SINGLE NUCLEOTIDE POLYMORPHISMS IN *LCAT, HMGCR, CTSZ* AND *TCF7L2* GENES WITH INFLUENCE ON MEAT QUALITY TRAITS IN CZECH LARGE WHITE PIGS

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ABSTRACT

In this part of our project we focused on some meat quality traits (cholesterol content in blood plasma and meat colour) and actual candidate genes for pork quality. We investigated the effects of 4 markers (*LCAT* - lecithin cholesterol acyltransferase, *HMGCR* - 3-hydroxy-3-methylglutaryl-CoA reductase, *CTSZ* - cathepsin Z and *TCF7L2* - transcription factor 7-like 2 genes) in commercial breed population of 83 Czech Large White pigs (sows). The blood and tissue samples (*m.longissimus lumborum et thoracis)* were obtained from one commercial herd, fed with the same diet. Animals were slaughtered at average 91.2 kg of live weight in the same abattoir. The measured phenotypes were cholesterol level in blood plasma (mmol/l) and L* (lightness), a* (redness), b* (yellowness) for meat colour determination. Genotypes of SNPs in candidate genes *LCAT*, *HMGCR*, *CTSZ*, *TCF7L2* were determined by previously reported PCR-RFLP assays. The statistical analysis was performed by the general linear model (GLM) by SAS for Windows 9.1.4.

All analysed polymorphisms were polymorphic in our population of Czech Large White, but allele *G* of LCAT gene was observed in very small rate. Our study revealed significant ($P<0.05$) association of c.266G>C polymorphism of *LCAT* gene to cholesterol level in blood plasma, genotype *GC* was associated with higher level of cholesterol, unfortunately no genotype *GG* was observed to verify the effect of allele *G* on higher cholesterol level in blood plasma, but no significant association between polymorphism c.807A>C of *HMGCR* and cholesterol level content, on the other hand high significant associations between SNP in *HMGCR* gene and meat colour characteristic were showed. But we observed no significant association of *CTSZ* gene and analysed traits. Our study revealed significant asscociation ($P<0.05$) of SNP c.646+154A>G in *TCF7L2* gene with cholesterol content in blood plasma (genotype *AA* was associated with lower cholesterol level) and also associations redness and yellowness of meat.

However number of pigs analysed in this part of our project is limited further investigation is required with higher number of pigs to confirm the associations and higher number of analysed traits.

Key words: *LCAT, HMGCR, TCF7L2, CTSZ*, Czech Large White pigs

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INTRODUCTION

Pork quality comprises a set of key fresh meat quality, processing, and sensory characteristics that are important for the future profitability and competitiveness of the swine industry. These include intramuscular fat content, cholesterol, ultimate pH, colour, water-holding capacity or drip loss, tenderness, cooking loss, and sensory traits involving taste. Improving meat quality genetically is difficult by standard selection methods, but possible if the genes responsible for meat quality variability are identified and mapped (Malek *et al*., 2001). Genetic markers associated with all these traits are of interest to the pig industry because, when used in combination with performance data, they may allow faster improvement of the traits of economic importance without decline of meat quality (Ramos *et al*., 2009).

In this part of our project we focused on some meat quality traits (cholesterol content in blood plasma and meat colour) and actual candidate genes for pork quality. We investigated the effects of 4 markers (*LCAT*, *HMGCR*, *CTSZ* and *TCF7L2* genes) in commercial breed population of Czech Large White pigs.

Porcine LCAT (lecithin cholesterol acyltransferase) is a soluble enzyme that converts cholesterol and licithins to cholesteryl esters and lysolecithins on the surface of high density lipoprotein (HDL) and plays an important role in lipoprotein metabolism, especially in the process termed ´reverse cholesterol transport´. This enzyme is synthesized in liver, but circulates in blood plasma as a complex with components of HDL and lack of LCAT causes accumulation of free cholesterol tissues and cholesterol level in the blood (Qiao *et al*., 2010). Porcine *LCAT* gene has been mapped on pig chromosome 6p13 by Frengen *et al*. (1997). In this study the single nucleotide polymorphism G/C in intron 1 at position 266 of *LCAT1* gene was analysed.

