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The *ACACA* and *SREBF1* genes are promising markers for pig carcass and performance traits, but not for fatty acid content in the longissimus dorsi muscle and adipose tissue

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ABSTRACT

Among functional and positional candidate genes for pig fatness traits, there are genes encoding *acetyl-CoA carboxylase alpha* (*ACACA*) and *sterol regulatory element binding transcription factor 1* (*SREBF1*). In our study, 5 known and 2 novel SNPs in the *ACACA* gene and 2 known and 6 novel polymorphisms in the *SREBF1* were found. The SNPs segregated as 6 and 10 haplotypes in *ACACA* and *SREBF1*, respectively. Two SNPs in *ACACA* were associated with fatness and performance traits in Polish breeds. In the *SREBF1* gene, two novel SNPs were associated with daily gain and one, within a potential intronic micro-RNA gene, showed an association with fatness traits. Breed-specific differences in the transcript level were observed for *ACACA* only. No correlation between the transcript levels and fatty acid compositions was found in muscle and fat tissues. We concluded that polymorphisms of the *ACACA* and *SREBF1* genes are promising markers for pig carcass and performance traits.

1. Introduction

Fatty acid content and lipid deposition traits are important factors influencing sensory, nutritional and technological properties of pork, and are related to fattening efficiency. The phenotypic variability of fatness traits depends significantly on the genetic background, including breed-specific gene pools. It is known that the heritability coefficient (h^2) for backfat thickness and intramuscular fat content is around 0.5 (Switonski, Stachowiak, Cieslak, Bartz, & Grzes, 2010), whereas for intramuscular fatty acid content it oscillates around 0.3 (Casellas et al., 2010).

Our study comprised two genes encoding proteins relevant to lipid metabolism that are positional and functional candidates potentially affecting porcine fatness and performance traits. The *ACACA* gene encodes acetyl-CoA carboxylase alpha, which catalyzes the carboxylation of acetyl-CoA to malonyl-CoA — a regulator of mitochondrial fatty-acid β-oxidation and affects the expression of hypothalamic neuropeptides modulating energy homeostasis and lipid metabolism (Diéguez, Vazquez, Romero, López, & Nogueiras, 2011; Wakil & Abu-Elheiga, 2009). The *SREBF1* gene encodes sterol regulatory element binding transcription factor 1 involved in adipocyte differentiation, as well as cholesterol and fatty acid synthesis. The active SREBF1 protein binds to sterol response elements (SRE) in the regulatory regions of many target genes involved in lipid metabolism, including *ACACA* (Horton, Goldstein, & Brown, 2002).

In mammals both genes are functionally linked, since the SREBF1 transcription factor regulates the expression of the *ACACA* gene and both were assigned by fluorescent in situ hybridization to the pig chromosome SSC12 (Magaña, Lin, Dooley, & Osborne, 1997). A precise localization of the *ACACA* gene on SSC12q12-13 (Kociucka, Cieslak, & Szczerbal, 2012) coincides with QTLs for average backfat thickness and birth body weight, the percentage of vaccenic, stearic, palmitic and palmitoleic fatty acids and the total percentage of saturated fatty acids in backfat and/or skeletal muscles (Liu et al., 2007, 2008; Muñoz et al., 2007; Quintanilla et al., 2011). The *SREBF1* gene was mapped to SSC12q15 (Szczerbal & Chmurzynska, 2008) where QTLs for backfat thickness, body weight at birth and carcass length were found (Liu et al., 2007, 2008). Taking the above into consideration we hypothesized that polymorphism and/or transcript level of the *ACACA* and *SREBF1* genes may contribute to phenotypic variation of carcass and performance traits in pigs.

The aims of the study were: 1) to search for polymorphism in the porcine *ACACA* and *SREBF1* genes, 2) to analyze their association with carcass and performance traits, 3) to determine their transcription start site, and 4) to investigate whether the muscle and adipose tissue transcript levels of both genes show breed-specific differences and are related to fatty acids and intramuscular fat content and other lipid deposition traits.

