

ASSOCIATION OF GENE CODING FOR MICROSOMAL **TRIGLYCERIDE TRANSFER PROTEIN (MTP) AND MEAT TEXTURE CHARACTERISTIC IN PIG***

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Abstract

The microsomal triglyceride transfer protein via participation in transport of neutral lipids between membrane vesicle is essential for assembly of chylomicrons, low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL). In human and pigs, it has been confirmed that mutations within MTTP locus affected lipid-transfer activity of this protein. The aim of the present study was to establish potential influences of ENSSSCP00000009789.2:p.Leu840Phe polymorphism on a panel of meat texture parameters measured in two muscles: m. longissimus lumborum and m. semimembranosus. The research performed on 410 pigs showed that investigated missense polymorphism was associated with meat texture profile parameters - TPA (hardness, cohesiveness, springiness, resilience, chewiness) as well as firmness and toughness estimated in loin muscle. In whole analyzed population, the meat of pigs with CC genotype was characterized by significantly the lowest value of TPA characteristic and this trend was also confirmed in two breeds (Pulawska and Large White pigs). In turn, the results obtained for firmness and toughness parameters in longissimus lumborum were not consistent across the different populations studied. Our research, in connection with previous studies, indicated that the MTTP gene may be considered as a candidate gene responsible for pork quality traits and pinpointed a need for further analysis in order to select useful genetic markers associated with meat quality parameters.

Key words: MTTP gene, lipid metabolism, polymorphism, pork, texture parameters

The microsomal triglyceride transfer protein (MTP), via participation in transport of neutral lipids between membrane vesicle, plays a critical role in the biosynthesis of beta-lipoproteins which are a main source of triglyceride-rich lipoproteins

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(Hussain et al., 2003). MTP protein is essential for assembly of chylomicrons, lowdensity lipoproteins (LDL), and very low-density lipoproteins (VLDL). Moreover, microsomal triglyceride transfer protein controls the biosynthesis of cluster of differentiation 1 proteins (CD1) as well as cholesterol ester (Hussain et al., 2012).

In human, mutations within *MTTP* locus affected MTP's lipid-transfer activity and are associated with deficiencies of B-48 and B-100 apolipoproteins in blood which causes abetalipoproteinemia disorder (Filippo et al., 2012; Miller et al., 2014). *MTTP* gene variants have been also related with plasma cholesterol concentration and body mass index (Ledmyr et al., 2002).

In pig, *MTTP* gene has been considered as one of candidate genes regulating lipid metabolism. In 2009, Estellé et al. (2009) showed that non-synonymous mutation located in exon 18 of the *MTTP* gene (c.2573T>C; p.Phe840Leu) affected the lipid transfer activity of this protein. Furthermore, the c.2573T>C mutation was significantly associated with the fatty acid composition of fat tissue in investigated pig populations. The recent research carried out by Renaville et al. (2015) also showed that polymorphism in *MTTP* locus affected carcass weight and weight loss during salting in dry-cured ham production.

In order to obtain the comprehensive information about association between *MTTP* gene variants and pork quality, potential influences of ENSSSCP00000009789.2:p. Leu840Phe polymorphism were evaluated on a panel of meat texture parameters measured in two important muscles: *m. longissimus lumborum* and *m. semimembranosus*.

Material and methods

The study was performed on three pig breeds - Landrace, Large White and Puławska pigs (n=201, 160, 49, respectively). Pigs were maintained at the same environmental (housing and feeding) conditions in the Pig Test Station of the National Research Institute of Animal Production in Chorzelów. Piglets (all gilts) entered the station at the age of about 12 weeks (average body weight of 20-26 kg) and then were fed *ad libitum* from 30 kg up to 100 (± 2.5) kg. After fattening period pigs were slaughtered according to the same procedure and right half-carcasses were dissected subsequent to 24-hour chilling at 4°C. Blood samples were collected into EDTA tubes during slaughter procedure, while muscle samples (m. longissimus lumborum and m. semimembranosus) were collected after dissection. For each pig WB (Warner-Bratzler shear force - firmness and toughness) and TPA (texture profile analysis - hardness, cohesiveness, springiness, resilience, chewiness) parameters were determined for both muscles. The muscle slices of a width of 3.5 cm (approximately 200 g) were cooked in a polyethylene bag in a water bath till the core reached the temperature of 80°C and then chilled for 24 h at 4°C. Parallel to the direction of muscle fiber, 2 cores (15 mm diameter) from each sample were cut and analyzed on Texture Analyser TA-XTplus (Stable Micro Systems, Godalming, UK) at room temperature. During WB shear force (N) analysis slices were sheared by WB triangular blade at 4.5 mm/s and TPA parameters were estimated by double compression procedure (cylinder – SMS P/25, base diameter 50 mm). The data was collected and analyzed by Texture Expert, version 1.20 software. All investigated texture parameters (hardness, cohesiveness, springiness, resilience and chewiness) were calculated according to the procedure previously described by Ropka-Molik et al. (2014).

