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Association of Missense *MTTP* Gene Polymorphism with Carcass Characteristics and Meat Quality Traits in Pigs

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ABSTRACT

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Microsomal triglyceride transfer protein, coded by *MTTP* gene, has multiple functions including participation in formation of chylomicrons, low-density lipoproteins, and very low-density lipoproteins. Therefore MTTP protein plays a key role in the transport of fats and cholesterol between membrane vesicles, which can be associated with lipid metabolism. In the present study, ENSSSCT00000010052.2:c.2518C>T (rs335896411) missense polymorphism (Leu>Phe) located in exon 18 of *MTTP* gene was investigated in order to estimate its potential association with production traits of pigs. The analysis was performed with five breeds (Duroc, Landrace, Large White, Pietrain, Pulawska pigs) and totally 678 pigs, for which the genotypes of c.2518C>T polymorphism were identified by the polymerase chain reaction–restriction fragment length polymorphism method. The present study showed a significant association of c.2518C>T polymorphism with carcass yield. When analyzing the whole population, *CC* homozygotes showed significantly higher carcass yield than heterozygotes ($P \leq 0.05$). Moreover, c.2518C>T single nucleotide polymorphism (SNP) affected pH measured in loin (*m. longissimus dorsi*) and ham (*m. semimembranosus*) 45 min after slaughter. For both parameters, the highest pH values were obtained for *CC* pigs, while the lowest for heterozygotes ($P \leq 0.05$). The SNP analyzed was also related with meat colour (yellowness intensity (b*)). Previous research confirmed that ENSSSCP00000009789.2:p.Leu840Phe polymorphism, via affecting MTTP protein activity, influences metabolism of fatty acids. Additionally, results obtained in the present study suggest that the analyzed missense mutation in porcine *MTTP* gene can be one of the potential genetic factors associated with meat quality (pork pH and colour) and carcass yield.

Keywords: microsomal triglyceride transfer protein; non-synonymous SNP; pork quality

In pigs, several quantitative trait loci (QTLs) related with growth traits, especially average daily body weight gain and fatness traits, have been mapped to chromosome 8 (Liu et al. 2007; Ai et al. 2012). Furthermore, common QTLs for both fatness and growth traits were found on SSC8 and

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one of them, related with abdominal fat weight, span a large region of this chromosome (22.2 – 108.9 cM; QTL #21252; http://goo.gl/twg8sl) (Ai et al. 2012). Porcine microsomal triglyceride transfer protein (*MTTP*) has been localized on the q-arm of SSC8 between *S0144* and *SW61* microsatellite within QTLs that affect abdominal fat weight, body weight gain, and fatty acid composition (Estelle et al. 2005).

MTTP encodes a large subunit of the heterodimeric microsomal triglyceride transfer protein, which has multiple functions (Hussain et al. 2003). MTTP protein participates in chylomicrons formation via engagement in lipidation of apoliprotein B (Brozovic et al. 2004). In addition, microsomal triglyceride transfer protein is essential for the production of transport lipoproteins such as low-density lipoproteins and very low-density lipoproteins (VLDL) and therefore plays a key role in transporting fats and cholesterol between membrane vesicles (Hussain et al. 2012). The research performed on *Mttp* knockout mice showed that heterozygous animals (*Mttp* +/–) had reduced apolipoprotein B (apoB100) and total cholesterol plasma levels compared to *Mttp* +/+ mice. On the other hand, complete deficiency of *Mttp* –/– gene was lethal, probably because of the lack of lipoprotein synthesis which results in an impaired capacity of the yolk sac to export lipids to growing embryo (Raabe et al. 1998). Leung et al. (2000) confirmed that the level of MTTP protein in endoplasmic reticulum is a critical determinant of lipoprotein secretion. In human, mutation in *MTTP* gene causes abetalipoproteinemia disease which results in deficiencies of the apolipoproteins B48 (apoB48) and B100 (apoB100) used in the synthesis and transport of chylomicrons and VLDL, respectively (Pons et al. 2011; Di Filippo et al. 2012). To date, in pigs polymorphisms within *MTTP* gene have been investigated only in terms of their impact on protein activity (Estelle et al. 2009).

