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The effect of gene *CTSL* on the quantitative and qualitative production traits of pork meat

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Abstract

Cathepsin L (*CTSL*) affects the proteolytic system of meat in pigs. Moreover, this gene belongs to the candidate genes for economically important traits in pigs. The objective of this study was to test the influence of mutation AJ315771:g.143C > T in the *CTSL* gene on qualitative and quantitative traits in pigs. The association analysis performed within this project tested the effect of the *CTSL* gene in 514 crossbred combinations. These pigs are commonly raised on commercial farms in the Czech Republic. No statistically significant differences were detected in qualitative traits. However, we identified a trend of allele *T* on increased fatness and the effect of allele *C* on lean meat.

CTSL, pig, meat, carcass traits, PCR - RFLP, gene

Introduction

The utility properties of livestock are affected by many genes whose mutual interaction, and the degree of their influence, are presently unknown. Consequently, a number of molecular methods are being developed in an effort to identify the sequences or genes that determine the livestock utility (the most recent involving SNP chips; more in the work of Ramos et al. 2009). These methods, based on DNA technologies and insights from the quantitative and population genetics lead to definitions of QTL areas (the areas containing the gene with a significant influence on a given production trait). The fact that a demonstrable impact of QTL on useful properties was found on practically all pig chromosomes inspired the creation of an international database of QTLdb (http://www.animalgenome.org/QTLdb/pig). The QTLdb website currently holds information on about 5.986 QTL areas for 581 utility traits (these results were published in 268 scientific papers). Six hundred and seven QTL were identified for production traits and 4.143 QTL for qualitative traits of pig meat. One of the genes detected in the QTL area is gene *CTSL.* Cathepsin L *(CTSL)* belongs to the family cathepsins *(CTSL, CTSB, CTSD, CTSH, CTSF* and *CTSZ)*, which is a group of genes that produce lysosomal proteinases that are important for the degradation of proteins. Their main role is in the *post-mortem* proteolysis of meat. These genes or their mutations may profoundly affect the qualitative properties of meat (Toldrá and Flores 1998). However, that proposition has not yet been confirmed by any of the published report known to us (Russo et al. 2002 and 2008; Fontanesi et al. 2010). On the contrary, Fontanesi et al. (2010) claim that the genes of the cathepsin family play an important role in the quantitative production traits of pigs. The authors found a statistically conclusive effect of gene *CTSL* on the average daily gain, the lean meat, and the backfat thickness ($P < 0.05$). Those results are consistent with the finding that gene *CTSL* was located in the QTL area for an average daily gain and the backfat thickness (Fontanesi et al. 2001 and 2010, Knott et al. 1998, Rohrer et al. 2006). These results, as well as the results of recent studies, suggest a new theory, which implies that cathepsin

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genes and their lysosomal proteinases have another important cell function in addition to the degradation of proteins.

The objective of this study was to verify the effect of gene *CTSL* on the quantitative and qualitative production traits of pork meat.

Materials and Methods

Animals

This study is based on a test of quantitative and qualitative production traits in 514 pigs (Table 1). Animals were raised and finished at the test station in Ploskov, Department of Special Zootechnology . Pigs weighted 25-30 kg when brought in, and were slaughtered when their average weight reached 108 kg. They were fed a mixture of four components (wheat, barley, soya extract mash and a mix of essential element supplements), *ad libitum* and/or in doses feed.

Carcass traits

Carcass dissection was done per Walster and Merkuse (1995). Dissection of the belly followed the methodology described by Stupka et al. (2004), lean meat was measured by the ZP method (on 506 pigs). The following qualitative traits were measured: electrical conductivity in ham and chop (45 minutes *post-mortem;* on 353 pigs), intramuscular fat content in the shoulder (on 162 pigs), in the neck (on 147 pigs), in the ham (on 99 pigs), in the chop (on 129 pigs); temperature (45 minutes *post-mortem*; on 415 pigs), pH (45 minutes *post-mortem;* on 403 pigs) and drip loss (on 136 pigs).

Genotyping

The genotype for mutation AJ315771:g.143C > T of the *CTSL* gene was determined on all tested pigs. The primers were taken from the work of Fontanesi et al. (2010). A reactive mix (25 µl) was prepared, containing 100 ng genomic DNA, standard PCR buffer, 1.5 mM $MgCl_2$, 200 µM from each dNTP, 10 pmol primer and 1.0 U Taq DNA polymerase (Fermentas, Prague, Czech Republic). Cycling conditions: 2 min at 95 °C, followed by 31 cycles: 94 °C (1 min), 57 °C (30 s), 72 °C (30 s) and final elongation 72 °C (7 min). The PCR product was cut by restrictive enzyme *Taq*I and fragments of the following length were obtained: allele *T*: 380 bp and allele *C*: 162 + 218 bp.

