (2016) demonstrated that the suppression of the *ECHS1* gene led to a decrease in fat deposition in the liver of broiler chickens. Notably, we identified a polymorphism in a total of 21 animals, and all of them exhibited allelic imbalance at this position. This genetic variant is positioned within a protein-coding region of the gene, causing a synonymous mutation. Therefore, the genotyped SNP could be in significant linkage disequilibrium with the causal mutation responsible of ASE.

The Uncoupling Protein 3 (*UCP3*) gene is implicated in pathways including β-oxidation of FAs and it has been involved in chemical reactions and pathways such as FA

metabolism process and adaptative thermogenesis (Han et al., 2012; Lin et al., 2017). This gene encodes a proton transport carrier that is distributed in the inner mitochondrial membrane, leading to the uncoupling of oxidation and ADP phosphorylation processes by reducing the H+ electrochemical gradient on both sides of the membrane. Consequently, this mechanism plays a crucial role in energy and heat production (Stelzer et al., 2016). In pigs, the 5' and 3' sequence control regions of the *UCP3* gene, along with the *AvaI* enzyme *loci*, have been significantly associated with fat metabolism (Knoll et al., 2008). In addition, a study conducted by Xing et al. (2021) found significant correlations between *UCP3* gene expression levels and fat deposition between two extreme groups of a Landrace pig cross. In our study, we identified two polymorphisms in a total of 21 and 3 animals, respectively. Notably, both SNPs exhibited allelic imbalance in all the animals at this position. . These SNPs, positioned downstream of the gene, could influence post-transcriptional regulation. The genotyped SNPs are likely to be in strong linkage disequilibrium with the causal mutation contributing to ASE.

The Lipin 1 (*LPIN1*) gene is an essential contributor to adipogenesis and additionally serves as a potent transcriptional co-activator of peroxisome-proliferator-activated receptors (PPARs), modulating lipid metabolism-related gene expression (Phan et al., 2004). The deficiency of *LPIN1* gene has been reported to prevent normal adipose tissue development and to reduce adipose tissue mass, while overexpression of this gene in either skeletal muscle or adipose tissue promotes adiposity in mice (He et al., 2009). Furthermore, a synonymous mutation in this gene was associated with the percentage of leaf fat and IMF in pigs (He et al., 2009). Additionally, Wang et al. (2011) demonstrated an upregulated expression of *LPIN1* in the *longissimus dorsi* (LD) muscle of Rongchang pigs compared to lean PIC pigs (PIC Swine Improvement Group, England, UK), and a higher expression of *LPIN1* expression in the LD muscle of Rongchang pigs displaying high IMF content relative to those exhibiting low IMF content. Another research in chickens established that miRNA-429 exerts suppressive effects on *LPIN1* via targeted binding, thereby disrupting the proper functioning of the *PPAR* signalling cascade (Chao et al., 2020). In previous studies of our group, we found that *LPIN1* gene expression in muscle is positively correlated with the ω6/ω3 ratio (Valdés-Hernández et al., 2023). Moreover, Criado-Mesas et al. (2020) detected three trans-eQTLs on SSC4, SSC7, and

SSC15, which influence the expression of *LPIN1* in the same population of this study (BC1_DU). Remarkably, we detected an SNP showing ASE, located in 3'UTR region, that was present in a total of 65 animals, with 64 of them (98.46%) displaying allelic imbalance. This result suggests that the genotyped SNP may exhibit strong linkage disequilibrium with the causal mutation responsible for ASE.

The Peroxiredoxin Like 2B (*PRXL2B*) gene has been involved in FA metabolism and prostaglandin biosynthesis (Stelzer et al., 2016). However, no studies in pigs and lipid metabolism pathways have been reported for this gene. We identified an SNP located in a 3'UTR region that exhibited ASE in 64 of 65 heterozygous animals (98.46%).

Regarding the Farnesyl-Diphosphate Farnesyl-transferase 1 (*FDFT1*), despite its association with cholesterol and lipid metabolism pathways (Stelzer et al., 2016), studies on this gene in pigs were not found. On the other hand, we observed a polymorphism located in a protein-coding region of the gene, resulting in a synonymous mutation associated with ASE in 57 of the 58 (98.3%) heterozygous animals. Thus, there is likely another causal mutation in strong linkage disequilibrium with the causal mutation responsible for ASE.

Another interesting candidate gene is the Patatin Like Phospholipase Domain Containing 2 (*PNPLA2*), which predominantly performs the first step in triglyceride hydrolysis (Stelzer et al., 2016). The *PNPLA2* gene is located in SSC2 and has been associated with a QTL region related to backfat thickness (Rattink et al., 2001). Moreover, a previous study performed in pigs have identified a polymorphism that was significantly associated with several economic traits such as subcutaneous fat thickness, visceral adipose tissue weight, lean meat percentage and loin eye traits (Dai et al., 2011). Furthermore, Dai et al. (2016) identified a total of four polymorphisms located in the promotor region of *PNPLA2* gene that were associated with growth and fat deposition traits in pigs, as well as Fu et al. (2020), who found a strong association between a high expression of *PNPLA2* gene and fat-related traits. Interestingly, this gene has been suggested as a candidate gene for a QTL detected in SSC2 with the abundance of C16:1n-9, C18:1n-9, and C18:2n-6, and four metabolic ratios, monounsaturated FAs (MUFA), polyunsaturated FAs (PUFA), MUFA/PUFA, and PUFA/SFA (saturated FAs) in a previous study conducted by Crespo-Piazuelo et al. (2020) in a 439 Iberian backcrossed pigs. Remarkably, we detected

a polymorphism located in the 3'UTR region in a total of 60 animals, of which 58 (96.67%) exhibited ASE. This result suggests that this SNP may be in strong linkage disequilibrium with the causal mutation contributing to ASE.

The Acyl-CoA Synthetase Long-Chain Family Member 1 (*ACSL1*) gene is involved in the long-chain FA import and signal transduction biological processes. Moreover, it plays a key role in the synthesis of long-chain acyl-CoA esters, FA degradation, and phospholipid remodelling (Widmann et al., 2011). Additionally, this gene has been associated with lipid metabolism and mitochondrial oxidation of FAs in pigs (X. Liu et al., 2015). Recently, Valdés-Hernández et al. (2023) reported an association between the *ACSL1* gene expression and the C18:2n-6/C18:3n-3 and ω6/ω3 ratios in the same population of this study (BC1_DU). Moreover, Zhao et al. (2023) suggested that the *ACSL1* gene plays a key role in affecting IMF deposition in Landrace cross populations, as well as Xu et al. (2018) found a differential gene expression of the *ACSL1* gene for IMF in Yorkshire pigs.

The Electron Transfer Flavoprotein Subunit Alpha (*ETFA*) gene is involved in catalyzing the first step of mitochondrial FA β-oxidation (Stelzer et al., 2016). Laforêt & Vianey-Saban (2010) established a connection between multiple acyl-CoA dehydrogenase deficiency and disorders in the *ETFA* gene expression. Unfortunately, no studies linking the *ETFA* gene to pig lipid metabolism have been reported. Nevertheless, further analysis are needed to investigate and determine if there is any relationship between this gene and lipid metabolism, as well as fatty acid composition, in pigs.

The investigation of allelic imbalance in genes associated with the regulation of porcine lipid metabolism and different production traits has not been extensively explored thus far. Only a limited number of studies have reported the correlation between ASE based on transcriptome analysis and lipid metabolism or meat quality traits. Our analysis revealed widespread ASE throughout the pig muscle transcriptome, similar to findings in previous studies in pigs (Liu et al., 2020; Stachowiak et al., 2018; Stachowiak & Flisikowski, 2019) and in other species such as mice (Crowley et al., 2015) and bovine (de Souza et al., 2020; Guillocheau et al., 2019).

Although other studies have used SNP calling from RNA-Seq data to detect ASE, which could potentially involve a larger number of studied SNPs, we opted to use the common

variants between the Axiom Porcine Genotyping Array 660K (*Affymetrix*) and the RNA-Seq data because this methodology ensures reduced errors in genotyping heterozygous individuals. For future studies, we believe that exploring epigenetic mechanisms, such as parental origin effects and DNA methylation, may provide additional insights into the regulation of ASE gene expression, which in turn affects economically significant traits. To gain a more comprehensive understanding of ASE patterns through pig transcriptome, it will be crucial to obtain supplementary data from various tissues and developmental stages. Additionally, in order to ascertain the presence of *cis*-regulatory elements in our findings, further investigation will be required through different approaches to mapping the causative *cis*-acting variants. These findings emphasize the importance of allelic expression patterns and underlying mechanisms, as several genes with crucial roles in diverse biological processes exhibited allelic imbalance.

Conclusions

We conducted an analysis of the global genetic regulation of gene expression in the *longissimus dorsi* muscle via ASE analysis. This research highlighted that allelic expression can affect genes associated with lipid metabolism and FA composition traits and may affect meat quality traits, but further research is needed to fully understand the underlying molecular mechanisms. Moreover, this study revealed the prevalence of ASE in several genes affected by multiple SNPs, with specific genes showing extensive variation in ASE patterns. In addition, the comparison of our results with the published study strengthens the evidence for the relevance of ASE analysis in identifying candidate genes linked to meat quality traits. In conclusion, the study of ASE could provide valuable insights into the genetic regulation of production traits in livestock. The identification of variants and genes associated with ASE sheds light on the molecular mechanisms influencing important production traits.

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GENERAL DISCUSSION

Chapter 4

Traditional and pure pig breeds are valuable reservoirs for meat production, as well as for their cultural, historical, and environmental significance. Nevertheless, modern intensive livestock production systems are predominantly dependent on highly productive global breeds, leading to concern among breeding companies due to the loss of genetic diversity (Hulsegge et al., 2019). To enhance growth, fatness, and meat quality traits, among other economically impactful characteristics, the development of improved strategies for genetic selection is crucial. Animal breeding emerged in the 18th century, involving a systematic selection of animals as progenitors to establish predetermined traits within populations (Kor & Waaij, 2014).

Meat quality, a complex trait shaped by consumer preferences, includes factors like food safety, animal welfare, FA composition, and sensory attributes (Webb & O'Neill, 2008; Wood et al., 1999). The genetic basis of meat quality traits is complex, influenced by several *loci* with small effects and a small number of genes with moderate impacts (Hayes & Goddard, 2001). Recent genetic researches have led to the characterization of several QTLs in pork complex traits, providing insights into their genetic architecture. The advent of high-throughput SNP genotyping arrays and NGS technologies has facilitated the discovery of causal genes and mutations linked to monogenic Mendelian traits, as well as the identification of genes associated with complex traits, although this remains a challenging task (Andersson & Georges, 2004). On the other hand, the integration of gene expression levels and epigenetic variations can improve predictive models for animal breeding, potentially enhancing overall model accuracy (MacKay et al., 2009). In recent years, the relationship between gene expression and the profile of FAs in muscle has shown increased attention. This attention is primarily attributed to the significant impact this relationship has on both meat quality parameters and human health considerations (Geiker et al., 2021; Huang & Ahn, 2019). Notably, muscle, being an important metabolically active tissue, serves as both an energy reservoir and a consumer of energy (Liu et al., 2015). Muscle is a tissue containing various components that significantly contribute to the overall quality and characteristics of meat (Listrat et al., 2016; Maltin et al., 2003), including proteins, fats, carbohydrates, vitamins, minerals, and water. Moreover, skeletal muscle assumes a key role in controlling lipid and glucose metabolism; it remains highly responsive to changes in the availability of glucose and

FAs (Morales et al., 2017). On the other hand, the lipid content and FA composition influence the tenderness, juiciness, and flavour of the meat (Maltin et al., 2003). Understanding and optimizing the composition of muscle is essential for producing meat products according to the consumer preferences and dietary requirements. Therefore, meat quality is a current topic of significant interest. Hence, the muscle FA composition is determined by a combination of dietary and genetic factors (Wood et al., 2008).

The identification of the causal genes within QTLs and their allelic variations remains challenging for complex traits. The success of QTL mapping depends on factors like recombination frequency and sample size (MacKay et al., 2009). Moreover, accurate gene mapping is essential for identifying candidate genes within QTLs and the causal polymorphisms of QTLs, which is crucial for understanding the molecular mechanisms underlying relevant phenotypic variations. Andersson et al. (1994), identified the firsts QTLs in domestic animals, including a QTL on SSC4 affecting fat deposition in pigs. Subsequently, several investigations with different breeds have been conducted to identify numerous QTLs distributed along the chromosomes and associated with important economic traits (Marklund et al., 1999).

In the IBMAP experimental population, several *loci* associated with growth, fatness, and meat quality traits have been identified through QTL mapping, GWAS approaches, microarrays, RNA-Seq, and systems genetics approaches, among other methodologies.

In previous studies in the IBMAP population, our group evaluated *FABP4* and *FABP5* genes as candidate genes for the QTL for growth and fatness (Estellé et al., 2006; Mercadé et al., 2006). Additionally, a GWAS study in the IBMAP BC1_LD identified eight regions associated with IMF composition (Ramayo-Caldas et al., 2012b). Furthermore, in a recent study performed by Crespo-Piazuelo et al. (2020), several QTLs related to FA composition and IMF were identified in 439 pigs having the Iberian breed in common. These QTLs were located in regions where *ELOVL6*, *ELOVL7*, *FADS2*, *FASN,* and *SCD* genes were mapped.

This PhD thesis aimed to identify genes and genetic variants regulating lipid metabolism and FA composition, and consequently influencing meat quality. Several molecular genetics and genomic technologies were utilized to reach this objective.

We conducted gene expression analysis of 45 lipid-related genes, previously identified in the IBMAP population, in pig muscle samples of animals from three experimental backcrosses. Furthermore, an allelic-specific expression analysis was conducted in the muscle transcriptome of the BC1_DU population to identify lipid-related genes exhibiting allelic imbalance. Finally, eGWAS using muscle RNA-Seq data of 129 animals from the BC1_DU population was performed with the aim of identifying eQTLs and exploring their relationship with lipid and FA metabolism pathways. In the next sections, the main results obtained are discussed.

4.1. Gene expression analysis in muscle of lipid-metabolism candidate genes by RT-qPCR

In eGWAS studies, the detection of significant associations between gene expression and genetic markers is possible due to linkage disequilibrium between the genotyped SNPs and the causal mutation (Teo et al., 2009). In addition, if the molecular variant is located close to the gene region of the transcript under investigation, the regulation is called *cis,* proximal or local eQTL, but if the polymorphisms associated with the variation in the transcript are elsewhere, it is called *trans* or distal eQTL (Rockman & Kruglyak, 2006). In relation to this, *cis*-eQTLs tend to have larger effects than *trans*-eQTLs. Moreover, some genomic regions are associated with the variation in the expression levels of many transcripts (eQTL hotspots); and the expression levels of many transcripts are usually highly correlated (Sieberts & Schadt, 2007). Moreover, eQTL identification can offer valuable insights into gene expression regulatory mechanisms, including gene network interactions. Therefore, accurate gene mapping and annotation are crucial for identifying candidate genes within eQTL regions.

In general, transcriptional regulation plays a key role in the regulation of genes associated with lipid metabolism, and unravelling the molecular mechanisms regulating their expression could enhance our comprehension of the genetic architecture of the FA composition in muscle (Hausman et al., 2009).

In the past few years, RNA-Seq analysis of adipose, muscle, and liver tissue transcriptomes in the BC1_LD population led to the identification of differentially expressed genes in animals with different FA composition (Corominas, et al., 2013a; Puig-Oliveras et al., 2014b, 2016; Ramayo-Caldas, et al., 2012a). In the functional analysis of the three tissues analyzed, the PPAR signalling pathway was identified among the overrepresented pathways, with observed differential expression of target genes for PPARs. These findings supported the significant role of gene expression variation and its genetic basis in influencing the genetic determinism of these traits.

RT-qPCR analysis was further used to analyse the gene expression levels in a large number of animals. Gene-specific eQTL analyses for various candidate genes were conducted within the IBMAP population, including *ACSL4*, *APOA2*, *ELOVL6*, *FABP4*, *FABP5*, and *IGF2* genes (Ballester et al., 2016; Ballester, et al., 2017a; Corominas et al., 2012; Corominas et al., 2013a; Puig-Oliveras et al., 2016; Revilla et al., 2018; Criado-Mesas et al., 2019, 2020).

Fluidigm microfluidic technology (Fluidigm; San Francisco, CA, USA) is an RT-qPCR-based method that uses integrated fluidic circuits (IFCs) comprising thousands of microfluidic valves and interconnected channels (Melin & Quake, 2007). By scaling down qPCR reactions from the typical 10-20 microliter range to a 10 nanoliter scale, Fluidigm microfluidic technology facilitates the execution of thousands of qPCR analyses in a single run, ensuring efficiency, high sensitivity and reproducibility (Melin & Quake, 2007; Spurgeon et al., 2008). Consequently, Fluidigm provides cost-effective and customizable arrays for gene expression profiling across a moderate number of animals. Furthermore, our group conducted similar investigations using the Fluidigm platform to assess mRNA expression levels of candidate genes involved in lipid metabolism in liver and adipose tissue of 111 and 115 BC1_LD animals, respectively (Ballester, et al., 2017; Revilla et al., 2018). Finally, Criado-Mesas et al. (2020) conducted a gene expression analysis of 45 candidate genes involved in lipid metabolism in skeletal muscle using data from Puig-Oliveras et al. (2016) for BC1_LD and new data from BC1_DU and BC1_PI populations.

In our study, the eGWAS was performed using a total of 38,488 SNPs mapped on the *Sscrofa* 11.1 assembly and the expression values of 45 additional candidate genes involved in lipid metabolism in porcine *longissimus dorsi* muscle in the animals that

Criado-Mesas et al. (2020) used. This study analysed the gene expression of candidate genes related to lipid metabolism that were previously identified in different works of our research group. In addition, a correlation analysis was performed including the FA composition of the same population, which enables the detection of co-expression patterns that enhance the identification of associations between candidate genes and FA composition.

In this study, we observed that *trans*-eQTLs (88.8%) were more prevalent than *cis*-eQTLs (11.2%) (Table 4.1). In previous gene expression studies conducted using the same technology for the analysis of different candidate genes in lipid metabolism, a similar pattern was observed regarding the proportion of *cis*- and *trans*-eQTLs in skeletal muscle. For example, Puig-Oliveras et al. (2016) identified a total of 19 eQTLs, of which three were located in *cis*-acting regions (15.8%) while 16 were in *trans*-acting regions (84.2%). Meanwhile, Criado-Mesas et al. (2020) found a total of 12 eQTLs, where two were located in *cis*-acting regions (16.7%) and 10 were in *trans*-acting regions (83.3%).

Comparing the results with other studies conducted within our group using the same technology but different tissues, the following outcomes were observed. Ballester, et al. (2017) analyzed candidate genes for lipid metabolism in the liver and identified a total of 7 eQTLs, with two located in *cis*-acting regions (21.4%) and five in *trans*-eQTL regions (78.6%). On the other hand, Revilla et al. (2018) conducted a similar analysis with different candidate genes using adipose tissue. In this case, they identified a total of 20 eQTLs, where three were located in *cis*-acting regions (10.5%) and 17 in *trans*-acting regions (89.5%).

Interestingly, in all the studies, the number of *cis*-eQTLs is consistently lower than that of *trans*-eQTLs. As we explained above, *cis*-eQTLs are defined as regions located near the regulated gene, while *trans*-eQTLs are usually situated farther away, which implies an extensive search area that leads to an increased likelihood of discovering significant associations. Furthermore, gene regulation is complex and many *trans*-acting factors may modulate gene expression. It is important to note that the prevalence of *cis* and *trans*-eQTLs can vary depending on factors such as tissue, population or specific genetic variants, which can be in linkage disequilibrium with the causal mutation (Kvamme et al., 2022).

Table 4.1. Summary of the articles published by our group using the Fluidigm platform, highlighting the number of chromosomal regions associated with gene expression phenotypes in different tissues and populations.

Reference	Puig-Oliveras *et* al. 2016	Ballester *et* al. 2017	Revilla *et* al. 2018	Criado-Mesas *et* al. 2020	Passols *et* al. In revision
Tissue	Muscle	Liver	Adipose tissue	Muscle	Muscle
Population	BC1_LD	BC1_LD	BC1_LD	BC1_DU, BC1_LD, BC1_PI	BC1_DU, BC1_LD, BC1_PI
Associated genes	*ACSM5, CROT, FABP3, FOS, HIF1AN, IGF2, MGLL, NCOA1, PIK3R1, PLA2G12A* and *PPARA*	*CROT, CYP2U1,DGAT2, EGF, FABP1, FABP5, PLA2G12A* and *PPARA*	*ACSM5, ELOVL5 FABP4, FADS2* and *SLC27A4*	*ACSM5, ACSS2, ATF3, DGAT2, FOS* and *IGF2*	*ANK2, APOE, ARNT, EGF, ELOVL6, FASN, GPAT3, NR1D2, PLIN1, PPAP2A, RORA, RXRA, UCP3*
Total genes with eQTLs	18	7	19	10	27
***Cis*-eQTLs**	3	2	3	2	3
***Trans*-eQTLs**	16	5	17	10	24

In the present work, the eGWAS identified 301 expression-SNPs located in 27 SSC regions and were associated with the expression of 18 candidate genes. The three *cis*-eQTLs identified were for the gene expression of *GPAT3, RXRA* and *UCP3*, none of which had been previously reported in the IBMAP population. Conversely, recent studies identified two polymorphisms located in the promoter region of *GPAT3* gene affecting the IMF content in Laiwu pigs (Ma et al., 2022) and growth traits in Duroc pigs (K. Wang

et al., 2017). On the other hand, a *cis*-eQTL was reported for the expression of *RXRA* gene in human adipose tissue (Orozco et al., 2018). In turn, *UCP3* has been studied using various methodologies in different tissues and traits, but no study has reported a *cis*-eQTL for this gene.

Interestingly, the strongest signal associated with *GPAT3* gene expression in our study was detected for the polymorphism rs81336088, which was located in a 134.73 – 135.60 Mb genomic region on SSC8 and containing eight SNPs with similar significance. This gene belongs to the lysophosphatidic acid acyltransferase protein family and is involved in pathways such as the triglyceride biosynthetic process through the conversion of glycerol-3-phosphate to lysophosphatidic acid in the synthesis of triacylglycerol, and pathways related with gluconeogenesis (Cao et al., 2006).

Concerning the *cis*-eQTL detected in the *RXRA* eGWAS results, the strongest signal was detected for the polymorphism rs81352834, which was located in a 270.27 – 274.01 Mb genomic region on SSC1 and contained a total of 11 SNPs. This gene is a nuclear receptor that mediates the biological effects of retinoids by their involvement in retinoic acid-mediated gene activation and is involved in the hormone-mediated signalling pathway, as well as the regulation of RNA transcription (Stelzer et al., 2016). Furthermore, this gene has been associated with the *PPARA* gene, forming the complex *PPARA-RXRA,* which increases FA β-oxidation in pigs (Vitali et al., 2018).

Finally, the third *cis*-eQTL identified in the current study is for the *UCP3* gene expression, where the polymorphism rs81413811, which was located in a 8.36 – 8.40 Mb genomic region on SSC9 and containing two SNPs. This gene belongs to the mitochondrial uncoupling proteins family and is implicated in pathways such as β-oxidation of FAs and adaptative thermogenesis (Han et al., 2012; Lin et al., 2017). In the present study, it is important to consider that the observed associations between gene expression and genetic markers might be influenced by the presence of linkage disequilibrium with the underlying causal mutation. However, it is important to conduct further investigations to validate and corroborate these findings.

On the other, a total of 24 *trans*-eQTLs affecting the expression of different target genes were detected, but we only identified candidate regulatory genes (*MLX, NRBF2, STAT1,*

STAT3, STAT4, STAT5 and *USF2*) that may affect the expression of the target genes in six of these *trans* eQTLs.

Furthermore, two *trans*-regulatory hotspots on SSC13 and SSC15 were detected. The *trans*-hotspot located in a 77.10 – 82.19 Mb genomic region on SSC13 containing 14 SNPs affects the expression of *APOE, ELOVL6, FASN* and *PLIN1* genes. In this region, the *PIK3CB* gene was mapped and could be a potential regulatory gene modulating the expression of several genes. *PIK3CB* was described to be involved in the PI3K-Akt-mTOR signalling pathway, which has been studied in different researches (Dibble, 2013; Liu et al., 2016). Moreover, the PI3K-Akt-mTOR signalling pathway participates in the decrease of intracellular lipid accumulation, along with a reduction in mRNA expression and protein content of genes involved in *de novo* FA synthesis in pigs (Y. Zhao et al., 2023), such as *FASN* gene. Hence, the *PIK3CB* gene emerge as a promising regulator to explain the differences in gene expression variations of *APOE, ELOVL6, FASN* and *PLIN1* associated with the SSC13 hotspot.

On the other hand, the second *trans*-regulatory hotspot located in a 93.18 – 94.92 Mb genomic region on SSC15 and containing six SNPs affects the expression of *APOE* and *FASN* genes. The *STAT1* and *STAT4* genes were mapped within this region. *STAT1* and *STAT4* genes belong to the STAT family and they could act as transcription activators (Stelzer et al., 2016). Interestingly, Zhang et al. (2019) found a relationship between the transcription factor *STAT1* and the regulation of genes associated with lipid metabolism and FA synthase genes in rats, such as *FASN.* Furthermore, Stephens et al. (1996) suggest that the *STAT1* gene can regulate adipogenesis and adipolysis in the adipocytes of different tissues. Therefore, the presence of *trans*-hotspots concurrently affecting the expression of both *FASN* and *APOE* genes suggests the influence of distinct genetic variants on their muscle-specific regulation. However, additional investigations are necessary to corroborate this hypothesis and validate the observed associations.

Gene expression and FA composition correlation network was performed using gene expression data from 89 genes (45 from this study and 44 from Criado-Mesas et al. (2020)), along with the relative amount of FA composition in the same animals. We want to highlight the significant role of *SCD* and *PPARG* genes, which exhibit numerous connections with other genes and are associated with FAs, particularly in the regulation

of oleic acid levels. In relation to this, in a study conducted by Shi et al. (2013) it was demonstrated that *PPARG* plays a crucial role in FA metabolism by regulating gene expression, including the *SCD* gene, which serves as the rate-limiting enzyme for MUFA synthesis in rodents. Additionally, *SCD* expression can be indirectly regulated in adipose tissue through *PPARG* activation (Walkey & Spiegelman, 2008). Conversely, Kadegowda et al. (2009) demonstrated that the activation of the *PPARG* gene in bovine mammary epithelial cells increases *SCD* gene expression. Moreover, *SCD* gene encodes a key rate-limiting enzyme in lipogenesis, which transform palmitic acid and stearic acid into palmitoleic and oleic (Meng et al., 2018).

4.2. Exploring muscle transcriptome through RNA-Sequencing

Using RNA-Seq data, our research group previously examined the transcriptomes of liver, adipose tissue, hypothalamus and muscle in two groups of BC1_LD animals with extreme muscle FA composition differences. These studies revealed that pigs with higher MUFA and SFA levels exhibited decreased FA oxidation in liver, increased *de novo* lipogenesis in adipose tissue, and may enhance uptake of FAs and glucose and increase the lipogenesis in muscle (Corominas, et al., 2013; Pérez-Montarelo et al., 2014; Puig-Oliveras et al., 2014; Ramayo-Caldas, et al., 2012). In this thesis, we studied the *longissimus dorsi* transcriptome in 129 BC1_DU animals. Using the power of RNA-Seq, two different protocols were employed to unravel the genetic basis of muscle transcriptome and its association with lipid metabolism and FA composition.

