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TRANSCRIPT VARIANTS OF A REGION ON SSC15 RICH IN QTLS ASSOCIATED WITH MEAT QUALITY IN PIGS*

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Abstract

A high meat percentage in the porcine carcass has been achieved as a result of selection, but it has contributed to a deterioration of pork quality. The level of intramuscular fat has significantly declined, the pork has lost its tenderness and drip loss in meat has substantially increased, which has led to a deterioration of meat flavour and its technological suitability. The recovery of good pork quality could be supported by the development of genetic markers enabling faster breeding progress. This study presents a method by using RNA-seq data that identifies new variants for a chromosome region rich in QTLs for pork quality and selects gene candidates for these traits. This work included two pig breeds: the Polish Landrace (PL) and Puławska (PUL), which differ in meat quality and fat content. The transcriptome profile was estimated for *semimembranosus* and *longissimus dorsi* muscles. Into variant calling analysis, transcripts of both muscles encoded by genes located in a region between microsatellites SW964 and SW906 (43-135.9 Mbp) in SSC15 were included. In total, 439 transcripts were searched, 2,800 gene variants were identified and 6 mutations with a high effect belonging to the frameshift variants were found (*ENSSSCG00000015976, ENSSSCG00000027516, WRN* and *XIRP2*). Moreover, several interesting significant missense variants in *PDLIM3, PLCD4* and *SARAF* genes were detected. These genes are recommended as candidates for meat quality; however they require further investigation in an association study.

Key words: pig, meat quality trait, RNA-seq, variant calling, SNP

Pork is the most widespread meat in the majority of countries around the world because pigs have relatively low breeding requirements and they reach slaughter age quickly. Nevertheless, the quality of pork had declined in recent decades, induced by the breeding treatments aimed at improving the meat percentage in the porcine carcasses. However, breeders did not pay enough attention to the intramuscular fat level or water holding capacity values, which strongly determine the meat taste and tenderness. Currently, there are efforts aimed at a recovery of the lost pork quality; however, traditional breeding treatments are time-consuming and expensive. There-

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fore, scientists are elaborating genetic markers which could support breeding programmes by indicating an animal with the appropriate phenotypic potential by an estimation of genetic traits in young individuals.

Unfortunately, the most interesting of the phenotypic traits of livestock are encoded by numerous genes. To narrow down the area of searching quantitative trait loci (QTLs) were designated, which describe chromosome regions strongly associated with farm animal traits and we can find them in the public datasets. The QTL regions are often huge areas, containing thousands of genes, therefore the search of a single genetic trait is a big challenge. However, such challenges were undertaken and in pigs, in the proximal end of chromosome SSC2, a QTL for loin weight without external fat was discovered (Andersson-Eklund et al., 1998). The detailed analysis identified that SNP, which is located in CpG islands of the imprinted *IGF2* gene was responsible for the effect observed for the QTL (van Laere et al., 2003). In the other study on SSC15, the QTLs for pH and meat colour were detected, in which a missense mutation in the *PRKAG3* gene affecting pH and meat colour was identified (Rothschild et al., 2002). Currently, next-generation sequencing approach is commonly used, including whole-genome sequencing (WGS) and genome-wide association study (GWAS) (Zhang et al., 2015; Sonah et al., 2015), which identify the whole set of genes influencing on phenotypic trait. Moreira et al. (2015) using WSG, searched the QTL region on GGA3 in chickens and found that a few loss-of-function variants of the *GGPS1, EGLN1, GNPAT*, *FAM120B* and *THBS2* genes were related to fat deposition. In turn, still another approach to identifying genetic traits associated with a meatiness in the QTL regions by using entropy analysis was tested by Borowska et al. (2014), who observed the effects of *MYOD1*:c.566G>C (SSC2), *TNNT3*:g.153T>C (SSC2) and *MYF6*:g.255T>C (SSC5) on meat content.

