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# The effect of cystatin B gene (*CSTB*) on productive traits in pigs*

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**Cystatin B gene is a candidate gene for carcass and meat quality traits of pigs and belongs to the family 1 of cysteine proteinase inhibitors. The enzyme is a cathepsin inhibitor and the proteolytic cystatin/cathepsin system plays an important role in the growth and development of muscles. Investigations presented here covered 707 pigs from different genetic groups reared in Poland. The aim of this study was to characterise the polymorphism of the *CSTB* gene identified with restriction endonuclease: *Taq*I and *Pvu*II, and to analyse the relation between the *CSTB* genotypes and carcass traits. All tested animals proved to be monomorphic at the *CSTB/Taq*I *locus*. All three possible genotypes were observed with regard to the second *CSTB/Pvu*II *locus*. In Polish Large White and Polish Landrace pigs the highest frequency was reported for *BB* homozygotes. The association between *CSTB* and carcass traits was found only in Polish Landrace pigs for the meat content of carcass, meat content of valuable cuts and weight of the loin.**

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Muscle deposition of carcass and meat quality are quantitative traits, which means that their final expression is affected by genetic and environmental factors. Muscular or hepatic expression genes have been found to correlate highly with growth or carcass traits in pigs [Pierzchała *et al*. 2011]. The genetic component is comprised of the expression of many genes, known as quantitative trait *loci* (QTLs). Currently, there are two methods of identification of the QTLs known in animals. One is evaluation of the effect of candidate genes polymorphisms on a trait of interest. In recent years several other genes were reported as candidate genes for pig carcass traits. These were reviewed by Urbański *et al*. [2003] and Terman *et al*. [2008].

An example of candidate genes for carcass traits are two genes involved in the proteolytic activity in dry cured hams: the cystatin B (*CSTB*) and the cathepsin B (*CTSB*) genes. Cystatin B is a member of family 1 (stefins) of cysteine protease inhibitors, discovered as an inhibitor of cathepsin B [Lenney *et al*. 1979]. The stefins were reported by Turk and Bode [1991] as one of the most important intracellular cathepsin inhibitors, that are distributed among various cell types and tissues. Cathepsin B, together with cathepsins L and D is one of the most abundant lysosomal proteinases in animal tissues. They are involved in the degradation of proteins mainly inside lysosomes, in the processing of several pro-enzymes and in antigen presentation [Turk *et al*. 2000].

The porcine cystatin B gene has been mapped to the end of chromosome 13 together with *S0290* and *S0291* microsatellites [Russo *et al*. 2002]. The polymorphisms in coding and non-coding regions of *CSTB* gene has also been reported by Russo *et al*. [2002]. Three of these point mutations are located in introns; the fourth is in exon 3. The latter mutation is a missense mutation that substitutes an aspartic codon for asparagine [Russo *et al*. 2002]. Furthermore *CSTB* and *CTSB* were used as markers in tests for association with several traits of economic importance in pig breeding [Russo *et al*. 2002].

The objective of this study was to evaluate the effect of two mutations of porcine *CSTB* gene on traits characterizing meat deposition in pig carcasses. One of these mutations is located in the intron, while the second in exon 3. These mutations are recognized by restriction endonucleases *Alu*I or *Pvu*II and *Taq*I, respectively.

## Material and methods

The investigations included a total of 707 gilts: two breeds – Polish Large White (PLW – 185 animals) and Polish Landrace (PL – 216 animals), one synthetic line L990 (216 animals) and 90 crossbreds of PLW x PL. Pigs of two breeds: PLW and PL and L990 were kept at the Pig Hybridization Centre in Pawłowice near Leszno Wielkopolskie of the National Research Institute of Animal Production, Cracow, Poland. Between 25 and 100 kg body weight (BW) the gilts were fed *ad libitum* the standard commercial mixed feed, and then slaughtered at 100 kg BW. Right carcass sides were divided into cuts and dissected into meat, fat and bone according to the procedure used in Polish Pig Testing Stations, as described by Różycki [1996].