In order to estimate the frequency of missense *MTTP* mutation (ENSSSCT00 000010052.2:c.2518C>T; ENSSSCP00000009789.2:p.Leu840Phe) DNA was isolated from 250 µl of whole blood with the use of Wizard Genomic Purification Kit (Promega, Madison, USA) according to the manufacturer's protocol. The genotypes were determined by PCR–RFLP method using primers: F CTCTGACCAGTGT-GAGGCAA and R ACCCAAAGTGTCACGTAGGT (441bp PCR product). The PCR was performed by using AmpliTaq Gold® 360 Master Mix (Applied Biosystems, ThermoFisher, USA) according to the protocol (at annealing temperature – 55°C) and Mastercycler nexus gradient thermocycler (Eppendorf, Germany). The obtained PCR products were digested at 37°C for 16 h using *Mlu*CI endonuclease (New England Biolabs, Canada) according to the manufacturer's protocol. After digestion, PCR fragments were separated on a 3% agarose gel (LabEmpire, Poland) and the obtained alleles were: C - 293, 148; T - 293, 84 and 64.

The association study was performed using GLM procedure and means were separated between groups with Tukey's test (SASv. 8.02), and the model was:

$$Y_{ijk} = \mu + b_i + g_j + (bg)_{ij} + e_{ijk}$$

where:

 Y_{ijk} – the observation, μ – the overall mean, b_i – the fixed effect of *i* breed, g_j – the fixed effect of *j* genotype group of *MTTP* gene, $(bg)_{ij}$ – interaction between g_j genotype group and breed (when significant), e_{ijk} – random error.

The interaction $(bg)_{ij}$ was included in model only when it was significant. For analysis performed separately for each breed the presented model was used, but without interaction between genotype group and breed.

Departures from the Hardy-Weinberg equilibrium were determined by using Court Lab – HW calculator – Michael H. Court (2005–2008) (Court and Michael, 2012). The differences in genotypic frequencies were evaluated by chi-square test.

Results

In two breeds – Landrace and Large White – the lowest frequency of CC genotype was observed, while the most abundant were homozygotes TT and het-

erozygotes (average 50.9% and 34.6%, respectively) (Table 1). On the other hand, in Puławska pigs the lowest number of animals with TT genotype and higher frequency of CC homozygote (more than 16% compared to other investigated breeds) were detected. Furthermore, chi-square test confirmed significant differences in genotypic frequencies between Landrace and Puławska pigs (P=0.0001) and Large White and Puławska (P=0.0001), while differences between Landrace and Large White populations were not significant (P=0.193). Analyzed populations were in Hardy-Weinberg equilibrium (Table 1). The preliminary statistical analysis showed the significance of interaction between breed and genotype for toughness estimated in *m. longissimus lumborum* and chewiness measured in *m. semimembranosus* muscle. Thus, GLM analysis was performed separately for each breed and for whole population.

	Genotypes			Alleles		
	CC	СТ	TT	С	Т	HWE
Landrace	0.16 (32)	0.467 (94)	0.373 (75)	0.39	0.61	0.77
Large White	0.131 (21)	0.55 (88)	0.319 (51)	0.41	0.59	0.07
Puławska	0.326 (16)	0.47 (23)	0.204 (10)	0.61	0.39	0.74

Table 1. Frequencies of genotypes and alleles of ENSSSCT00000010052.2:c.2518C>T polymorphism in *MTTP* gene

The numbers of animals in each group are shown in brackets; HWE P-value – Hardy-Weinberg Equilibrium.