A highly interesting region was found on SSC15 between microsatellites *SW964* and *SW906* (43–135.9Mbp, Pig Quantitative Trait Locus database [Pig QTLdb]), which accumulates QTLs for pork quality such as juiciness score, meat colour (a*, b*, L*), pH after 24 h in ham and loin, cooking and drip loss, firmness, PSE meat, shear force, tenderness score, thawing loss and intramuscular fat and also growth traits such as daily gain, feed conversion and feed intake (Pig QTLdb). Few genes located in this region were previously analysed in the context of their effect on pig traits. Cho et al. (2005) noticed that a mutation in the *MSTN* gene increases the eye muscle area without decreasing the backfat thickness. The *IGFBP5* gene was analysed in terms of the effect on fat deposition (Fan et al., 2009). Piórkowska et al. (2014) identified a large insertion in a promoter region of the *ABCA12* gene affecting meat brightness. In turn, Ropka-Molik et al. (2015) analysing *MARCH4* gene, observed that *ENSSSCG00000016176:g.576T>G* mutation in the promoter region is related to pH levels, while the *ENSSSCT00000017613.2:c.675+5C>A* polymorphism to meat colour.

The aim of the present study was to identify interesting gene variants in the SSC15 region, which could be associated with important pig traits, particularly with meat quality. The investigation was performed by including transcripts of two porcine muscles: the *semimembranosus* and *longissimus dorsi* collected from pigs that significantly differ in meat quality traits.

Material and methods

Animals, libraries construction and sequencing

The study was conducted on 16 gilts: Polish Landrace (n=8, PL) and Puławska (n=8, PUL) and two types of muscle samples (*semimembranosus* and *longissimus dorsi*). The pigs were not related; they came from different farms and were selected from a huge population in order to achieve the greatest disparity between meat quality traits. The pigs were maintained in the Pig Test Station according to the same feeding and housing conditions. They were delivered to the test station as piglets and fed *ad libitum* from 30 up to 100 (±2.5) kg of weight, then were starved for 24 h before slaughter. Stunning with high-voltage electric tongs was followed by exsanguination. After 24-h chilling at 4°C, the half-carcasses were measured. The carcass traits according to Tyra and Żak (2013) were measured and the meat texture parameters and pH for the *longissimus lumborum* (LL) and *semimembranosus* (S) muscles according to Ropka-Molik et al. (2014) were determined. The water holding capacity, IMF and meat colour were measured in the *longissimus dorsi* by the Grau-Hamm method (Hamm, 1986), and according to Piórkowska et al. (2013). Muscle samples for molecular analysis were collected immediately after dissection, stabilised by RNAlater solution (Ambion) and stored at −20°C.

RNA was extracted from both porcine muscles using TRI Reagent (Applied Biosystems) according to the manufacturer's protocol. In addition, the RNA was purified by a bead method (Agencourt RNAClean XP kit) and ribosomal RNA was removed by Ribo-Zero Gold rRNA Removal Kit (Human/Mouse/Rat) (Epicentre). cDNA libraries preparation was performed according to the TruSeq RNA Sample Preparation Kit v2 (Illumina) and each library has been indexed with a unique adaptor. The quality and quantity of RNA, depleted-RNA and cDNA libraries were assessed by the Qubit Fluorometer (Invitrogen) and TapeStation 2200 system (Agilent). The final concentration of the cDNA libraries was normalised to 10 nM, diluted to 2 nM and pooled together according to a cluster generation protocol, loaded into a v3 Illumina flowcell (16 samples) and clustered by cBot (Illumina) using a TruSeq SR Cluster Kit v3-cBot-HS. Single-end sequencing, with a read length of 81 bp in four technical replicates, was performed on the HiScanSQ System using TruSeq SBS Kit v3-HS chemistry (Illumina).

Transcript variants identification

The FastQC tool was used for quality control of the raw sequences. Then, Flexbar was used to remove adapters and poly-A sequences, selecting reads with a quality score ≥20 and a fragment size reads ≥36 bp. Filtered sequences were aligned to the *Sus scrofa* genome (Sscrofa10.2 assembly) with STAR aligner (Dobin et al., 2013). Then, Picard tools and GATK (McKenna et al., 2010) were used to split reads containing Ns in their CIGAR string, base quality score recalibration, INDEL realignment, duplicate removal, and SNP and INDEL performed. The used standard hard filtering parameters were FisherStrand (FS) value>50, QualByDepth (QD) <2.0, RMSMappingQuality (MQ)<40, Quality<40 and a SnpCluster filter. Next, identified variants were analysed by SnpEff 4.1b and Variant Effect Predictor (Ensembl) to

indicate, based on their genome annotation: which of the genes are associated, their type and prediction of their function (Cingolani et al., 2012). The most important variants were validated by Sanger methods.