Association Analysis

The association analysis for quantitative and qualitative production traits of pork meat was composed of a different number of individuals (phenotypic values in each group were measured in a random part of the population or not measured at all). The effect of mutation g.143C > T of gene *CTSL* was analyzed using the UNIVARIATE, MEANS, GLM (type IV) procedures (SAS, 9.1 Institute). This model included genotype *CSTL* (individuals with genotype *TT* were excluded because of low occurrence; 3 pigs), crossbred combination, sex, and nutrition as a fixed factor. The carcass weight was used as a regressive coefficient. The following model was used:

$Y_{ijklmn} = \mu + genotype_i + crossbred_j + sex_k + nutrition_l + \beta_m + e_{ijklmn}$, where

Y_{ijklmn} = slaughter value trait; µ = overall average; $genotype_i$ = effect of genotype of gene *CTSL* (i = 1, 2); $crossbred_j$ = crossbred combination effect (k = 1, 2, 3, 4, 5, 6, 7, 8, 9); sex_k = sex effect (k = 1, 2); $nutrition_l$ = nutritional effect (l = 1, 2); β_m= carcass weight of animal m; e_{ijklmn}= random residual.

Results and Discussion

A fragment of expected length of 380 bp was obtained (allele *T* was not cut - 380 bp, and allele *C* was cut into two fragments, 162 and 218 bp). The frequency of genotypes in this study was *TT* = 3, *CT* = 65 and *CC* = 446. Given the low frequency of genotype *TT*, these pigs were excluded from the association analysis.

Although the *CTSL* gene belongs to the cathepsin family, which is responsible for a production of lysosomal proteins that are involved in the *post-mortem* proteolysis of meat, this study could not confirm a statistically significant effect of mutation g.143C > T of gene *CTSL* on the qualitative traits of meat (Table 2). These results agree with the studies of Russo et al. (2002 and 2008) and Fontanesi et al. (2010). Our results also correlate with the findings of Fontanesi et al. (2010), who claim to have discovered the effect of gene *CTSL* on growth rate, the lean meat, and the backfat thickness. The authors mention a statistically effect of allele *T* on greater fat accumulation and allele *C* on a higher lean meat. This study detected a significant influence of mutation g.143C > T of the *CTSL* gene on the lean meat in favor of allele *C*, $P < 0.03$ (Table 2). Conversely, the trend toward greater fatness was found with allele *T* in terms of intramuscular fat content, fat content in belly 1,

Table 1. List of hybrid combinations used in the association study

Group	Crossbred Combinations	Number of Pigs
1	(BUxL)x(PnxBO), (BUxL)xPIC	71 (35 + 36)
2	(BUxL)xPn	46
3	BU, BUxL, (BUxL)xPn	68 (23 + 21 + 24)
4	PICxFH	62
5	(BUxL)x(BOxD)	71
6	(BUxL)x(HxPn)	70
7	(BUxL)x(Pn)	65
8	(BUxL)x(BOxPn)	61

BU - Large White; L - Landrace; H - Hampshire; FH - French Hybrid; PN - Pietrain; PIC - Pig Improvement Company; D - Duroc; BO - Large White sire line

2 and 3, and the fat coverage of main meat parts (Table 2, some date not shown).

Also evident from Table 2 is that a significant factor in this association analysis was found to be the *crossbred combination* influence. However, it was not possible to carry out the association analysis for (BuxL) xPn with 135 pigs of one combination, because all the pigs were carriers of allele *C* only. It is therefore probable that there was a shift in the selection of the Pietrain pig (Pn; a pig with above-average musculature) toward a higher frequency of the *C* alleles. Conversely, the *TT* genotype was also found in a crossbred combination where the C position was occupied by a Durok or a Hampshire, plus a series of *CT* heterozygots (33 *CT* and 3 *TT* out of 141 pigs). Those findings correlate with the study of Fontanesi et al. (2010). These and the foregoing results once again suggest a theory that the family of cathepsin genes and lysosomal proteinases have an important cellular function other than the *post-mortem* proteolysis of meat. This theory is supported by studies of Fontanesi et al. (2001 and 2010), Knott et al. (1998) and Rohrer et al. (2006), who assert that gene *CTSL* is situated in the QTL area for the average daily gain and backfat thickness. We therefore consider a continued testing of this mutation essential, particularly for quantitative production traits of pigs. Since only a few scientific studies of this mutation has been published, it is impossible to say if it could be a causal mutation. It appears likely that it is not, but rather a mutation linked with a causal mutation (LD marker; linkage disequilibrium).