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2. Material and methods

2.1. Animals, sample collection and analyzed traits

All animal procedures performed for the purpose of the study were approved by the Local Ethical Commission on Experiments on Animals at the Poznan University of Life Sciences, Poznan, Poland. For the analyses of gene expression and fatty acid content we included 85 unrelated gilts representing four breeds — Pietrain (N = 17), Duroc (N = 23), Polish Large White (PLW, N = 23) and Polish Landrace (PL, N = 22). All animals were kept under the same environmental conditions at a local Pig Testing Station (Pawlowice), fed ad libitum with the same commercial mix fodder, slaughtered at about 100 kg (SD = 1.8), and dissected.

Blood and tissue samples – the longissimus dorsi muscle (LD), visceral and subcutaneous (SC) fat (at 10th vertebra) – were collected at slaughter and stored at −20 °C for lipid content analysis, or −80 °C (1 g of LD, visceral and SC fat) for transcript level studies. Fatty acid content was measured by gas chromatography and expressed as a weight percentage of total fatty acids. Details concerning fatty acid content measurements can be found elsewhere (Klensporf-Pawlik et al., 2012).

For the association analysis with fatness and performance traits another 242 PL and 191 PLW gilts were included. The gilts descended from 55 sires. The size of a sire group ranged from 1 to 15 gilts. The following 14 traits were considered: abdominal fat weight, backfat thickness (BFT) measured at 7 points, loin backfat weight, intramuscular fat percentage (IMF), meat content of primal cuts (%), loin muscle area, average daily gain and feed conversion ratio. BFT measurement was taken after slaughter with a ruler over the shoulder, over the back, at the sacrum (points I, II and III), and at points C1 (on a vertical line extending from the height of the longissimus dorsi muscle) and K1 (on a vertical line extending from a side edge of the longissimus dorsi). IMF was measured using the SOXTEC AVANTI 2050 automatic extraction system (Foss Tecator, Hoganas, Sweden) and analyzed in the log-transformed scale to reduce skewness. Post-weaning average daily gain was calculated for the fattening period from 25 to 100 kg live weight. Feed conversion ratio was calculated as a ratio of the consumed feed (kg) and weight gain during the fattening period (kg).

2.2. DNA extraction, sequencing and genotyping

Genomic DNA was extracted using a Perfect gDNA Blood Mini Isolation Kit (A&A Biotechnology, Gdynia, Poland). Comparative sequencing (in reference to GenBank sequences: EU168399 and NM214157 for *ACACA* and *SREBF1* genes, respectively) of PCR products was applied for polymorphism screen in a panel of 100 pigs. In this group 85 gilts were used for gene expression study (see Section 2.1) and additional 15 unrelated gilts representing the same breeds (4 Duroc, 3 Pietrain, 4 PL and 4 PLW pigs) for initial polymorphism searching.

PCR primers were designed to amplify a fragment of 3′UTR region of the *ACACA* gene and three fragments (exon 6 with a fragment of intron 6, a fragment of intron 17 and a fragment of exon 19 with 293 bp of 3′UTR) of the *SREBF1* gene (Supplementary Table 1). Prior to sequencing the amplicons were purified with Exonuclease I and Thermosensitive Alkaline Phosphatase (Fermentas, Vilnius, Lithuania). The BigDye Terminator3.1 Sequencing Kit (Applied Biosystems, Foster City, CA) was used for dideoxynucleotide incorporation by the PCR amplification. DNA-sequencing products were purified on a Sephadex G-50 (Sigma Aldrich, St. Luis, MO) and analyzed by capillary electrophoresis on a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Genotyping for the association analysis was performed by RFLP for *ACACA* SNP c.*99A>T (*EcoO109*I, Fermentas, Vilnius, Lithuania) and *RYR1* SNP c.1843C>T (*Hha*I, Fermentas, Vilnius, Lithuania), ASA-PCR (allele-specific-PCR) for *ACACA* SNP c.*195C>A and sequencing for all SNPs in the *SREBF1* gene, using primers listed in Supplementary Table 1. The Haploview software (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview) was used to estimate haplotype frequencies. For the detection of the putative microRNA target sequences the Micro Inspector v1.5 (http://bioinfo.uni-plovdiv.bg/microinspector/) was applied.