The present research focused on porcine *MTTP* gene because it has an important function in microsomal triglyceride transfer protein and localization of this gene in QTL is associated with fatty acid composition and growth traits. *MTTP* gene was proposed as potentially associated with porcine production characteristics, thus the aim of the present study was the analysis of polymorphism within porcine *MTTP* gene and the estimation of its association with selected slaughter, growth, and meat quality traits.

MATERIAL AND METHODS

Animals and samples. The analysis was performed with five breeds (Duroc: 38, Landrace: 273, Large White: 245, Pietrain: 54, Pulawska: 72 pigs) on a total of 678 pigs, all maintained under the same housing and feeding conditions in the Pig Test Station of the National Research Institute of Animal Production in Pawłowice, Chorzelów and Mełno according to the SKURTCH procedure. Pigs were the offspring of 164 boars (4.20 individuals per one sire) and 435 sows (1.58 individuals per one mother). The family structure for all analyzed pig breeds is presented in Supplementary Table S1. All pigs were fed *ad libitum* from 30 up to 100 kg (± 2.5 kg), they were individually penned, and weighed daily throughout the fattening period. Then, pigs were slaughtered according to the same procedure, exsanguinated in horizontal position, and after 24-hour chilling at 4°C right half-carcasses were dissected. The exact procedure of pig maintaining, estimation of growth traits, and dissection were described in detail by Ropka-Molik et al. (2015). The carcass characteristics (carcass yield (%), weight of loin (kg), weight of ham without skin and bone (kg), loin eye area (cm^2), lean meat percentage, weight of primary cuts (kg), average backfat thickness (cm)) and growth traits (test daily gain, feed : gain ratio, number of days on test, age at slaughter) were collected for all pigs, while meat quality traits were assessed for 595 animals. The meat quality traits analyzed were: meat colour (L* – lightness, a* – redness, b*– yellowness, parameters determined using Minolta CR-310 spectrophotometer (Minolta, Japan)), pH in *Longissimus dorsi* and *Semimembranosus* muscles (measured at 45 min (pH_{45}) and 24 h (pH_{24}) after slaughter), and water holding capacity (WHC) (measured by the Grau–Hamm method). Intramuscular fat content (IMF) was estimated by the Soxhlet method (Oczkowicz et al. 2012).

Genotyping of MTTP polymorphism. The DNA was isolated from whole blood collected into EDTA tubes with the use of Wizard Genomic Purification Kit (Promega, USA) according to the manufacturer's protocol. In the present study, non-synonymous ENSSSCT00000010052.2:c.2518C>T –

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rs335896411 (ENSSSCP00000009789.2:p.Leu840Phe) polymorphism located in exon 18 of the *MTTP* gene was analyzed. Genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method using the following primers: F: 5'–CTCTGACCAGTGTGAGGCAA–3' and R: 5'–ACCCAAAGTGTCACGTAGGT–3' (ENSSSCG00000009179). The obtained PCR product (441 bp) was digested by *Mlu*CI endonuclease and separated on 3% agarose gel (alleles *C* – 293 and 148 bp; *T* – 293, 84, and 64 bp).

Statistical analysis. The association between the analyzed traits and the investigated single nucleotide polymorphism (SNP) was evaluated using the GLM procedure of the SAS software (Statistical Analysis System, Version 8.02, 2001) with Tukey's test, and the model included:

$$Y_{ijkl} = \mu + g_j + f_j + h_k + (fh)_{jk} + \alpha(x_{ijk}) + e_{ijkl}$$

where:

Y_{ijkl} = measured trait
μ = overall mean for the trait
g_j = fixed effect of the i^{th} year of slaughter
f_j = fixed effect of the j^{th} genotype group of *MTTP* gene
h_k = fixed effect of the k^{th} breed
$(fh)_{jk}$ = interaction between f_k genotype group and breed (when significant)
$\alpha(x_{ijk})$ = covariate with weight of half-carcass for all investigated slaughter traits
e_{ijkl} = random error

The Hardy-Weinberg equilibrium was estimated by using Court Lab – HW calculator (Court and Michael 2012).