Crossbreds were kept in Broniewo and Pruszcz farms (Kujawy region). Both maintenance and feeding were similar for all animals and remained in accordance with obligatory standards. At a BW of about 105 kg the animals were slaughtered and right carcass-sides were dissected according to Walstra and Merkus [1995]. This material was used to characterise the polymorphism in the porcine *CSTB* gene. We did not analyse the relations between the polymorphism in *CSTB*/*Pvu*II *locus* and productive traits in these animals.

Blood samples were withdrawn from the external jugular vein of each gilt with a disposable syringe (MONOVETTE, SARSTEDT) using EDTA as an anticoagulant. DNA isolation from blood leukocytes was performed according to Kawasaki [1990]. The *RYR1*/*HinP*1 genotypes were identified using sequence of primers after Kamiński *et al*. [2001].

The analyses of the *CSTB* gene polymorphisms were performed using following primer sequences:

*CYSB1* pair , F: 5' - GAAGGCTGGGCGTGTCATC - 3';
R: 5'- GGTCAAGGGCTTGTTCTCGTG - 3';
*CYSB2* pair: F: 5'- GTTCCAGGTTCAAGTTGACGATGTC - 3';
R: 5'- GGTCTGGTAGCTGGACAAGG - 3' [Russo *et al*. 2002].

The PCR samples of 10 µl contained 5 µl RedTaq™ ReadyMix™ PCR Reaction Mix (SIGMA), 0.2 µl of each primer (0.2 µM), 3.6 µl water (SIGMA) and 1 µl DNA (20 mg/µl). The DNA amplification reactions were performed on Tetrad PTC-225 Thermocycler (MJ RESEARCH, WATERTAWN, USA) at 95°C for 2 min, followed by 35 cycles of 94°C for 45 s, 64°C for 30 s, and 72°C for 45 s, followed by a final extension step at 72°C for 10 min, and completed by cooling for 10 min at 4°C. To perform the RFLP analyses the PCR fragment obtained with primers *CYSB1* were digested with 5U of the *Pvu*II restriction endonuclease (NEW ENGLAND BIOLABS, Frankfurt am Main, Germany) and the PCR fragment obtained with primer *CYSB2* were digested with *Taq*I restriction endonuclease (NEW ENGLAND BIOLABS, Frankfurt am Main, Germany). Five µl of the PCR reaction mixture was added to 5 µl of a reaction mix containing 5U of restriction endonuclease. Digestion was carried out overnight at 37°C and DNA fragments were analysed in a 4 % agarose gel (0.9 g. agarose NuSieve GTG Agarose, CAMBREX BIOSCIENCE, USA and 0.3 g agarose SIGMA Aldrich, Steinheim, Germany) with ethidium bromide in 1x TBE at a constant current of 50 mA.

Statistical evaluation of the results was performed for each breed separately using the least squares method of the GLM procedure [SAS 8.2, 2002] according to the following model:

$$Y_{ijkl} = \mu + G_i + RYR1_j + O_k + \beta(x_{ijkl} - x) + e_{ijkl}$$

where:

$Y_{ijkl}$ – trait measured on *ijkl*-th animal;

$\mu$ – overall mean;

$G_i$ – effect of *CSTB* genotype;

$RYR1_j$ – fixed effect of *RYR1* genotype;

$O_k$ – sire effect;

$\beta(x_{ijkl} - x)$ – linear regression for weight of right carcass side (for traits and age at slaughter for growth rate traits);

$e_{ijkl}$ – random error.

Age at slaughter and weight of right carcass side were included as covariates.

## Results and discussion

The first step of this study was to identify *CSTB* genotypes by PCR-RFLP method. Studied was the frequency of genotypes at *loci CSTB*/*Pvu*II and *CSTB*/*Taq*I. Three possible genotypes were observed at the mutation site recognized by *Pvu*II endonuclease With regard to mutation recognized by *Taq*I no polymorphism was observed; the analysed material occurred monomorphic. The frequency of genotypes at *CSTB*/*Pvu*II *locus* in tested pigs breeds is shown in Table 1. In PLW, PL and L990 gilts observed was the lowest frequency of *AA* (from 0.44% in PL to 12.6% in L990), and the highest of *BB* genotypes (from 53.71% in L990 to 83.55% in PL). In crossbred pigs the lowest frequency was characteristic for *AB* genotype (15.45%) and the highest for *BB* genotype (65.55%) (Tab. 1).