The research performed on 410 pigs showed present that ENSSSCT00000010052.2:c.2518C>T (p.Phe840Leu) polymorphism was associated with firmness and toughness (Table 2) as well as with all analyzed TPA texture parameters (Table 3) estimated in loin muscle. The results obtained for firmness and toughness parameters in longissimus lumborum were not consistent across the different populations studied. In Puławska pigs, cooked loin muscle of both homozygous pigs had the highest firmness and toughness traits, while in Large White pigs the highest values were obtained for CT and TT genotypes (P<0.05) (Table 2).

The meat (*m. longissimus lumborum*) of pigs with CC genotype was characterized by significantly the lowest value of hardness, cohesiveness, springiness, resilience and chewiness and this trend was also confirmed in Large White breed and in Puławska only for cohesiveness and resilience (Table 3). The opposite trend was observed in cooked *semimembranosus* muscle in Large White pigs: CC homozygotes were characterized by the highest value of chewiness, springiness and chewiness (P<0.05) (Table 4).

Texture traits	Genotypes LMS±S.E.	Large White (160)	Landrace (201)	Puławska (49)	Total (410)
Firmness	CC	74.9±3.17 b	74.3±1.90	77.4±4.62 a	75.2±2.31
m. longissimus lumborum	CT	84.1±2.34 a	78.1±1.99	66.5±3.12 b	79.3±1.44
	TT	80.7±2.64 ab	79.1±2.26	81.0±5.39 a	79.8±1.63
Toughness	CC	176.1±9.43 b	180.1±9.97	191.4±10.6 a	181.5±5.94
m. longissimus lumborum	CT	208.9±7.16 a	180.1 ± 5.00	157.8±8.36 b	189.9±4.12
	TT	190.6±7.25 ab	184.2±5.30	195.4±13.0 a	187.4±4.09
Firmness	CC	87.9±7.25	82.3±3.60	84.3±5.38	84.4±3.04
m. semimembranosus	CT	88.9±2.65	86.8±2.26	81.7±4.59	87.5±1.63
	TT	82.7±3.05	87.1±2.77	86.4±8.92	85.4±1.99
Toughness	CC	223.6±20.5	201.6±8.44	192.7±11.6	206.2±8.03
m. semimembranosus	CT	216.8±6.93	205.2±5.57	191.4±10.6	208.6±4.14
	TT	205.3±8.39	208.9 ± 5.56	202.6±15.2	207.1±4.51

Table 2. The association of ENSSSCP00000009789.2:p.Leu840Phe MTTP polymorphism and selected shear-force meat parameters measured in cooked *longissimus lumborum* and *semimembranosus* muscles

Values (LSM \pm S.E.) with different letters show significant differences between genotypes (a, b = P \leq 0.05). Firmness and toughness parameters are shown as N/mm/s.

Texture traits	Genotypes	Large White	Landrace	Puławska	Total
	LMS±S.E.	(160)	(201)	(49)	(410)
Hardness (N)	CC	6.29±0.57 B	7.11±0.70	7.60±0.85	6.97±0.40 b
	CT	8.69 ±0.41 A	7.14±0.35	6.44±0.55	7.73±0.25 ab
	TT	9.77±0.62 A	7.21±0.45	8.36±1.25	8.47±0.36 a
Springiness (mm)	CC	0.67±0.02 B	0.68±0.01	0.67±0.01	0.67±0.009 b
	СТ	0.71±0.06 A	0.68 ± 0.01	0.69 ± 0.01	0.69±0.004 a
	TT	0.72±0.01 A	0.68±0.01	0.71 ± 0.01	0.70±0.005 a
Cohesiveness	CC	0.62±0.01 b	0.61±0.01	0.61±0.01 b	0.61±0.008
	СТ	0.65±0.01 ab	0.63±0.01	0.64 ±0.01 a	0.64 ± 0.004
	TT	0.66±0.01 a	0.61±0.01	0.65±0.01 a	$0.64{\pm}0.005$
Chewiness	CC	2.80±0.30 B	3.14±0.35	3.24 ±0.42	3.06±0.20
	СТ	4.25±0.23 A	3.23±0.18	3.04±0.29	3.64±0.13
	TT	4.64±0.29 A	3.23±0.23	4.08±0.68	3.98±0.18
Resilience	CC	0.27±0.007 b	0.26±0.01	0.26±0.01 b	0.26±0.004
	СТ	0.28±0.024 ab	0.27±0.02	0.27±0.01 ab	0.27±0.002
	TT	0.29±0.05 a	0.26±0.01	0.28±0.01 a	0.28±0.003