RESULTS AND DISCUSSION

In pig, the coding sequences of *MTTP* gene have been previously investigated in order to estimate the potential application of identified polymorphisms in breeding selection. The present study shows the differences in frequencies of c.2518C>T alleles between the analyzed pig populations. The most abundant in Landrace and Large White breeds was allele *T* (Phe; 0.63, 0.61, respectively), while in Duroc pigs the highest frequency was observed for allele *C* (Leu; 0.90) (Table 1). Analyzing porcine *MTTP* gene, Estelle et al. (2009) showed that one of the 16 detected SNPs caused the amino acid substitution p.Leu840Phe and was localized in a conserved residue of the lipid transfer domain of microsomal triglyceride transfer protein. Furthermore, authors indicated opposite frequencies for both alleles (*C* and *T*) between Duroc and Landrace pigs, similarly as in our study. The estimated frequency of allele *C* in paternal population was 0.83 (Iberian Guadyerbas), while in maternal breed it was 0.17 (Landrace IBMAP). In our research a trend was observed that the breeds used in crossbreed as a dam-line (Landrace, Large White) had opposite frequencies of *MTTP* alleles compared to sire-line breed (Duroc) (Table 1).

Additionally, Estelle et al. (2009) confirmed that NM_214185:c.2573T>C polymorphism (c.2518C>T) is significantly related with the lipid transfer activity of the MTTP protein and affected fatty acid composition traits, mainly the percentage of oleic and palmitoleic acids content in subcutaneous fat. In human, some reports showed that polymorphisms in *MTTP* gene increased predisposition to higher accumulation of fat in liver in patients with Hepatitis C virus (Zampino et al. 2008). Another *MTTP* mutation affects total cholesterol levels and is related with a risk of cardiovascular disease (di Giuseppe et al. 2013).

The presented association study performed on selected pure breeds showed a significant association of c.2518C>T SNP with several pork quality traits: pH measures in *Longissimus dorsi* and S*emimembranosus* muscles 45 min after slaughter and meat colour – yellowness (Table 2). The meat of pigs with *CC* (Leu) genotype was characterized by the highest pH estimated in both muscles. An analogous trend was also observed for Pietrain pigs, when the analysis was performed for each breed separately (Table 2). The c.2518C>T polymorphism

Table 1. Genotype and allele frequencies of ENSSSCT-00000010052.2:c.2518C>T polymorphisms in *MTTP* gene in different pig breeds

Breed	Genotypes			Alleles		HWE
	CC	*CT*	*TT*	*C*	*T*	
Landrace	0.15	0.44	0.41	0.37	0.63	0.35
Large White	0.13	0.53	0.34	0.39	0.61	0.08
Pietrain	0.24	0.64	0.12	0.55	0.45	0.03
Duroc	0.82	0.15	0.03	0.90	0.10	0.30
Pulawska	0.36	0.44	0.20	0.58	0.42	0.44

HWE = Hardy-Weinberg equilibrium, *P*-value

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was associated with meat colour – the meat of pigs with *TT* and *CC* genotypes was characterized by higher yellowness intensity (b*) compared to that of *CT* heterozygotes. On the other hand, the influence of the analyzed SNP on IMF was observed only for Pulawska pigs, where significantly highest values of this parameter were shown for *CC* homozygotes ($P \leq 0.05$). Interestingly, *CC* Pulawska pigs had the lowest average backfat thickness (without significance) (Supplementary Table S2). According to Estelle et al. (2009), the *CC* (Leu) pigs showed an increase of MTTP activity compared to opposite homozygotes and both other genotypes when the analysis was performed together for *CT* and *TT* animals. The authors suggested that p.Leu840Phe polymorphism, identified within important MTTP protein domain, was more informative and better explained the association with fatty acid composition than the QTL model.