The first study involved the ASE analysis, which identified genetic variants showing allelic imbalance within candidate genes associated with lipid metabolism and FA composition. Concurrently, the eGWAS analysis was conducted, aiming to identify eQTLs and unravel their complex role in lipid metabolism and FA composition pathways. The eQTL identification can deep also into the gene expression regulation mechanisms through gene network interactions. In general, genes involved in lipid metabolism are regulated at transcriptional level, and the study of the molecular mechanisms controlling its

expression will help to understand the genetic basis of FA composition in muscle tissue (Hausman et al., 2009).

To identify relevant genetic variants in genes that present allelic imbalance, an ASE analysis was performed and a total of 2,146 SNPs associated to 1,621 genes were detected in at least three animals. Furthermore, gene ontology filtering was applied to keep those variants affecting genes related to lipid and FA metabolism pathways. Finally, a total of 52 genes related to these pathways remained for further analysis. As previously mentioned, among several contributing factors to ASE, one of the most common causes is the presence of polymorphisms at regulatory sites within the same genetic region, commonly known as *cis*-eQTLs. These polymorphisms can potentially affect specific transcription factor binding sites, resulting in alterations to the binding affinity and subsequently influencing the transcription rate of the respective allele (de Souza et al., 2020; Jaenisch & Bird, 2003). In recent years, numerous studies have employed RNA-Seq data from various tissues to enhance our comprehension of ASE (Bruscadin et al., 2021; de Souza et al., 2020; Gregg et al., 2010; Liu et al., 2020; Pinter et al., 2015; Stachowiak et al., 2018; Stachowiak & Flisikowski, 2019; Wu et al., 2015).

On the other hand, eGWAS studies were performed and a total of 2,678 eQTLs for the expression of 854 genes were identified. Among the 854 significantly associated genes, 101 were associated with lipid and FA metabolism pathways. The majority of the eQTLs were categorized as *trans*-eQTLs, comprising 2,058 regions, whereas 620 regions exhibited a *cis*-effect. In accordance with the study of candidate genes via qPCR, the number of eQTLs located in *trans* is higher than the eQTLs found in *cis*. As previously mentioned, it is important to emphasize that the classification of *cis*- and *trans*-eQTLs depends on the selected *cis*-regions window (Ponsuksili et al., 2010), which affects the categorization of regions as either *cis* or *trans*. Moreover, these findings align with numerous studies, as research involving model organisms has consistently identified a higher number of *trans*-eQTLs compared to *cis*-eQTLs (Cánovas et al., 2012; Cheung & Spielman, 2009; Gilad et al., 2008; Hasin-Brumshtein et al., 2014; Leal-Gutiérrez et al., 2020; Liu et al., 2020; Ponsuksili et al., 2008, 2010).

After applying two different protocols for gene expression studies to the same set of animals and RNA-Seq data, we proceeded to identify those genes that were detected by both methodologies across the two studies.

Overlap analysis between ASE-associated genes and *cis*-eQTL genes identified 135 common genes (Figure 4.1). This limited overlap between the two approaches can be attributed to different factors. First, the number of markers involved in both studies is very different. In eGWAS, 405K SNPs from the Affymetrix DNA chip were employed, while in the ASE analysis only 13,113 common SNPs between RNA-Seq variant calling and the DNA chip were used. Secondly, the variants used in the eGWAS were located mostly within intronic and intergenic regions, whereas the variants employed in the ASE analysis were situated mainly in coding regions because belong to the muscle transcriptome. Third, some low-expressed genes were excluded from the ASE analysis but kept for eQTL analysis. Fourth, both approaches may be prone to detecting different signals not caused by *cis*-regulatory variants. For example, spurious *cis*-eQTL signals can result from copy number variations (Christopher et al., 2013) or splicing mutations (Lalonde et al., 2011), while spurious ASE signals can result from imprinting (Maroilley et al., 2017) or from allelic mapping bias.

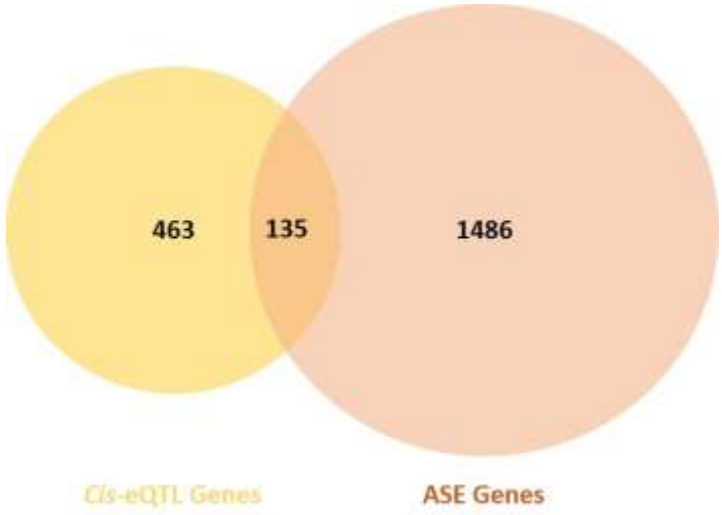

Figure 4.1. Venn diagram of *cis*-eQTL genes and ASE genes.

Fifth, while the eGWAS approach uses the gene expression information from all the animals, the ASE analysis is focused only on the heterozygous animals, comparing the expression of the two alleles within the same individual and ensuring a higher accuracy of the results (Kang et al., 2016).

Hence, ASE analysis can provide complementary and more precise mapping results than *cis*-eQTL analysis, and combining these two approaches can provide more reliable results. The expression level of genes acts as a molecular phenotype, bridging the connection between genes and phenotypes. In these studies, we observed significantly expressed genes belonging to pathways of lipid and FA composition metabolism. Specifically, the ASE study highlighted 52 genes and the eGWAS approach identified 75 *cis*-regulatory genes associated with lipid and FA composition metabolism pathways. These genes may be involved in meat quality trait variations and could be used in future studies, although further work is needed to validate our hypothesis.

Interestingly, we detected six candidate genes (*ACSM5, CBR4, ECHDC3, EPHX2, LIPE* and *PDPN*) that were identified by the two approaches and that were associated with lipid and FA composition metabolism pathways (Figure 4.2). The common genes identified by ASE and eGWAS analysis are discussed below.

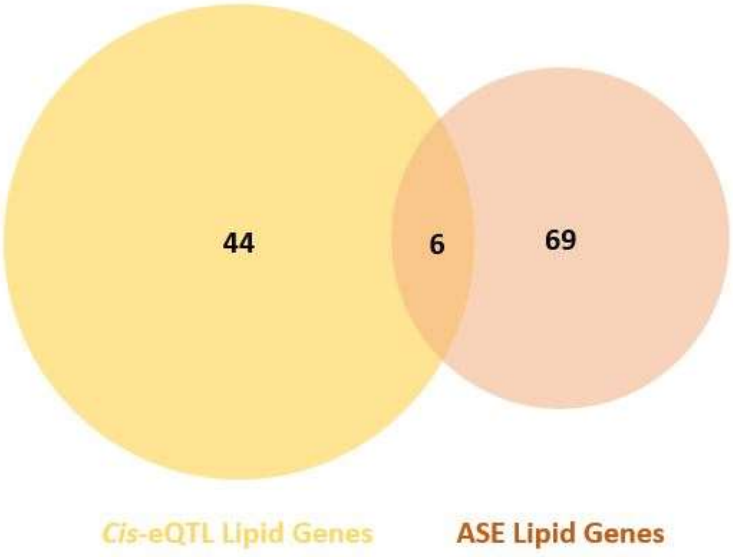

Figure 4.2. Venn diagram of *cis*-eQTL and ASE lipid genes.

The Acyl-CoA Synthetase Medium-Chain Family Member 5 (*ACSM5*) gene is an acyl-CoA synthase involved in a preliminary step of the FA β-oxidation pathway. This enzyme catalyses the activation of FAs by CoA to produce an acyl-CoA, and is then introduced in the mitochondria through the CPT system (Stelzer et al., 2016). This gene was reported in previous studies of our group (Puig-Oliveras et al., 2016; Revilla et al., 2018; Criado-Mesas et al., 2020). Puig-Oliveras et al. (2016) identified a *cis*-eQTL region in muscle, where the *ACSM5* proximal promoter region was amplified and sequenced in a subset of ten BC1_LD animals and three polymorphisms were identified (rs323520560, rs339587799, rs331702081). Furthermore, Criado-Mesas et al. (2020) genotyped the most significant SNP for *ACSM5* gene expression (rs331702081) in the muscle tissue of BC1_DU and BC1_PI animals. As a result, the rs331702081 was the most significantly associated SNP with the muscle *ACSM5* gene expression in the BC1_DU and BC1_PI populations and explained approximately 40% of the phenotypic variance. On the other hand, Revilla et al. (2018) reported that the rs331702081 was also the most significantly associated SNP with the *ACSM5* gene expression in adipose tissue in BC1_LD animals. They also identified two transcription factors (*ARNT* and *STAT6*) that bind only when the A allele is present were identified. On the other hand, in our eGWAS study, we identified six significant SNPs within the same eQTL, with one SNP located at position 25279054 on SSC3 being the most significant for *ACSM5* expression. Furthermore, through ASE analysis, we identified only one significantly associated SNP (rs322578425). Unfortunately, the eGWAS and ASE studies did not identify common SNPs between them. Nevertheless, we could suggest that the detected polymorphisms may be in linkage disequilibrium with the causal mutation.

The gene encoding Carbonyl reductase type-4 (*CBR4*) has been reported as the factor responsible for the final two enzymatic reactions following the formation of 3-Oxoacyl-acp, in the pathway of *de novo* FA synthesis leading to IMF deposition, in pig *longissimus thoracis* muscle (Cai et al., 2020). In our eGWAS investigation, we detected a total of 17 SNPs situated within the same region. Notably, the SNP located at position 21052693 on SSC14 exhibited the highest significance concerning *CBR4* expression. Additionally, in our ASE analysis, we identified the SNP rs343238244, which displays allelic-specific expression for the *CBR4* gene. However, there was no overlap in SNPs discovered

between the eGWAS and ASE studies. Thus, the identified polymorphisms may be in linkage disequilibrium with the causal mutation.

ECHDC3 gene encodes the enoyl-CoA hydratase domain containing 3, a mitochondrial enzyme that has a crotonase-like domain similar to enoyl-CoA hydratase (Rahul et al., 2015). Furthermore, *ECHDC3* is assumed to be involved in β-oxidation, the most important and well-known pathway for FA oxidation (Linster et al., 2011; Miura, 2013). In this study, a total of 17 significant SNPs were detected within the same eQTL in the eGWAS, with an SNP located at position 60383846 on SSC10 being the most significant in relation to *ECHDC3* gene expression. Concerning the ASE analysis, two significantly associated SNPs (rs346355799 and rs81477697) were identified. Interestingly, both studies identified the same polymorphism (rs81477697), located in a protein-coding region and causing a synonymous mutation. It would be of interest to further investigate this SNP as a potential candidate to elucidate the molecular mechanisms of *ECHDC3* gene expression.

The Epoxide Hydrolase 2 (*EPHX2*) gene is involved in FA metabolism (Stelzer et al., 2016). In a study conducted by Gondret et al. (2012), a gene expression analysis was performed between juvenile pre-obese pigs compared with Large White lean pigs. In this study, they observed an overexpression of the *EPHX2* gene in juvenile pre-obese pigs, in accordance with increased expression and greater total activity of *EPHX2* in adipose tissue that were observed during obesity development in humans (Taeye et al., 2010). Moreover, Piórkowska et al. (2018) identified differential expression of the *EPHX2* gene between Polish Landrace and Pulawska pigs using RNA-Seq analysis. In our eGWAS study, we identified six significant SNPs located within the same eQTL, with an SNP located at 11211355 on SSC14 being the most significant for *EPHX2* gene expression. On the other hand, through the ASE study, we identified only one significant SNP (rs322829130), but the eGWAS and ASE studies did not find any common SNPs. Hence, the detected polymorphisms could be in linkage disequilibrium with the causal mutation.

Finally, the Lipase E (*LIPE*) gene it is involved in the hydrolysis of triglycerides and diglycerides stored in muscle to free FAs. *LIPE* gene is located in a QTL region linked to meat sensory quality in pigs (Pena et al., 2013). A previous study conducted by Puig-

Oliveras et al. (2014) found a differential gene expression of *LIPE* gene among pigs phenotypically extreme for FA composition. In addition, an investigation performed on Laiwu pigs has demonstrated a negative association between *LIPE* expression and IMF content (H. Wang et al., 2020). Zappaterra et al. (2016) identified a key role of LIPE enzyme in IMF hydrolysis in pig skeletal muscle. Furthermore, a differential gene expression in *LIPE* gene was observed between a cohort of 12 Wujin pigs characterized by high IMF deposition and a group of 12 Landrace pigs that displayed low IMF deposition (Zhao et al., 2009). In this study, the eGWAS identified two significant SNPs located within the same region. Notably, the SNP located at 49382024 on SSC6 exhibited the highest significance concerning *LIPE* expression. On the other hand, the ASE analysis identified the SNP rs328830166 as the most significant SNP displaying allelic-specific expression. However, there was no overlap in SNPs identified through the eGWAS and ASE studies.

The ASE and eGWAS protocols performed in these researches converged with a principal purpose: to elucidate the genetic variants and genomic regions that regulate the key pathways of lipid metabolism and FA composition. Therefore, the combination of ASE and eGWAS protocols forms a strong base for upcoming research endeavours, facilitating deeper exploration into the complex genetic landscape that influences lipid metabolism and FA composition.

4.3. Future perspectives and challenges

Over the years, the advance of NGS technologies has been remarkable, leading to a substantial reduction in sequencing costs and making it achievable to sequence the complete genome of selected animals and to initiate large-scale sequencing projects to deepen our understanding of genetic variations across different populations. Furthermore, the transcriptome analysis was extended to a broader range of animals, diverse tissues within each animal and to single cells. Conversely, there has been a remarkable surge of interest in high-throughput “omics” technologies, encompassing genomics, transcriptomics, epigenomics, proteomics, metagenomics and metabolomics,

among others. These technologies are being increasingly applied within the domain of animal production, as highlighted by MacKay et al. (2009). Enhancing the analysis of complex traits requires integrating data from different "omics" and animal phenotypes using systems biology methods. Our group is working on a new investigation into the interrelation between pig growth, fatness, microbiome and immunity. Additionally, proteomics sheds light on disease mechanisms and protein applications, and the study of epigenomics could help us to understand the gene expression regulation.

In the current study, we conducted a muscle gene expression analysis using qPCR for 45 candidate lipid metabolism genes. Multiple polymorphisms located in *cis*-eQTL regions associated with the expression of *GPAT3*, *RXRA*, and *UCP3* were identified. Nevertheless, further validations are necessary to confirm whether these polymorphisms are indeed the causal mutations. Moreover, a comprehensive investigation into the transcription factors *PIK3CB, STAT1* and *STAT4* mapped within the hotspot regions of the 3BCs would be crucial to confirm if these regulatory genes have the potential to modulate the expression of several genes.

In the context of the RNA-Seq analysis, we have identified several eQTLs as well as genetic variants associated with candidate genes related to FA composition and lipid metabolism pathways. However, further analyses are necessary to confirm our hypothesis and validate the potential causal mutations identified through the eGWAS and ASE analyses. In future research, it would be interesting to delve deeper into the genes that are common between ASE and eGWAS analysis. Moreover, genotype polymorphisms significantly linked to the expression of candidate genes involved in lipid metabolism and FA composition, as well as exploring their potential connection to meat quality traits, could help to improve animal breeding programs. In addition, investigations focusing on the study of epigenetics may aid in understanding the molecular mechanisms of gene regulation. Interestingly, emerging technologies like cell cultures and luciferase reporter assays, protein binding assays, transcription factor binding studies or the CRISPR-Cas9 system may facilitate the validation of causal mutations associated with eQTLs and ASE studies.

CONCLUSIONS

Chapter 5

1. The skeletal muscle expression profile of 45 candidate genes for lipid metabolism and fatty acid composition were investigated using microfluidic arrays and real-time qPCR in 354 pigs representing three different genetic backgrounds. The eGWAS analysis identified 24 *trans*-acting eQTLs affecting the genes *ANK2, APOE, ARNT, EGF, ELOVL6, FASN, GPAT3, NR1D2, PLIN1, PPAP2A, RORA, RXRA* and *UCP3*, as well as three *cis*-acting eQTLs influencing the expression of *GPAT3, RXRA* and *UCP3* genes.

2. A *trans*-eQTL hotspot located in SSC13 is regulating the expression of *APOE, ELOVL6, FASN* and *PLIN1* genes, where a potential regulatory gene (*PIK3CB*) was mapped. On the other hand, a *trans*-eQTL located in SSC15 is controlling the *APOE* and *FASN* gene expression, where the transcription activators *STAT1* and *STAT4* were mapped.

3. A co-expression analysis of 89 lipid metabolism candidate genes in muscle and the correlation between gene expression and FA composition revealed the regulatory role of *FABP5, PPARG, SCD* and *SREBF1* genes in modulating the levels of α-linolenic, arachidonic, and oleic acids.

4. The muscle transcriptome of 129 Iberian × Duroc crossbreed pigs was studied by RNA-Seq. An ASE analysis identified 2,146 ASE-SNPs associated with the expression of 1,621 genes. Fifty-two of these genes were involved in lipid metabolism and FA composition pathways and may be associated with meat quality traits.

5. The correlation analysis between the expression of 52 lipid metabolism candidate genes with ASE and the FA composition in muscle suggested a role of *ABHD5, ACSL1* and *NUDT19* genes in regulating the levels of arachidonic, dihomo-ɣ-linolenic, gondoic and oleic acids.

6. An eGWAS performed with the muscle RNA-Seq data of 129 Iberian × Duroc crossbreed pigs identified 620 *cis*-eQTLs associated with the expression of 598 genes and 2,058 *trans*-eQTLs linked with the expression of 604 genes.

7. In the eGWAS analysis, 101 genes with eQTLs were linked to lipid metabolism pathways, 30 genes displayed *cis*-eQTLs, 26 exhibited *trans*-eQTLs, and 45 had both *cis*- and *trans*-eQTLs.

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ANNEXES

Chapter 7

7.1. Supplementary material Paper I: "Identification of genomic regions, genetic variants and gene networks regulating candidate genes for lipid metabolism in pig muscle"

Table S1: SNPs genotyped by Taqman OpenArray and their chromosomal positions

SNP ID	Gene name	Chr	position	ref	alt	position *Sscrofa 11,1*
ACACA.I23	*ACACA*	12	38724977	G	A	12:38724977-38724977
ACSL4	*ACSL4*	23	89763094	A	G	23:89763094-89763094
ACSL4.3UTR	*ACSL4*	23	89760895	G	A	23:89760895-89760895
ANK2	*ANK2*	8	109335876	A	G	8:109335876-109335876
CPT1A.2.4268130.AG	*CPT1A*	2	4268130	A	G	2:4268130-4268130
CPT1A.2.4279213.AG	*CPT1A*	2	4279213	G	A	2:4279213-4279213
ELOVL1.3UTR	*ELOVL1*	6	167885980	C	T	6:167885980-167885980
ELOVL1.6.167880944.CT	*ELOVL1*	6	167880944	C	T	6:167880944-167880944
ELOVL1.I	*ELOVL1*	6	167884207	C	T	6:167884207-167884207
ELOVL1.P	*ELOVL1*	6	167880497	T	C	6:167880497-167880497
ELOVL6.1408CT	*ELOVL6*	8	112186423	G	A	8:112186423-112186423
ELOVL6.1922CT	*ELOVL6*	8	112186937	A	G	8:112186937-112186937
ELOVL6.394GA	*ELOVL6*	8	112038663	A	G	8:112038663-112038663
ELOVL6.480	*ELOVL6*	8	112038577	T	C	8:112038577-112038577
ELOVL6.533	*ELOVL6*	8	112038523	T	C	8:112038523-112038523
ELOVL6.574	*ELOVL6*	8	112038483	C	T	8:112038483-112038483
ELOVL6.8.112038338.CT	*ELOVL6*	8	112038338	T	C	8:112038338-112038338
ELOVL6.8.112038405.AG	*ELOVL6*	8	112038405	G	A	8:112038405-112038405
ELOVL6.8.112039363.AG	*ELOVL6*	8	112039363	G	A	8:112039363-112039363
ELOVL6.8.112187085.CG	*ELOVL6*	8	112187085	C	G	8:112187085-112187085
ELOVL6.E4	*ELOVL6*	8	112180004	C	T	8:112180004-112180004
ELOVL7.629AG	*ELOVL7*	16	39680655	G	A	16:39680655-39680655
ELOVL7.E10	*ELOVL7*	16	39589566	G	A	16:39589566-39589566
ELOVL7.P	*ELOVL7*	16	39680072	A	G	16:39680072-39680072
FABP4.3UTR	*FABP4*	4	55100821	G	A	4:55100821-55100821
FABP4.4.55100943.GA	*FABP4*	4	55100943	A	G	4:55100943-55100943
FABP4insC	*FABP4insC*	4	55096733	I	D	4:55096733-55096733
FADS1	*FADS1*	2	9747629	C	T	2:9747629-9747629
FADS1.2.9733962.AG	*FADS1*	2	9733962	A	G	2:9733962-9733962
FADS1.2.9733986.AC	*FADS1*	2	9733986	A	C	2:9733986-9733986
FADS1.2.9734036.AG	*FADS1*	2	9734036	A	G	2:9734036-9734036
FADS1.2.9734294.AG	*FADS1*	2	9734294	A	G	2:9734294-9734294
FADS1.2.9747858.CT	*FADS1*	2	9747858	T	C	2:9747858-9747858
FADS1.3UTR	*FADS1*	2	9749025	T	C	2:9749025-9749025
FADS1.P	*FADS1*	2	9734553	T	C	2:9734553-9734553
FADS2.2.9633104.AT	*FADS2*	2	9633104	A	T	2:9633104-9633104
FADS2.2.9633749.CG	*FADS2*	2	9633749	G	C	2:9633749-9633749
FADS2.2.9654959.AC	*FADS2*	2	9654959	A	C	2:9654959-9654959
FADS2.2.9667336.CT	*FADS2*	2	9667336	C	T	2:9667336-9667336
FADS2.E6	*FADS2*	2	9642011	G	T	2:9642011-9642011
FADS2.E8	*FADS2*	2	9635779	T	C	2:9635779-9635779
FADS2.P	*FADS2*	2	9667306	C	T	2:9667306-9667306
FADS2.P2	*FADS2*	2	9667513	T	C	2:9667513-9667513
FADS2.P3	*FADS2*	2	9667427	G	A	2:9667427-9667427

FADS3	*FADS3*	2	9621709	A	G	2:9621709-9621709
FADS3.2.9606685.AC	*FADS3*	2	9606685	C	A	2:9606685-9606685
FADS3.2.9607176.AG	*FADS3*	2	9607176	A	G	2:9607176-9607176
FASN.12.923305.TC	*FASN*	12	923305	T	C	12:923305-923305
FASN.12.924947.CT	*FASN*	12	924947	C	T	12:924947-924947
FASN.12.935308.GA	*FASN*	12	935308	A	G	12:935308-935308
FASN.12.937358.GA	*FASN*	12	937358	G	A	12:937358-937358
FASN.P	*FASN*	12	919403	T	C	12:919403-919403
GPAT3.3UTR	*GPAT3*	8	134913000	A	G	8:134913000-134913000
GPAT3.P	*GPAT3*	8	134976964	C	T	8:134976964-134976964
LPL.14.4122395.AG	*LPL*	14	4122395	G	A	14:4122395-4122395
NR1D2.3UTR	*NR1D2*	13	10789348	T	C	13:10789348-10789348
NR1D2.E3	*NR1D2*	13	10770067	A	G	13:10770067-10770067
PLIN1.7.55237057.AG	*PLIN1*	7	55237057	G	A	7:55237057-55237057
SLC27A1.2.60205069.CT	*SLC27A1*	2	60205069	T	C	2:60205069-60205069
SREBF2.5.6758255.CA	*SREBF2*	5	6758255	A	C	5:6758255-6758255
THBS1.E6	*THBS1*	1	131742930	C	T	1:131742930-131742930
THBS1.E9	*THBS1*	1	131739520	C	T	1:131739520-131739520
THBS1.P	*THBS1*	1	131746388	G	T	1:131746388-131746388
USF1	*USF1*	4	89395154	D	I	4:89395154-89395154
USF1.P2	*USF1*	4	89394426	C	G	4:89394426-89394426

Table S2: Primer sequences of the 45 candidate genes.