2.3. RNA extraction, cDNA synthesis, real-time PCR and 5′RACE

Total RNA was extracted from the LD muscle and SC fat using Tripure Isolation Reagent (Roche, Basel, Switzerland) according to the manufacturer's protocol. The RNA quantity and purity were measured using NanoDrop 2000 (Thermo Scientific, Waltham, MA). Approximately 2.5 μg RNA was used for cDNA synthesis. Samples of RNA were incubated with a set of random hexamers and oligodT$_{(15)}$ (Roche, Basel, Switzerland) at 70 °C/10 min, then a mixture of dNTP (Roche, Basel, Switzerland), AMV Reverse Transcriptase (EUR$_X$, Gdansk, Poland) and Protector RNase Inhibitor (Roche, Basel, Switzerland) was added. After 2 h incubation at 37 °C the AMV enzyme was inactivated at 94 °C/5 min. The real-time PCR was performed on a Light Cycler 2.0 (Roche, Basel, Switzerland). The cyclophilin A (*PPIA*) and *β-actin* (*ACTB*) genes were used as references for the normalization of data. Real-time PCR primers were designed to anneal to adjacent exons or exon–exon junctions (Supplementary Table 1). Each 10 μl reaction mixture consisted of 2 μl cDNA, 1.5 μl LightCycler Fast Start DNA Master^{PLUS} SYBR Green I kit (Roche, Basel, Switzerland) and 0.5 mM forward and reverse primers. The real-time PCR conditions were 95 °C/10 min pre-denaturation, followed by 45 cycles of 95 °C/10 s, 62 °C/5 s and 72 °C/8 s, and the final extension at 72 °C/1 min. The amplified fragment specificity was confirmed by the melting curve and product length analysis. The relative quantification of the mRNA level was performed in duplicates based on a Second Derivative Maximum Method. Appropriate *ACACA*, *SREBF1*, *PPIA* and *ACTB* standards were included in each real-time PCR analysis. Standard curves were designed as 10-fold dilutions of the appropriate PCR product in the range of 10–0.0001 aM. The abundance of the *ACACA* and *SREBF1* gene transcripts was then normalized to a geometric mean of two reference genes — *PPIA* and *ACTB* (Vandesompele et al., 2002).

The 5′RACE analysis was performed using a FirstChoice RLM-RACE Kit (Ambion Foster City, CA) according to the manufacturer's protocol using total RNA extracted from SC and visceral fat of PL pigs and the primers used for amplification of cDNA 5′ ends are listed in Supplementary Table 1.

2.4. Statistical analysis

Transcript level was transformed to the logarithmic scale to reduce skewness. Next the transcript level, fatty acid level and phenotypes were checked for outliers using the *rm.outliers* procedure within the R statistical environment.

Means for production records (N = 433) were compared between genotype groups in univariate models. For across-breed analysis the model included genotype at the *RYR1* locus (2 levels: CC and CT at position 1843), tested SNP genotype, breed and breed-by-SNP interactions, as well as linear covariates of animal age at slaughter and carcass weight. Sire was included as a random effect. The *RYR1* gene was included in the statistical model due to its proven effect on multiple production traits, however, it was not verified in the current study. There were 57 heterozygous gilts (46 PL and 11 PLW), whereas homozygous TT were found in PL only and due to a low number (9) were excluded from the association study.

The relative transcript level (in log scale) of the *ACACA* and *SREBF1* genes recorded in a smaller panel of 85 pigs was analyzed to characterize the possible differences among 4 breeds and 2 tissues (SC and LD). The two genes were considered separately, each under a fixed model that included 3 terms: breed, tissue and breed-by-tissue interaction.