The investigated polymorphism in *MTTP* gene was associated with carcass yield ($P \leq 0.05$) (Table 3). Furthermore, *CC* homozygotes showed the lowest average backfat thickness compared to *TT* pigs (whole population: $P \leq 0.08$, Large White pigs: $P \leq 0.05$) (Supplementary Table S2). As described previously by Estelle et al. (2009), backcross pigs

Table 2. Association between c.2518C>T polymorphism and several meat quality traits in different pig breeds

Meat quality traits	Genotype	Pulawska (n = 72)	Large White (n = 245)	Pietrain (n = 54)	Landrace (n = 273)	Whole population (n = 644)
WHC (%)	*CC*	33.18 ± 1.57	36.36 ± 2.16	27.93 ± 1.82	37.15 ± 1.60	33.76 ± 1.28
	CT	35.25 ± 1.40	36.95 ± 1.63	30.55 ± 1.17	37.73 ± 1.23	35.17 ± 1.22
	TT	35.76 ± 1.82	35.35 ± 1.78	30.29 ± 2.81	37.41 ± 1.10	34.21 ± 1.26
a*	*CC*	54.35 ± 0.37$^{a\bullet}$	53.28 ± 0.11	52.57 ± 1.03	56.85 ± 0.70	54.47 ± 0.48
	CT	54.08 ± 0.33^{ab}	53.01 ± 0.18	53.10 ± 0.61	56.73 ± 0.60	54.32 ± 0.45
	TT	53.10 ± 0.41^{b}	53.20 ± 0.40	53.22 ± 1.47	56.25 ± 0.57	54.01 ± 0.47
L*	*CC*	16.87 ± 0.21	16.16 ± 0.57	17.71 ± 0.71	15.92 ± 0.48	16.30 ± 0.33
	CT	16.64 ± 0.19	16.00 ± 0.43	17.88 ± 0.43	16.11 ± 0.41	16.22 ± 0.32
	TT	16.91 ± 0.23	16.47 ± 0.47	15.97 ± 1.02	16.01 ± 0.39	16.41 ± 0.33
b*	*CC*	2.36 ± 0.13	2.78 ± 0.66^{ab}	5.44 ± 1.01	4.54 ± 0.56	3.11 ± 0.41^{ab}
	CT	2.31 ± 0.12	2.37 ± 0.50^{b}	5.18 ± 0.60	4.15 ± 0.48	2.68 ± 0.39^{b}
	TT	2.36 ± 0.15	3.67 ± 0.55^{a}	3.69 ± 1.44	4.34 ± 0.45	3.33 ± 0.40^{a}
$pH_{45\ (LD)}$	*CC*	6.28 ± 0.03	6.44 ± 0.07	6.57 ± 0.10	6.42 ± 0.02	6.44 ± 0.04^{a}
	CT	6.28 ± 0.03	6.36 ± 0.05	6.33 ± 0.06	6.29 ± 0.01	6.37 ± 0.04^{b}
	TT	6.29 ± 0.04	6.38 ± 0.06	6.43 ± 0.12	6.39 ± 0.01	6.38 ± 0.04^{ab}
$pH_{24\ (LD)}$	*CC*	5.64 ± 0.01	5.56 ± 0.04	5.57 ± 0.03	5.64 ± 0.03	5.60 ± 0.02
	CT	5.63 ± 0.01	5.52 ± 0.03	5.61 ± 0.02	5.60 ± 0.02	5.57 ± 0.02
	TT	5.62 ± 0.02	5.52 ± 0.03	5.60 ± 0.04	5.62 ± 0.02	5.57 ± 0.02
$pH_{45\ (SEMI)}$	*CC*	6.24 ± 0.03	6.37 ± 0.06	6.51 ± 0.09$^{a\bullet}$	6.35 ± 0.03	6.39 ± 0.04^{a}
	CT	6.26 ± 0.03	6.31 ± 0.05	6.27 ± 0.05^{b}	6.30 ± 0.02	6.33 ± 0.04^{b}
	TT	6.26 ± 0.03	6.33 ± 0.05	6.24 ± 0.11^{ab}	6.28 ± 0.02	6.34 ± 0.04^{ab}
$pH_{24\ (SEMI)}$	*CC*	5.68 ± 0.03	5.65 ± 0.04	5.66 ± 0.04	5.66 ± 0.02	5.66 ± 0.02
	CT	5.63 ± 0.03	5.66 ± 0.03	5.67 ± 0.02	5.64 ± 0.01	5.65 ± 0.02
	TT	5.64 ± 0.03	5.65 ± 0.03	5.61 ± 0.04	5.64 ± 0.01	5.64 ± 0.02
IMF (%)	*CC*	1.86 ± 0.04^{a}	1.84 ± 0.06	1.77 ± 0.09	1.82 ± 0.05	1.98 ± 0.03
	CT	1.74 ± 0.04^{b}	1.92 ± 0.04	1.92 ± 0.06	1.80 ± 0.04	1.99 ± 0.02
	TT	1.80 ± 0.05^{ab}	1.91 ± 0.05	1.85 ± 0.13	1.82 ± 0.03	1.99 ± 0.03