Gene	Orientation	Primer
ACACA	fw	TCTCATCCAAACAGAGGGAACA
ACACA	rv	ATGAGTCATGCCATAGTGGTTGA
ACLY	fw	TATCTCCGGCCTCTTCAATTTCTA
ACLY	rv	CGCCGTCTTTGGTCACTACAA
ACSL4	fw	CCCACTTTGCAATCTGCTACTG
ACSL4	rv	CTCCACCAGACAGCATCATACG
ADIPOQ	fw	GTACCCCAGGCCGTGATG
ADIPOQ	rv	CCCTTAGGACCAGTAAGACCTGTATCT
ADIPOR1	rv	GCAATCCCTGAATAATCCAGTTTG
ADIPOR1	fw	GAAGGTGGTGTTTGGGATGTTCT
ADIPOR2	rv	GTCAGGCAGCACATCGTGAG
ADIPOR2	fw	GGAAAGAATGGAAGAGTTCGTTTGT
AGPAT2	fw	CATGGTCAGGGAGAAGCTCAA
AGPAT2	rv	GCCAGGTAGAAGGCACCTTTC
ANK2	fw	GTGGATTCTGCTACGAAGAAAGG
ANK2	rv	AAGGACTTTGACAACTTCTGCTTGT
APOE	fw	GGGTGCAGTCCCTGTCTGAC
APOE	rv	CTCTCCTCTATCAGCTCCGTCAG
ARNT	fw	TCTAATGATAAGGAGCGGTTTGC
ARNT	rv	TATGATTTTCCCTGGCGAGTCT
CD36	fw	GGTCCTTACACGTACAGAGTTCGTT
CD36	rv	CCATTGGGCTGTAGGAAAGAGA
CIITA	fw	GCAGGCTGTTGTGCGACAT
CIITA	rv	TGGTCCAGTTCCGCGATAC
CPT1A	fw	CCTGAAGGTGCTGCTCTCCTA
CPT1A	rv	CTCACCATCATCATCCAGATCTTG
CYP2U1	fw	AGAGAAAACAGTGCTCCAAGGGTAT
CYP2U1	rv	TGGCTGGGTCTCTGTGTACTGA
EGF	fw	AACGGGAATGCCACTTGTGT
EGF	rv	CCTTCCAAGTCAATCCTAAAGATACTG
ELOVL1	fw	CATTGAGCTGATGGACACAGTGA
ELOVL1	rv	GCGTGGAAAGAGCCCATTC
ELOVL5	fw	CCTCTCGGCTGGCTGTACTT
ELOVL5	rv	CCTTCTTGTTGTAGGTCTGGATGTAG
ELOVL6	fw	AGCAGTTCAACGAGAACGAAGCC
ELOVL6	rv	TGCCGACCGCCAAAGATAAAG
ELOVL7	fw	GTACAGGTTATTCGTTTCGATGTGA
ELOVL7	rv	CAGGTGCGTACCATCCTCAGT
ESRRA	fw	CAAGAGCATCCCAGGCTTCTC
ESRRA	rv	CACCCAACACCAATACCTCCAT
ETFDH	fw	AGTGGAATTTTGGCAGCAGAA

ETFDH	rv	GTTACATGGAGTCCTATTGTCTTCGA
FABP4	fw	TAAGTTGGTGGTGGAATGTATCATG
FABP4	rv	AGAGTGTTGTAGAGTTCGATCCAAAC
FADS1	fw	CCTTGTGAGGAAGTATATGAGCTCTCT
FADS1	rv	TCATCTGTCAGCTCTTTATTCTTAGTCG
FADS2	fw	TCCACCGCGACCTTGATTTA
FADS2	rv	TCGGTGATCTCAGAGTTCTTGGT
FADS3	fw	CCAGCACCTCTACTTCTTCCTGAT
FADS3	rv	CATGTATGCCAGATTTTCCACTTC
FASN	fw	CGTGGGCTACAGCATGATAGG
FASN	rv	CTGGGCCCCTTGAAGTCA
GPAT3	fw	TCCTGCCTCTCAGGGTTACCT
GPAT3	rv	AACTGTCCAACCAGGGTGGTT
HADH	fw	AGCTCTTCAAGAGGCTGGACAA
HADH	rv	TTGTGATCTGCAAAGAGGAAGTG
HADHA	fw	CTTTGCTGACCAGAACCCATATT
HADHA	rv	GAGTATTTACCTTTGAATTGGGTGAGT
LPL	fw	GTGCTCAGATGCCCTACAAAGTC
LPL	rv	GGTTGGTGTGGGTATCACTCTCA
ME1	fw	ATGATCGAGGGCATGTTGCT
ME1	rv	AGTCACCACGACAGCCTTGAT
NR1D2	fw	GCTTGTGAAGGCTGTAAGGGT
NR1D2	rv	CTGACATCTGTTCCTATTCATTCTCATT
NR1H2	fw	CATCCACCATTGAGATCATGCT
NR1H2	rv	CATCCTTGCTGTAGGTGAAGTCTTT
NRF1	fw	CACGTTTGCTTAGGAAACTTCGA
NRF1	rv	GGGTTTGGAGGGTGAGATACAA
PDK4	fw	AGCTGCTGGACTTCGGTTCA
PDK4	rv	GCTAGCCTCACAGGCAACTCTT
PLIN1	fw	CCTCCAGTATCCTCCTGAAAAGATC
PLIN1	rv	GAGCTCGCAGCCAGCTAGAG
PNPLA2	fw	CTTCACCGTCCGCTTGCT
PNPLA2	rv	GCATCACCAGGTACTGGCAGAT
PPAP2A	fw	GGCCACTCTTCATTCTCCATGTAC
PPAP2A	rv	AGGCCCACGTAAATGGATACAG
RORA	fw	TCAGAACAATACCGTGTACTTTGATG
RORA	rv	AAAGTCTTCACAACCTAAGGATTTGAAG
RXRA	fw	CAACAAGGACTGCCTGATCGA
RXRA	rv	TCCTCCTGCACGGCTTCA
SLC27A1	fw	TCACTCGGCAGGGAACATC
SLC27A1	rv	CGGCTGGCTGAAAACTTCTT
SREBF2	fw	GTACCGCTCCTCCATCAATGA
SREBF2	rv	AAAACACCAGACTTGTGCATCTTG
THBS1	fw	CATCCGCAAAGTGACTGAAGAG
THBS1	rv	TGGACTCCGTTGTGGTAGCA

UCP3	fw	AAGTACAGCGGGACGATGGA
UCP3	rv	TGTTGGGCAGAATTCCTTTCC
USF1	fw	CCCTTATTCCCCGAAGTCAGA
USF1	rv	GCGGCGTTCCACTTCATTAT

Table S3: Variant Effect Predictor of the 258 significant eSNPs found in the eGWAS results for 18 candidate genes.

chr	position	rs	Allele	Consequence	IMPACT	SYMBOL	Gene ID
1	29427957	rs80824504	A	intergenic_variant	MODIFIER	-	-
1	31162256	rs80801544	G	synonymous_variant	LOW	*TAAR9*	ENSSSCG00000004189
1	39158009	rs81312346	A	intergenic_variant	MODIFIER	-	-
1	270278445	rs81352831	A	intron_variant	MODIFIER	*NCS1*	ENSSSCG00000039311
1	270313674	rs81352834	G	intron_variant	MODIFIER	*NCS1*	ENSSSCG00000039311
1	270868251	rs81319709	G	intron_variant	MODIFIER	*ABL1*	ENSSSCG00000005706
1	270888682	rs81334938	A	intron_variant	MODIFIER	*ABL1*	ENSSSCG00000005706
1	270920337	rs81474173	A	3_UTR_variant	MODIFIER	*FIBCD1*	ENSSSCG00000005707
1	271413237	rs81353004	A	intron_variant	MODIFIER	*PRRC2B*	ENSSSCG00000005715
1	271626303	rs81353058	G	intergenic_variant	MODIFIER	-	-
1	271944489	rs80812614	A	intergenic_variant	MODIFIER	-	-
1	273032673	rs81311731	A	intron_variant	MODIFIER	*ADAMTS13*	ENSSSCG00000021241
1	273242436	rs80827620	G	intron_variant	MODIFIER	*SARDH*	ENSSSCG00000005740
1	274019182	rs81319360	G	intron_variant	MODIFIER	*COL5A1*	ENSSSCG00000005751
10	5508391	rs81428136	G	intergenic_variant	MODIFIER	-	-
11	25626653	rs80965301	A	intron_variant	MODIFIER	*MTRF1*	ENSSSCG00000009436
12	20659791	rs81432406	A	intron_variant	MODIFIER	*ZNF385C*	ENSSSCG00000028051
12	20697851	rs81432416	A	intron_variant	MODIFIER	*ZNF385C*	ENSSSCG00000028051
12	20727551	rs81300859	A	upstream_variant	MODIFIER	*NKIRAS2*	ENSSSCG00000017418
12	20752782	rs81214864	A	synonymous_variant	LOW	*DNAJC7*	ENSSSCG00000017419
12	20767619	rs81260973	A	synonymous_variant	LOW	*ODAD4*	ENSSSCG00000017415
12	38329499	rs81305582	G	intergenic_variant	MODIFIER	-	-
12	53995960	rs81437196	G	intron_variant	MODIFIER	*PIK3R5*	ENSSSCG00000017991

13	24228663	rs80853212	G	intergenic_variant	MODIFIER	-	-
13	24253641	rs81443771	G	intergenic_variant	MODIFIER	-	-
13	52057695	rs80909668	G	intergenic_variant	MODIFIER	-	-
13	58821305	rs80909709	G	intron_variant	MODIFIER	-	ENSSSCG00000045742
13	61140536	rs81446211	A	intron_variant	MODIFIER	*ITPR1*	ENSSSCG00000023437
13	77105634	rs80831731	G	intron_variant	MODIFIER	*PPP2R3A*	ENSSSCG00000033185
13	77509075	rs81446940	A	intron_variant	MODIFIER	*STAG1*	ENSSSCG00000011652
13	77618140	rs81309969	A	intron_variant	MODIFIER	*STAG1*	ENSSSCG00000011652
13	77708756	rs81446946	A	intron_variant	MODIFIER	*STAG1*	ENSSSCG00000011652
13	78889869	rs81280406	G	intron_variant	MODIFIER	*DZIP1L*	ENSSSCG00000027467
13	78891871	rs81478270	G	intron_variant	MODIFIER	*DZIP1L*	ENSSSCG00000027467
13	80847255	rs81242540	A	intron_variant	MODIFIER	*CLSTN2*	ENSSSCG00000011666
13	81420530	rs322937606	A	intron_variant	MODIFIER	*CLSTN2*	ENSSSCG00000011666
13	81440526	rs80946349	A	intron_variant	MODIFIER	*CLSTN2*	ENSSSCG00000011666
13	81488325	rs80916261	G	intergenic_variant	MODIFIER	-	-
13	81561592	rs80942072	G	intron_variant	MODIFIER	*TRIM42*	ENSSSCG00000011668
13	81604531	rs81316562	G	intron_variant	MODIFIER	-	ENSSSCG00000050095
13	81965754	rs81447187	A	upstream_variant	MODIFIER	*PXYLP1*	ENSSSCG00000011670
13	82197802	rs80959349	C	downstrea_variant	MODIFIER	*ZBTB38*	ENSSSCG00000022073
13	178001286	rs80815933	A	intron_variant	MODIFIER	*ROBO2*	ENSSSCG00000012002
13	200778735	rs80961068	G	intron_variant	MODIFIER	*TTC3*	ENSSSCG00000012062
14	2722953	rs80785221	G	intergenic_variant	MODIFIER	-	-
14	2758196	rs80929308	G	intergenic_variant	MODIFIER	-	-
14	53689369	rs80920722	G	intron_variant	MODIFIER	*RYR2*	ENSSSCG00000010142
14	55291076	rs81450834	C	intron_variant	MODIFIER	*NID1*	ENSSSCG00000026819
14	56474441	rs80792689	A	intergenic_variant	MODIFIER	-	-
14	56910185	rs80811825	A	intron_variant	MODIFIER	*SLC35F3*	ENSSSCG00000010162

14	57880301	rs80967642	A	upstream_variant	MODIFIER	-	ENSSSCG00000052826
14	58293291	rs80912739	A	intron_variant	MODIFIER	*SIPA1L2*	ENSSSCG00000010169
14	58329122	rs80912264	A	intron_variant	MODIFIER	*SIPA1L2*	ENSSSCG00000010169
14	58410680	rs80966169	G	intergenic_variant	MODIFIER	-	-
14	59497467	rs80909629	A	intron_variant	MODIFIER	*TTC13*	ENSSSCG00000010180
14	61288545	rs80881665	A	intergenic_variant	MODIFIER	-	-
14	61318065	rs80837939	A	intron_variant	MODIFIER	*RET*	ENSSSCG00000010199
14	61429021	rs80810391	G	intron_variant	MODIFIER	*RASGEF1A*	ENSSSCG00000010201
14	61453852	rs80788284	A	intron_variant	MODIFIER	*RASGEF1A*	ENSSSCG00000010201
14	61489779	rs80941880	C	intron_variant	MODIFIER	*RASGEF1A*	ENSSSCG00000010201
14	61501367	rs80830365	A	intron_variant	MODIFIER	*RASGEF1A*	ENSSSCG00000010201
14	63488234	rs80815692	C	intron_variant	MODIFIER	-	ENSSSCG00000010212
14	63515463	rs80845884	A	intron_variant	MODIFIER	-	ENSSSCG00000010212
14	63574363	rs80860167	G	intron_variant	MODIFIER	-	ENSSSCG00000010212
14	63629712	rs80827664	G	intron_variant	MODIFIER	-	ENSSSCG00000010212
14	63687970	rs80892476	A	intron_variant	MODIFIER	-	ENSSSCG00000010212
14	63934099	rs80806131	G	intron_variant	MODIFIER	-	ENSSSCG00000010212
14	63957872	rs80991169	A	intron_variant	MODIFIER	-	ENSSSCG00000010212
14	64067892	rs80862647	G	intron_variant	MODIFIER	-	ENSSSCG00000010212
14	64162795	rs80874321	G	intron_variant	MODIFIER	-	ENSSSCG00000010212
14	64191356	rs326097302	C	intron_variant	MODIFIER	-	ENSSSCG00000010212
14	64593845	rs80863241	A	intergenic_variant	MODIFIER	-	-
14	64606897	rs80998583	A	intergenic_variant	MODIFIER	-	-
14	64714395	rs80943509	G	intergenic_variant	MODIFIER	-	-
14	64797427	rs80998166	A	intergenic_variant	MODIFIER	-	-
14	64823635	rs80909042	G	intergenic_variant	MODIFIER	-	-
14	64856186	rs80911891	A	intergenic_variant	MODIFIER	-	-

14	64944114	rs81450907	G	intergenic_variant	MODIFIER	-	-
14	64977875	rs81450909	C	intergenic_variant	MODIFIER	-	-
14	65293748	rs344057444	G	intergenic_variant	MODIFIER	-	-
14	65330288	rs80893603	G	non_coding	MODIFIER	-	ENSSSCG00000059800
14	65416111	rs80783805	C	intron_variant	MODIFIER	*ARID5B*	ENSSSCG00000010219
14	65594891	rs80927833	G	3_UTR_variant	MODIFIER	*ARID5B*	ENSSSCG00000010219
14	65622385	rs80844711	A	intergenic_variant	MODIFIER	-	-
14	65655137	rs80855882	A	upstream_variant	MODIFIER	-	ENSSSCG00000045063
14	65812365	rs80823092	A	intergenic_variant	MODIFIER	-	-
14	65849180	rs80825364	A	upstream_variant	MODIFIER	-	ENSSSCG00000010221
14	66131302	rs80919409	G	intron_variant	MODIFIER	-	ENSSSCG00000045125
14	66164114	rs80823440	A	intron_variant	MODIFIER	-	ENSSSCG00000045125
14	66217364	rs80790504	A	intron_variant	MODIFIER	-	ENSSSCG00000045125
14	66346934	rs80857308	G	intergenic_variant	MODIFIER	-	-
14	66411511	rs332630058	G	intergenic_variant	MODIFIER	-	-
14	66463802	rs80992681	A	intron_variant	MODIFIER	-	ENSSSCG00000050044
14	66511894	rs80790167	A	intergenic_variant	MODIFIER	-	-
14	66601592	rs80892283	G	intron_variant	MODIFIER	*NRBF2*	ENSSSCG00000024081
14	66650724	rs80806783	A	synonymous_variant	LOW	*JMJD1C*	ENSSSCG00000010226
14	66774513	rs80784829	G	intron_variant	MODIFIER	*JMJD1C*	ENSSSCG00000010226
14	67039033	rs80929206	A	intron_variant	MODIFIER	*REEP3*	ENSSSCG00000036476
14	67093823	rs80883807	A	intergenic_variant	MODIFIER	-	-
14	67147012	rs80917091	A	intergenic_variant	MODIFIER	-	-
14	67179643	rs80953180	A	intergenic_variant	MODIFIER	-	-
14	67221608	rs80855993	G	intergenic_variant	MODIFIER	-	-
14	67243311	rs80842796	A	intergenic_variant	MODIFIER	-	-
14	67269867	rs80958297	A	intergenic_variant	MODIFIER	-	-

14	67334411	rs80955072	A	intergenic_variant	MODIFIER	-	-
14	67342053	rs80935088	A	intergenic_variant	MODIFIER	-	-
14	67409551	rs80993577	A	non_coding	MODIFIER	-	ENSSSCG00000055826
14	67429030	rs81001094	G	intergenic_variant	MODIFIER	-	-
14	67443330	rs80867759	A	upstream_variant	MODIFIER	-	ENSSSCG00000057010
14	67864215	rs80782492	A	intergenic_variant	MODIFIER	-	-
14	67985525	rs80898366	G	intergenic_variant	MODIFIER	-	-
14	68132098	rs343967944	A	intergenic_variant	MODIFIER	-	-
14	68569202	rs80836807	G	intergenic_variant	MODIFIER	-	-
14	68649520	rs80910560	C	intergenic_variant	MODIFIER	-	-
14	68668578	rs80941150	A	intergenic_variant	MODIFIER	-	-
14	68712952	rs80869833	C	intergenic_variant	MODIFIER	-	-
14	68966102	rs329548237	G	intergenic_variant	MODIFIER	-	-
14	69037864	rs80791549	G	intergenic_variant	MODIFIER	-	-
14	69076616	rs327510211	G	intergenic_variant	MODIFIER	-	-
14	69112868	rs80927258	G	intergenic_variant	MODIFIER	-	-
14	69290179	rs80882860	A	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	69302116	rs324206983	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	69555273	rs81450994	A	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	69576192	rs81450997	A	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	69589926	rs81451001	A	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	69672751	rs80906784	A	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	69721367	rs80980794	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	69743473	rs80968726	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	69814326	rs80912747	C	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	69865089	rs80782356	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	69888948	rs80916352	A	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480

14	69965414	rs80901895	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70036197	rs80909540	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70068946	rs80934118	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70099607	rs80878343	A	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70113426	rs80997255	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70175904	rs80858025	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70216594	rs80798857	A	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70250940	rs80974369	A	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70268234	rs320089046	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70282756	rs80954290	A	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70304464	rs80826444	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70316991	rs80991185	A	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70334131	rs80943996	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70360906	rs80943789	G	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	70394534	rs81320173	A	intron_variant	MODIFIER	*CTNNA3*	ENSSSCG00000036480
14	71463045	rs81267540	G	intron_variant	MODIFIER	*PBLD*	ENSSSCG00000025876
14	71530810	rs80964645	A	intron_variant	MODIFIER	*RUFY2*	ENSSSCG00000010239
14	71578222	rs80905037	A	intron_variant	MODIFIER	*DNA2*	ENSSSCG00000010240
14	71651905	rs80961472	A	upstream_variant	MODIFIER	*SLC25A16*	ENSSSCG00000022303
14	71926501	rs81451202	G	upstream_variant	MODIFIER	*STOX1*	ENSSSCG00000010245
14	71996220	rs80956623	G	intron_variant	MODIFIER	*DDX50*	ENSSSCG00000010246
14	72025065	rs330051345	A	synonymous_variant	LOW	*DDX50*	ENSSSCG00000010246
14	72086975	rs80850507	A	intron_variant	MODIFIER	*KIFBP*	ENSSSCG00000010248
14	72160465	rs80970697	G	intergenic_variant	MODIFIER	-	-
14	72304198	rs80895291	A	intron_variant	MODIFIER	*HKDC1*	ENSSSCG00000010252
14	72454977	rs80984932	A	intron_variant	MODIFIER	*HK1*	ENSSSCG00000010253
14	72657517	rs81322238	C	intergenic_variant	MODIFIER	-	-

14	72681841	rs81306475	A	intergenic_variant	MODIFIER	-	-
14	73455311	rs80998089	A	intron_variant	MODIFIER	*PALD1*	ENSSSCG00000036446
14	73549191	rs80952995	C	intron_variant	MODIFIER	*ADAMTS14*	ENSSSCG00000010272
14	73915912	rs80972489	C	upstream_variant	MODIFIER	*U6*	ENSSSCG00000063002
14	73938018	rs80858413	A	intergenic_variant	MODIFIER	-	-
14	74005557	rs80916750	G	intergenic_variant	MODIFIER	-	-
14	82129423	rs80799556	A	upstream_variant	MODIFIER	*MAT1A*	ENSSSCG00000010337
14	82159674	rs80890286	G	intron_variant	MODIFIER	*DYDC1*	ENSSSCG00000061214
14	102414475	rs80856108	C	intergenic_variant	MODIFIER	-	-
15	74207778	rs80874418	G	intergenic_variant	MODIFIER	-	-
15	74223043	rs80875726	C	downstream_variant	MODIFIER	-	ENSSSCG00000048880
15	74235458	rs80850172	A	intergenic_variant	MODIFIER	-	-
15	93185092	rs81301298	G	intron_variant	MODIFIER	*GULP1*	ENSSSCG00000016033
15	94478426	rs333806503	G	intron_variant	MODIFIER	*C2orf88*	ENSSSCG00000033941
15	94737785	rs81478831	G	intron_variant	MODIFIER	-	ENSSSCG00000041649
15	94765099	rs81478871	C	intron_variant	MODIFIER	-	ENSSSCG00000041649
15	94839330	rs337294454	G	intron_variant	MODIFIER	-	ENSSSCG00000041649
15	94923471	rs80816554	G	intron_variant	MODIFIER	*HIBCH*	ENSSSCG00000016049
16	21884754	rs81457035	G	intron_variant	MODIFIER	*SLC1A3*	ENSSSCG00000016841
16	22998327	rs81345264	G	intergenic_variant	MODIFIER	-	-
17	33547292	rs80956481	G	intergenic_variant	MODIFIER	-	-
18	8938737	rs81477067	A	downstream_variant	MODIFIER	-	ENSSSCG00000041343
2	113333168	rs81362780	C	intron_variant	MODIFIER	*FBXL17*	ENSSSCG00000014190
2	145424076	rs81366970	A	intergenic_variant	MODIFIER	-	-
3	126863515	rs81224446	A	intron_variant	MODIFIER	*IAH1*	ENSSSCG00000008640
4	111702541	rs81380400	G	intron_variant	MODIFIER	-	ENSSSCG00000041066
4	119798655	rs80985433	G	non_coding	MODIFIER	-	ENSSSCG00000045465

5	27631915	rs81334652	G	intergenic_variant	MODIFIER	-	-
5	28327581	rs80819392	G	intron_variant	MODIFIER	*SRGAP1*	ENSSSCG00000029815
6	17459517	rs81391557	C	intergenic_variant	MODIFIER	-	-
6	45156998	rs337498531	G	synonymous_variant	LOW	*KMT2B*	ENSSSCG00000002895
6	45447973	rs81395741	A	intron_variant	MODIFIER	*WDR62*	ENSSSCG00000002923
6	45460146	rs329941598	A	splice_donor_variant	LOW	*WDR62*	ENSSSCG00000002923
6	45595002	rs81325388	G	intron_variant	MODIFIER	*ZNF146*	ENSSSCG00000025355
6	45671421	rs81395814	A	intergenic_variant	MODIFIER	-	-
6	45923403	rs81226690	A	intron_variant	MODIFIER	*ZNF566*	ENSSSCG00000029170
6	46126442	rs81250717	A	intron_variant	MODIFIER	*ZNF829*	ENSSSCG00000063057
6	46387903	rs81476008	G	intron_variant	MODIFIER	*ZNF569*	ENSSSCG00000029420
6	99307882	rs81347503	A	intergenic_variant	MODIFIER	-	-
6	99339946	rs81337232	G	intergenic_variant	MODIFIER	-	-
6	99580947	rs81277378	A	intron_variant	MODIFIER	*PTPRM*	ENSSSCG00000028805
6	129477727	rs81476249	A	intergenic_variant	MODIFIER	-	-
6	129734771	rs81391421	G	intron_variant	MODIFIER	*TTLL7*	ENSSSCG00000003760
6	132846185	rs81391604	A	intergenic_variant	MODIFIER	-	-
7	10724105	rs81000135	G	downstream_variant	MODIFIER	-	ENSSSCG00000052083
7	90911258	rs340169919	A	intron_variant	MODIFIER	*GPHN*	ENSSSCG00000002285
7	91673905	rs80940210	A	intron_variant	MODIFIER	*RAD51B*	ENSSSCG00000028877
7	92399480	rs80896235	G	intergenic_variant	MODIFIER	-	-
7	105856850	rs81223355	G	intergenic_variant	MODIFIER	-	-
7	105892246	rs80823303	A	downstream_variant	MODIFIER	-	ENSSSCG00000060880
8	22813218	rs81301715	C	intergenic_variant	MODIFIER	-	-
8	25554848	rs80812211	A	intergenic_variant	MODIFIER	-	-
8	39669734	rs81477007	A	intergenic_variant	MODIFIER	-	-
8	79507941	rs80840907	A	non_coding	MODIFIER	-	ENSSSCG00000055701

8	79839602	rs81401770	A	intergenic_variant	MODIFIER	-	-
8	79853747	rs81401771	G	intergenic_variant	MODIFIER	-	-
8	124785626	rs81404194	G	intergenic_variant	MODIFIER	-	-
8	131373563	rs81247816	C	intergenic_variant	MODIFIER	-	-
8	131452301	rs81211121	G	missense_variant	MODERATE	*NUDT9*	ENSSSCG00000053724
8	134933342	rs81269758	A	intron_variant	MODIFIER	*GPAT3*	ENSSSCG00000009233
9	8362141	rs81413811	G	intron_variant	MODIFIER	*UCP2*	ENSSSCG00000014833
9	8406364	rs81413868	A	intron_variant	MODIFIER	*C2CD3*	ENSSSCG00000014835
9	28657220	rs81259127	A	intergenic_variant	MODIFIER	-	-
9	138952691	rs81419738	G	intergenic_variant	MODIFIER	-	-
8	134733478	rs81339371	A	intergenic_variant	MODIFIER	-	-
8	134763128	rs81331248	A	intergenic_variant	MODIFIER	-	-
8	134933342	rs81269758	A	intron_variant	MODIFIER	*GPAT3*	ENSSSCG00000009233
8	135212871	rs81405675	A	intron_variant	MODIFIER	-	ENSSSCG00000009239
8	135491186	rs81336088	G	intron_variant	MODIFIER	*SEC31A*	ENSSSCG00000009244
8	135550523	rs81344869	A	intron_variant	MODIFIER	*SCD5*	ENSSSCG00000009245
8	135604963	rs81328785	A	intron_variant	MODIFIER	*SCD5*	ENSSSCG00000009245
8	135607348	rs81339189	G	intron_variant	MODIFIER	*SCD5*	ENSSSCG00000009245

7.2. Supplementary material Paper II: "Expression quantitative trait loci (eQTL) identification and its relationship with lipid metabolism in pig muscle"

Table S1: Gene ontology biological terms of eQTL–associated genes.