Functional annotation

The genes containing the variants occurring only in one breed that were considered into the functional analysis included missense mutations, frameshift variants, INDELs, 5'UTR variants and 3'UTR mutations. They were imputed to the metabolic pathways by using the Panther Geneontology Classification System, Kyoto Encyclopedia of Genes and Genomes (KEGG) and GeneMANIA software.

Results

Animals

In the present investigation, it has been attempted to find genetic variants associated with pork quality, which could be used in selection independently of pig breed. The breeds used in this work differ significantly in pork quality traits such as values of water holding capacity and texture parameters. Moreover, the PL pigs are characterised by better growth performances and meat content in the carcass, which is a consequence of breeding treatments (Table 1).

Table 1. The parameters of traits in two studied pig breeds

Traits	PL (n=8)		PUL (n=8)	
	mean	SD	mean	SD
Backfat thickness (cm)	1.18 a	0.17	1.64 b	0.40
Meat percentage (%)	63.04 a	1.95	59.5 b	2.80
IMF (%)	1.08	0.12	1.19	0.21
Water holding capacity	41.50 A	5.11	28.32 B	1.24
Loin				
pH_{45}	6.33	0.18	6.32	0.19
pH_{24}	5.63	0.05	5.60	0.06
firmness by WB (cooked, N)	122.58 A	14.7	57.7 B	3.21
toughness by WB (cooked, N)	277.07 A	37.6	140.97 B	14.2
hardness by TPA (cooked, N)	6.95	2.7	5.01	2.05
Ham				
pH_{45}	6.30	0.12	6.26	0.16
pH_{24}	5.64	0.04	5.62	0.05
firmness by WB (cooked, N)	89.92 a	10.17	75.13 b	6.83
toughness by WB (cooked, N)	203.91	39.72	180.30	35.86
hardness by TPA (cooked, N)	9.30	3.84	8.25	3.88

Abbreviations: SD – standard deviation, BF – backfat thickness, MP – meat percentage , IMF – intramuscular fat, WHC – water holding capacity, TPA – texture parameter analysis, WB – Warner-Bratzler, PL – Polish Landrace, PUL – Puławska. Values with the same superscripts show significant differences between genotypes (A, B = $P<0.01$; a, b = $P<0.05$).

Transcript variants of chromosome 15 region between microsatellites SW964 and SW906

The transcripts of chromosome 15 (SSC15) region, amounted to over 400 for both muscles and breeds. The variants previously known and submitted to NCBI constituted approximately 90% of all those detected. In total, 8,419 mutations were detected, which then were filtered. A few filters were applied: Snp Cluster – removing SNP located in the cluster (Quinn et al., 2013), FisherStrand (FS) value>50, QualByDepth (QD) <2.0, RMSMappingQuality (MQ)<40 and Quality<40. The SNP cluster filter deleted nearly 39% of mutations located in the intergenic regions, 31% frameshift variants and 25% of the intronic regions. 429 variants were deleted by the 'Quality' filter included mainly in non-exonic region. In turn, the FS, MQ and QD filters deleted only 175 variants. After the filtering, 2,850 variants remained for the Puławska pigs and 2,953 for the Polish Landrace. Among them, the single nucleotide polymorphisms (SNPs) prevailed over the INDEL variants. 3'UTR regions were rich in mutations contrary to the 5'UTR sequence. Nevertheless, in the 5'UTR regions, 4 variants generating the premature start codon in the PL pigs were identified. Numerous mutations in the intergenic regions and introns were identified, which could confirm that many genes have not been imputed yet or numerous unknown alternative transcripts exist. In addition, it was observed that heterozygosity was higher in the PL pig groups than in the PUL gilts (19.8% and 15.3%, respectively, Table 2).

Table 2. Characteristic of region on chromosome 15 (43,100,000–140,400,000) rich in QTLs associated with pork quality in the context of identified mutations

Mutation (variant)	Count	
	PUL	PL
Total	2.850	2.953
Known variant (%) (SNPdb NCBI)	90.2	89.6
Heterozygotes (%)	15.3	19.8
Analysed transcript	405	399
Insertion	162	161
Deletion	143	149
SNP	2.545	2.643
High effect	9	9
Low effect	358	390
Moderate effect	124	133
Missense variant	123	159
Silent	349	376
3'UTR	471	553
5'UTR premature start codon	2	4
5'UTR	29	30
Frameshift variant	4	4
Downstream gene	1.144	1.211
Upstream gene (included promoter region)	239	280
Intergenic region	1.265	1.267
Intronic	1.228	1.228
Exonic	500	538
Splice region	17	24

Abbreviation: PL – Polish Landrace pigs, PUL – Puławska pigs; High, low, moderate effects are the powerful effect, for example frame shift variants are included as high effect variants.