The HMGCR (3-hydroxy-3-methylglutaryl-CoA reductase) is rate-limiting enzyme in de novo biosynthesis of cholesterol, this enzyme catalyses step which converts HMG-CoA into mevalonate (Friesen and Rodwell, 2004). The polymorphisms on the human *HMGCR* gene have been associated with changes in plasma cholesterol and triglyceride levels and in pigs *HMGCR* gene displays a relationship with not only lipid serum traits but also with commercionally important pig meat quality trait (Tong *et al*., 2004, Canovas *et al*., 2010). Porcine *HMGCR* gene has been mapped on SSC2 and the single nucleotide polymorphism *HMGCR*:c.807A>C situated in exon 9 was studied in our study.

Cathepsins are lysosomal proteinases with a broad spectrum of functions and high cathepsin activities of porcine skeletal muscle have been correlated to defects with excessive meat softness or dark colour (Russo *et al*., 2008). *CTSZ* (cathepsin Z) gene is located in QTL for meat quality on SSC17 and impacted on meat colour, with less favourable genotype for growth being associated with darker meat, both visula scoring of meat colour and the objective measure of meat colour (Russo *et al*., 2008, Ramos *et al*., 2009, Fan *et al*., 2010). The single nucleotide polymorphism *CTSZ*:g.557A>G (p.Arg64Lys) in exon 2 was analysed.

TCF7L2 (transcription factor 7-like 2) has been implicated in glucose homeostasis through the regulation of pro-glucagon gene expression, which encodes glucagon-like peptide 1 in intestinal cells (Shu *et al*., 2008). The *TCF7L2* has been identified as one of the most promising candidates associated with type II diabetes in humans (Grant *et al*., 2006). In pigs *TCF7L2* gene is located on porcine chromosome 14 and *TCF7L2* mutations were associated with backfat or meat colour traits (Du *et al*., 2009, Fan *et al*., 2010). For our analysis we used polymorphism *TCF7L2*:c.646+154A>G in intron 10 which may be in linkage disequilibrium with causative variant with additive effects on backfat traits and total lip percentage (Du *et al*., 2009).

MATERIALS AND METHODS

Blood and tissue samples

The blood and tissue samples were obtained from 83 Czech Large White purebred pigs (sows) from one commercial herd, fed with the same diet. Animals were slaughtered at average 91.2 kg of live weight in the same abattoir.

Immediately after slaughter the blood of animals was collected and blood plasma was prepared by spinning a tube of fresh blood with heparin in a centrifuge and was stored at -20°C until cholesterol level measuring. A portion of blood was stored with EDTA at 8°C until genomic DNA purification. Automated purification of DNA was realised by QIAmp DNA Mini Kit (QIAGEN, Hilden, Germany) on QIAcube® (QIAGEN, Hilden, Germany). Purified DNA was stored at -20°C until SNPs genotyping. The tissue samples of *m.longissimus lumborum et thoracis* were collected from each individual after slaugther during the cutting.

SNP genotyping

Single nucleotide polymorphisms in candidate genes *LCAT*, *HMGCR*, *CTSZ*, *TCF7L2* and their PCR-RFLP assays were previously reported by Qiao *et al*. (2010), Canovas *et al.* (2010), Ramos *et al*. (2009) and Fan *et al.* (2010), respectively. Detailed information about these SNPs and respective PCR-RFLP genotyping approach are listed in Table 1. DNA fragments (visualised by ethidium bromide) after digestion were separated on 2-3% agarose gels after the electrophoresis.

Tab. 1 Detailed information about 4 SNPs in 4 candidate genes analysed in this study

Gene	SNP	Position	Primer sequence (5′-3′)	Tm (°C)	Restriction enzyme	PCR-RFLP pattern (bp)	Ref.
LCAT	c.266G>C	Intron 1	GCTCCTCAATGTGCTCTTC CATCTAGCGTGGCTTTCC	64	*Pvu*II	535/ 193+342	Qiao *et al.* (2010)
HMGCR	c.807A>C	Exon 9	CAAATCCTGTTACTCAGAGAG CAGGAGCATAGCGTGTTATG	56	*Hha*I	650/ 450+200	Canovas *et al.* (2010)
CTSZ	g.557A>G	Exon 2	GGCATTTGGGGCATCTGGG ACTGGGGGATGTGCTGGTT	62	*Alw*NI	330/ 260+70	Ramos *et al.* (2009)
TCF7L2	c.646+154 A>G	Intron 10	AGAAAGGAAAGGGTGCAGGT GCGATAACTTGTCAGCACGA	60	*Bsr*I	314/ 192+122	Fan *et al.* (2010)

Analysed traits and statictical analysis

The measured phenotypes were cholesterol level in blood plasma (mmol/l) and L* (lightness), a* (redness), b* (yellowness) for meat colour determination. Cholesterol level in blood plasma was determined on Thermo Scientific* Konelab 20XT Clinical Chemistry Analyzer (Thermo Scientific, Bremen, Germany). Meat colour was determined by spectrophotometry on CM-3500d (KONICA MINOLTA) according to CIELAB (L*, a*, b*).