 Table 3. The association of MTTP gene polymorphism and TPA parameters measured in cooked longissimus lumborum muscle

Values (LSM±S.E.) with different letters show significant differences between genotypes (A, B = P \leq 0.01; a, b = P \leq 0.05).

Texture traits	Genotypes	Large White	Landrace	Puławska	Total
	LMS±S.E.	(160)	(201)	(49)	(410)
Hardness (N)	CC	13.2±0.99 a	10.3±0.83	9.10±0.81	10.9±0.55
	CT	10.6±0.43 b	9.91±0.46	10.1±0.76	10.2±0.29
	TT	10.7±0.61 b	41.2±18.6	9.70±0.77	27.5±10.3
Springiness (mm)	CC	0.73±0.01 a	0.72±0.01	0.71±0.01	0.72±0.007
	CT	0.72±0.01 a	0.71±0.01	0.72±0.01	0.72±0.004
	TT	0.68±0.01 b	0.72±0.01	0.74±0.02	0.71±0.009
Cohesiveness	CC	0.62±0.01	0.62±0.01	0.64±0.02	0.62±0.009
	CT	0.70±0.06	0.62±0.01	0.64±0.01	0.66±0.031
	TT	0.64±0.01	0.61±0.01	0.63±0.01	0.62±0.005
Chewiness	CC	6.19±0.51 a	4.71±0.41	4.24±0.49	5.06±0.28
	CT	5.01±0.24 b	4.50±0.24	4.91±0.49	4.77±0.16
	TT	5.18±0.31 ab	5.25±0.26	4.65±0.45	5.18±0.19
Resilience	CC	0.26±0.006	0.24±0.006	0.25±0.007	0.25±0.004
	CT	0.27±0.004	0.26±0.004	0.27±0.001	0.26±0.002
	TT	0.28±0.005	0.25±0.004	0.26±0.008	0.26±0.003

Table 4. The association of MTTP gene polymorphism and selected TPA parameters measured in cooked semimembranosus muscle

Values (LSM \pm S.E.) with different letters show significant differences between genotypes (a, b = P \leq 0.05).

Discussion

The gene coding for microsomal triglyceride transfer protein has been proposed as a candidate gene responsible for lipid metabolism. In pig, based on *MTTP* gene localization within QTL (Quantitative trait loci) associated with fatness traits (abdominal fat weight, fatty acid composition), *MTTP* gene has been deliberated as a potential genetic factor related with fat deposition traits (Estellé et al., 2005, 2009).

the present research. frequency of genotypes of missense In ENSSSCT00000010052.2:c.2518C>T polymorphism within exon 18 of MTTP gene was determined in three different pig breeds. Two pure breeds (Landrace and Large White) are used in breeding programs as dam-lines and are characterized by good growth rate and high feed conversion efficiency. On the other hand, Puławska pigs are a native breed and compared to Landrace and Large White had meat with a higher protein and intramuscular fat content, lower drip loss and cooking loss (Florowski et al., 2006; Szyndler-Nędza et al., 2010). In the present study, in both dam line breeds the lowest number of pigs with CC genotype and the highest of TT genotype were observed. The opposite trend was established in native Puławska breeds. The reverse distribution of MTTP genotypes and alleles in native breed may be related with the lack of selection pressure focused on improving lean meat content and decreasing of fat deposition in carcass which also negatively affected pork quality. Renaville et al. (2015), analyzing the same *MTTP* gene polymorphism, showed similar genotype frequencies for traditional hybrid Landrace × Large White breeds as in the present study: the amount of CC homozygotes was 17%, while the opposite homozygotes

was 30%. On the other hand, authors detected the opposite genotypes distribution in population obtained by crossbreeding with Duroc pigs.