WHC = water holding-capacity, meat colour: L* = lightness, a*= redness, b*= yellowness; pH_{45} and pH_{24} = pH measured 45 min and 24 h after slaughter in *Longissimus dorsi* (LD) or *Semimembranosus* (SEMI) muscles, IMF = intramuscular fat content values (Least Squares Means ± standard errors) with different superscripts show significant differences between genotypes ($^{a,b}P \leq 0.05$; $^{a,b\bullet}P \leq 0.1$ – trends)

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Table 3. Basic statistical characteristics of slaughter traits and the association of c.2518C>T polymorphism with traits estimated for the whole pig population analyzed (n = 678)

Slaughter traits	Mean ± SD	Genotype	Mean (LSM ± SE)
Carcass yield (%)	76.94 ± 2.50	*CC*	76.99 ± 0.20^{a}
		CT	76.70 ± 0.19^{b}
		TT	76.86 ± 0.20^{ab}
Weight of loin (kg)	7.74 ± 0.78	*CC*	7.55 ± 0.09
		CT	7.66 ± 0.09
		TT	7.64 ± 0.09
Weight of ham without bone and skin (kg)	9.31 ± 0.68	*CC*	9.47 ± 0.09
		CT	9.42 ± 0.09
		TT	9.35 ± 0.09
Loin eye area (cm^2)	53.63 ± 7.16	*CC*	55.14 ± 0.89
		CT	54.05 ± 0.86
		TT	54.64 ± 0.89
Average backfat thickness (cm)	1.34 ± 0.35	*CC*	1.33 ± 0.05$^{b\bullet}$
		CT	1.36 ± 0.05^{ab}
		TT	1.40 ± 0.05^{a}
Weight of primary cuts (kg)	24.22 ± 1.98	*CC*	24.38 ± 0.27
		CT	24.29 ± 0.26
		TT	24.34 ± 0.27
Lean meat percentage	61.65 ± 3.56	*CC*	62.20 ± 0.47
		CT	62.01 ± 0.45
		TT	61.79 ± 0.47

LSM = Least Squares Means, SD = standard deviation, SE = standard error

means with superscripts differ significantly between genotypes ($^{a,b}P \leq 0.05$, $^{a,b\bullet}P \leq 0.1$ – trends)

with *CC* genotypes showed significantly higher lipid transfer activity of MTTP protein compared to other genotypes. Our results indicated that c.2518C>T polymorphism affected porcine fatness traits such as backfat thickness and IMF content in selected breeds. This observation confirmed that the analyzed SNP is responsible for lipid management and fatty acid distribution in pigs. The present research did not confirm the association of c.2518C>T polymorphism with growth traits.

CONCLUSION

In conclusion, the obtained results suggest that missense mutation c.2518C>T in *MTTP* gene can be one of the potential genetic factors related with pork pH and colour. The analyzed ENSSSCP00000009789.2:p.Leu840Phe polymorphism, via affecting MTTP protein activity, affects metabolism of fatty acids and in a consequence, thickness of subcutaneous or intramuscular fat. The proposed missense polymorphism in *MTTP* gene may be one of the important factors that impact carcass yield and meat quality in pigs. Therefore, in the future this research should be performed on a larger population in order to assess the potential use of c.2518C>T polymorphism in pig breeding selection.

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