Table 3. The gene variants observed only in one breed and common mutations for the particular breeds dependent on gene function

Mutation	Gene	
	PUL	PL
Frameshift variant (high effect)	*WRN (novel variant, c.4519dupT)* *ENSSSCG00000015976 (novel variant, c.104dupA)*	*XIRP2(novel variant, c.283dupA)* *ENSSSCG00000027516 (novel variant, c.227dupA)*
Deleterious missense variants	*PLEKHA3 (rs343019152)* *CWC22 (rs81216911)* *ENSSSCG00000016195 (novel variant, c.344G>A, p.Ser115Asn)* *PDLIM3(rs322071455)* *WRN (rs81216606, rs323024274)* *UPP2(rs330764298)*	*ENSSSCG00000028320 (rs697823262)* *UBXN8 (rs340700656, rs81211431)* *PLCD4 (rs790932767)*
Splice region	*ANKRD37 (rs328763355)* *UPP2 (rs340886638)* *NDUFS1 (rs335007263)* *ENSSSCG00000022460 (novel variant)*	*SARAF (rs327729020)* *NOSTRIN (rs346403633)* *SSB (rs694246434)* *GORASP2 (rs336057591)* *RBM45 (rs318955054)* *PRKAG3 (rs337622813)* *CNPPD1(rs344993319)*

Abbreviation: PL – Polish Landrace pigs, PUL – Puławska pigs. In parenthesis the number of identified mutations.

In the functional analysis were included only the genes with mutations occurring in the one breed.

Table 4. The genes with unique mutations for the particular breeds dependent on the localisation

Mutation	Gene	
	PUL	PL
5'UTR	*SARAF (rs329633346, rs318985243)*	*TTI2(rs330531820)*
	HS6ST1 (novel variant)	*GTF2E2 (rs81214721)*
	PPIG (rs707933668)	*ENSSSCG00000021721 (rs788624862)*
	PHOSPHO2(rs701490034)	*ATP5G3 (rs55619110)*
	DCAF17 (rs324567047)	*METTL21A (rs321084713)*
	DYNC1I2 (novel variant)	*TMEM169 (rs323937836)*
	MRPL44 (rs337279159)	*ENSSSCG00000024508 (rs339508010)*
	ENSSSCG00000016152 (rs327265517)	*TNS1 (rs343007950)*
		ANKZF1 (rs325692013)
3'UTR	*ITGAV (11)*	*MAP2 (5)*
	COQ10B (4)	*ACADL (4)*
	RFTN2 (16)	*TTI2 (5)*
	TRAK2 (5)	
	WDR12 (8)	
	IGFBP5 (4)	
	CNPPD1 (5)	
	DES (4)	
	CHPF (6)	
	ASB5 (10)	
	ACSL1 (3)	
	CFAP97 (12)	
	TM2D2 (7)	
	ENSSSCG00000026608(7)	
	FGFR1 (5)	
	WHSC1L1 (9)	
	PLPP5 (11)	
	DDHD2 (8)	
	EIF4EBP1 (6)	
	ADGRA2 (13)	
	BRF2 (12)	
	PROSC (13)	
	LEPROTL1 (21)	
	SARAF (4)	
	LIMS2 (6)	
	PRPF40A (8)	
	PPIG (6)	
	TLK1 (6)	
	HNRNPA3 (7)	
	ITGA4 (13)	

Abbreviations: PL – Polish Landrace pigs, PUL – Puławska pigs. In parenthesis the number of identified mutations.

Identified gene variants

Numerous mutations were identified for only one breed. In the Puławska, which is characterized by thicker backfat and better meat quality values (Table 1), 286 gene variants were observed. The majority were located in the 3`UTR regions. Two of

the detected gene variants belong to frameshift variants (in genes: *WRN* and *ENSSSCG00000015976*), 6 to deleterious missense mutations (*PLEKHA3*, *CWC22*, *ENSSSCG00000016195*, *PDLIM3, WRN, UPP2*) and 4 in the splice regions (*ANKRD37, UPP2*, *NDUFS1, ENSSSCG00000022460*). In turn, for the Polish Landrace only 32 variants were discovered, among them being a new interesting frameshift variant in the *XIRP2* gene, a variant in the splice region of the *SARAF* gene and deleterious missense mutations in the *PLCD4* gene (Table 3 and 4). The sequence data has been submitted to the GEO (accession no. GSE75707, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE75707) and gene variants to NCBI SNPdb (in progress).