In 2009, Estellé et al. identified p.Phe840Leu mutation in MTTP gene which modified amino acid content in conserved region of the lipid transfer domain and in vitro affected lipid transfer activity of MTP protein. This mutation has been deliberated as a possible causal factor associated with fatty acid composition in pig. On the other hand, it has been proven that fatty acid concentration and composition are closely related with melting point of lipid and as a result affected firmness (hardness) of meat (Wood et al., 2003). Our study indicated that ENSSSCT00000010052.2:c.2518C>T (p.Phe840Leu) polymorphism was significantly associated with a panel of TPA parameters (hardness, cohesiveness, springiness, resilience and chewiness) as well as shear-force traits (firmness and toughness). In Large White breeds, the lowest value of all investigated TPA characteristics measured in cooked loin muscle was obtained for CC pigs. A similar trend was also observed for cohesiveness and resilience in Puławska breed. The analysis performed on whole population (410 animals) also confirmed the obtained results. In turn, in Large White pigs, the opposite trend was observed for semimembranosus muscle: the meat of CC homozygotes was characterized by the highest value of hardness, springiness and chewiness.

Estellé et al. (2009) showed that the Phe840 amino acid residue is highly conservative across different mammalian species, and thus the identified p.Phe840Leu polymorphism (NM 214185:c.2573T>C) is critical for activity of microsomal triglyceride transfer protein. Authors confirmed that pigs with CC (LeuLeu) genotype were characterized by an increase in lipid transfer activity when compared to other CT and TT animals. In vitro research confirmed that overexpression of MTP protein level in liver cells significantly increased secretion of VLDL triglycerides and apolipoprotein B (Tietge et al., 1999). In turn, the hepatic secretion of apoB-containing VLDL is one of the most important determinants of triglycerides, LDL cholesterol, and apoB levels in the plasma (Griffin and Zampelas, 1995). In pig, several studies showed that fatty acid composition and fat deposition traits affected meat quality (Wood and Enser, 1997; Wood et al., 2008). In the present study, loin muscle (longissimus lumborum) of CC homozygotes had the lowest value of all analyzed TPA texture parameters in Large White and Puławska breeds and for the whole population. The previous study established also that meat of CC pigs (longissimus lumborum) had lower shear force (WBSF) parameters (P<0.05) compared to TT pigs. Furthermore, Phe840Leu polymorphism was related with carcass weight and weight loss during salting (P<0.05) (Renaville et al., 2015). Additionally, Fontanesi et al. (2012) showed a significant effect of MTTP gene on back fat thickness.

In the present study, the significant impact of *MTTP* gene on firmness and toughness parameters estimated in *m. semimembranosus* was not observed. Moreover, the obtained effect of *MTTP* polymorphism on TPA traits differs between both analyzed muscles. The reason of these discrepancies can be related with different fiber-type distribution and metabolic properties that may occur in both muscles. According to study performed by Herault et al. (2014) the porcine *m. semimembranosus* muscle is composed of highest percentage of intermediate fast-twitch type IIa fibers and showed higher oxidative capacity compared to *longissimus* muscle. Moreover, it has

been proven that proportion of individual fiber types (especially percentage of type I fiber) strongly affects intramuscular fat content (main factor determining meat quality), due to an increase of triglycerides deposition in muscle cells (Gao and Zhao, 2009; Bereta et al., 2014). These may be a main reason of obtained differences in the effect of *MTTP* gene on meat quality traits depending on muscle type.

In summary, the above results suggested that p.Phe840Leu polymorphism in *MTTP* gene, through modifying of microsomal triglyceride transfer protein activity, affected pork quality traits such as texture parameters (both WBS and TPA features). Such quality characteristics of pork are a major factor determining technological value of meat and should be improved according to consumer preferences. Our research indicated that investigated polymorphism within *MTTP* gene may be considered as a candidate gene responsible for pork quality traits and pinpointed a need for further analysis in order to select useful genetic markers associated with meat quality parameters.

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