Conclusions

The study presented herein did not confirm the effect of mutation AJ315771:143C > T of the *CTSL* gene on the qualitative traits of port meat. However, it did find the trend of allele *T* on greater fat deposition and a statistically conclusive effect of allele *C* allele on greater

Table 2. Association analysis for mutation g.143C > T of gene *CTSL* and pig traits

Performance traits	*CT* (LSM ± SE)	*CC* (LMS ± SE)	P Value	G	V	Carcass weight
IM fat content in belly (%)	1.91 ± 0.17	1.76 ± 0.09	0.29	*		
IM fat content in neck (%)	4.53 ± 0.38	4.12 ± 0.20	0.27	**		
IM fat content in shoulder (%)	2.36 ± 0.16	2.41 ± 0.08	0.75	**		
IM fat content in ham (%)	4.18 ± 0.46	3.40 ± 0.25	0.08	**		
Temperature (°C)	34.83 ± 0.38	35.00 ± 0.21	0.62	**		**
pH	6.39 ± 0.05	6.38 ± 0.03	0.71	**	**	**
Drip loss (%)	9.91 ± 0,68	9.86 ± 0.32	0.95	**	**	**
Electrical conductivity in ham (mS^{-1})	3.96 ± 0.15	3.88 ± 0.07	0.6	**		
Electrical conductivity in chop (mS^{-1})	4.13 ± 0.17	4.10 ± 0.07	0.86	**		**
Lean meat ZP (%)	57.42 ± 0.49	58.37 ± 0.29	0.03	**	**	**

IM-intramuscular; LSM - least square mean; SE – standard error; allele *C* (162+218 bp) allele T (380 bp);
G - crossbred combination; V - nutrition; * $p < 0.05$; ** $p < 0.001$.

formation of muscle tissue. Allele *T* in the pig population tested was detected at a very low frequency, and it did not occur at all in the hybrid combinations that had only the above-average muscled Pietrain breed in the C position. It is therefore probable that gene *CTSL* has another important biological function aside from the *post-mortem* proteolysis of meat.

The utility characteristics of livestock are influenced by many genes and, to some extent, by external environmental factors. The genes that have a major impact on these quantitative traits are in quantitative trait locus (QTL), which is a relatively large area of many candidate genes. Molecular genetics strives to find a mutation of a candidate gene (ideally a causal one) which has a significant impact on the economic utility of livestock. Such mutations are then used in breeding programs within the marker-assisted selection (MAS). One of these candidates could be gene *CTSL*. Even though this gene is associated primarily with a *post-mortem* proteolysis of meat, its influence on the qualitative properties has not been confirmed by our study or any other published study that we know of. However, our study did find a trend of allele *T* on the formation of adipose tissue and the trend of allele *C* on a greater muscle formation ability, which as been confirmed by the latest scientific papers published on the subject.

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References

Fontanesi L, Speroni C, Buttazzoni L, Scotti E, Nanni Costa L, Davoli R, Russo V 2010: Association between cathepsin L (CTSL) and cathepsin S (CTSS) polymorphisms and meat production and carcass traits in Italian Large White pigs. Meat Science **85**: 331 – 338

Fontanesi L, Davoli R, Yerle M, Zijlstra C, Bosma AA, Russo V 2001: Regional localization of the porcine cathepsin H (CTSH) and cathepsin L (CTSL) genes. Animal Genetics **32**: 321 – 323

Knott SA, Marklund L, Haley CS, Andersson K, Davies W, Ellegren H et al 1998. Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and Large White pigs. Genetics **149**: 1069 – 1080

Ramos AM, Crooijmans RP, Affara NA, Amaral AJ, Archibald AL et al. 2009: Design of a high density SNP genotyping assay in the pig uasing SNPs identified and charaterized by next generation sequencing technology. Plos One, e6524

Rohrer GA, Thallman RM, Shackelford S, Wheeler T, Koohmaraie MA 2006: Genome scan for loci affecting pork quality in a Duroc-Landrace F2 population. Animal Genetics **37**: 17 – 27

Russo V, Fontanesi L, Davoli R, Nanni Costa L, Cagnazzo M, Buttazzoni L et al 2002: Investigation of candidate genes for meat quality in dry-cured ham production: the porcine cathepsin B (CTSB) and cystatin B (CSTB) genes. Animal Genetics **33**: 123 – 131

Russo V, Fontanesi L, Scotti E, Beretti F, Davoli R, Nanni Costa L et al. 2008: Single nucleotide polymorphisms in several porcine cathepsin genes are associated with growth, carcass, and production traits in Italian Large White pigs. Journal of Animal Science **86**: 3300 – 3314

SAS® Propriety Software Release 9.1 of the SAS® system for Microsoft® Windows®. SAS Institute Inc., Cary, NC., 2001

Stupka R, Šprysl M, Pour M 2004: Analysis of the formation of the belly in relation to sex. Czech Journal of Animal Science **49**: 64 – 70

Toldrá F, Flores M 1998: The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. Critical Reviews in Food Science and Nutrition **38**: 331 – 352

Walstra P, Merkus GSM 1995: Procedure for assessment of the lean meat percentage as a consequence of the new EU reference dissection method in pig carcass classification. DLO- Research Institute for Animal Science and Health Research Branch, Zeist, The Netherlands, 1 – 22