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Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Preliminary observations on the effect of calpastatin gene (*CAST*) polymorphism on carcass traits in pigs*

Jolanta Kurył[1], Wojciech Kapelański[2], Mariusz Pierzchała[1], Salomea Grajewska[2], Maria Bocian[2]

[1] Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec, 05-552 Wólka Kosowska, Poland

[2] Department of Pig Breeding, University of Technology and Agriculture, Mazowiecka 28, 85-084 Bydgoszcz, Poland

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The aim of the study was to characterize the polymorphism of calpastatin (*CAST*) gene identified with three restriction enzymes (*Hinf*I, *Msp*I, *Rsa*I) in several pig breeds and lines bred in Poland and to evaluate the relation between the *CAST* genotypes and carcass traits. The analyses covered a total of 294 fatteners of Polish Landrace (PL), Pietrain (P), Zlotnicka Spotted (ZS), Torhyb [P × (Polish Large White × PL)] and Stamboek (Dutch Large White × Dutch Landrace). P pigs appeared to be monomorphic at each of *loci* considered, *i.e. CAST*/*Hinf*I, *CAST*/*Msp*I and *CAST/Rsa*I, whereas all three genotypes at these *loci* were observed only in ZS and Stamboek pigs. An association between genotypes at *locus CAST* and carcass traits was analysed on 39 Stamboek castrated males free of *RYR1*^{T} gene. Genotypes *DD* at *locus CAST*/*Msp*I and *EF* at *locus CAST*/*Rsa*I proved less fatty (thinnest backfat and lower weight of backfat with skin in loin) than two remaining genotypes at each of these *loci*. Moreover, genotypes *DD* at *locus CAST*/*Msp*I and *EE* at *locus CAST*/*Rsa*I were the most advantageous for eye-muscle area when compared to the remaining genotypes at these *loci*. It is concluded that *CAST* gene may be considered as a candidate gene for pig carcass quality. However, further studies are needed on more numerous animal material covering breeds (e.g. Large White) with all possible genotypes at *loci* considered in this report.

KEY WORDS: calpastatin gene / candidate gene / carcass quality / gene polymorphism / pig

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Most traits of economic importance in farm animals are polygenic (quantitative traits *loci* – QTLs) and influenced by non-genetic environmental factors. Initial molecular genetic studies detecting QTLs for carcass and meat quality have used either whole genomic scan or candidate gene approach. Candidate genes are selected on the basis of known relationship between physiology and production trait and are tested as putative QTLs. The following genes were considered as candidate genes for carcass quality in pigs: *GH* [Nielsen *et al*. 1995, Knorr *et al*. 1997, Křenková *et al*. 1998, Pierzchała *et al*. 1999, Kurył *et al*. 2003], *GHRH* [Pierzchała *et al*. 2002], *IGF1* [Pierzchała *et al*. 2002], *IGF2* [Jeon *et al*. 1999, Nezer *et al*. 1999], *PIT1* (Yu *et al*. 1995, Stančeková *et al*. 1999, Kurył and Pierzchała 2001, Brunsch *et al*. 2002], *LEP* [Jiang and Gibson 1999, Kennes *et al*. 2001, Kulig *et al*. 2001, Kurył *et al*. 2003], *MYOG* [Te Pas *et al*. 1999, Cieślak *et al*. 2000 and 2002, Kurył *et al*. 2002], *MYF3* [Cieślak *et al*. 2000 and 2002, Kurył *et al*. 2002] and *MYF5* [Te Pas *et al*. 1999, Cieślak *et al*. 2002, Kurył *et al*. 2002]. Conflicting data presented by various authors indicate that the relationship between variants of some genes and carcass traits has not been fully resolved or that identified gene mutations were not causal mutations for the differences observed in the traits level.