FDR	nGenes	Assoc.	Pathway	Genes
1,50E-08	92	1079	Small molecule metabolic process	*MCAT ACO2 SLC25A17 ADSL ALDH5A1 AARS2 PLA2G7 FAH EFL1 ARG2 RDH11 APRT LDHD LIPE APOE NMNAT1 PEX7 GATM CLN6 NAXE GBA SARS1 PEX13 GALM ACOX3 NAAA COQ2 PTK2B EPHX2 CBR4 ACADS AIFM2 PCBD1 ADK MTARC2 EPHX1 PYCR2 HACL1 OXSM ACAA1 NME6 NIT2 BAD ASRGL1 LARS1 RRM1 ASNS LPGAT1 ATIC CYP27A1 FARSB AASS FAM3C PRPSAP2 DHCR7 PTGR1 BDH2 ACO1 ALDH4A1 THNSL2 COQ9 ALDH1A2 TARS2 ADCY5 FH ADI1 GDE1 AKR1A1 PON3 DHFR MLYCD MGST2 LOC110261142 AK2 NUDT16 SLC39A14 PNPO GPX1 LOC110255953 ATP5F1C COQ7 ATP5F1D ITPA INSIG2 ATPSCKMT RBKS PHYH ERLIN2 GCSH LRP2 CBS IPMK*
7,05E-06	52	536	Oxoacid metabolic process	*MCAT ACO2 SLC25A17 ALDH5A1 AARS2 FAH ARG2 LDHD PEX7 GATM SARS1 PEX13 ACOX3 NAAA CBR4 ACADS MTARC2 EPHX1 PYCR2 HACL1 OXSM ACAA1 NIT2 ASRGL1 LARS1 ASNS LPGAT1 CYP27A1 FARSB AASS PTGR1 BDH2 ACO1 ALDH4A1 THNSL2 ALDH1A2 TARS2 FH ADI1 AKR1A1 PON3 DHFR MLYCD MGST2 LOC110261142 GPX1 LOC110255953 INSIG2 PHYH*
7,28E-06	51	527	Carboxylic acid metabolic process	*MCAT ACO2 SLC25A17 ALDH5A1 AARS2 FAH ARG2 LDHD PEX7 GATM SARS1 PEX13 ACOX3 NAAA CBR4 ACADS EPHX1 PYCR2 HACL1 OXSM ACAA1 NIT2 ASRGL1 LARS1 ASNS LPGAT1 CYP27A1 FARSB AASS PTGR1 BDH2 ACO1 ALDH4A1 THNSL2 ALDH1A2 TARS2 FH ADI1 AKR1A1 PON3 DHFR MLYCD MGST2 LOC110261142 GPX1 LOC110255953 INSIG2 PHYH ERLIN2*
1,15E-05	52	555	Organic acid metabolic process	*MCAT ACO2 SLC25A17 ALDH5A1 AARS2 FAH ARG2 LDHD PEX7 GATM SARS1 PEX13 ACOX3 NAAA CBR4 ACADS MTARC2 EPHX1 PYCR2 HACL1 OXSM ACAA1 NIT2 ASRGL1 LARS1 ASNS LPGAT1 CYP27A1 FARSB AASS PTGR1 BDH2 ACO1 ALDH4A1 THNSL2 ALDH1A2 TARS2 FH ADI1 AKR1A1 PON3 DHFR MLYCD MGST2 LOC110261142 GPX1 LOC110255953 INSIG2 PHYH ERLIN2 GCSH*
0,000634	79	1134	Organonitrogen compound biosynthetic process	*ADSL RXYLT1 MOCS1 MRPL14 AARS2 GALNT16 APRT LSM14A APOE NMNAT1 ZDHHC18 RMND1 GATM ALG2 ST6GALNAC4 PTDSS1 PRKDC GBA SARS1 PLCB1 TUFM PCBD1 ADK COX15 PYCR2 DAPK1 NME6 ATG7 PIGP LTO1 LARS1 ASNS EIF2D ATIC FARSB MOCS2 MRPL22 MPDU1 B3GAT3 KHDRBS1 ACO1 LOC100738836 TARS2 PIGX ADCY5 SECISBP2 MRPL9 LOC100739087 ADI1 EXTL1 RPS9 DHFR MLYCD MGST2 LOC110261142 AK2 MRPL37 EIF1AX POGLUT1 B3GNT8 CERS6 PNPO MRPL12 DHDDS ATP5F1C RPL13A ZDHHC14 ATP5F1D ATPSCKMT DPH2 MTIF3 DHX33 PIGH PIGG LOC100519675 CBS*

0,000634	32	310	Small molecule biosynthetic	*MCAT APRT APOE GATM GBA COQ2 PTK2B CBR4 PCBD1 ADK PYCR2 OXSM ASNS LPGAT1 CYP27A1 FAM3C DHCR7 THNSL2 COQ9 ALDH1A2 ADI1 AKR1A1 DHFR MLYCD MGST2 SLC39A14 PNPO COQ7 INSIG2 ERLIN2 CBS IPMK*
0,000634	19	130	Organic acid catabolic process	*SLC25A17 ALDH5A1 FAH LDHD PEX7 PEX13 ACOX3 ACADS HACL1 ACAA1 ASRGL1 AASS BDH2 ALDH4A1 THNSL2 AKR1A1 PON3 PHYH*
0,00079	51	631	Cellular lipid metabolic process	*MCAT NAGA SLC25A17 PLA2G7 RDH11 APOE PEX7 SMPD2 CLN6 ALG2 PTDSS1 GBA PLCB1 PEX13 ACOX3 NAAA COQ2 EPHX2 CBR4 ACADS PSAP EPHX1 PIP4K2A HACL1 OXSM ACAA1 PIGP LPGAT1 MPDU1 PTGR1 BDH2 SERINC2 THNSL2 ALDH1A2 PIGX PLCD1 GDE1 MLYCD MGST2 SOCS6 CERS6 DHDDS GPX1 D2HGDH INSIG2 PHYH PIGH ERLIN2 PIGG CAV3*
0,00136	62	849	Lipid metabolic process	*MCAT NAGA SLC25A17 LOC100626199 PLA2G7 RDH11 MBTPS1 LIPE APOE PEX7 SMPD2 CLN6 ALG2 PTDSS1 LYN GBA PLCB1 PEX13 ACOX3 NAAA COQ2 PTK2B EPHX2 CBR4 ACADS PSAP EPHX1 PIP4K2A HACL1 OXSM ACAA1 PIGP GDPD5 LPGAT1 CYP27A1 FAXDC2 MPDU1 DHCR7 PTGR1 BDH2 SERINC2 FADS1 THNSL2 ALDH1A2 PIGX PLCD1 GDE1 MLYCD MGST2 SOCS6 CERS6 DHDDS GPX1 D2HGDH INSIG2 ERG28 PHYH PIGH ERLIN2 PIGG*
0,00136	18	128	Carboxylic acid catabolic process	*SLC25A17 ALDH5A1 FAH LDHD PEX7 PEX13 ACOX3 ACADS HACL1 ACAA1 ASRGL1 AASS BDH2 ALDH4A1 AKR1A1 PON3 PHYH GCSH*
0,00136	6	12	Antigen processing and presentation	*TAPBPL ZBTB22 B2M IDE ERAP1 ERAP2*
0,002542252	23	203	Small molecule catabolic process	*SLC25A17 ALDH5A1 FAH LDHD APOE PEX7 PEX13 ACOX3 ACADS HACL1 ACAA1 BAD ASRGL1 CYP27A1 AASS BDH2 ALDH4A1 THNSL2 AKR1A1 PON3 RBKS PHYH GCSH*
0,003968	5	9	Antigen processing MHC class I	*ZBTB22 B2M IDE ERAP1 ERAP2*
0,004109	21	182	Cellular amino acid metabolic	*ALDH5A1 AARS2 FAH ARG2 SARS1 PYCR2 NIT2 ASRGL1 LARS1 ASNS FARSB AASS ALDH4A1 THNSL2 TARS2 ADI1 DHFR LOC110261142 LOC110255953 GCSH CBS*
0,004900	76	1168	Regulation of catalytic activity	*CD4 DDX11 APAF1 TBC1D7 RGMA SIPA1L1 APOE PIH1D1 DVL1 DFFA MAP3K4 GNB5 LYN GBA SORT1 BCAR3 TPX2 SRC MSH2 RASGRP3 SCARB2 SFRP2 ABCE1 PTK2B PSAP BCCIP AIDA DAPK1 PIP4K2A CCNY RFC4 BAD TRPT1 ECSIT DNAJB1 AP3B1 SH3RF2 LARS1 SIRT3 PAK1 HDAC9 WRN COPS8 SSBP1 ZNF622 CCDC125 STRADA TOM1L1 RABEP1 B3GAT3 MSH6 NET1 UBXN1 RIPK2 DVL2 MON1A EPHB3 RASIP1 DHFR PSENEN PLAUR SLC39A14 BOK BAG2 GPX1 SH3BP4 CHP2 ATPSCKMT EREG SERPINB6 PPP1R2 SLPI*
0,005313	11	59	Antigen processing and presentation	*TAPBPL SLA-8 SLA-DRB1 ZBTB22 B2M GBA IDE AP3B1 ERAP1 ERAP2 RAB4A*

0,005905	82	1299	Cellular catabolic process	*NAGA SLC25A17 ALDH5A1 PLA2G7 FAH RGMA LDHD APOE DVL1 DFFA MFN2 PEX7 SMPD2 CLN6 RNF20 VPS28 GBA VPS16 RBCK1 WDR24 AUP1 PEX13 ACOX3 SCARB2 USP12 PTK2B EPHX2 ACADS LGALS8 IDE XPNPEP1 ZRANB1 DAPK1 AGTPBP1 PIP4K2A HACL1 NGLY1 ACAA1 TEX264 ATG7 ASRGL1 SH3RF2 ZFAND2B AASS GAA BDH2 UBXN1 PNRC2 RIPK2 FASTK ALDH4A1 THNSL2 CARHSP1 TMEM259 SECISBP2 FBXL7 RASIP1 AKR1A1 PON3 ACHE DDA1 MLYCD MGST2 SOCS6 NUDT16 GNA12 BOK VTI1A PSMA7 BAG2 GPX1 SH3BP4 HNMT ITPA DNASE1 SIAH1 RNF34 PHYH ERLIN2*
0,00769	11	62	Lipid oxidation	*SLC25A17 PLA2G7 PEX7 PEX13 ACOX3 ACADS HACL1 ACAA1 BDH2 MLYCD PHYH*
0,009315	5	11	Antigen processing	*ZBTB22 B2M IDE ERAP1 ERAP2*
0,010277	21	198	Fatty acid metabolic process	*MCAT SLC25A17 PEX7 PEX13 ACOX3 NAAA CBR4 ACADS EPHX1 HACL1 OXSM ACAA1 LPGAT1 PTGR1 BDH2 THNSL2 MLYCD GPX1 INSIG2 PHYH ERLIN2*
0,010309	14	101	Aerobic respiration	*ACO2 NDUFB9 MSH2 IDE UQCRC1 SIRT3 SDHA COQ9 FH ATP5F1C ATP5F1D SURF1 ATPSCKMT NDUFS8*
0,011516	11	66	Monocarboxylic acid catabolic	*SLC25A17 LDHD PEX7 PEX13 ACOX3 ACADS HACL1 ACAA1 BDH2 AKR1A1 PHYH*
0,012550	76	1220	Cellular response to stress	*DDX11 DEK TDP2 GNL1 CMTR1 SRF RGMA ARG2 MFN2 TXNDC12 MAP3K4 B2M LYN PRKDC GBA CHD1L SLC25A24 MCM8 PLCB1 SRC NFATC2 WDR24 AUP1 MSH2 SFRP2 PTK2B AIFM2 ANKRD1 BCCIP MTARC2 AIDA DAPK1 TEX264 NEK4 FANCD2 ATG7 BAD DNAJB1 PIK3R2 HDAC3 SH3RF2 LARS1 PAK1 SDHD ASNS INTS7 PRIMPOL WRN ZNF622 RAD1 ERCC8 MSH6 NET1 UBXN1 RSL1D1 SCARF1 RIPK2 BDKRB2 TMEM259 HUS1 PTPRS NDNF DHFR BOK GPX1 SMARCAL1 INSIG2 RWDD3 PPP4R2 RNF34 SERPINB6*
0,012550	18	159	Carboxylic acid biosynthetic process	*MCAT GATM CBR4 PYCR2 OXSM ASNS LPGAT1 CYP27A1 THNSL2 ALDH1A2 ADI1 AKR1A1 DHFR MLYCD MGST2 INSIG2 ERLIN2 CBS*
0,012550	3	3	Positive regulation of transcription	*MRTFA SRF MICAL2*
0,012725	18	160	Organic acid biosynthetic process	*MCAT GATM CBR4 PYCR2 OXSM ASNS LPGAT1 CYP27A1 THNSL2 ALDH1A2 ADI1 AKR1A1 DHFR MLYCD MGST2 INSIG2 ERLIN2 CBS*
0,014881	97	1671	Intracellular signal transduction	*CD4 TBC1D7 NFKBIL1 SIPA1L1 CDH13 LSM14A APOE PIH1D1 DVL1 MFN2 PDPN SSX2IP TXNDC12 RPS6KA2 MAP3K4 ARHGAP18 ZWILCH LYN PRKDC GBA BCAR3 PLCB1 RBCK1 SRC NFATC2 WDR24 MSH2 RASGRP3 SFRP2 EDNRB DOCK5 KCTD9 PTK2B DUSP29 ANKRD1 SHOC2 AIDA CDC42BPA DAPK1 BRK1 BAD TRIM44 WDR83 TMEM38A HBEGF HDAC3 SH3RF2 LARS1 ANAPC15 PAK1 ZW10 INTS7 COPS8 ASB1 ZNF622 RAD1 CCDC125 SEC14L1 NEK8 MSH6 LOC100513863 NOC2L RIPK2 SH3BP5 TEAD2 DVL2 LOC100738836 BDKRB2*

				PLCD1 ADCY5 HUS1 RASIP1 NDNF CD2AP PRKCZ CHML SOCS6 PLAUR GNA12 SLC39A14 BOK AKIP1 GPX1 TBXA2R SH3BP4 CHP2 C1QBP RAC1 RAB4A RFXANK SIAH1 AXL DHX33 CAV3 HERPUD1 LRP2 CBS
0,014881	53	775	Protein transport	*PICK1 CAPRIN2 MBTPS1 APOE MFN2 PEX7 VPS28 SORT1 VPS16 TOMM34 AUP1 PEX13 SCARB2 COG2 ANKRD1 GGA2 MIA3 HACL1 ATG7 COPB2 BAD PIK3R2 CLTB AP3B1 HDAC3 SYTL2 ICA1 ZFAND2B STRADA SNX11 TOM1L1 C12H17orf75 RABEP1 B3GAT3 ZFAND1 MON1A LMAN2L ADCY5 COG4 SLC9B2 SAMM50 ARCN1 CD2AP CHML VTI1A CHP2 RAB4A RPH3AL E2F3 HERPUD1 LRP2*
0,014881	35	441	Regulation of cellular response to stress	*DDX11 DEK RGMA ARG2 TXNDC12 MAP3K4 B2M LYN PRKDC PLCB1 SFRP2 PTK2B AIFM2 ANKRD1 AIDA NEK4 BAD HDAC3 SH3RF2 ZNF622 UBXN1 RSL1D1 SCARF1 RIPK2 BDKRB2 TMEM259 PTPRS DHFR BOK GPX1 INSIG2 PPP4R2 SPIRE1 CAV3 HERPUD1*
0,014881	6	20	Quinone metabolic process	*COQ2 CBR4 AIFM2 COQ9 AKR1A1 COQ7*
0,014881	5	13	Antigen processing	*ZBTB22 B2M IDE ERAP1 ERAP2*
0,015780	14	109	Alpha-amino acid metabolic	*ALDH5A1 FAH ARG2 PYCR2 NIT2 ASRGL1 ASNS AASS ALDH4A1 THNSL2 ADI1 DHFR GCSH CBS*
0,016599	89	1512	Catabolic process	*NAGA SLC25A17 ALDH5A1 NEU1 PLA2G7 FAH RGMA LDHD LIPE APOE DVL1 DFFA MFN2 PEX7 SMPD2 CLN6 RNF20 VPS28 GBA PLCB1 VPS16 RBCK1 WDR24 AUP1 PEX13 ACOX3 SCARB2 USP12 PTK2B EPHX2 ACADS LGALS8 IDE XPNPEP1 ZRANB1 DAPK1 AGTPBP1 PIP4K2A HACL1 NGLY1 ACAA1 TEX264 ATG7 BAD ASRGL1 SH3RF2 CYP27A1 ZFAND2B AASS GAA BDH2 PTER UBXN1 PNRC2 RIPK2 FASTK ALDH4A1 THNSL2 CARHSP1 TMEM259 SECISBP2 FBXL7 RASIP1 AKR1A1 PON3 ACHE DDA1 MLYCD MGST2 SOCS6 NUDT16 GNA12 BOK VTI1A PSMA7 BAG2 GPX1 SH3BP4 HNMT ITPA DNASE1 SIAH1 RNF34 RBKS PHYH ERLIN2 GCSH*
0,016599	10	60	Fatty acid oxidation	*SLC25A17 PEX7 PEX13 ACOX3 ACADS HACL1 ACAA1 BDH2 MLYCD PHYH*
0,019242	21	215	Positive regulation of proteolysis	*APAF1 RGMA APOE DVL1 CLN6 LYN GBA SRC SFRP2 PTK2B DAPK1 AGTPBP1 BAD SPON1 SH3RF2 RIPK2 TMEM259 PSENEN BOK BAG2 HERPUD1*
0,019485	40	544	Response to nitrogen compound	*ITPR2 DDX11 CIB2 CDH13 APLP1 APOE GNB5 PRKDC SORT1 BCAR3 PLCB1 SRC AUP1 PPP2R2A MTARC2 MIA3 ZEB1 PIP4K2A ECHDC3 TMEM38A PIK3R2 DIAPH1 LARS1 HDAC9 P2RX5 UBXN1 RIPK2 LOC100738836 TMEM259 ATP2B1 ADCY5 ACHE PRKCZ SLC39A14 TRIM16 SH3BP4 ERLIN2 HERPUD1 CBS SNX6*
0,020440	27	315	Monocarboxylic acid metabolic process	*MCAT SLC25A17 ALDH5A1 LDHD PEX7 GATM PEX13 ACOX3 NAAA CBR4 ACADS EPHX1 HACL1 OXSM ACAA1 LPGAT1 CYP27A1 PTGR1 BDH2 THNSL2 ALDH1A2 AKR1A1 MLYCD GPX1 INSIG2 PHYH*

0,020772	44	622	Regulation of hydrolase activity	*DDX11 APAF1 TBC1D7 RGMA SIPA1L1 PIH1D1 DFFA GNB5 LYN SORT1 BCAR3 SRC RASGRP3 SCARB2 SFRP2 ABCE1 PSAP DAPK1 BAD DNAJB1 SH3RF2 LARS1 HDAC9 WRN CCDC125 RABEP1 NET1 UBXN1 RIPK2 DVL2 EPHB3 RASIP1 PSENEN PLAUR SLC39A14 BOK GPX1 SH3BP4 CHP2 SERPINB6 PPP1R2 SLPI*
0,020772	41	567	Protein localization to organelle	*MBTPS1 RUVBL2 PIH1D1 DVL1 MFN2 PEX7 ZWILCH VPS28 SORT1 SRC TOMM34 SUN1 AUP1 PEX13 MSH2 SCARB2 MIA3 HACL1 PACS1 PIK3R2 AP3B1 DIAPH1 HDAC3 C2CD3 ZW10 WRN ZFAND2B TMEM98 RABEP1 DLG4 MON1A FAM149B1 ARL6 SAMM50 CD2AP SH3BP4 CHP2 INSIG2 MARCHF5 E2F3 HERPUD1*
0,020772	37	493	Response to organonitrogen compound	*ITPR2 DDX11 CIB2 CDH13 APLP1 APOE GNB5 PRKDC SORT1 BCAR3 SRC AUP1 PPP2R2A MIA3 ZEB1 PIP4K2A ECHDC3 TMEM38A PIK3R2 DIAPH1 LARS1 HDAC9 P2RX5 UBXN1 RIPK2 LOC100738836 TMEM259 ATP2B1 ADCY5 PRKCZ SLC39A14 TRIM16 SH3BP4 ERLIN2 HERPUD1 CBS SNX6*
0,024476	56	862	Protein localization	*PICK1 CAPRIN2 MBTPS1 APOE RUVBL2 PIH1D1 MFN2 PEX7 VPS28 SORT1 VPS16 TOMM34 AUP1 PEX13 SCARB2 COG2 ANKRD1 GGA2 MIA3 HACL1 ATG7 COPB2 BAD PIK3R2 CLTB AP3B1 HDAC3 SYTL2 ICA1 ZFAND2B STRADA SNX11 TOM1L1 C12H17orf75 RABEP1 B3GAT3 DLG4 ZFAND1 MON1A LMAN2L ADCY5 COG4 SLC9B2 SAMM50 ARCN1 CD2AP CHML VTI1A CHP2 RAB4A RPH3AL E2F3 HERPUD1 LRP2 SNX6*
0,024476	32	408	Regulation of proteolysis	*APAF1 RGMA APOE PIH1D1 DVL1 CLN6 LYN GBA SRC SFRP2 PTK2B DAPK1 AGTPBP1 BAD SPON1 SH3RF2 C2CD3 TMEM98 UBXN1 RIPK2 TMEM259 DDA1 PSENEN PLAUR GNA12 BOK BAG2*
0,025131	27	322	Organophosphate biosynthetic process	*ADSL MOCS1 NMNAT1 PTDSS1 PTK2B ADK PIP4K2A NME6 PIGP RRM1 ATIC MOCS2 PRPSAP2 PIGX ADCY5 MLYCD AK2 SOCS6 PNPO DHDDS ATP5F1C ATP5F1D ATPSCKMT PIGH PIGG IPMK*
0,025245	100	1778	Cellular component assembly	*PARVB TTLL1 TAPBPL AMIGO2 POC1B TBC1D7 SRF EFL1 CDH13 TMEM231 IRX3 LSM14A APOE PIH1D1 FUZ DVL1 WRAP73 MFN2 SF3A3 SSX2IP EPS15 ARHGAP18 B2M POLR1E PSMD5 NDUFB9 NDUFAF6 PRKDC GBA TPX2 SRC CIAO1 AUP1 FAM161A FAM98A TAF1B REEP4 KCTD9 PTK2B CBR4 LOC100512926 HPS1 BCCIP AIDA MSRB2 PIP4K2A ARPC4 BRK1 IQCB1 CDKL5 BAD CKAP5 DNAJB1 PIK3R2 NDUFA2 DIAPH1 HDAC3 RIC3 RRM1 C2CD3 PAK1 ZW10 EIF2D COPS8 ZYX ABCA7 NOC2L DLG4 SNRPG BBS2 TEAD2 ALDH1A2 EPHB3 FAM149B1 ARL6 NDUFA11 FHOD3 SAMM50 PTPRS RASIP1 SCLT1 PRKCZ CCDC28B BOK C1QBP RAC1 JCHAIN ATP5F1D SURF1 MFAP4 DNAH17 NDUFS8 EML2 LRRC8D DHX33 SSBP3 COX20 SPIRE1 CAV3 TMEM199*
0,025245	3	4	Negative regulation of membrane	*SRC BOK CAV3*
0,025245	3	4	Asparagine metabolic process	*NIT2 ASRGL1 ASNS*
0,027570	109	1979	Phosphate-containing	*ADSL TAB1 CD4 MOCS1 PLA2G7 EFL1 APOE PIH1D1 DVL1 CDK11B NMNAT1 RPS6KA2 MAP3K4 SMPD2 MUSK PTDSS1 LYN PRKDC NAXE GBA CDC14A BCAR3 RPAP2 PLCB1 TPX2 SRC TOP1 EEF2K RPIA NAAA SFRP2 CDK20 PPP2R2A PTK2B EPHX2 ADK DUSP29 BCCIP AIDA CDC42BPA DAPK1 PIP4K2A CCNY OXSM*

			compound process	*NME6 NEK4 PIGP CDKL5 GRK2 BAD TRPT1 HTATIP2 PGLS HBEGF HDAC3 SH3RF2 RRM1 PAK1 ATIC COPS8 ZNF622 MOCS2 FAXDC2 STRADA TOM1L1 NEK8 PRPSAP2 PPA2 SERINC2 RIPK2 FASTK THNSL2 DVL2 EPHB3 PIGX BDKRB2 PLCD1 ADCY5 PTPRS RASIP1 GDE1 PRKCZ MLYCD AK2 SOCS6 NUDT16 PLAUR PNPO DHDDS DUSP23 ATP5F1C CHP2 RAC1 ATP5F1D ITPA ATPSCKMT RBKS EREG AXL PPP1R2 PIGH TWSG1 PIGG CAV3 CBS IPMK*
0,028350	76	1279	Organic substance catabolic	*NAGA SLC25A17 ALDH5A1 NEU1 PLA2G7 FAH RGMA LDHD LIPE APOE DVL1 DFFA PEX7 SMPD2 CLN6 RNF20 VPS28 GBA PLCB1 RBCK1 AUP1 PEX13 ACOX3 SCARB2 USP12 PTK2B EPHX2 ACADS IDE XPNPEP1 ZRANB1 AGTPBP1 HACL1 NGLY1 ACAA1 ATG7 BAD ASRGL1 SH3RF2 CYP27A1 ZFAND2B AASS GAA BDH2 UBXN1 PNRC2 FASTK ALDH4A1 THNSL2 CARHSP1 TMEM259 SECISBP2 FBXL7 AKR1A1 PON3 ACHE DDA1 MLYCD MGST2 SOCS6 NUDT16 GNA12 PSMA7 BAG2 GPX1 HNMT ITPA DNASE1 SIAH1 RNF34 RBKS PHYH ERLIN2 GCSH HERPUD1 TMEM199*
0,030171	44	640	Cellular response to oxygen-	*ITPR2 DDX11 NFKBIL1 CIB2 RDH11 GRAMD1A APLP1 RUVBL2 PIH1D1 GNB5 LYN PRKDC BCAR3 PLCB1 SRC PTK2B ANKRD1 DAPK1 ZEB1 PIP4K2A ECHDC3 BAD PIK3R2 LARS1 HDAC9 NET1 RIPK2 ZFAND1 TEAD2 ALDH1A2 LOC100738836 NFKBIB ATP2B1 ADCY5 SLC9B2 AKR1A1 ACHE PRKCZ DHFR SLC39A14 SH3BP4*
0,031177	109	1991	Phosphorus metabolic process	*ADSL TAB1 CD4 MOCS1 PLA2G7 EFL1 APOE PIH1D1 DVL1 CDK11B NMNAT1 RPS6KA2 MAP3K4 SMPD2 MUSK PTDSS1 LYN PRKDC NAXE GBA CDC14A BCAR3 RPAP2 PLCB1 TPX2 SRC TOP1 EEF2K RPIA NAAA SFRP2 CDK20 PPP2R2A PTK2B EPHX2 ADK DUSP29 BCCIP AIDA CDC42BPA DAPK1 PIP4K2A CCNY OXSM NME6 NEK4 PIGP CDKL5 GRK2 BAD TRPT1 HTATIP2 PGLS HBEGF HDAC3 SH3RF2 RRM1 PAK1 ATIC COPS8 ZNF622 MOCS2 FAXDC2 STRADA TOM1L1 NEK8 PRPSAP2 PPA2 SERINC2 RIPK2 FASTK THNSL2 DVL2 EPHB3 PIGX BDKRB2 PLCD1 ADCY5 PTPRS RASIP1 GDE1 PRKCZ MLYCD AK2 SOCS6 NUDT16 PLAUR PNPO DHDDS DUSP23 ATP5F1C CHP2 RAC1 ATP5F1D ITPA ATPSCKMT RBKS EREG AXL PPP1R2 PIGH TWSG1 PIGG CAV3 CBS IPMK*
0,031177	26	313	Mitochondrion organization	*MFN2 NDUFB9 NDUFAF6 GBA MGME1 TOMM34 AIFM2 AGTPBP1 ATG7 BAD AP3B1 NDUFA2 PRIMPOL SSBP1 CHCHD3 NDUFA11 SAMM50 PLAUR BOK GPX1 ATP5F1D SURF1 NDUFS8 COX20*
0,031177	9	56	Fatty acid catabolic process	*SLC25A17 PEX7 PEX13 ACOX3 ACADS HACL1 ACAA1 BDH2 PHYH*
0,031615	14	122	Lipid modification	*SLC25A17 PLA2G7 APOE PEX7 GBA PEX13 ACOX3 EPHX2 ACADS HACL1 ACAA1 BDH2 MLYCD PHYH*
0,031666	6	25	Antigen processing	*TAPBPL ZBTB22 B2M IDE ERAP1 ERAP2*
0,033606	59	943	Protein-containing complex	*TAPBPL APOE PIH1D1 SF3A3 EPS15 ARHGAP18 B2M POLR1E PSMD5 NDUFB9 NDUFAF6 PRKDC GBA TPX2 SRC TAF1B KCTD9 PTK2B CBR4 LOC100512926 AIDA MSRB2 ARPC4 BRK1 BAD CKAP5 PIK3R2 NDUFA2 DIAPH1 RIC3 RRM1 PAK1 ZW10 EIF2D COPS8 ABCA7 DLG4 SNRPG TEAD2 ALDH1A2 NDUFA11 FHOD3 SAMM50 RASIP1 PRKCZ BOK RAC1 JCHAIN ATP5F1D SURF1 DNAH17 NDUFS8 EML2 LRRC8D DHX33 SSBP3 COX20 SPIRE1 TMEM199*