The statistical analysis was performed by the general linear model (GLM) by SAS for Windows 9.1.4. The genotypes of relevant genes (G_{i-l}) were used as fixed effects. The model used to analyze the data was assumed to be: $y_{ijklm} = \mu + G_i + G_j + G_k + G_l + e_{ijklm}$, where y_{ijklm} is the observation or the trait, μ is the population mean, $G_{i, j, k, l}$ is the effect of i, j, k, l-th genotype, e_{ijklm} is the random residue.

RESULTS AND DISCUSSION

Genotypes frequencies

All analysed polymorphisms were polymorphic in our population of Czech Large White, but allele *G* of *LCAT* gene was observed in very small rate and genotype *GG* of *LCAT* gene was not determined (see Tab. 2). Qiao *et al*. (2010) characterized this SNP in porcine *LCAT* gene and revealed also very low frequency of alelle *G* in Yorkshire or Landrace population (allele *G* in Yorkshire was not observed) however allele *G* was in majority in Meishan population used in their study. So it is possible analogous tendency of low occurence of one allele in western breeds but high rate of the same allele in Chinese breeds (Shan *et al*., 2009, Xu *et al*., 2010). Fan *et al*. (2010) published similar results of Yorkshire pigs as our frequencies in Czech Large White pigs of *TCFL7L2* and *CTSZ* genes. Russo *et al*. (2008) studied polymorphisms in cathepsins genes in different pig populations – they revealed no allele *G* of *CTSZ* gene in Meishan, but in western breeds (Duroc, Pietrain, Hampshire and Italian Large White) they observed the occurence of allele *G* and the results of Italian Large White were similar to our investigation. In *HMGCR* polymorphism c.807A>C Canovas *et al*. (2010) published intermediate frequency of allele *G* for Large White compared to no segregation of allele *G* in other population where either allele *A* (Iberian, Duroc lines) or allele *G* (Mesihan pigs) was fixed. Our results were similar to results of Canovas *et al*. (2010) in Large White.

Tab. 2 Frequency of genotypes, number of observations and the allele frequency for different markers used in this study

Gene	Genotype frequency (no. of animals)			Allele frequency	
HMGCR	AA	AC	CC	A	C
	39.76 (33)	51.81 (43)	8.43 (7)	0.66	0.34
TCF7L2	AA	AG	GG	A	G
	12.05 (10)	56.63 (47)	31.33 (26)	0.40	0.60
CTSZ	AA	AG	GG	A	G
	13.25 (11)	68.67 (57)	18.07 (15)	0.48	0.52
LCAT	CC	GC	GG	C	G
	91.57 (76)	8.43 (7)	0	0.96	0.04

Associations of genotypes with the traits

Table 3 shows results corresponding to the association study between polymorphisms in candidate genes (*LCAT, HMGCR, CTSZ, TCF7L2)* and cholesterol content and meat colour characteristic in Czech Large White population.

Qiao *et al*. (2010) studied *LCAT* gene and revealed that SNP c.266G>C in intron 1 was significantly associated with ratio of lean fat, leaf fat weight or carcass lenght in Yorshire, Landrace and Meishan pigs. *LCAT* gene or lecithin cholesterol acyltransferase is a key enzym of reverse cholesterol transport, converts cholesterol and lecithins to cholesteryl esters and lysolecithins on the surface of high density lipoproteins and a lack of *LCAT* activity would be lead to accumulation of free cholesterol in the tissues, *LCAT* is a key enzyme in cholesterol homeostasis and regulating its transport in blood (Qiao *et al*., 2010). Our study revealed significant ($P<0.05$) association of c.266G>C polymorphism to cholesterol level in blood plasma, genotype *GC* was associated with higher level of cholesterol, unfortunately no genotype *GG* was observed to verifying the effect of allele *G* on higher cholesterol level in blood plasma.