**Table 1**. Frequency of genotypes and alleles at *locus CSTB*/*Pvu*II in pigs of tested genetic groups

| Genetic group | N | Frequency of genotypes (%) | | | | | | Frequency of alleles | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *CSTB*/*Pvu*II | | | *RYR1*/*Hin*P1I | | | *CSTB*/*Pvu*II | | *RYR1*/*Hin*P1I | |
| | | *AA* | *AB* | *BB* | *CC* | *CT* | *TT* | *A* | *B* | *C* | *T* |
| PLW | 185 | 10.92 | 15.3 | 73.77 | 91.2 | 7.8 | 1.0 | 0.19 | 0.81 | 0.95 | 0.05 |
| PL | 216 | 0.44 | 16.00 | 83.55 | 73.3 | 25.8 | 0.9 | 0.08 | 0.92 | 0.86 | 0.14 |
| L990 | 216 | 12.66 | 33.62 | 53.71 | 31.02 | 61.11 | 7.9 | 0.29 | 0.71 | 0.61 | 0.39 |
| PLWxPL | 90 | 20.95 | 15.45 | 65.55 | 97.1 | 2.9 | 0.00 | 0.29 | 0.71 | 0.98 | 0.02 |

PLW – Polish Large White; PL – Polish Landrace; L990 – synthetic line.

Russo *et al*. [2002] has defined the frequency of alleles at *CSTB*/*Pvu*II *locus* in 423 animals of 11 pig breeds – Large White, Landrace, Duroc, Belgian Landrace, Hamsphire, Pietrain, Meishan, Cinta Senese, Casertana, Calabrese and Nero di Sicilia. In their study all pigs except Meishan had lower frequency of *B* alleles (from 0.72% in Nero di Sicilia to 0.97% in Casertana) when compared to *A* allele. Solely Meishan pigs were found monomorphic as 100% of tested animals had *A* allele. In the present study a reverse situation was observed: in all pigs tested the higher frequency of *B* allele was reported.

Due to the fact that our earlier studies as well as those by other authors showed that *RYR1* genotype significantly affects carcass traits and may modify the effect of other genes [Kurył *et al*. 1998 – a review], we have included the effect of *RYR1* genotype into our statistical model. This frequency was very different in each breed. In PLW x PL crosses, PLW and PL breeds the frequency of *CC* heterozygotes was from very high to high (97.1%, 91.2% and 73.3%, respectively). In L990 gilts we observed the inverse situation. Only 30% of animals within this group were free of $RYR1^T$ allele. The frequency of *CT* heterozygotes was 61.1%, and 7.9% for *TT* homozygotes (Tab. 1).

Associations between *CSTB* gene variants and carcass traits were analysed only for *CSTB*/*Pvu*II *locus*. A significant relation was found between several carcass traits and the *CSTB* genotype only in PL pigs. The results are shown in Table 2. The tested mutation in the intron 3 of *CSTB* gene appeared to be significant for the meat content of carcass, meat content of valuable cuts and weight of the loin. *AA* homozygotes showed the highest values of these traits compared to second of *BB* homozygotes and *AB* heterozygotes. Heterozygotes showed the lowest weight of loin and of the meat content of valuable cuts compared to both homozygotes. Such phenomenon observed earlier for certain human genes was named a "negative" (or "positive") heterosis and was initially described by Coming and MacMurray [2000]. They suggested that if the regulation of the gene is dose-dependent, the presence of regulatory sequence in a heterozygous state could modify the gene function. Similar associations were observed in our earlier studies regarding family of *MyoD* genes [Cieślak *et al*. 2002, Urbański *et al*. 2005, 2006, Wyszyńska-Koko *et al*. 2006].