0,033606	42	610	Organophosphate metabolic	*ADSL MOCS1 PLA2G7 EFL1 NMNAT1 SMPD2 PTDSS1 NAXE PLCB1 RPIA NAAA PTK2B ADK PIP4K2A OXSM NME6 PIGP BAD PGLS RRM1 ATIC MOCS2 PRPSAP2 SERINC2 PIGX PLCD1 ADCY5 GDE1 MLYCD AK2 SOCS6 NUDT16 PNPO DHDDS ATP5F1C ATP5F1D ITPA*
0,033606	18	184	Alcohol metabolic process	*RDH11 LIPE APOE CLN6 GBA NAAA PTK2B EPHX2 CBR4 PCBD1 CYP27A1 DHCR7 GDE1 AKR1A1 DHFR INSIG2 ERLIN2 IPMK*
0,033606	15	138	Membrane lipid metabolic	*NAGA SMPD2 CLN6 GBA NAAA PSAP PIGP SERINC2 PIGX MGST2 CERS6 D2HGDH PIGH PIGG*
0,036301	105	1919	Cellular component biogenesis	*PARVB TTLL1 TAPBPL AMIGO2 POC1B TBC1D7 SRF EFL1 CDH13 TMEM231 IRX3 LSM14A APOE PIH1D1 FUZ DVL1 WRAP73 MFN2 SF3A3 SSX2IP EPS15 ARHGAP18 B2M POLR1E PSMD5 NDUFB9 NDUFAF6 PRKDC GBA TPX2 SRC CIAO1 AUP1 FAM161A FAM98A TAF1B SDAD1 GTF3A REEP4 KCTD9 PTK2B CBR4 LOC100512926 HPS1 BCCIP AIDA MSRB2 PIP4K2A ARPC4 BRK1 IQCB1 CDKL5 LTO1 BAD CKAP5 DNAJB1 PIK3R2 NDUFA2 DIAPH1 HDAC3 RIC3 RRM1 C2CD3 PAK1 ZW10 EIF2D COPS8 ZYX ABCA7 NOC2L DLG4 SNRPG BBS2 TEAD2 ALDH1A2 EPHB3 EMG1 FAM149B1 ARL6 NDUFA11 FHOD3 SAMM50 PTPRS RASIP1 SCLT1 PRKCZ CCDC28B BOK C1QBP RAC1 JCHAIN ATP5F1D SURF1 MFAP4 DNAH17 NDUFS8 EML2 LRRC8D DHX33 SSBP3 COX20 SPIRE1 CAV3*
0,040149	44	655	Carbohydrate derivative metabolic process	*NAGA ADSL RXYLT1 EFL1 GALNT16 APRT NMNAT1 CLN6 ALG2 ST6GALNAC4 GBA PLCB1 RPIA CBR4 ADK NGLY1 OXSM NME6 PIGP BAD PGLS RRM1 MPDU1 PRPSAP2 B3GAT3 PIGX ADCY5 AKR1A1 NDNF EXTL1 MLYCD AK2 POGLUT1 NUDT16 B3GNT8 DHDDS ATP5F1C D2HGDH ATP5F1D ITPA ATPSCKMT PIGH PIGG*
0,040938	18	188	Lipid catabolic process	*NAGA SLC25A17 PLA2G7 LIPE APOE PEX7 SMPD2 GBA PLCB1 PEX13 ACOX3 ACADS HACL1 ACAA1 CYP27A1 BDH2 MGST2 PHYH*
0,041389	44	657	Positive regulation of catalytic activity	*CD4 DDX11 APAF1 TBC1D7 RGMA APOE PIH1D1 MAP3K4 GNB5 LYN BCAR3 TPX2 SRC MSH2 SFRP2 PTK2B PSAP DAPK1 CCNY RFC4 BAD DNAJB1 LARS1 SIRT3 PAK1 WRN COPS8 SSBP1 ZNF622 CCDC125 STRADA TOM1L1 RABEP1 B3GAT3 MSH6 NET1 RIPK2 DVL2 DHFR PSENEN BOK CHP2 ATPSCKMT EREG*
0,042061	64	1062	Nitrogen compound transport	*SLC25A17 PICK1 CAPRIN2 MBTPS1 APOE MFN2 PEX7 VPS28 SLC25A32 SORT1 SLC25A24 VPS16 TOMM34 AUP1 PEX13 SCARB2 COG2 PSAP ANKRD1 GGA2 MIA3 HACL1 ATG7 COPB2 BAD PIK3R2 CLTB AP3B1 HDAC3 SYTL2 SDHD ICA1 ZFAND2B SEC14L1 STRADA SNX11 TOM1L1 C12H17orf75 RABEP1 B3GAT3 KHDRBS1 ZFAND1 SLC36A4 MON1A LMAN2L ADCY5 COG4 SLC9B2 SLC1A4 SAMM50 ARCN1 CD2AP CHML VTI1A SLC47A1 CHP2 RAB4A RPH3AL LRRC8D E2F3 HERPUD1 LRP2 SNX6*
0,042068	14	128	Cellular respiration	*ACO2 NDUFB9 MSH2 IDE UQCRC1 SIRT3 SDHA COQ9 FH ATP5F1C ATP5F1D SURF1 ATPSCKMT NDUFS8*
0,042061	10	73	Lipoprotein biosynthetic process	*APOE ZDHHC18 GBA ATG7 PIGP PIGX ZDHHC14 PIGH PIGG*

0,042068	10	73	Alcohol biosynthetic process	*APOE GBA PTK2B PCBD1 CYP27A1 DHCR7 DHFR INSIG2 ERLIN2 IPMK*
0,042061	3	5	Modulation by virus of host cellular process	*CD4 ATG7 BAD*
0,04206	3	5	Neurotransmitter catabolic process	*ALDH5A1 ACHE HNMT*
0,043987	98	1787	Cellular localization	*CAPRIN2 CD4 EXOC2 MBTPS1 CDH13 APOE RUVBL2 PIH1D1 DVL1 MFN2 ZDHHC18 SSX2IP EPS15 KIF2C PEX7 ZWILCH VPS28 SEC31B LYN NAXE SORT1 VPS16 SRC TOMM34 SUN1 AUP1 PEX13 MSH2 FAM98A SDAD1 SCARB2 PTK2B PSAP GGA2 MIA3 AGTPBP1 PIP4K2A HACL1 COPB2 LSG1 PACS1 AP5B1 BAD HTATIP2 TMEM38A PIK3R2 CLTB AP3B1 DIAPH1 HDAC3 RIC3 C2CD3 SYTL2 ZW10 WRN ZFAND2B GAA STRADA SNX11 TOM1L1 TMEM98 C12H17orf75 VPS53 RABEP1 B3GAT3 KHDRBS1 DLG4 ZFAND1 DVL2 MON1A FAM149B1 ARL6 ADCY5 SLC9B2 SAMM50 UBE2O CD2AP PRKCZ CHML DNAJC5 SLC39A14 VTI1A ATP5F1C SH3BP4 CHP2 RAC1 BTBD8 ATP5F1D INSIG2 ATPSCKMT RPH3AL SPIRE1 MARCHF5 E2F3 CAV3 HERPUD1 SNX6*
0,043987	55	881	Intracellular transport	*CAPRIN2 MBTPS1 MFN2 SSX2IP EPS15 PEX7 VPS28 SEC31B SORT1 VPS16 SRC TOMM34 SUN1 AUP1 PEX13 SDAD1 SCARB2 GGA2 MIA3 AGTPBP1 PIP4K2A HACL1 COPB2 LSG1 AP5B1 HTATIP2 PIK3R2 CLTB AP3B1 HDAC3 SYTL2 ZW10 ZFAND2B STRADA SNX11 TOM1L1 C12H17orf75 VPS53 B3GAT3 KHDRBS1 ZFAND1 MON1A SAMM50 UBE2O CHML VTI1A ATP5F1C CHP2 BTBD8 ATP5F1D ATPSCKMT RPH3AL E2F3 HERPUD1 SNX6*
0,043987	4	11	Neurotransmitter metabolic process	*ALDH5A1 AGTPBP1 ACHE HNMT*
0,046420	43	646	Peptide metabolic process	*MRPL14 AARS2 GSTA4 LSM14A APOE RMND1 PRKDC SARS1 TUFM IDE XPNPEP1 DAPK1 LTO1 SPON1 LARS1 EIF2D FARSB MRPL22 ABCA7 KHDRBS1 ACO1 LOC100738836 TARS2 SECISBP2 MRPL9 LOC100739087 RPS9 MGST2 LOC110261142 MRPL37 EIF1AX MRPL12 GPX1 RPL13A LOC100153094 DPH2 MTIF3 CLIC5*
0,047184	65	1091	Cellular protein localization	*CAPRIN2 CD4 MBTPS1 APOE RUVBL2 PIH1D1 DVL1 MFN2 ZDHHC18 PEX7 ZWILCH VPS28 SORT1 VPS16 SRC TOMM34 SUN1 AUP1 PEX13 MSH2 SCARB2 GGA2 MIA3 HACL1 COPB2 PACS1 PIK3R2 CLTB AP3B1 DIAPH1 HDAC3 RIC3 C2CD3 SYTL2 ZW10 WRN ZFAND2B STRADA SNX11 TOM1L1 TMEM98 C12H17orf75 RABEP1 B3GAT3 DLG4 ZFAND1 DVL2 MON1A FAM149B1 ARL6 SAMM50 CD2AP PRKCZ CHML VTI1A SH3BP4 CHP2 INSIG2 RPH3AL MARCHF5 E2F3 CAV3 HERPUD1 SNX6*
0,047184	64	1070	Protein-containing complex subunit	*TAPBPL PLA2G7 APOE PIH1D1 SF3A3 EPS15 KIF2C ARHGAP18 B2M POLR1E PSMD5 NDUFB9 NDUFAF6 PRKDC GBA VPS16 TPX2 SRC TAF1B KCTD9 PTK2B CBR4 LOC100512926 AIDA MSRB2 ARPC4 BRK1 BAD CKAP5 MICAL2 PIK3R2 NDUFA2 DIAPH1 RIC3 RRM1 PAK1 ZW10 EIF2D COPS8 ABCA7 DLG4 SNRPG ZFAND1*

				TEAD2 ALDH1A2 NDUFA11 FHOD3 SAMM50 RASIP1 PRKCZ BOK RAC1 JCHAIN ATP5F1D SURF1 DNAH17 NDUFS8 EML2 LRRC8D DHX33 SSBP3 COX20 SPIRE1 TMEM199
0,049797	50	788	Cellular amide metabolic process	*MRPL14 AARS2 GSTA4 LSM14A APOE RMND1 SMPD2 CLN6 PRKDC GBA SARS1 TUFM NAAA IDE XPNPEP1 DAPK1 OXSM LTO1 SPON1 LARS1 EIF2D FARSB MRPL22 ABCA7 KHDRBS1 ACO1 LOC100738836 TARS2 SECISBP2 MRPL9 LOC100739087 RPS9 MLYCD MGST2 LOC110261142 MRPL37 EIF1AX CERS6 MRPL12 GPX1 RPL13A LOC100153094 DPH2 MTIF3 CLIC5 DHX33*

Table S2: eQTLs associated to genes related to lipid metabolism pathways.

Gene ID	Gene Name	Chr	Ch eQTL	Start eQTL	Stop eQTL	eQTL size (Mb)	Nº SNPs	p. adjust	Location
ENSSSCG00000023121	*ABCA7*	2	2	60589918	69102367	8,5124	4	0,00029353	trans
ENSSSCG00000023121	*ABCA7*	2	2	73453753	74827486	1,3737	4	5,11E-05	trans
ENSSSCG00000011250	*ACAA1*	13	13	15848476	18414160	2,5656	4	0,00131419	trans
ENSSSCG00000011250	*ACAA1*	13	13	20033485	24337792	4,3043	26	0,00125889	cis
ENSSSCG00000009916	*ACADS*	14	14	40864510	44398797	3,5342	4	0,00041694	cis
ENSSSCG00000038184	*ACBD7*	10	10	40462382	49903571	9,4411	47	2,74E-16	cis
ENSSSCG00000038184	*ACBD7*	10	10	50576866	51445177	0,8683	5	6,37E-06	trans
ENSSSCG00000030303	*ACHE*	3	14	432200	9195207	8,7630	18	0,00224071	trans
ENSSSCG00000030303	*ACHE*	3	9	3159142	4771690	1,6125	3	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	1	6611958	7598242	0,9862	3	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	14	10656366	19718046	9,06168	7	0,00343177	trans
ENSSSCG00000030303	*ACHE*	3	3	11587100	16958979	5,3718	3	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	7	21421263	26195369	4,7741	8	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	14	21634659	23452185	1,8175	4	0,00343177	trans
ENSSSCG00000030303	*ACHE*	3	15	25005982	27162434	2,1564	3	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	18	41012551	49476286	8,4637	5	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	13	41650270	45990306	4,3400	4	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	17	61220172	62669040	1,4488	3	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	6	73024568	74738668	1,7141	3	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	8	86047751	88866381	2,818	3	0,00224071	trans
ENSSSCG00000030303	*ACHE*	3	2	97835035	99949356	2,1143	3	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	6	112516434	118833836	6,3174	6	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	3	123785748	126603096	2,8173	4	5,73E-05	trans

ENSSSCG00000030303	*ACHE*	3	6	134456535	139944576	5,4880	4	0,00343177	trans
ENSSSCG00000030303	*ACHE*	3	1	224595193	227687180	3,0919	3	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	1	235415859	239098038	3,6821	4	0,00446804	trans
ENSSSCG00000030303	*ACHE*	3	1	270825814	273317908	2,4920	3	0,00389503	trans
ENSSSCG00000008724	*ACOX3*	8	8	1763716	2523676	0,7599	3	0,00257145	cis
ENSSSCG00000026453	*ACSM5*	3	3	2795124	7031147	4,2360	4	7,45E-07	trans
ENSSSCG00000026453	*ACSM5*	3	3	16974611	19563865	2,5892	6	1,37E-05	trans
ENSSSCG00000026453	*ACSM5*	3	3	30021463	33333074	3,3116	11	6,47E-08	trans
ENSSSCG00000026453	*ACSM5*	3	3	20313793	29533135	9,2193	33	4,09E-11	cis
ENSSSCG00000026453	*ACSM5*	3	3	47145545	48929412	1,7838	4	1,95E-10	trans
ENSSSCG00000026453	*ACSM5*	3	3	56243978	59043269	2,7992	4	0,00040872	trans
ENSSSCG00000026453	*ACSM5*	3	3	60335995	64491165	4,155	8	4,02E-07	trans
ENSSSCG00000027952	*ADCY5*	13	6	102133603	109302717	7,1691	4	0,00883741	trans
ENSSSCG00000025578	*ALDH1A2*	1	2	130367	9705128	9,5747	32	1,37E-07	trans
ENSSSCG00000025578	*ALDH1A2*	1	2	10105523	13693405	3,5878	15	4,99E-05	trans
ENSSSCG00000001090	*ALDH5A1*	7	7	20180481	23045761	2,865	4	6,74E-05	trans
ENSSSCG00000001090	*ALDH5A1*	7	7	19231980	19998274	0,7662	4	2,19E-06	cis
ENSSSCG00000005383	*ALG2*	1	1	240493437	248808890	8,3154	22	4,66E-06	cis
ENSSSCG00000010461	*ANKRD1*	14	1	200615239	203534334	2,9190	4	0,00619948	trans
ENSSSCG00000003088	*APOE*	6	2	31021	9705128	9,6741	33	2,55E-07	trans
ENSSSCG00000003088	*APOE*	6	2	10780223	12817790	2,0375	11	0,00011518	trans
ENSSSCG00000022282	*BDH2*	8	8	112093530	118791546	6,6980	32	9,79E-10	cis
ENSSSCG00000040643	*CAV3*	13	13	21109158	22987068	1,877	5	0,00482237	trans
ENSSSCG00000040643	*CAV3*	13	13	60254113	68613104	8,3589	34	0,00020311	cis
ENSSSCG00000040643	*CAV3*	13	13	53261312	59955569	6,6942	12	0,00020311	trans
ENSSSCG00000040643	*CAV3*	13	13	70407839	79483278	9,0754	8	0,00045073	trans
ENSSSCG00000009717	*CBR4*	14	14	20005183	28879459	8,8742	91	4,19E-10	cis

ENSSSCG00000009717	*CBR4*	14	14	6565596	9910644	3,3450	12	9,48E-05	trans
ENSSSCG00000009717	*CBR4*	14	14	10709166	19909136	9,199	30	1,42E-06	trans
ENSSSCG00000009717	*CBR4*	14	14	30692558	39593724	8,9011	39	6,34E-06	trans
ENSSSCG00000009717	*CBR4*	14	14	40699456	48819666	8,120	26	2,69E-06	trans
ENSSSCG00000009717	*CBR4*	14	14	50086705	59710483	9,6237	17	0,00041411	trans
ENSSSCG00000009717	*CBR4*	14	14	60047761	67830352	7,7825	17	0,00086539	trans
ENSSSCG00000009717	*CBR4*	14	14	72896281	79737901	6,841	4	0,00206381	trans
ENSSSCG00000009717	*CBR4*	14	14	80445521	89980342	9,5348	7	0,00110651	trans
ENSSSCG00000032715	*CERS6*	15	15	71219292	79731723	8,5124	46	2,60E-13	cis
ENSSSCG00000032715	*CERS6*	15	15	46410413	47916445	1,5060	5	5,61E-05	trans
ENSSSCG00000032715	*CERS6*	15	15	53092200	59381909	6,2897	16	2,56E-05	trans
ENSSSCG00000032715	*CERS6*	15	15	60064644	69733377	9,6687	20	1,56E-11	trans
ENSSSCG00000032715	*CERS6*	15	15	80451217	89957332	9,5061	16	8,84E-10	trans
ENSSSCG00000032715	*CERS6*	15	15	90291366	99534160	9,2427	27	1,13E-06	trans
ENSSSCG00000032715	*CERS6*	15	15	100320220	100959088	0,6388	4	1,89E-05	trans
ENSSSCG00000004957	*CLN6*	1	1	166280392	168100452	1,820	3	0,00111986	cis
ENSSSCG00000009238	*COQ2*	8	9	3159142	4771690	1,6125	3	0,00836854	trans
ENSSSCG00000009238	*COQ2*	8	3	11587100	16958979	5,3718	3	0,00836854	trans
ENSSSCG00000009238	*COQ2*	8	7	21421263	26195369	4,7741	4	0,00836854	trans
ENSSSCG00000009238	*COQ2*	8	14	23177233	24087811	0,9105	3	0,00825467	trans
ENSSSCG00000009238	*COQ2*	8	15	25005982	27162434	2,1552	3	0,00836854	trans
ENSSSCG00000009238	*COQ2*	8	18	41012551	49476286	8,4635	4	0,00836854	trans
ENSSSCG00000009238	*COQ2*	8	13	41650270	45990306	4,3436	4	0,00836854	trans
ENSSSCG00000009238	*COQ2*	8	13	72253216	79769186	7,517	3	0,00836854	trans
ENSSSCG00000009238	*COQ2*	8	6	72568258	73753725	1,1467	4	0,00825467	trans
ENSSSCG00000009238	*COQ2*	8	2	97835035	99949356	2,1321	3	0,00836854	trans
ENSSSCG00000009238	*COQ2*	8	6	112516434	118833836	6,3402	6	0,00836854	trans

ENSSSCG00000009238	*COQ2*	8	6	134456535	139944576	5,4041	5	0,00548151	trans
ENSSSCG00000009238	*COQ2*	8	6	140617232	144961195	4,3963	4	0,00548151	trans
ENSSSCG00000009238	*COQ2*	8	1	224595193	228744721	4,1528	4	0,00836854	trans
ENSSSCG00000009238	*COQ2*	8	1	235415859	239098038	3,6179	4	0,00836854	trans
ENSSSCG00000034655	*COQ7*	3	3	20455934	29682113	9,2179	61	4,31E-07	cis
ENSSSCG00000034655	*COQ7*	3	3	30484700	37152428	6,6628	42	7,66E-08	trans
ENSSSCG00000034655	*COQ7*	3	3	42898823	46127196	3,2373	10	0,00017333	trans
ENSSSCG00000034655	*COQ7*	3	3	50252339	57727116	7,4777	4	0,00650657	trans
ENSSSCG00000025284	*COQ9*	6	4	122208096	128901152	6,6056	3	0,00851586	trans
ENSSSCG00000016199	*CYP27A1*	15	15	120104268	126883889	6,7621	13	5,00E-08	cis
ENSSSCG00000003006	*CYP2B22*	6	6	21478949	28045497	6,5548	16	1,93E-07	trans
ENSSSCG00000003006	*CYP2B22*	6	6	30116182	39488584	9,3402	29	6,04E-09	trans
ENSSSCG00000003006	*CYP2B22*	6	6	40021993	49502086	9,4093	32	1,92E-11	cis
ENSSSCG00000003006	*CYP2B22*	6	6	50527170	59837138	9,3968	12	1,92E-11	trans
ENSSSCG00000003006	*CYP2B22*	6	6	60315681	69943201	9,652	26	1,04E-10	trans
ENSSSCG00000003006	*CYP2B22*	6	6	70163903	78299598	8,1395	36	4,71E-10	trans
ENSSSCG00000003006	*CYP2B22*	6	6	80309424	88476975	8,1651	25	1,80E-06	trans
ENSSSCG00000003006	*CYP2B22*	6	6	90703532	97779354	7,0722	25	1,96E-05	trans
ENSSSCG00000003006	*CYP2B22*	6	6	100489838	109221647	8,7309	5	0,00050501	trans
ENSSSCG00000021181	*DHCR7*	2	2	2248563	9277022	7,0459	7	0,0014364	cis
ENSSSCG00000033516	*DHDDS*	6	6	90613843	93719999	3,1056	6	0,00106958	trans
ENSSSCG00000004216	*ECHDC1*	1	1	35632737	39310966	3,6729	4	0,00011116	cis
ENSSSCG00000011119	*ECHDC3*	10	10	60057927	69358131	9,3204	94	3,38E-13	cis
ENSSSCG00000011119	*ECHDC3*	10	11	23964222	24228339	0,2117	3	6,59E-06	trans
ENSSSCG00000011119	*ECHDC3*	10	10	50711436	59910972	9,1996	95	1,92E-11	cis
ENSSSCG00000001000	*ECI2*	7	17	10392395	13779280	3,3865	4	0,00640175	trans
ENSSSCG00000009666	*EPHX2*	14	14	10060923	19892646	9,8313	96	4,14E-25	cis

ENSSSCG00000009666	*EPHX2*	14	14	3990532	9921904	5,9312	66	5,82E-13	trans
ENSSSCG00000009666	*EPHX2*	14	14	20034521	29032159	8,9978	56	4,31E-10	trans
ENSSSCG00000038185	*EREG*	8	8	70009168	79312596	9,3038	104	1,36E-18	cis
ENSSSCG00000038185	*EREG*	8	8	60137650	69933436	9,7986	81	1,92E-15	cis
ENSSSCG00000038185	*EREG*	8	8	30107874	39913677	9,8053	34	2,43E-08	trans
ENSSSCG00000038185	*EREG*	8	8	40311619	49102510	8,7901	29	2,53E-12	trans
ENSSSCG00000038185	*EREG*	8	8	50859088	59871066	9,0118	24	1,25E-11	trans
ENSSSCG00000036494	*ERG28*	7	7	90122140	99611741	9,4801	28	5,41E-09	cis
ENSSSCG00000036494	*ERG28*	7	7	89239806	89995039	0,7553	4	0,00012413	trans
ENSSSCG00000036494	*ERG28*	7	7	101496790	107798274	6,3014	4	0,00108283	trans
ENSSSCG00000039480	*ERLIN2*	15	15	53315375	58916239	5,6064	3	0,00070986	trans
ENSSSCG00000024015	*FADS1*	2	2	10638551	12412070	1,7719	8	1,26E-05	trans
ENSSSCG00000024015	*FADS1*	2	2	2250135	9990081	7,7346	27	2,89E-10	cis
ENSSSCG00000017068	*FAXDC2*	16	16	60179062	69744783	9,5621	44	1,32E-08	cis
ENSSSCG00000017068	*FAXDC2*	16	16	57547564	59282387	1,7323	3	0,00838209	trans
ENSSSCG00000017068	*FAXDC2*	16	16	70061020	78773660	8,764	43	4,93E-06	trans
ENSSSCG00000006522	*GBA*	4	4	90685994	99951603	9,2609	42	2,43E-07	cis
ENSSSCG00000006522	*GBA*	4	4	80073050	89938421	9,8671	6	0,00157315	trans
ENSSSCG00000006522	*GBA*	4	4	100099034	107654595	7,5561	14	8,57E-06	trans
ENSSSCG00000033727	*GPX1*	13	13	23970077	29909827	5,935	15	1,01E-10	trans
ENSSSCG00000033727	*GPX1*	13	13	30074120	39466034	9,3914	23	5,80E-17	cis
ENSSSCG00000002279	*GPX2*	7	7	80469999	89995039	9,524	67	5,07E-12	cis
ENSSSCG00000002279	*GPX2*	7	7	67661072	69784027	2,1255	10	9,74E-08	trans
ENSSSCG00000002279	*GPX2*	7	7	70005048	79574171	9,5623	7	0,00013808	trans
ENSSSCG00000002279	*GPX2*	7	7	90010642	94955808	4,9466	51	3,72E-14	trans
ENSSSCG00000021973	*GPX8*	16	16	32792270	39439991	6,6721	15	0,00038598	cis
ENSSSCG00000009305	*GTF3A*	11	11	4454263	5073761	0,6498	5	8,19E-05	cis