Discussion

The usefulness of RNAseq data in the variant calling analysis was tested in the present study. RNAseq is the method used in order to estimate differentially expressed genes (DEGs) between the investigated groups. However, this analysis delivers the complete transcript sequences, which could be used to search for the genetic markers. The pigs included in this work are characterised by significant differences in the values of water holding capacity and meat texture parameters, which could be helpful in the investigation of biomarkers associated with these traits. The meat of Polish Landrace exhibits a low quality, because over the last decades the selection was targeted to increase meat content without preserving proper IMF content and tenderness. The Puławska belongs to the indigenous breed i.e. it is not a component of breeding, thus it has not been subjected to the selection. The Puławska, as the native breed, is more disease-resistant and tolerates adverse environmental conditions (Szyndler-Nędza et al., 2010).

The frameshifts are interesting gene variants because they lead to the formation of modified proteins, which probably have impaired function (Finno et al., 2015). In the Puławska pigs, the new frameshift variant in the *ENSSSCG00000015976* gene was detected, which is the *KIAA1715* ortholog. The human *KIAA1715* gene is associated with abnormality in limb development (Dlugaszewska et al., 2006). The *KIAA1715* gene encodes the protein which interacts with the lysophosphatidic acid receptor 1 (LPAR1). The LPAR1 is the transmembrane protein involved in the Rho signalling pathway, activates the G protein subunit alpha 12 and 13 (GNA12, GNA13) proteins and interacts with the Rho guanine nucleotide exchange factor 12 (LGAR) affecting RhoA. The ras homolog family member A (RHOA) through profiline (PFN) and fibronectin 1 (FN1), is implicated in actin polymerization and nuclearisation (Dubash et al., 2007), and through PIP5K and ROCK plays a role in cytoskeleton organization (Ivetic et al., 2004), the biological process determining the myofibrillar network, and subsequently meat quality (Huff-Lonergan and Lonergan, 2005). The next frameshift mutation was detected in *XIRP2* gene but only in the Polish Landrace pigs. This gene contains a Xin Actin-Binding Repeat and plays a role in the actin cytoskeleton organisation, which is responsible for the assembly or disassembly of cytoskeletal structures (Sinn et al., 2002). Moreover, the XIRP2 protein binds filamin, which

in turn is involved in two important pathways: MAPK signalling and focal adhesion, which determine actin polymerisation and cell proliferation. Kesireddy (2011), when carrying out the dissertation study on murine skeletal muscle cells, observed that XIRP2 is closely related to the Xin protein. Therefore, the deficiency of the Xin protein could be supplemented by XIRP2. However, the lack of both proteins results in the development of thinner myotubes that are differentiated to a less advanced degree when compared to wild type cells. Thus, the frameshift variant of the *XIRP2* gene occurring only in Polish Landrace could be associated with texture parameters due the involvement of *XIRP2* in actin organisation. The *XIRP2* gene is one of the proposed candidates implicated in pork quality. However, this requires the confirmation in the further association study. Next frameshift variant observed in the Polish Landrace was detected in the *ENSSSCG00000027516* encoding protein corepressor interacting with RBPJ (*CIR1*), which interacts with histone deacetylase 1 (HDACT1) inhibiting interaction between PPARA and CREB, the main relation in adipogenesis. It was proved that some muscle-secreted proteins are involved in adipogenesis in local fat tissue (Trayhurn et al., 2011), which could be associated with IMF content and so with meat flavour. In addition, the HDACT1 inhibits MEF2 (Yan et al., 2014), which is one of the main transcription factors during the slow muscle fibre development, which could be related to meat drip loss and texture parameters (Waritthitham et al., 2010). The significant missense mutation was found in *PDLIM3* gene (rs322071455) in the Puławska pigs. The change *Arg*268*Ser* is located in the zing-finger LIM domain, which is believed to play a role in cytoskeletal organisation, organ development and oncogenesis (Kadrmas et al., 2004). PDLIM3 is considered to be potentially involved in the cytoskeletal assembly by colonising with alpha-actinin-2 at the Z-lines of skeletal muscles (Xia et al., 1997). Its involvement in cytoskeleton organisation recommends the *PDLIM3* gene as the candidate for meat texture. In turn, significant missense mutation was observed in the *PLCD4* gene (*rs790932767)* in the Polish Landrace. This mutation is located in 13 exon, which encodes PLC-like phosphodiesterase, TIM beta/alpha-barrel, C2 and phospholipase C, phosphatidylinositol-specific Y domains. Phospholipase C delta 4 *(PLCD4)* activates Ca^{2+} ion in the leptin signalling pathway, which induces the activity of NPY neurons and feed intake. Phospholipase C catalyses reaction DAG and IP3, which through PKC alpha/beta and PPARA affect fatty acid metabolism (Parekh et al., 2000). Therefore, the mutation in the *PLCD4* gene is recommended as the candidate gene associated with IMF content and fat content.