**Table 2**. Least squares means and their standard errors (±) for carcass traits in Polish Landrace pigs as related to *CSTB locus*

| *Locus* | Genotype | MCVC (%) | WL (kg) | MCC (%) |
|---|---|---|---|---|
| *CSTB*/*Pvu*II | *AA* | 69.59±1.93 | 6.85±0.11 | 60.03±0.75 |
| | *AB* | $67.91^{A}$±1.12 | $6.70^{A}$±0.10 | $59.24^{A}$±0.72 |
| | *BB* | $68.034^{B}$±0.98 | $6.78^{B}$±0.13 | $58.24^{B}$±0.83 |

MCVC – meat content of valuable cuts; WL – weight of loin; MCC – meat content of carcass.
$^{AB}$Within columns means bearing different superscripts differ significantly at P≤0.01.

The effect of the *RYR1* genotype on productive traits within PLW and PL gilts analysed in this study occurred to be non-significant. In L990 the effect of *RYR1* genotype was identified on the weight of sirloin (P<0.05), weight of ham (P<0.01), meat content of valuable cuts (P<0.01) and of the whole carcass (P<0.01) – Table 3.

In the studies by Russo *et al*. [2002] only *locus* of the *AA* and *BB* genotypes of CSTB were detected. The numbers of animals with these genotypes were 28 and 192, respectively. The analysis for this *locus* indicated its significant association (P=0.0102)

**Table 3**. Least squares means and their standard errors (±) for carcass traits in L990 pigs as related to *RYR1 locus*

| *Locus* | Genotype | WH (kg) | WS (kg) | MCC (%) | MCVC (%) |
|---|---|---|---|---|---|
| *RYR1/HinP1I* | *CC* | $8.44^{A}$±0.1 | $0.33^{a}$±0.01 | $57.1^{A}$±0.4 | $64.9^{A}$±0.4 |
| | *CT* | $8.54^{A}$±0.1 | $0.33^{a}$±0.01 | $57.7^{A}$±0.3 | $65.4^{A}$±0.3 |
| | *TT* | $9.06^{B}$±0.1 | $0.36^{b}$±0.01 | $60.3^{B}$±0.8 | $68.0^{B}$±0.8 |

WH – weight of ham ; WS – weight of sirloin; MCC – meat content of carcass; MCVC – meat content of valuable cuts.
$^{AB}$Within columns means bearing different superscripts differ significantly: capital letters – P≤0.01; small letters – P≤0.05.

with average daily gain (ADG). The *BB* genotype showed a favourable effect on this trait, the value of which (least squares mean±SE) was 764.0±12.9 for the *AB* genotypes and 800.0±5.7 in case of *BB* genotypes, respectively [Russo *et al*. 2002].

The *CSTB locus* has been localized to the distal portion of Sscr13 q by linkage. Pennacchio *et al*. [1996] has located the human orthologous gene on Hsap 21q22.3, confirming conservation of syntheny between the region of Sscr 13 and Hsap21 regions [Goureau *et al*. 1996]. Several earlier studies have indicated the presence of a QTLs in this region for ADG and birth weight [Andersson *et al*. 1994, Yu *et al*. 1999, Bidanel *et al*. 2001]. These data may suggest that the chromosomal region in question can affect growth in pigs. Also, the data of our study might suggest association between *CSTB* and ADG for which the less frequent genotype has a less favourable effect on the trait. Russo *et al*. [2000] indicated a general trend in negative genetic correlations between cathepsin B activity (CBA) and backfat thickness (BFT) and between CBA and ADG. This makes their report interesting as BFT and ADG are traits associated with the *CSTB* and *CTSB* polymorphisms.

Presented results indicate that analysing the mutations of the porcine *CSTB* gene may be useful in selection aimed at improving the value of traits characterizing carcass meat deposition The relationship between genotype and trait value in our study in one breed may also be true for other pig breeds. Continuation of similar studies in the future, on the other pig groups could shed more light on the association of *CSTB* gene with valuable pig traits.

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