ENSSSCG00000011192	*HACL1*	13	13	1192168	3840014	2,6846	25	1,18E-09	cis
ENSSSCG00000014362	*HBEGF*	2	1	200615239	203534334	2,9095	4	0,00394618	trans
ENSSSCG00000014388	*HDAC3*	2	2	140112767	149932954	9,87	56	9,00E-19	cis
ENSSSCG00000014388	*HDAC3*	2	2	124373882	129794043	5,4201	23	1,80E-07	trans
ENSSSCG00000014388	*HDAC3*	2	2	130010906	139969310	9,9584	76	3,32E-14	trans
ENSSSCG00000014388	*HDAC3*	2	2	150453670	151069847	0,6177	4	0,00481912	trans
ENSSSCG00000008700	*HGFAC*	8	8	94954	9879388	9,7834	104	6,27E-54	cis
ENSSSCG00000040863	*IPMK*	14	15	131509060	135576599	4,0639	3	0,00471972	trans
ENSSSCG00000000555	*ITPR2*	5	2	150429223	150787156	0,3533	3	0,0008121	trans
ENSSSCG00000002712	*LDHD*	6	6	12017393	13391574	1,3781	4	0,00221513	cis
ENSSSCG00000003018	*LIPE*	6	6	34068719	37673437	3,6018	4	0,00234119	trans
ENSSSCG00000003018	*LIPE*	6	6	50225490	59162423	8,9333	12	2,38E-05	trans
ENSSSCG00000003018	*LIPE*	6	6	41081581	49683839	8,6058	22	1,55E-06	cis
ENSSSCG00000003018	*LIPE*	6	6	65162482	67059160	1,8968	5	0,00158894	trans
ENSSSCG00000003018	*LIPE*	6	6	129013882	129734771	0,7209	3	0,00548377	trans
ENSSSCG00000015603	*LPGAT1*	9	9	130496818	131223186	0,7268	3	0,00627658	cis
ENSSSCG00000040746	*LRP2*	15	15	53771172	58204639	4,4367	6	0,00180194	trans
ENSSSCG00000040746	*LRP2*	15	15	60064644	69733377	9,6683	13	3,73E-05	trans
ENSSSCG00000040746	*LRP2*	15	15	71219292	79989640	8,7708	18	8,65E-05	cis
ENSSSCG00000040746	*LRP2*	15	15	80548747	89957332	9,4085	8	3,73E-05	trans
ENSSSCG00000040746	*LRP2*	15	15	90897104	99004182	8,1078	14	0,00020059	trans
ENSSSCG00000006250	*LYN*	4	6	112652485	116074407	3,4222	9	0,00833259	trans
ENSSSCG00000002682	*MBTPS1*	6	6	144582	8563612	8,403	34	0,00012684	cis
ENSSSCG00000000031	*MCAT*	5	5	1981418	9918532	7,9314	13	1,11E-05	cis
ENSSSCG00000005720	*MED27*	1	1	271192849	272001272	0,8023	3	0,00035304	cis
ENSSSCG00000035098	*MED4*	11	11	2433514	9253861	6,8247	11	0,00029236	trans
ENSSSCG00000031736	*MGST2*	8	8	81182315	89576800	8,3985	26	4,93E-07	cis

ENSSSCG00000031736	*MGST2*	8	8	90014762	99770507	9,7545	42	1,98E-07	trans
ENSSSCG00000031736	*MGST2*	8	8	100109142	109892961	9,7819	42	1,98E-07	trans
ENSSSCG00000031450	*MLYCD*	6	6	220075	3455626	3,2351	3	0,0067229	trans
ENSSSCG00000017955	*MPDU1*	12	12	50653261	54595235	3,9474	4	3,78E-05	cis
ENSSSCG00000025106	*NA*	3	3	53934692	59613342	5,665	7	1,19E-08	cis
ENSSSCG00000009489	*NA*	11	4	110635436	111307734	0,6798	3	0,00366284	trans
ENSSSCG00000027013	*NA*	2	2	31408370	39977173	8,5603	10	1,99E-06	trans
ENSSSCG00000027013	*NA*	2	2	60117952	69717346	9,5994	28	2,35E-08	cis
ENSSSCG00000027013	*NA*	2	2	40202282	49691674	9,4892	28	2,88E-07	trans
ENSSSCG00000027013	*NA*	2	2	50111296	59568130	9,4534	14	2,35E-08	trans
ENSSSCG00000027013	*NA*	2	2	70220543	79992453	9,791	22	2,35E-08	trans
ENSSSCG00000027013	*NA*	2	2	80200688	89168756	8,9688	13	6,62E-07	trans
ENSSSCG00000027013	*NA*	2	2	100452179	105402679	4,905	3	0,00998969	trans
ENSSSCG00000017608	*NA*	12	12	30020452	39814922	9,447	52	7,28E-09	cis
ENSSSCG00000017608	*NA*	12	12	22565138	29828287	7,2649	47	7,72E-09	trans
ENSSSCG00000017608	*NA*	12	12	50653261	52088566	1,4305	4	0,00486292	trans
ENSSSCG00000003909	*NA*	6	8	70726141	72055494	1,3353	3	0,00077883	trans
ENSSSCG00000013900	*NA*	2	2	31408370	39791103	8,3733	7	0,00046702	trans
ENSSSCG00000013900	*NA*	2	2	40202282	49402057	9,1975	13	8,34E-05	trans
ENSSSCG00000013900	*NA*	2	2	60438014	69717346	9,2732	18	8,34E-05	trans
ENSSSCG00000013900	*NA*	2	2	81345790	82567688	1,2298	3	0,00243	trans
ENSSSCG00000013900	*NA*	2	2	50111296	59167984	9,0588	7	8,34E-05	cis
ENSSSCG00000035293	*NA*	2	2	31021	9909640	9,8719	137	3,13E-24	cis
ENSSSCG00000035293	*NA*	2	2	10004690	16899791	6,8901	55	6,72E-11	trans
ENSSSCG00000003451	*NA*	6	6	73007048	74191500	1,1852	3	0,000145	cis
ENSSSCG00000034049	*NA*	12	12	51766735	59981770	8,2135	56	5,51E-15	cis
ENSSSCG00000034049	*NA*	12	12	60011199	61406036	1,3937	11	3,08E-14	trans

ENSSSCG00000036224	*NA*	3	3	50089886	59343289	9,2503	52	6,95E-15	cis
ENSSSCG00000036224	*NA*	3	3	21792771	27496564	5,7793	6	0,0002239	trans
ENSSSCG00000036224	*NA*	3	3	48023480	49795973	1,7493	3	0,00320056	trans
ENSSSCG00000036224	*NA*	3	3	60040017	63004901	2,9884	4	2,09E-06	trans
ENSSSCG00000036229	*NA*	15	15	132514678	136079026	3,5648	3	0,00089357	trans
ENSSSCG00000008973	*NAAA*	8	8	70068935	79995913	9,9278	73	7,01E-15	cis
ENSSSCG00000008973	*NAAA*	8	8	20196669	29946585	9,7496	15	3,62E-05	trans
ENSSSCG00000008973	*NAAA*	8	8	30030826	39836160	9,8054	40	4,79E-08	trans
ENSSSCG00000008973	*NAAA*	8	8	40001725	48516038	8,5113	28	1,03E-07	trans
ENSSSCG00000008973	*NAAA*	8	8	52127966	59871066	7,71	16	3,13E-08	trans
ENSSSCG00000008973	*NAAA*	8	8	60137650	69838204	9,7054	43	1,30E-09	trans
ENSSSCG00000000050	*NAGA*	5	5	5258684	8610495	3,3511	8	7,00E-05	cis
ENSSSCG00000001418	*NEU1*	7	7	20139894	28847227	8,7033	25	2,77E-13	cis
ENSSSCG00000001418	*NEU1*	7	7	31996106	32376442	0,3836	3	0,00122529	cis
ENSSSCG00000014157	*NR2F1*	2	2	85778573	89821405	4,0432	8	0,00088318	trans
ENSSSCG00000014157	*NR2F1*	2	2	90978857	99745941	8,7684	6	0,00160622	trans
ENSSSCG00000014157	*NR2F1*	2	2	100316206	101426587	1,1181	6	0,00088318	cis
ENSSSCG00000016715	*OSBPL3*	18	18	40436598	49973261	9,5363	28	2,89E-05	cis
ENSSSCG00000016715	*OSBPL3*	18	18	50568550	52713756	2,1406	6	0,00086557	trans
ENSSSCG00000011215	*OXSM*	13	13	12045596	13103797	1,0501	7	1,31E-06	cis
ENSSSCG00000029070	*PAFAH1B3*	6	6	42291460	49731358	7,4398	10	7,40E-12	cis
ENSSSCG00000008387	*PEX13*	3	15	131509060	136079026	4,5666	4	1,44E-05	trans
ENSSSCG00000004160	*PEX7*	1	1	18266607	19239794	0,9737	3	0,00039888	trans
ENSSSCG00000004160	*PEX7*	1	1	20875482	28160470	7,2848	14	0,00021955	cis
ENSSSCG00000039244	*PHYH*	10	10	37332232	39613493	2,281261	3	0,00313804	trans
ENSSSCG00000039244	*PHYH*	10	10	41620166	48686436	7,027	4	2,47E-07	cis
ENSSSCG00000039991	*PIGG*	8	8	94954	2933071	2,8117	4	4,02E-07	cis

ENSSSCG00000039351	*PIGH*	7	7	90010642	99694015	9,6873	29	7,31E-09	cis
ENSSSCG00000039351	*PIGH*	7	7	84255797	89995039	5,7342	13	7,31E-09	trans
ENSSSCG00000012061	*PIGP*	13	2	42180092	46036027	3,8535	3	0,00328345	trans
ENSSSCG00000026934	*PIGX*	13	13	133264172	137918150	4,6538	6	4,12E-06	cis
ENSSSCG00000026934	*PIGX*	13	13	125143843	125244490	0,1047	3	0,00070633	trans
ENSSSCG00000026934	*PIGX*	13	13	140676615	148426734	7,7519	3	0,00312343	trans
ENSSSCG00000011079	*PIP4K2A*	10	10	44552304	48612344	4,060	6	0,00010627	trans
ENSSSCG00000011079	*PIP4K2A*	10	10	61628730	63383491	1,7541	4	2,20E-07	trans
ENSSSCG00000011079	*PIP4K2A*	10	10	51216665	59163109	7,9464	19	2,20E-07	cis
ENSSSCG00000001723	*PLA2G7*	7	7	40334301	42007620	1,6739	4	2,23E-06	cis
ENSSSCG00000032434	*PLAUR*	6	6	41610731	49634384	8,0233	6	6,42E-12	trans
ENSSSCG00000032434	*PLAUR*	6	6	51113588	51245746	0,1328	3	2,49E-05	trans
ENSSSCG00000032434	*PLAUR*	6	6	64775780	66491010	1,715	5	1,88E-12	trans
ENSSSCG00000007056	*PLCB1*	17	17	13301787	18240168	4,9381	11	8,96E-05	cis
ENSSSCG00000027550	*PLCD1*	13	13	20033485	29969767	9,9362	159	4,65E-25	cis
ENSSSCG00000027550	*PLCD1*	13	13	10021776	19973632	9,9516	125	3,63E-21	trans
ENSSSCG00000027550	*PLCD1*	13	13	30163762	39508051	9,3449	23	4,92E-06	trans
ENSSSCG00000027550	*PLCD1*	13	13	40328305	41350386	1,0221	3	0,00038289	trans
ENSSSCG00000027550	*PLCD1*	13	13	50924481	58467874	7,5433	8	0,00096663	trans
ENSSSCG00000027550	*PLCD1*	13	13	60992646	68337650	7,3454	21	5,73E-05	trans
ENSSSCG00000027550	*PLCD1*	13	13	70386551	76414124	6,0273	7	0,00037447	trans
ENSSSCG00000027550	*PLCD1*	13	13	81033862	85030228	3,996366	5	0,00142343	trans
ENSSSCG00000032313	*POGLUT1*	13	13	82225347	89912139	7,6792	14	4,96E-06	trans
ENSSSCG00000032313	*POGLUT1*	13	13	90063046	99323417	9,2671	25	5,29E-06	trans
ENSSSCG00000032313	*POGLUT1*	13	13	140802462	148030316	7,2274	15	1,34E-06	cis
ENSSSCG00000032313	*POGLUT1*	13	13	122565180	129052123	6,6943	8	2,26E-05	trans
ENSSSCG00000032313	*POGLUT1*	13	13	130394931	138906814	8,5883	20	1,87E-06	trans

ENSSSCG00000032313	*POGLUT1*	13	13	150709829	159490085	8,7856	9	8,39E-05	trans
ENSSSCG00000032313	*POGLUT1*	13	13	161439503	167507958	6,0685	4	0,00026133	trans
ENSSSCG00000029515	*PON3*	9	9	70027518	79609079	9,5815	52	9,64E-38	cis
ENSSSCG00000029515	*PON3*	9	9	21101886	28426018	7,3232	25	1,19E-06	trans
ENSSSCG00000029515	*PON3*	9	9	30748549	39872533	9,1284	28	1,00E-06	trans
ENSSSCG00000029515	*PON3*	9	9	40115762	49797975	9,6813	59	2,29E-08	trans
ENSSSCG00000029515	*PON3*	9	9	50180939	59782190	9,6051	57	6,96E-14	trans
ENSSSCG00000029515	*PON3*	9	9	60331043	69948308	9,6175	49	3,25E-14	trans
ENSSSCG00000029515	*PON3*	9	9	80784102	88155132	7,373	6	2,99E-09	trans
ENSSSCG00000029515	*PON3*	9	9	90956417	99995397	9,098	37	3,12E-25	trans
ENSSSCG00000029515	*PON3*	9	9	100356931	109214318	8,8587	35	3,59E-10	trans
ENSSSCG00000029515	*PON3*	9	9	110028935	117693067	7,6132	13	8,80E-05	trans
ENSSSCG00000010281	*PSAP*	14	14	73900332	79567277	5,6945	16	0,0007832	cis
ENSSSCG00000006089	*PTDSS1*	4	11	22583147	25959904	3,3757	4	0,00513309	trans
ENSSSCG00000006089	*PTDSS1*	4	1	261347674	266716391	5,3617	3	0,00221455	trans
ENSSSCG00000021386	*PTGR1*	1	1	251386126	253752980	2,3664	12	1,32E-06	cis
ENSSSCG00000036039	*RAB4A*	14	14	53980065	59558918	5,5853	21	0,00031527	trans
ENSSSCG00000036039	*RAB4A*	14	14	60028498	69236370	9,2872	21	0,00031527	cis
ENSSSCG00000036039	*RAB4A*	14	14	71387515	73900332	2,5117	8	0,00045335	trans
ENSSSCG00000035090	*RAC1*	3	3	1813273	5507280	3,6907	15	6,40E-05	cis
ENSSSCG00000002296	*RDH11*	7	7	90243662	99936352	9,699	43	2,03E-08	cis
ENSSSCG00000002296	*RDH11*	7	7	83211722	89991274	6,7952	17	2,33E-06	trans
ENSSSCG00000002296	*RDH11*	7	7	100027071	106933131	6,606	17	7,82E-06	trans
ENSSSCG00000023585	*SERINC2*	6	6	80764182	89896435	9,1253	19	8,78E-05	cis
ENSSSCG00000023585	*SERINC2*	6	6	90587791	97931290	7,3499	14	8,70E-05	trans
ENSSSCG00000017818	*SERPINF1*	12	4	105127069	107790356	2,6637	3	7,72E-05	trans
ENSSSCG00000000072	*SLC25A17*	5	5	10347960	10976362	0,8402	3	0,00411791	trans

ENSSSCG00000000072	*SLC25A17*	5	5	3354087	8379751	5,0664	12	6,20E-11	cis
ENSSSCG00000004408	*SMPD2*	1	2	22468845	25369747	2,9002	3	0,00497716	trans
ENSSSCG00000004408	*SMPD2*	1	1	72714511	79943446	7,2235	19	0,00021677	cis
ENSSSCG00000004408	*SMPD2*	1	1	80224111	86673286	6,4475	12	0,0001807	trans
ENSSSCG00000004408	*SMPD2*	1	1	95066737	99720951	4,6214	6	0,00029956	trans
ENSSSCG00000004408	*SMPD2*	1	1	100630729	103352719	2,799	5	0,00186306	trans
ENSSSCG00000005628	*ST6GAAC4*	1	10	42291757	45104014	2,8157	3	0,00660646	trans
ENSSSCG00000025568	*TEAD2*	6	6	60080423	64472806	4,3983	6	0,00107441	trans
ENSSSCG00000025568	*TEAD2*	6	6	53269495	59058869	5,7874	13	0,00054513	cis

Table S3: eQTLs associated to genes related to lipid metabolism pathways.

FDR	nGenes	Pathway Genes	Pathway	Genes
3,25E-45	54	849	Lipid metabolic process	*MCAT NAGA SLC25A17 PLA2G7 RDH11 MBTPS1 LIPE APOE PEX7 SMPD2 CLN6 ALG2 PTDSS1 LYN GBA PLCB1 PEX13 ACOX3 NAAA COQ2 EPHX2 CBR4 ACADS PSAP PIP4K2A HACL1 OXSM ACAA1 PIGP LPGAT1 CYP27A1 FAXDC2 MPDU1 DHCR7 PTGR1 BDH2 SERINC2 FADS1 THNSL2 ALDH1A2 PIGX PLCD1 MLYCD MGST2 CERS6 DHDDS GPX1 INSIG2 ERG28 PHYH PIGH ERLIN2 PIGG CAV3*
1,37E-18	20	198	Fatty acid metabolic process	*MCAT SLC25A17 PEX7 PEX13 ACOX3 NAAA CBR4 ACADS HACL1 OXSM ACAA1 LPGAT1 PTGR1 BDH2 THNSL2 MLYCD GPX1 INSIG2 PHYH ERLIN2*
5,04E-17	26	527	Carboxylic acid metabolic process	*MCAT SLC25A17 ALDH5A1 LDHD PEX7 PEX13 ACOX3 NAAA CBR4 ACADS HACL1 OXSM ACAA1 LPGAT1 CYP27A1 PTGR1 BDH2 THNSL2 ALDH1A2 PON3 MLYCD MGST2 GPX1 INSIG2 PHYH ERLIN2*
6,71E-17	26	536	Oxoacid metabolic process	*MCAT SLC25A17 ALDH5A1 LDHD PEX7 PEX13 ACOX3 NAAA CBR4 ACADS HACL1 OXSM ACAA1 LPGAT1 CYP27A1 PTGR1 BDH2 THNSL2 ALDH1A2 PON3 MLYCD MGST2 GPX1 INSIG2 PHYH ERLIN2*
1,06E-12	11	62	Lipid oxidation	*SLC25A17 PLA2G7 PEX7 PEX13 ACOX3 ACADS HACL1 ACAA1 BDH2 MLYCD PHYH*
2,82E-11	10	60	Fatty acid oxidation	*SLC25A17 PEX7 PEX13 ACOX3 ACADS HACL1 ACAA1 BDH2 MLYCD PHYH*
4,96E-10	9	56	Fatty acid catabolic process	*SLC25A17 PEX7 PEX13 ACOX3 ACADS HACL1 ACAA1 BDH2 PHYH*
4,66E-09	9	72	Cholesterol metabolic process	*LIPE APOE CLN6 GBA EPHX2 CYP27A1 DHCR7 INSIG2 ERLIN2*
2,63E-06	6	43	Fatty acid beta-oxidation	*SLC25A17 PEX7 ACOX3 ACADS ACAA1 BDH2*
0,004620501	4	62	Triglyceride metabolic process	*APOE GPX1 INSIG2 CAV3*

7.3. Supplementary material Paper III: "Deciphering allele-specific expression in muscle transcriptome of Duroc crossbreed pigs from RNA-Seq data"

Table S1: Gene ontology biological terms of genes affected by allele-specific expression.

Enrichment FDR	Nº Genes	Pathway Genes	Enrichment	Pathway	Genes
6,01E-08	45	220	2,880843	Muscle system proc,	*MYBPC1 PI16 SLC8A3 SETD3 MTOR MYOM1 SMAD4 TPM1 FBXO32 ATP1B1 CASQ1 LMNA GJA5 MKKS TNNC2 SULF2 ATP2A1 ACTN2 MYOZ1 PRKG1 LMCD1 CSRP3 MYOD1 SMAD5 ATP2B4 BIN1 PRKAG3 ANXA6 SCN4A CAMTA2 MYH3 ENO1 TRPM4 SCN1B TNNI1 CACNA1S TNNT1 LOC100620992 KCNE3 MAP2K3 GSN*
7,15E-08	71	447	2,230227	Actin cytoskeleton organization	*PARVB PACSIN2 MICAL3 WHAMM SETD3 ARHGAP35 MTOR MAD2L2 LATS1 ARHGAP18 SMAD4 TPM1 ARPC5L SDCBP F11R CASQ1 STRIP1 DLC1 MKKS ACTR2 CYRIA LIMCH1 PDGFRA GAB1 AKAP11 RGCC SORBS3 ARPC3 ACTN2 MYOZ1 LDB3 CDC42BPA ITGB1 NRP1 ATP2C1 NISCH BCL6 AMOT CSRP3 JMY RASA1 CTNNA1 DIAPH1 ABLIM3 PPARGC1B INPPL1 ARAP1 PAK1 JAM3 BIN1 XIRP2 NCKAP1 ARPC2 MKLN1 LMOD2 BAIAP2 FOXJ1 GRB2 ITGB3 TNNT1 FHOD1 FHOD3 SSH3 MYPN PALLD AVIL ACTN1 EMP2 GSN RFLNB*
3,30E-08	77	490	2,214536	Actin filament-based proc,	*PARVB PACSIN2 MICAL3 WHAMM CCDC88C SETD3 ARHGAP35 MTOR MAD2L2 LATS1 ARHGAP18 SMAD4 TPM1 ARPC5L SDCBP F11R CASQ1 GJA5 STRIP1 DLC1 MKKS ACTR2 CYRIA LIMCH1 PDGFRA GAB1 AKAP11 RGCC SORBS3 ARPC3 ACTN2 MYOZ1 LDB3 CDC42BPA ITGB1 NRP1 ATP2C1 NISCH BCL6 AMOT CSRP3 JMY RASA1 CTNNA1 DIAPH1 ABLIM3 PPARGC1B INPPL1 ARAP1 PAK1 JAM3 BIN1 XIRP2 NCKAP1 ARPC2 MKLN1 LMOD2 BAIAP2 FOXJ1 GRB2 ITGB3 TRPM4 SCN1B TNNT1 FHOD1 FHOD3 SSH3 MYPN KCNE3 PALLD AVIL ACTN1 EMP2 GSN*
2,16E-07	86	607	1,961466	Protein modification by small protein conjugation or removal	*HECTD1 ASB2 AMFR MIB2 UBE4B MAD2L2 PINK1 UFL1 HSPA5 VPS28 FBXO32 OTUD6B WWP1 VCPIP1 USP7 EPAS1 OTUD4 UCHL3 MYCBP2 CUL4A ANAPC5 KLHL22 TNKS2 UCHL5 NFX1 BMI1 KLHL40 DCAF1 GNL3 PDZRN3 USP13 ATG3 UBE2A BRCC3 DDB1 PRPF19 DDB2 PRMT3 USP47 FBXL17 CDC23 RNF14 RPS3 USP28 USP2 IVNS1ABP NFE2L2 USP40 COPS8 ASB1 UBE3C UBE2D4 RNF216 CNOT4 FEM1B UBR5 TRPM4 PJA2 RNF6 AIMP2 USP20 TRIP12 USP25 RNF19B SPSB2 USPL1 NXN ASB10*

					USP24 ANAPC7 LTN1 PTTG1IP ANAPC13 COPS7B UBE2K TOR1A UBE2R2 U2AF2 MAD2L1 NSMCE1 UBE2B PCGF2 MARCHF5 CYLD ISG15
1,20E-07	100	736	1,914091	Reg, of organelle organization	*PPP1R10 WHAMM TERF2IP ARHGAP35 EXOSC10 MTOR PINK1 NEXN MAP3K4 LATS1 ARHGAP18 SMAD4 ARPC5L NBN SDCBP F11R LMNA SETDB1 SH3GLB1 ODF2L DLC1 MKKS ERCC4 ACTR2 FEZ2 CYRIA RNF4 EGF RGCC MYCBP2 SORBS3 BNIP3L ARPC3 MTMR3 ACTN2 PPIF TNKS2 ARMH3 STN1 HNRNPU NRP1 DYNC1LI1 PDCD6IP GNL3 ATG3 NAT10 JMY RASA1 DCP2 APC CDC23 DIAPH1 ARAP1 RPS3 PAK1 CEP295 JAM3 BIN1 NCKAP1 XRCC5 ARPC2 MAD2L1BP LMOD2 BAIAP2 GRB2 ITGB3 CALCOCO2 TOM1L1 ZNF207 TAOK1 GPSM2 CEP135 CCSAP LOC100624559 PHLDB1 DHX36 RALB FHOD1 STX5 FHOD3 SSH3 MFF SMG1 PIP4K2B PARL SDCCAG8 G3BP1 MAD2L1 TMEM33 GSN SMCR8 RANGRF FIS1 UBE2B MARCHF5 NUPR1 CYLD NUP62 TSC1*
6,05E-12	170	1299	1,844232	Cellular catabolic proc,	*DERA FAH FURIN ABHD2 ETFA LGMN ATG2B CSNK2A2 AMFR BCAT2 UBE4B DFFA EXOSC10 MTOR MFN2 UBR4 PINK1 ECE1 AGO1 NT5C1A NPC1 ACADM CPT2 ZYG11B NRDC FAF1 LATS1 UFL1 HERC1 RAD23B HSPA5 ZER1 CRAT VPS28 AGO2 EIF3E MTDH WWP1 SDCBP ATF6 CSDE1 AGL SH3GLB1 VPS16 HM13 WIPI2 ERCC4 USP7 RAB1A PSME4 ERLEC1 FEZ2 LPIN1 RNF4 LNX1 GRSF1 SCARB2 CNOT6L EGF TET2 WDFY3 PAN3 ESD UCHL3 CUL4A BNIP3L EPHX2 CLU HSPB8 MTMR3 LGALS8 ZSWIM8 IDE ATE1 UCHL5 HNRNPU AGTPBP1 CUL2 UPF2 NGLY1 STT3B PDCD6IP ABHD5 ARL8B ACAD11 USP13 ATG3 LAMP2 PNPLA2 MUS81 CAPN1 DDB1 PRPF19 CAT USP47 EIF4G2 FBXL17 DCP2 HINT1 TMEM41B SMPD1 USP28 USP2 ATP2B4 SSB NFE2L2 USP40 HIBADH RETREG1 MTREX FOXK2 PSMC5 PLEKHM1 OSBPL7 CALCOCO2 TAF15 EPG5 PFKM RNF216 CNOT4 ZHX2 NEU3 DCP1A OPTN USP20 USP25 ALDH4A1 RCN3 DHX36 STX12 RALB SPSB2 UBE2Z IRS1 ENPP4 DNAJC3 CARHSP1 NUDT15 ABCD3 RB1CC1 CUL5 PDE2A USP24 PCYOX1 LTN1 PTTG1IP CDADC1 SMG1 PIP4K2B PARL UBE2K ATG12 TOR1A MTMR9 PDE4C CST3 FITM2 ECHS1 SMCR8 CACUL1 TRIB1 FIS1 UBE2B ERLIN2 PSMA2 NUPR1 CYLD ISG15*
1,20E-07	114	881	1,823956	Intracellular transport	*NUP50 POLDIP3 SCYL2 ATXN1 MAPK14 WHAMM VIPAS39 CCDC88C CILK1 AP2A1 MFN2 PINK1 NPC1 STX7 BACH2 HSPA5 VPS28 SNX16 SEC31B COPA LMNA RAB13 SNX27 SORT1 VPS16 HM13 KCNB1 SUN1 STX4 TBC1D10B USP7 TEX261 ACTR2 RAB1A ERLEC1 SCARB2 ARFIP1 SEC24B BNIP3L ACTN2 TOMM20 SEC23IP MIA3 HNRNPU DENND1B AGTPBP1 KIF5B ARL8B SEC13 COPG1 SEC61A1 DNAJC13 LSG1 LAMP2 THOC2 CRY2 HTATIP2 CLTB HSPA4 SAR1B FAM160A2 NUP98 RAB6A PTPN14 BIN1 SSB INSIG1 CREB3L2 TNPO3 VPS41 GGA3 TOM1L1 AP2B1 VPS53 UBE2G1 KIF13A EPG5 UBR5 SLC25A30 KPNA3 HOOK3 STX12 SNX17 CSE1L FHOD1 SRP19 STX5 FYTTD1*