Tab. 3 Association analysis between LCAT, HMGCR, CTSZ, TCF7L2 genes and meat colour and cholesterol level in blood plasma

Marker	Analysed traits (LSM (Least square mean value) ± SE (standard error))			
	Cholesterol content (mmol/l)	L*	a*	b*
HMGCR				
AA (33)[1]	2.65±0.16	55.52±1.04	2.13±0.85a	11.19±0.62
AC (43)	2.59±0.16	56.58±1.02	4.3±0.83a	12.77±0.61a
CC (7)	2.54±0.25	58.23±1.62	2.76±1.32	12.68±0.97a
TCF7L2				
AA (10)	2.32±0.22b	56.63±1.42	2.95±1.16	12.13±0.85
AG (47)	2.81±0.15b	57.23±0.98	4.15±0.80b	12.84±0.59b
GG (26)	2.65±0.17	56.45±1.08	2.10±0.88b	11.66±0.65b
CTSZ				
AA (11)	2.72±0.22	56.86±1.38	2.59±1.13	11.94±0.83
AG (57)	2.58±0.16	57.51±1.02	3.07±0.83	12.46±0.61
GG (15)	2.48±0.19	55.95±1.19	3.54±0.97	12.23±0.71
LCAT				
CC (76)	2.32±0.12b	57.64±0.78	2.31±0.63	12.04±0.47
GC (7)	2.87±0.24b	55.91±1.54	3.83±1.25	12.39±0.96

b - significant difference beetween genotypes (P<0,05), a - high significant difference between genotypes (P<0,01), cholesterol content in plasma (mmol/l), meat colour characteristic L* (lightness), a* (redness), b* (yellowness)
1 – number of animals with mentioned genotype

HMGCR gene is the rate-limiting enzyme in the biosynthesis of cholesterol. Canovas *et al.* (2010) revealed that allele *A* of c.807A>C in HMGCR gene showed significant association with intramuscular fat content or with higher oleic and lower linoleic acid level in fat so they supposed that *HMGCR* could be shown as an interesting candidate gene assisted selection in commercial important meat quality. Our study revealed no significant association between polymorphism c.807A>C and cholesterol level content, on the other hand high significant associations (P<0.01) between SNP in *HMGCR* gene and meat colour characteristic (redness and yellowness) were showed. *HMGCR* gene has been intensively studied in human and its polymorphisms were associated with changes in plasma or triglyceride levels and statits, known as *HMGCR* inhibitors, are the standard treatment for hypercholesterolemic patient (Tong *et al.*, 2004, Osborne *et al.*, 2004). Porcine *HMGCR* gene was presented as a source of genetic variation for traits related to serum lipid level levels and fat deposition in pigs. In physiological function of *HMGCR* gene there is no obvious reason of meat colour influencing. These results suggest there is common pathway or network regulating fatness of energy balance.

Cathepsin Z and SNP g.557A>G within it and polymorphism c.646+154A>G in *TCF7L2* were associated with both visual meat colour scoring and objective L-value measure of meat colour in purebred Yorkshire (Fan *et al*., 2010). In addition Ramos *et al*. (2009) revealed impact of g.557GG of *CTSZ* on slower growth and darker meat colour. In fact, Ramos *et al*. (2009) mentioned that g.557A was associated with faster growth, but to the contrary to this study Russo *et al.* (2008) observed opposite tendency in Italian Large White and allele g.557G was the preferred allele for daily gain. It indicated that the effects of *CTSZ* gene may differ between different populations. But we observed no significant association of *CTSZ* gene and analysed traits. In addition to associations with meat colour *TCF7L2* gene has been identified as promising candidate associated with type II diabetes in humans (Grant *et al*., 2006). In pigs mutations were associated with backfat and meat colour (Du *et al*., 2009). Our study revealed significant association ($P<0.05$) of SNP c.646+154A>G in *TCF7L2* gene with cholesterol content in blood plasma (genotype *AA* was associated with lower cholesterol level) and also associations redness and yellowness of meat. Interestingly the LSM of heterozygotes *AG* was always higher than those of homozygotes for this SNP.

CONCLUSION

In summary, for the present study, we analysed previously reported polymorphisms of *LCAT, HMGCR, CTSZ, TCF7L2* actual candidate genes in population of Czech Large White pigs. We revealed significant associations between *LCAT* and *TCF7L2* genes and cholesterol level and associations between *HMGCR* and *TCF7L2* and redness and yellowness of meat. These results are very interesting because they suggest possible involvement of *HMGCR* gene in physiological pathway influenced with meat colour. However number of pigs analysed is limited further investigation is required among other populations of pigs to confirm the associations between *HMGCR* polymorphism and meat colour and the others previously reported associations.

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