In the Polish Landrace the splice region variant was observed for *PRKAG3* (rs337622813). This variant is localised in the splice region between exons 9 and 10. However, there is no evidence for existing *PRKAG3* isoform without exon 9 or 10. Therefore, this polymorphism probably does not disturb the splicing and rs337622813 variant have probably another important regulatory role or is linked to another marker. Nevertheless, PRKAG3 is involved in the numerous pathways determining pork quality such as insulin signalling, AMPK development, leptin signalling via PI3K-dependent etc, because it affects expression of the *FGRF1, MAP2K6, PPARGC1A, SREBF1, LEP, CDH5* and *CTNNA1* genes. In previous study it was observed that polymorphisms in the *PRKAG3* gene are associated with drip loss,

tenderness and pH (Kavar et al., 2009), meat yellowness and shear force (Bertić et al., 2013). Therefore this gene should be further analysed.

The second approach in the present study was to identify the unique variants in the regulatory regions of genes for the pigs of both breeds. The 5'UTR region is located upstream of an initial codon and is important during transcription due to its regulating the binding of the initiation complex. It also interacts with 3'UTR by bound protein to prepare the mRNA loop, which is essential during the translation process. In the Puławska pig, two mutations in the 5'UTR region of store-operated calcium entry-associated regulatory factor (*SARAF*) gene were observed. SARAF interact with nuclear factor kappa B subunit 1 (NFKB1), which participates in numerous pathways: inter alia it inhibits PPARG which is associated with adipose cell differentiation and glucose uptake. Moreover, SARAF plays a role in calcium ion transport and regulation of store-operated Ca^{2+} entry (Palty et al., 2012). It interacts with the stromal interaction molecule 1 (STIM1), which is described as a Ca^{2+} sensor essential for Ca^{2+} store depletion-triggered and interacting with Orai1, which is the component of the Ca^{2+} channel (CRAC). They participate in a store-operated Ca^{2+} channel function (Giachini et al., 2011). Thus, the *SARAF* gene could be taken into consideration as a determinant of pig traits associated with calcium transport, such as pig traits determined *postmortem*. In the PL pigs, among the genes with the unique mutation in the 5'UTR region, the *TNS1* gene could be associated with porcine meat quality, because tensin 1 interacts with the F actin complex (Lo et al., 1994), which is involved in actin polymerisation and nucleation and these processes determine meat tenderness (Pérez-Juan et al., 2007).

Conclusions

In summary, this study indicates a new panel of gene variants that could be associated with pork quality, because their genes affect the protein playing a role in actin polymerisation, the process determines meat texture and water holding capacity (*TNS1*, *SARAF, PDLIM3, KIAA1715, XIRP2)*. The present study found polymorphisms in new genes which are involved in the adipogenesis process, thus they could determine the IMF and fat content (*PLCD4, CIR1*) in pigs. However, the effects of the identified variants should be tested in an association study including numerous pig populations.

Compliance with ethical standards

Conflict of interest: None of the authors has a financial or other relationship with other people or organizations that may inappropriately influence this work.

Ethical approval: The research will be performed on biological material derived from pigs maintained and slaughtered in the Test Pig Station (National Research Institute of Animal Production). In the Test Station pigs are slaughtered, dissected and after carcass evaluation, meat is standard intended for consumption. Therefore, our research does not require the approval of Animal Experimentation committee.

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