					TUBA8 KPNA1 ABCD3 IPO7 GOSR1 IPO13 RHOBTB3 SSR3 PTTG1IP TXNIP MFF ABRA KPNA6 SEC22C SRSF10 HNRNPA2B1 EMP2 ATP5PB VAPA RANGRF FIS1 YIPF5 NUP62 NDE1 HERPUD1 TSC1
1,55E-12	193	1512	1,798719	Catabolic proc,	*PPARA DERA NEU1 FAH FURIN ABHD2 ETFA PKM LGMN ATG2B CSNK2A2 AMFR MMP2 LIPE BCAT2 UBE4B DFFA EXOSC10 MTOR MAD2L2 MFN2 UBR4 PINK1 ECE1 AGO1 NT5C1A NPC1 ACADM CPT2 ZYG11B NRDC FAF1 LATS1 UFL1 HERC1 RAD23B HSPA5 ZER1 CRAT VPS28 AGO2 OC90 EIF3E MTDH WWP1 SDCBP ATF6 CSDE1 AGL SH3GLB1 VPS16 HM13 WIPI2 ERCC4 USP7 RAB1A PSME4 ERLEC1 FEZ2 LPIN1 RNF4 LNX1 GRSF1 SCARB2 CNOT6L EGF TET2 WDFY3 PAN3 ESD UCHL3 MYCBP2 CUL4A BNIP3L EPHX2 CLU HSPB8 MTMR3 LGALS8 ZSWIM8 IDE ATE1 UCHL5 BPNT1 HNRNPU AGTPBP1 ITGB1 CUL2 UPF2 NGLY1 STT3B PDCD6IP ABHD5 ARL8B ACAD11 USP13 ATG3 LAMP2 PNPLA2 MUS81 CAPN1 DDB1 PRPF19 CAT USP47 EIF4G2 FBXL17 DCP2 APC HINT1 TMEM41B SMPD1 MTMR2 USP28 USP2 ATP2B4 SSB NFE2L2 CYP27A1 PRKAG3 USP40 PRKAG2 HIBADH RETREG1 MTREX FOXK2 PSMC5 PLEKHM1 PSMD3 FBXL20 OSBPL7 CALCOCO2 TAF15 EPG5 PFKM RNF216 CNOT4 ZHX2 NEU3 EGFR ENO1 DCP1A OPTN USP20 USP25 ALDH4A1 RCN3 DHX36 STX12 RALB SPSB2 UBE2Z IRS1 ENPP4 DNAJC3 STX5 CARHSP1 NUDT15 TIPARP ABCD3 RB1CC1 CUL5 PDE2A USP24 PCYOX1 LTN1 PTTG1IP CDADC1 SMG1 BPNT2 PIP4K2B PARL UBE2K ATG12 TOR1A MTMR9 MAD2L1 PDE4C CST3 FITM2 ECHS1 SMCR8 CACUL1 TRIB1 FIS1 UBE2B ERLIN2 PSMA2 NUPR1 CYLD ISG15 HERPUD1*
4,03E-07	110	863	1,79047	Macromolecule catabolic proc,	*FURIN LGMN CSNK2A2 AMFR UBE4B DFFA EXOSC10 MAD2L2 UBR4 AGO1 ZYG11B NRDC FAF1 LATS1 UFL1 RAD23B HSPA5 ZER1 VPS28 AGO2 EIF3E WWP1 SDCBP CSDE1 AGL SH3GLB1 HM13 USP7 PSME4 ERLEC1 RNF4 LNX1 GRSF1 CNOT6L EGF PAN3 UCHL3 MYCBP2 CUL4A BNIP3L CLU ZSWIM8 IDE ATE1 UCHL5 HNRNPU AGTPBP1 CUL2 UPF2 NGLY1 STT3B PDCD6IP USP13 LAMP2 MUS81 CAPN1 DDB1 PRPF19 USP47 FBXL17 DCP2 APC USP28 USP2 SSB NFE2L2 USP40 MTREX PSMC5 PSMD3 FBXL20 OSBPL7 TAF15 PFKM RNF216 CNOT4 ZHX2 EGFR DCP1A USP20 USP25 RCN3 DHX36 SPSB2 UBE2Z DNAJC3 STX5 CARHSP1 NUDT15 TIPARP CUL5 USP24 PCYOX1 LTN1 PTTG1IP SMG1 UBE2K TOR1A MAD2L1 CST3 CACUL1 TRIB1 UBE2B ERLIN2 PSMA2 NUPR1 CYLD ISG15 HERPUD1*
2,02E-07	118	933	1,782311	Cytoskeleton organization	*PARVB PACSIN2 MICAL3 DST WHAMM SETD3 ARHGAP35 MTOR MAD2L2 MACF1 NEXN LATS1 ARHGAP18 SMAD4 TPM1 ARPC5L SDCBP F11R CASQ1 LMNA STRIP1 DLC1 MKKS SUN1 SBDS SEPTIN1 ACTR2 CEP68 CYRIA RNF4 LIMCH1 PDGFRA ARHGAP10 GAB1 AKAP11 RGCC MYCBP2 SORBS3 ARPC3 ACTN2 MYOZ1 LDB3 SLK*

					ABLIM1 CDC42BPA HNRNPU KIF24 SVIL ITGB1 NRP1 ATP2C1 PDCD6IP NISCH BCL6 AMOT NAT10 CSRP3 JMY RASA1 APC CTNNA1 DIAPH1 ABLIM3 PPARGC1B INPPL1 ARAP1 C2CD3 RPS3 PAK1 CHORDC1 CEP295 JAM3 BIN1 XIRP2 MAP3K20 NCKAP1 ARPC2 MKLN1 LMOD2 BAIAP2 FOXJ1 GRB2 ITGB3 ZNF207 TAOK1 DES GPSM2 CEP135 CCSAP RHOU RAF1 HOOK3 PHLDB1 TNNT1 FHOD1 FHOD3 SSH3 MYPN PALLD AVIL DAG1 TOR1A SDCCAG8 MAD2L1 ACTN1 EMP2 GSN TACC2 FITM2 TTLL11 RFLNB RANGRF UBE2B
1,13E-08	151	1220	1,744295	Cellular response to stress	*PPARA YBX3 SLC38A2 PPP1R10 KLHL31 MAPK14 ZFYVE26 SUSD6 SLC8A3 FOXN3 CCDC88C TERF2IP AMFR MMP2 PPP1R15A MTOR MAD2L2 MFN2 PINK1 MAP3K4 BCLAF1 RRAGD UFL1 TPM1 FAN1 RAD23B HSPA5 SETX OXR1 NBN VCPIP1 PRKDC RCSD1 ATF6 LMNA SLC25A24 SH3GLB1 HM13 RBL1 WIPI2 STX4 ATP2A1 ERCC4 USP7 CDIP1 ACTR2 VRK2 PSME4 ERLEC1 EPAS1 DDX1 LETM1 RELL1 PDGFRA ABRAXAS1 RGCC TMTC4 CUL4A BNIP3L CLU HSPB8 MTMR3 PPIF TGFB2 STT3B OXSR1 LAMB2 NEK4 USP13 BCL6 BACH1 PDK3 UBE2A BRCC3 MUS81 DDB1 PRPF19 DDB2 MYOD1 USP47 THBS4 APC RAD50 LARS1 PPARGC1B RPS3 PAK1 CHORDC1 USP28 PDK4 ATF3 WRN PDK1 MAP3K20 NFE2L2 XRCC5 INSIG1 CREB3L2 CBX3 MTREX GRB2 NFE2L1 CBX1 TAOK1 EGFR ENO1 FEM1B UBR5 PJA2 NET1 OPTN SCARF1 MLH1 TRIP12 USP25 RCN3 DHX36 FGF1 RALB DNAJC3 INIP PDK2 LPCAT3 NUDT15 HUS1 RB1CC1 TMBIM6 PTTG1IP PRRX1 CHCHD6 SMG1 SWI5 KIN ARL6IP5 MAP2K3 TOR1A CXCL12 NSMCE1 TMEM33 LRRC8D TRIB1 UBE2B PCGF2 ERLIN2 CD34 NUPR1 CYLD SOD2*
2,38E-08	155	1279	1,707874	Organic substance catabolic proc,	*PPARA DERA NEU1 FAH FURIN ABHD2 ETFA PKM LGMN CSNK2A2 AMFR LIPE BCAT2 UBE4B DFFA EXOSC10 MAD2L2 UBR4 ECE1 AGO1 NT5C1A ACADM CPT2 ZYG11B NRDC FAF1 LATS1 UFL1 RAD23B HSPA5 ZER1 CRAT VPS28 AGO2 OC90 EIF3E WWP1 SDCBP CSDE1 AGL SH3GLB1 HM13 USP7 PSME4 ERLEC1 LPIN1 RNF4 LNX1 GRSF1 SCARB2 CNOT6L EGF TET2 PAN3 ESD UCHL3 MYCBP2 CUL4A BNIP3L EPHX2 CLU ZSWIM8 IDE ATE1 UCHL5 BPNT1 HNRNPU AGTPBP1 CUL2 UPF2 NGLY1 STT3B PDCD6IP ABHD5 ACAD11 USP13 LAMP2 PNPLA2 MUS81 CAPN1 DDB1 PRPF19 USP47 FBXL17 DCP2 APC HINT1 SMPD1 MTMR2 USP28 USP2 ATP2B4 SSB NFE2L2 CYP27A1 PRKAG3 USP40 PRKAG2 HIBADH MTREX FOXK2 PSMC5 PSMD3 FBXL20 OSBPL7 TAF15 PFKM RNF216 CNOT4 ZHX2 NEU3 EGFR ENO1 DCP1A USP20 USP25 ALDH4A1 RCN3 DHX36 SPSB2 UBE2Z IRS1 ENPP4 DNAJC3 STX5 CARHSP1 NUDT15 TIPARP ABCD3 CUL5 PDE2A USP24 PCYOX1 LTN1 PTTG1IP CDADC1 SMG1 BPNT2 UBE2K TOR1A MAD2L1 PDE4C CST3 FITM2 ECHS1 CACUL1 TRIB1 UBE2B ERLIN2 PSMA2 NUPR1 CYLD ISG15 HERPUD1*

3,92E-07	132	1097	1,690602	Cellular macromolecule localization	*PACSIN2 ILRUN MAPK14 HECTD1 MMP14 MPP5 VIPAS39 TTC7B TERF2IP PPP1R15A AP2A1 EXOSC10 MFN2 PINK1 NPC1 LATS1 STX7 PYGO1 HSPA5 VPS28 SNX16 VCPIP1 ATP1B1 F11R COPA LMNA RAB13 SNX27 RAP1A SORT1 SH3GLB1 VPS16 HM13 KCNB1 SUN1 WIPI2 STX4 POLR1A CEP68 ERLEC1 DDX1 SCARB2 ARFIP1 EGF SEC24B PAQR3 AKAP11 BNIP3L ACTN2 TOMM20 VCL TNKS2 ZFYVE27 MIA3 HNRNPU KIF5B ITGB1 NRP1 ATP2C1 GNL3 SEC13 COPG1 SEC61A1 AMOT LAMP2 PACS1 PRPF19 CRY2 CSRP3 CLTB APC HSPA4 SAR1B CTNNA1 DIAPH1 RAB6A C2CD3 JAM3 ATP2B4 FAM126A PTPN14 WRN SSB INSIG1 TNPO3 VPS41 FOXJ1 GGA3 TOM1L1 AP2B1 KIF13A GPSM2 EGFR UBR5 DCP1A OPTN SLC25A30 KPNA3 CTCF HOOK3 STX12 SNX17 CSE1L DVL2 MFSD1 SRP19 NVL FAM149B1 TUBA8 KPNA1 IPO7 IPO13 SSR3 PTTG1IP TXNIP ARL5B MFF ABRA RTN4 TOR1AIP1 ARL6IP5 DCLK1 KPNA6 DAG1 TOR1A EMP2 VAPA RANGRF FIS1 MARCHF5 NUP62 HERPUD1*
5,55E-09	204	1787	1,608797	Cellular localization	*NUP50 PACSIN2 POLDIP3 SCYL2 ATXN1 ITPR3 ILRUN MAPK14 WHAMM HECTD1 MMP14 MPP5 VIPAS39 TTC7B CCDC88C TRIP11 CILK1 TERF2IP PPP1R15A AP2A1 EXOSC10 MTOR MFN2 PLEKHM2 PINK1 NPC1 LATS1 STX7 BACH2 PYGO1 HSPA5 VPS28 SNX16 SEC31B VCPIP1 ATP1B1 MPC2 F11R COPA CASQ1 LMNA RAB13 SNX27 RAP1A SORT1 SH3GLB1 VPS16 HM13 KCNB1 SUN1 WIPI2 RABGEF1 STX4 TBC1D10B SEPTIN1 ATP2A1 USP7 POLR1A DCTN1 EXOC6B TEX261 ACTR2 RAB1A CEP68 ERLEC1 FAM98A DDX1 LETM1 SCARB2 ARFIP1 EGF SEC24B PAQR3 AKAP11 BNIP3L ACTN2 TOMM20 VCL TNKS2 ZFYVE27 SEC23IP MIA3 HNRNPU DENND1B AGTPBP1 KIF5B ITGB1 NRP1 ATP2C1 GNL3 ARL8B SEC13 COPG1 SEC61A1 DNAJC13 LSG1 CDK16 AMOT LAMP2 THOC2 PACS1 PRPF19 CRY2 HTATIP2 CSRP3 CLTB APC HSPA4 SAR1B CDC23 CTNNA1 DIAPH1 FAM160A2 NUP98 RAB6A C2CD3 JAM3 ATP2B4 FAM126A PTPN14 BIN1 WRN SSB INSIG1 CREB3L2 TNPO3 VPS41 FOXJ1 UNC13D GGA3 ITGB3 PLEKHM1 FBXL20 TOM1L1 AP2B1 VPS53 UBE2G1 DHRS7C KIF13A EPG5 GPSM2 EGFR UBR5 DCP1A OPTN SCN1B SLC25A30 KPNA3 CTCF RAF1 HOOK3 STX12 SNX17 CSE1L DVL2 MFSD1 FHOD1 SRP19 STX5 NVL SNCG FAM149B1 FYTTD1 TUBA8 TRDN KPNA1 ABCD3 IPO7 GOSR1 KCNE3 IPO13 TMBIM6 RHOBTB3 SSR3 PTTG1IP TXNIP ARL5B MFF ABRA RTN4 TOR1AIP1 ARL6IP5 DCLK1 KPNA6 SEC22C DAG1 TOR1A MAD2L1 SRSF10 HNRNPA2B1 EMP2 ATP5PB VAPA RANGRF FIS1 UBE2B YIPF5 MARCHF5 TMEM38B NUP62 NDE1 HERPUD1 TSC1 P2RX4*
1,91E-07	166	1455	1,608679	Reg, of cellular component organization	*PACSIN2 ACVR1B SCYL2 PPP1R10 MAPK14 PI16 WHAMM MMP14 TERF2IP ARHGAP35 PPP1R15A EXOSC10 MTOR MAD2L2 PDPN PLEKHM2 PINK1 ADGRL2 NEXN FAF1 MAP3K4 LATS1 ARHGAP18 SMAP1 SMAD4 VLDLR ARPC5L SETX VPS28 NBN SDCBP*

					F11R LMNA ADAM15 SETDB1 RAP1A SH3GLB1 ODF2L DLC1 MKKS PFDN4 RABGEF1 ERCC4 ACTR2 FEZ2 FAM98A CYRIA RNF4 ADD1 ATP8A1 EGF PAQR3 RGCC MYCBP2 SEMA4D SORBS3 BNIP3L CLU ARPC3 MTMR3 ACTN2 AGT VCL PPIF TNKS2 ZFYVE27 ARMH3 STN1 SLK TGFB2 HNRNPU NRP1 DYNC1LI1 PDCD6IP SEMA3G GNL3 BCL6 ATG3 NAT10 CAPRIN1 MYOD1 USP47 EIF4G2 NCLN SMARCA4 JMY RASA1 DCP2 APC CDC23 ETF1 DIAPH1 SMPD1 ARAP1 RPS3 PAK1 FZD4 CHORDC1 CEP295 JAM3 BIN1 NCKAP1 ITGAV FN1 XRCC5 ARPC2 MAD2L1BP LMOD2 IFRD1 MYO10 BAIAP2 GRB2 ITGB3 PLEKHM1 CALCOCO2 TOM1L1 ZNF207 TAOK1 ANKRD13B UBE2G1 GSPT1 GPSM2 NEU3 EGFR CEP135 ENO1 SEMA7A SCN1B CCSAP SCARF1 RAF1 LOC100624559 PHLDB1 SPART DHX36 RALB FHOD1 STX5 FHOD3 SSH3 AVIL MFF RTN4 SMG1 PIP4K2B PARL DAG1 TOR1A SDCCAG8 SACS G3BP1 MAD2L1 TMEM33 CST3 GSN SLC25A33 SMCR8 RANGRF FIS1 UBE2B MARCHF5 NUPR1 CYLD NUP62 TSC1
2,17E-07	164	1439	1,606104	Protein localization	*PACSIN2 ARFGAP3 PICK1 ILRUN MAPK14 HECTD1 MMP14 MPP5 VIPAS39 TTC7B TERF2IP PPP1R15A AP2A1 MFN2 PLEKHM2 PINK1 MYOM1 NPC1 JAK1 FAF1 LATS1 STX7 UFL1 PYGO1 KTN1 VLDLR HSPA5 VPS28 SNX16 VCPIP1 ATP1B1 MPC2 F11R COPA LMNA RAB13 SNX27 RAP1A SORT1 SH3GLB1 VPS16 HM13 KCNB1 SUN1 WIPI2 STX4 POLR1A CEP68 ERLEC1 DDX1 SCARB2 ARFIP1 EGF SEC24B PAQR3 AKAP11 MYCBP2 BNIP3L CLU ACTN2 TOMM20 VCL TNKS2 ZFYVE27 TGFB2 MIA3 HNRNPU KIF5B ITGB1 NRP1 ATP2C1 GNL3 ARL8B SEC13 COPG1 SEC61A1 BCL6 CDK16 AMOT LAMP2 PACS1 PRPF19 CRY2 CSRP3 CLTB MCC APC HSPA4 SAR1B CTNNA1 DIAPH1 FAM160A2 RAB6A C2CD3 CEP295 SNX19 JAM3 ATP2B4 FAM126A PTPN14 WRN SSB INSIG1 TNPO3 VPS41 FOXJ1 GGA3 PECAM1 ITGB3 TOM1L1 AP2B1 KIF13A PFKM GPSM2 EGFR CEP135 UBR5 DCP1A OPTN SLC25A30 KPNA3 CTCF RAF1 HOOK3 RCN3 STX12 SNX17 CSE1L DVL2 IRS1 MFSD1 SRP19 TIMM50 NVL SNCG FAM149B1 LMAN2L TUBA8 KPNA1 IPO7 IPO13 SSR3 PTTG1IP TXNIP ARL5B MFF ABRA RTN4 TOR1AIP1 ARL6IP5 DCLK1 KPNA6 DAG1 TOR1A EMP2 VAPA RANGRF NPNT FIS1 MARCHF5 NUP62 HERPUD1 KCNJ11*
5,99E-08	188	1671	1,585885	Intracellular signal transduction	*PPARA TNS2 YBX3 WNK1 PPP1R10 KLHL31 MAPK14 HOMER2 IQGAP1 AKAP13 FOXN3 CCDC88C TERF2IP NFAT5 ARHGAP35 MTOR MAD2L2 MFN2 PDPN PINK1 RALBP1 MYOM1 JAK1 RPS6KA2 MAP3K4 LATS1 BCLAF1 ARHGAP18 RRAGD SMAD4 CTNNAL1 SETX MTDH NBN SDCBP PRKDC ATP1B1 F11R CASQ1 SETDB1 RAP1A VCAM1 TGFBR3 CCN1 DLC1 TTI1 RALGAPB DOK5 RABGEF1 ATP2A1 CDIP1 VRK2 RASGRP3 DDX1 RELL1 PDGFRA EGF PAQR3 CUL4A RASA3 SEMA4D SORBS3 KCTD9 CLU PITPNM2 MAPK1 KLHL22 SIPA1L2 AGT MYOZ1 DUSP29 PPIF PRKG1 DNMBP TGFB2 RGS7 CDC42BPA*

					ITGB1 NRP1 DYNC1LI1 OXSR1 NISCH LMCD1 SEC13 BCL6 AMOT CAT CSRP3 TEAD1 USP47 RASA1 MEF2C APC HINT1 LARS1 SMPD1 RPS3 PAK1 FZD4 USP28 ATP2B4 IVNS1ABP ATF3 GRB10 PDK1 MAP3K20 GPR155 NFE2L2 NCKAP1 ITGAV FN1 PLCD4 COPS8 ASB1 MAD2L1BP PRKAG2 ASB15 GRB2 PRKCA PLEKHM1 ZNF207 KSR1 TAOK1 ASB8 GPSM2 DAPK2 DOCK9 EGFR ENO1 FEM1B KBTBD2 TRPM4 PDGFRB PJA2 SEMA7A CASTOR2 NKIRAS1 RHOU RAF1 DHX36 FGF1 RALB SPSB2 DVL2 IRS1 SLC15A4 TMEM106A SH3BP5L PDK2 HDAC7 HUS1 RB1CC1 ASB10 PDE2A RASGEF1B TMBIM6 NDRG2 ARHGEF6 PTTG1IP ABRA CDC42SE1 RTN4 SIK2 ARL6IP5 PARL DCLK1 DAG1 MAP2K3 EIF4EBP2 CXCL12 MAD2L1 NSMCE1 CAVIN4 SMCR8 PERP TRIB1 RANGRF NPNT FIS1 UBE2B TMEM38B NUPR1 CYLD NUP62 RND3 HERPUD1 TSC1 P2RX4
5,99E-08	193	1728	1,574754	Macromolecule localization	*PPARA PACSIN2 ARFGAP3 POLDIP3 PICK1 ILRUN MAPK14 FURIN HECTD1 MMP14 MPP5 VIPAS39 TTC7B TERF2IP PPP1R15A AP2A1 EXOSC10 MFN2 PLEKHM2 PINK1 MYOM1 NPC1 JAK1 FAF1 LATS1 STX7 UFL1 PYGO1 KTN1 VLDLR HSPA5 VPS28 OC90 SNX16 VCPIP1 ATP1B1 MPC2 F11R COPA LMNA RAB13 SNX27 RAP1A SORT1 SH3GLB1 VPS16 HM13 KCNB1 SUN1 WIPI2 STX4 POLR1A CEP68 ERLEC1 MFSD2B DDX1 ATP8A1 SCARB2 ARFIP1 EGF SEC24B PAQR3 AKAP11 MYCBP2 BNIP3L CLU PITPNM2 ACTN2 TOMM20 VCL TNKS2 ZFYVE27 TGFB2 EPRS1 MIA3 HNRNPU KIF5B ITGB1 NRP1 ATP2C1 ABHD5 GNL3 ARL8B SEC13 COPG1 SEC61A1 BCL6 CDK16 AMOT LAMP2 THOC2 PNPLA2 PACS1 PRPF19 CRY2 CSRP3 SLC27A1 CLTB MCC DCP2 APC HSPA4 SAR1B CTNNA1 DIAPH1 FAM160A2 RAB6A C2CD3 FZD4 CEP295 SNX19 JAM3 ATP2B4 FAM126A PTPN14 ACSL1 WRN SSB ITGAV INSIG1 TNPO3 VPS41 FOXJ1 GGA3 ABCA5 ABCA6 PECAM1 ITGB3 STAT5B TOM1L1 AP2B1 KIF13A NRIP1 PFKM GPSM2 EGFR CEP135 UBR5 DCP1A OPTN SLC25A30 KPNA3 CTCF RAF1 HOOK3 RCN3 STX12 SNX17 CSE1L DVL2 IRS1 MFSD1 SRP19 TIMM50 NVL SNCG FAM149B1 LMAN2L FYTTD1 TUBA8 LPCAT3 KPNA1 ABCD3 IPO7 PRPF6 IPO13 SSR3 PTTG1IP TXNIP ARL5B MFF ABRA TMEM159 RTN4 TOR1AIP1 ARL6IP5 DCLK1 KPNA6 DAG1 TOR1A HNRNPA2B1 EMP2 FITM2 VAPA RANGRF NPNT FIS1 MARCHF5 NUP62 HERPUD1 KCNJ11 TSC1*
4,56E-07	209	1979	1,488316	Phosphate-containing compound metabolic proc,	*PPARA PWP1 ACVR1B TNS2 DERA WNK1 SCYL2 KLHL31 MAPK14 KCTD20 MOCS1 ADGRF5 IQGAP1 PEAK1 PKM THTPA SLC8A3 PTPN21 TTC7B CCDC88C CILK1 TERF2IP VAC14 CSNK2A2 PPP1R15A MTOR MAD2L2 PINK1 NT5C1A JAK1 CMPK1 TESK2 RPS6KA2 MAP3K4 LATS1 PGM3 SMAD4 ALPK2 VLDLR PIGO AK1 ST3GAL1 OC90 NBN PKIA SDCBP PRKDC MPC2 AMPD1 CDC14A TGFBR3 CCN1 DLC1 GPAT4 RBL1 RABGEF1 PHKG1 EEF2K RPIA DUSP11 VRK2 EFEMP1 PDGFRA EGF PAQR3 PAN3 RGCC SEMA4D EPHX2 CLU GRK3 MTMR3 MAPK1 AGT DUSP29 SAMD8 PRKG1 SLK TGFB2 BPNT1*

					COQ8A CDC42BPA HNRNPU NRP1 PFKFB3 OXSR1 ACVR2B ABHD5 PFKFB4 NEK4 UMPS ATP5PO ADARB1 PDK3 CDK16 OCRL PPP6R3 TRPT1 CRY2 HTATIP2 SLC27A1 MAML1 THBS4 SERINC5 CKMT2 APC HINT1 SMAD5 PPARGC1B CAMK2A SMPD1 INPPL1 RPS3 PAK1 FZD4 CHORDC1 MTMR2 ATP2B4 CDK18 PDK4 FAM126A NAMPT PTPN14 RPS6KC1 GRB10 DCTD ACSL1 PDK1 MAP3K20 FZD7 ATIC FN1 XRCC5 PLCD4 PRKAG3 COPS8 ILKAP PRKAG2 NADK2 NDUFS4 PLPP1 FOXK2 PRKCA PECAM1 ITGB3 NME2 TOM1L1 KSR1 KIAA0100 TAOK1 PFKM DAPK2 EGFR ENO1 AMPD3 PDGFRB SEMA7A SERINC2 RAF1 FGF1 RALB DVL2 DNAJC3 TIMM50 PIGS FARP1 PDK2 LPCAT3 NUDT15 IL6R BMP2K SSH3 TAF7 RB1CC1 GDE1 PDE2A PGAP4 SMG1 BPNT2 SIK2 PIP4K2B DBNDD2 UBE2K DCLK1 MAP2K3 PIGL DUSP13 MTMR6 MTMR9 CKM PDE4C EMP2 PTDSS2 ATP5PB FITM2 SMCR8 CACUL1 TRIB1 NPNT UBE2B IP6K3 CAMK2B TMEM38B NUPR1 NUP62
4,56E-07	210	1991	1,486407	Phosphorus metabolic proc,	*PPARA PWP1 ACVR1B TNS2 DERA WNK1 SCYL2 KLHL31 MAPK14 KCTD20 MOCS1 ADGRF5 IQGAP1 PEAK1 PKM THTPA SLC8A3 PTPN21 TTC7B CCDC88C CILK1 TERF2IP VAC14 CSNK2A2 PPP1R15A MTOR MAD2L2 PINK1 NT5C1A JAK1 CMPK1 TESK2 RPS6KA2 MAP3K4 LATS1 PGM3 SMAD4 ALPK2 VLDLR PIGO AK1 ST3GAL1 OC90 NBN PKIA SDCBP PRKDC MPC2 AMPD1 CDC14A TGFBR3 CCN1 DLC1 GPAT4 RBL1 RABGEF1 PHKG1 EEF2K RPIA DUSP11 UGP2 VRK2 EFEMP1 PDGFRA EGF PAQR3 PAN3 RGCC SEMA4D EPHX2 CLU GRK3 MTMR3 MAPK1 AGT DUSP29 SAMD8 PRKG1 SLK TGFB2 BPNT1 COQ8A CDC42BPA HNRNPU NRP1 PFKFB3 OXSR1 ACVR2B ABHD5 PFKFB4 NEK4 UMPS ATP5PO ADARB1 PDK3 CDK16 OCRL PPP6R3 TRPT1 CRY2 HTATIP2 SLC27A1 MAML1 THBS4 SERINC5 CKMT2 APC HINT1 SMAD5 PPARGC1B CAMK2A SMPD1 INPPL1 RPS3 PAK1 FZD4 CHORDC1 MTMR2 ATP2B4 CDK18 PDK4 FAM126A NAMPT PTPN14 RPS6KC1 GRB10 DCTD ACSL1 PDK1 MAP3K20 FZD7 ATIC FN1 XRCC5 PLCD4 PRKAG3 COPS8 ILKAP PRKAG2 NADK2 NDUFS4 PLPP1 FOXK2 PRKCA PECAM1 ITGB3 NME2 TOM1L1 KSR1 KIAA0100 TAOK1 PFKM DAPK2 EGFR ENO1 AMPD3 PDGFRB SEMA7A SERINC2 RAF1 FGF1 RALB DVL2 DNAJC3 TIMM50 PIGS FARP1 PDK2 LPCAT3 NUDT15 IL6R BMP2K SSH3 TAF7 RB1CC1 GDE1 PDE2A PGAP4 SMG1 BPNT2 SIK2 PIP4K2B DBNDD2 UBE2K DCLK1 MAP2K3 PIGL DUSP13 MTMR6 MTMR9 CKM PDE4C EMP2 PTDSS2 ATP5PB FITM2 SMCR8 CACUL1 TRIB1 NPNT UBE2B IP6K3 CAMK2B TMEM38B NUPR1 NUP62*

Table S2: ASE-SNPs affecting candidate lipid genes. The ASE proportion is the percentage of heterozygous that present allelic imbalance for a given SNP.

SNP rs	Gene ID	Gene Name	ASE Individuals (p.value < 0,05)	Total heterozygous tested	ASE proportion (%)	Consequence
rs328486316	ENSSSCG00000028620	*ABCD3*	9	25	36	Downstream
rs81217189	ENSSSCG00000011297	*ABHD5*	5	32	15,63	Synonymous
rs196959825	ENSSSCG00000011629	*ACAD11*	15	58	25,86	3' UTR
rs323056426	ENSSSCG00000023354	*ACAD9*	13	74	17,57	Synonymous
rs81211675	ENSSSCG00000003776	*ACADM*	74	74	100	Synonymous
rs345294790	ENSSSCG00000017337	*ACBD4*	3	40	7,5	5' UTR
rs55618935	ENSSSCG00000015784	*ACSL1*	20	21	95,24	3' UTR
rs322578425	ENSSSCG00000026453	*ACSM5*	5	10	50	3' UTR
rs196952262	ENSSSCG00000015755	*AGPAT5*	9	70	12,86	Missense
rs346053510	ENSSSCG00000015755	*AGPAT5*	15	70	21,43	3' UTR
rs80997895	ENSSSCG00000010184	*AGT*	55	83	66,27	Synonymous
rs343238244	ENSSSCG00000009717	*CBR4*	27	36	75	Downstream
rs345224133	ENSSSCG00000003851	*CPT2*	8	36	22,22	Synonymous
rs322980308	ENSSSCG00000005671	*CRAT*	6	34	17,65	Intron
rs332617618	ENSSSCG00000010780	*CYP2E1*	29	62	46,77	Synonymous
rs55618570	ENSSSCG00000024484	*DEGS1*	11	63	17,46	Synonymous
rs346355799	ENSSSCG00000011119	*ECHDC3*	32	47	68,09	3' UTR
rs81477697	ENSSSCG00000011119	*ECHDC3*	35	56	62,5	Synonymous
rs80915078	ENSSSCG00000038086	*ECHS1*	21	21	100	Synonymous
rs332100640	ENSSSCG00000001000	*ECI2*	3	12	25	Intron
rs322829130	ENSSSCG00000009666	*EPHX2*	35	42	83,33	Synonymous

rs80813406	ENSSSCG00000001862	*ETFA*	30	33	90,91	Synonymous
rs333455646	ENSSSCG00000013072	*FADS2*	6	49	12,24	3' UTR
rs81215802	ENSSSCG00000026044	*FDFT1*	57	58	98,28	Synonymous
rs343615786	ENSSSCG00000007019	*GPAT4*	23	42	54,76	3' UTR
rs335455698	ENSSSCG00000025729	*IRS1*	38	69	55,07	Synonymous
rs328830166	ENSSSCG00000003018	*LIPE*	42	54	77,78	Missense
rs324455395	ENSSSCG00000008624	*LPIN1*	2	5	40	Intron
rs81378004	ENSSSCG00000008624	*LPIN1*	11	22	50	Intron
rs338458270	ENSSSCG00000008624	*LPIN1*	18	26	69,23	Synonymous
rs326383148	ENSSSCG00000008624	*LPIN1*	4	28	14,29	Intron
rs345575817	ENSSSCG00000008624	*LPIN1*	64	65	98,46	3' UTR
rs341613198	ENSSSCG00000001556	*MAPK14*	18	32	56,25	3' UTR
rs322149848	ENSSSCG00000010107	*MED15*	15	60	25	3' UTR
rs330721142	ENSSSCG00000029989	*MED28*	35	60	58,33	3' UTR
rs345605839	ENSSSCG00000035098	*MED4*	56	66	84,85	Synonymous
rs196956362	ENSSSCG00000035098	*MED4*	56	83	67,47	3' UTR
rs80901314	ENSSSCG00000034290	*MED6*	30	86	34,88	Missense
rs330731389	ENSSSCG00000003413	*MTOR*	16	80	20	3' UTR
rs81219366	ENSSSCG00000036294	*NUDT19*	9	34	26,47	Downstream
rs335350933	ENSSSCG00000015334	*PDK4*	22	55	40	Intron
rs323993804	ENSSSCG00000015334	*PDK4*	22	60	36,67	Intron
rs322819411	ENSSSCG00000003451	*PDPN*	21	57	36,84	3' UTR
rs339524839	ENSSSCG00000012841	*PNPLA2*	58	60	96,67	3' UTR
rs326772027	ENSSSCG00000000006	*PPARA*	1	6	16,67	Intron
rs81231121	ENSSSCG00000000006	*PPARA*	27	67	40,3	Downstream
rs342853730	ENSSSCG00000014437	*PPARGC1B*	16	36	44,44	Upstream
rs80917802	ENSSSCG00000006703	*PRKAB2*	58	73	79,45	Synonymous

rs338572921	ENSSSCG00000006703	*PRKAB2*	47	74	63,51	3' UTR
rs81310741	ENSSSCG00000016432	*PRKAG2*	2	3	66,67	Intron
rs329891306	ENSSSCG00000016432	*PRKAG2*	6	14	42,86	Intron
rs327471058	ENSSSCG00000016432	*PRKAG2*	6	28	21,43	Synonymous
rs320642043	ENSSSCG00000016200	*PRKAG3*	1	5	20	Intron
rs1112162843	ENSSSCG00000016200	*PRKAG3*	26	60	43,33	Intron
rs322383150	ENSSSCG00000026554	*PRXL2B*	64	65	98,46	3' UTR
rs325140087	ENSSSCG00000022655	*PTGES2*	24	66	36,36	3' UTR
rs344694929	ENSSSCG00000004029	*QKI*	17	65	26,15	Downstream
rs337851590	ENSSSCG00000016367	*RNPEPL1*	18	20	90	5' UTR
rs331043783	ENSSSCG00000016367	*RNPEPL1*	27	65	41,54	3' UTR
rs80782536	ENSSSCG00000001720	*SLC25A27*	3	11	27,27	3' UTR
rs320952513	ENSSSCG00000013879	*SLC27A1*	12	62	19,35	3' UTR
rs333695749	ENSSSCG00000000272	*SP1*	5	32	15,63	Downstream
rs332553125	ENSSSCG00000017406	*STAT5B*	10	21	47,62	Downstream
rs345880217	ENSSSCG00000013399	*TEAD1*	48	56	85,71	Downstream
rs319491855	ENSSSCG00000013399	*TEAD1*	60	70	85,71	Synonymous
rs321574540	ENSSSCG00000014834	*UCP3*	3	3	100	Downstream
rs336634583	ENSSSCG00000014834	*UCP3*	11	17	64,71	Intron
rs326806805	ENSSSCG00000014834	*UCP3*	18	18	100	Downstream
rs81389195	ENSSSCG00000003570	*WDTC1*	22	26	84,62	Synonymous

Table S3: Common ASE-SNPs were identified between our study and the one conducted by Liu et al. (2020) in Duroc x Luchuan pigs, focusing on candidate genes related to meat quality.

Chr	Position	SNP rs	Consequence	Ensembl ID	Gene name
14	74735219	rs333684092	downstream_variant	ENSSSCG00000010278	-
15	121489462	rs334957944	intergenic_variant	-	-
1	2551985	rs55618867	synonymous_variant	ENSSSCG00000032916	-
4	30712844	rs80853234	synonymous_variant	ENSSSCG00000031728	*ABRA*
14	54671108	rs335002364	intron_variant	ENSSSCG00000010144	*ACTN2*
4	118192174	rs338066881	synonymous_variant	ENSSSCG00000006872	*AGL*
6	77374418	rs337463568	3_UTR_variant	ENSSSCG00000024752	*ALDH4A1*
4	105875649	rs80886011	synonymous_variant	ENSSSCG00000006754	*AMPD1*
4	90341948	rs55618847	downstream_gene_variant	ENSSSCG00000006391	*ATP1A2*
4	90358204	rs337150379	synonymous_variant	ENSSSCG00000006391	*ATP1A2*
10	23506975	rs337736193	missense_variant	ENSSSCG00000024784	*CACNA1S*
10	23533274	rs344595791	synonymous_variant	ENSSSCG00000024784	*CACNA1S*
2	151251114	rs81367920	3_UTR_variant	ENSSSCG00000014443	*CAMK2A*
4	90277066	rs326395928	3_UTR_variant	ENSSSCG00000006390	*CASQ1*
2	89814154	rs333166681	synonymous_variant	ENSSSCG00000014128	*CKMT2*
16	72204077	rs81212760	synonymous_variant	ENSSSCG00000039763	*CMBL*
2	15387579	rs341370139	upstream_variant	ENSSSCG00000013243	*DDB2*
4	105891070	rs80806741	upstream_variant	ENSSSCG00000006755	*DENND2C*
12	54653333	rs81213334	synonymous_variant	ENSSSCG00000017996	*DHRS7C*
14	141343802	rs80915078	synonymous_variant	ENSSSCG00000038086	*ECHS1*
6	148854583	rs81215882	synonymous_variant	ENSSSCG00000003812	*EFCAB7*
5	18296730	rs325084555	synonymous_variant	ENSSSCG00000032028	*EIF4B*
13	138883473	rs335347548	3_UTR_variant	ENSSSCG00000011885	*FBXO40*
4	99331757	rs343127577	synonymous_variant	ENSSSCG00000035429	*HJV*
7	30563851	rs333189515	3_UTR_variant	ENSSSCG00000001527	*ILRUN*
4	117944377	rs80795413	synonymous_variant	ENSSSCG00000006867	*LRRC39*
15	79328240	rs328301396	intron_variant	ENSSSCG00000015960	*MAP3K20*
14	76456694	rs345277231	upstream_variant	ENSSSCG00000010304	*MYOZ1*
15	686266	rs325077707	synonymous_variant	ENSSSCG00000016397	*NEB*
15	687431	rs321740884	synonymous_variant	ENSSSCG00000016397	*NEB*
15	694683	rs326317941	synonymous_variant	ENSSSCG00000016397	*NEB*
6	54598842	rs329151980	upstream_variant	ENSSSCG00000024823	*RCN3*
8	30694399	rs337912544	synonymous_variant	ENSSSCG00000061243	*RPL9*
9	9626505	rs55619120	synonymous_variant	ENSSSCG00000014855	*RPS3*
1	268576015	rs80830222	3_UTR_variant	ENSSSCG00000005636	*SLC25A25*
2	8894947	rs81312355	synonymous_variant	ENSSSCG00000022404	*SLC3A2*
3	57870098	rs334447146	3_UTR_variant	ENSSSCG00000008215	*SMYD1*
5	63840701	rs80868263	downstream_variant	ENSSSCG00000025460	*SPSB2*
1	239455864	rs345128403	synonymous_variant	ENSSSCG00000063066	*TMOD1*

1	39525860	rs327383186	synonymous_variant	ENSSSCG00000027613	*TRDN*
12	40021728	rs81434826	synonymous_variant	ENSSSCG00000017717	*UNC45B*
5	61360959	rs55619150	3_UTR_variant	ENSSSCG00000000633	*YBX3*
4	118196368	rs80809403	synonymous_variant	ENSSSCG00000006872	*AGL*
17	48028661	rs320512199	upstream_variant	ENSSSCG00000007424	*TNNC2*
14	15010065	rs81215802	synonymous_variant	ENSSSCG00000026044	*FDFT1*
13	64993726	rs318581283	3_UTR_variant	ENSSSCG00000011538	*LMCD1*
4	81912941	rs81213257	synonymous_variant	ENSSSCG00000006296	*ATP1B1*
13	138887967	rs324947205	synonymous_variant	ENSSSCG00000011885	*FBXO40*
3	55686851	rs333083882	3_UTR_variant	ENSSSCG00000058408	*COA5*
5	100764373	rs340349360	synonymous_variant	ENSSSCG00000026533	*MYF6*
18	48526014	rs81215877	synonymous_variant	ENSSSCG00000028523	*HUS1*
6	68960180	rs709011514	intron_variant	ENSSSCG00000033497	*RERE*
6	135351740	rs81217583	5_UTR_variant	ENSSSCG00000003768	*NEXN*
12	24315213	rs1112301778	3_UTR_variant	ENSSSCG00000017525	*NFE2L1*
1	268569552	rs335457302	synonymous_variant	ENSSSCG00000005636	*SLC25A25*
8	39128611	rs338676518	3_UTR_variant	ENSSSCG00000058015	*SGCB*
12	5305600	rs81434107	upstream_variant	ENSSSCG00000017187	*FOXJ1*
13	26208896	rs340940976	3_UTR_variant	ENSSSCG00000011286	*KLHL40*
14	108948871	rs340123986	missense_variant	ENSSSCG00000010522	*ANKRD2*
14	77677549	rs334467231	upstream_variant	ENSSSCG00000010319	*SAMD8*
2	10832146	rs340758760	synonymous_variant	ENSSSCG00000013110	*TMEM109*
6	54244744	rs45435515	synonymous_variant	ENSSSCG00000003154	*GYS1*
14	77660653	rs318806339	3_prime_UTR_variant	ENSSSCG00000035105	-
12	23344596	rs1108115245	3_prime_UTR_variant	ENSSSCG00000033291	*PIP4K2B*
3	5117830	rs55618502	5_prime_UTR_variant	ENSSSCG00000024245	*AIMP2*
2	116878437	rs81213697	synonymous_variant	ENSSSCG00000022048	*REEP5*
13	16892960	rs81243380	3_UTR_variant	ENSSSCG00000038607	*GADL1*
2	7921760	rs81213209	missense_variant	ENSSSCG00000021620	*STIP1*
12	5281032	rs327576749	synonymous_variant	ENSSSCG00000017186	*RNF157*
14	141257121	rs342495519	missense_variant	ENSSSCG00000010774	*ZNF511*
13	65130787	rs81215798	synonymous_variant	ENSSSCG00000056902	*CAV3*
X	41845691	rs81213062	synonymous_variant	ENSSSCG00000012269	*CDK16*
6	55328951	rs319771677	downstream_variant	ENSSSCG00000055583	-
6	52840574	rs335469124	3_UTR_variant	ENSSSCG00000003107	*ARHGAP35*
4	366159	rs55618482	synonymous_variant	ENSSSCG00000005904	*VPS28*
14	76450783	rs333025427	intron_variant	ENSSSCG00000010304	*MYOZ1*
14	81877940	rs80855699	downstream_variant	ENSSSCG00000010331	*ANXA11*
3	23887869	rs81313849	intron_variant	ENSSSCG00000007839	*EEF2K*
6	95478637	rs328326902	intron_variant	ENSSSCG00000003662	*NT5C1A*
18	48734613	rs342453650	downstream_variant	ENSSSCG00000016722	*UBE2D4*
7	45967451	rs80834158	downstream_variant	ENSSSCG00000049456	-
3	56780533	rs323205258	intron_variant	ENSSSCG00000008200	*ANKRD23*
13	202767758	rs81212247	synonymous_variant	ENSSSCG00000062206	*PSMG1*

8	120797165	rs81403966	missense_variant	ENSSSCG00000057637	*C4orf54*
15	121431187	rs326201198	intron_variant	ENSSSCG00000020785	*DES*
10	32442287	rs81423443	synonymous_variant	ENSSSCG00000033196	*MYORG*
14	77663270	rs321324707	3_UTR_variant	ENSSSCG00000035105	-
4	86929049	rs345715585	intergenic_variant	-	-
1	881098	-	3_UTR_variant	ENSSSCG00000004012	*THBS2*
9	21749745	rs327689182	intron_variant	ENSSSCG00000014924	*CTSC*
11	71191392	rs81212427	synonymous_variant	ENSSSCG00000042169	-
10	10187869	rs323573076	upstream_variant	ENSSSCG00000010829	*MTARC1*
4	96007274	rs55618485	synonymous_variant	ENSSSCG00000031053	*S100A1*
4	122234335	rs333866332	3_UTR_variant	ENSSSCG00000039854	*TLCD4*
14	141258454	rs339733512	intron_variant	ENSSSCG00000010774	*ZNF511*
9	113875098	rs331765803	downstream_variant	ENSSSCG00000021059	*ADORA1*
10	56088388	rs81213201	synonymous_variant	ENSSSCG00000011101	*ITGB1*
2	88776183	rs1109433572	synonymous_variant	ENSSSCG00000014117	*THBS4*
18	1751306	rs337423366	synonymous_variant	ENSSSCG00000016411	*NOM1*
7	31859476	rs341613198	3_UTR_variant	ENSSSCG00000001556	*MAPK14*
1	242319854	rs336222829	3_UTR_variant	ENSSSCG00000037954	*CAVIN4*
4	85345636	rs80783545	synonymous_variant	ENSSSCG00000006328	*RXRG*
12	53486525	rs328962961	synonymous_variant	ENSSSCG00000038726	*RANGRF*
2	5059013	rs331196147	missense_variant	ENSSSCG00000012905	*TMEM134*
10	39885762	rs332787631	downstream_variant	ENSSSCG00000056325	-
10	32443725	rs336979398	3_UTR_variant	ENSSSCG00000033196	*MYORG*
1	65875605	rs80924453	synonymous_variant	ENSSSCG00000004347	*FBXL4*
7	114176385	rs10720191	3_UTR_variant	ENSSSCG00000002452	*LGMN*
12	39995516	rs318972369	intron_variant	ENSSSCG00000017717	*UNC45B*
3	125269117	rs333133716	3_UTR_variant	ENSSSCG00000026161	*E2F6*
12	561604	rs345060363	3_UTR_variant	ENSSSCG00000017125	*FOXK2*
6	63337751	rs324952295	5_UTR_variant	ENSSSCG00000033735	-
10	56099889	rs81213197	synonymous_variant	ENSSSCG00000011101	*ITGB1*
1	229928827	rs319792426	non_coding_transcript	ENSSSCG00000044318	-
3	106077747	rs332838197	synonymous_variant	ENSSSCG00000008508	*FAM98A*
18	55465655	rs339749290	missense_variant	ENSSSCG00000016772	*VPS41*
7	24958150	rs80929898	missense_variant	ENSSSCG00000001456	-
5	63654591	rs81214778	synonymous_variant	ENSSSCG00000060530	*C1R*
14	20705168	rs328487846	synonymous_variant	ENSSSCG00000029331	*PALLD*
13	72353100	rs80817166	synonymous_variant	ENSSSCG00000011622	*KBTBD12*
12	55358735	rs344933137	intron_variant	ENSSSCG00000018007	*MYH3*
2	147500131	rs338568085	synonymous_variant	ENSSSCG00000014411	*LARS1*
14	11342815	rs55619081	synonymous_variant	ENSSSCG00000009668	*CLU*
6	51709386	rs1112593947	intron_variant	ENSSSCG00000036132	*CKM*
12	57235477	rs344875842	missense_variant	ENSSSCG00000028465	*ELAC2*
5	63743600	rs337519120	missense_variant	ENSSSCG00000027205	*LPCAT3*
8	120796517	rs81403965	synonymous_variant	ENSSSCG00000057637	*C4orf54*
13	72300643	rs331383534	intron_variant	ENSSSCG00000011622	*KBTBD12*

14	125183673	rs320582474	synonymous_variant	ENSSSCG00000010652	*FHIP2A*
10	56293972	rs337749140	synonymous_variant	ENSSSCG00000011102	*NRP1*
14	74941734	rs318297800	intron_variant	ENSSSCG00000010283	*SPOCK2*
1	72834539	rs81218519	missense_variant	ENSSSCG00000004374	*QRSL1*
5	23105446	rs699050767	intron_variant	ENSSSCG00000029571	*AVIL*
2	8291013	rs323903055	Downstream_variant	ENSSSCG00000031666	*ZFTA*
7	115221148	rs702721383	intron_variant	ENSSSCG00000002467	*ASB2*
1	72835324	rs81218520	synonymous_variant	ENSSSCG00000004374	*QRSL1*
18	6045394	rs322111153	intron_variant	ENSSSCG00000029241	*ASB10*
6	74852222	rs345945726	downstream_variant	ENSSSCG00000003461	-

Table S4: ASE-SNPs identified in this study are located within the QTL regions previously identified in the BC1_DU animals by Crespo-Piazuelo et al. (2020).

SNP rs	Consequence	Impact	Symbol	Gene ID	Start region Crespo et al., 2020	End region Crespo et al., 2020	Phenotype	Candidate genes QTL Crespo et al., 2020
rs323450134	downstream_variant	MODIFIER	*NRIP1*	ENSSSCG00000021038	175539436	181652057	C20:3(n-3)	*LIPI; NRIP1; ssc-let-7c*
rs330744970	missense_variant	MODERATE	*NRIP1*	ENSSSCG00000021038	175539436	181652057	C20:3(n-3)	*LIPI; NRIP1; ssc-let-7c*
rs81217196	synonymous_variant	LOW	*USP25*	ENSSSCG00000024623	175539436	181652057	C20:3(n-3)	*LIPI; NRIP1; ssc-let-7c*
rs332508992	downstream_variant	MODIFIER	*USP25*	ENSSSCG00000024623	175539436	181652057	C20:3(n-3)	*LIPI; NRIP1; ssc-let-7c*
rs332617618	synonymous_variant	LOW	*CYP2E1*	ENSSSCG00000010780	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs80915078	synonymous_variant	LOW	*ECHS1*	ENSSSCG00000038086	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs339733512	intron_variant	MODIFIER	*ZNF511*	ENSSSCG00000010774	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs342495519	missense_variant	MODERATE	*ZNF511*	ENSSSCG00000010774	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs1108330410	intron_variant	MODIFIER	*CALHM2*	ENSSSCG00000010600	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs342555189	downstream_variant	MODIFIER	*MFSD13A*	ENSSSCG00000010584	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs323476599	synonymous_variant	LOW	*PITX3*	ENSSSCG00000010578	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs339794165	3_UTR_variant	MODIFIER	*ARMH3*	ENSSSCG00000010571	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs341378114	3_UTR_variant	MODIFIER	*OGA*	ENSSSCG00000010569	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs326425328	3_UTR_variant	MODIFIER	*FBXW4*	ENSSSCG00000010566	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs343387045	upstream_variant	MODIFIER	*TWNK*	ENSSSCG00000030428	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs705003269	intron_variant	MODIFIER	-	ENSSSCG00000049992	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs790970169	intron_variant	MODIFIER	-	ENSSSCG00000049992	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs334275765	missense_variant	MODERATE	*SEC31B*	ENSSSCG00000006165	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs340093540	3_UTR_variant	MODIFIER	*DNMBP*	ENSSSCG00000010544	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs330856166	3_UTR_variant	MODIFIER	*DNMBP*	ENSSSCG00000010544	109946218	114621937	C18:1(n-9)/C18:0*; MUFA/SFA	*ELOVL3; SCD*
rs339594448	synonymous_variant	LOW	*CD93*	ENSSSCG00000007116	30061857	32867849	C20:0	*ABHD12; ACSS1; PANK2*

rs81211078	synonymous_variant	LOW	*CST3*	ENSSSCG00000037360	30061857	32867849	C20:0	*ABHD12; ACSS1; PANK2*
rs321168302	3_UTR_variant	MODIFIER	*ATRN*	ENSSSCG00000007153	30061857	32867849	C20:0	*ABHD12; ACSS1; PANK2*
rs318844839	3_UTR_variant	MODIFIER	*ATRN*	ENSSSCG00000007153	30061857	32867849	C20:0	*ABHD12; ACSS1; PANK2*
rs81217421	synonymous_variant	LOW	*VPS16*	ENSSSCG00000007167	30061857	32867849	C20:0	*ABHD12; ACSS1; PANK2*

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Chapter 8

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