The rate and extent of skeletal muscle growth ultimately depends mainly on three factors: rate of muscle protein synthesis, rate of muscle protein degradation, and the number and size of skeletal muscle cells. Recent studies have shown that calpain activity is required for myoblast fusion [Balcerzak 1995, Barnoy 1997] and cell proliferation in addition to cell growth [Mellegren 1997]. The calpain system may also affect the number of skeletal muscle cells (fibres) in domestic animals by altering rate of myoblast proliferation and modulating myoblast fusion. A number of studies have shown that the calpain system is also important in normal skeletal muscle growth. Increased rate of skeletal muscle growth can result from a decreased rate of muscle protein degradation, and this is associated with a decrease in activity of the calpain system, due principally to a large increase in calpastatin activity [Goll *et al*. 1998 – a review]. These observations suggest that genes coding for calpains and calpastatin may be considered as candidate genes for lean content of carcass in pigs.

Ernst *et al*. [1998] amplified the porcine *CAST* gene fragment encompassing part of exon 6, intron 6 and part of exon 7, and identified RFLP polymorhism with restriction endonucleases *Hinf*I, *Msp*I, *Rsa*I. They mapped *CAST* gene to porcine chromosome 2.

The purpose of the present investigation was to analyse the polymorphism of the *CAST* gene in several pig breeds and lines raised in Poland and to evaluate its effect on carcass traits.

Material and methods

The study covered 294 fatteners of the following breeds and lines: Polish Landrace (PL I and PL II), Pietrain (P), Zlotnicka Spotted (ZS), Torhyb I and Torhyb II [P × (Polish Large White × PL)] and Stamboek (Dutch Large White × Dutch Landrace),

The animals were fattened up to approx. 105 kg live body weight according to feeding standards, at the AGRO-WRONIE farm, Wronie near Toruń, Poland.

From all animals blood samples were drawn into test-tubes containing K_2EDTA, and kept at -20°C or, for longer storage, at -70°C. Slaughtered were 39 Stamboek castrated males free of *RYR1*^{T} gene. After 24 h cooling their left carcass-sides were dissected according to Walstra and Merkus [1996]. Apart from dissection, the meat content of carcasses was estimated with the ultrasound procedure and presented as UFOM (%). Carcass traits examined are listed in Table 2.

Genomic DNA was isolated according to Kanai *et al*. [1994]. Genetic variants of *CAST* gene were identified according to Ernst *et al.* [1998] using three restriction endonucleases (*Hinf*I, *Msp*I and *Rsa*I).

Data analysis was performed using GLM procedure of the statistical package SAS 8.2 [2001] according to the following model:

$$Y_{ijk} = \mu + g_i + \beta(m_{ij} - \bar{m}) + e_{ijk}$$

where:

Y_{ijk} – *ijk*-th observation of a given trait;

μ – mean value of a trait;

g_i – effect of *i*-th *CAST* genotype on a trait (*i = 1 ... 9*);

β – regression coefficient of covariate trait *m* (cold right carcass-side);

e_{ijk} – random error.

Results and discussion

Polymorphism in *CAST* gene

The *CAST* genotypes identified by PCR/RFLP method were characterized by the occurrence of restriction DNA fragments consistent with the results of Ernst *et al* [1998].

The frequency of genotypes at *loci CAST*/*Hinf*I, *CAST*/*Msp*I and *CAST/Rsa*I in the tested pig breeds and lines is shown in Table 1. A total of 294 pigs were tested. P pigs appeared to be monomorphic at these *loci*. All three possible genotypes at each of *loci* considered were present only in ZS and Stamboek pigs. A similar distribution of *CAST* genotypes among several pig breeds was reported by Ernst *et al*. [1998]. In their study P pigs were found monomorphic at all three restriction sites, and distribution of genotypes among Landrace pigs appeared similar to these given in Table 1 of the present report. Moreover, all three genotypes at each restriction site of the *CAST* gene (*CAST*/*Hinf*I, *CAST*/*Msp*I and *CAST/Rsa*I) were observed among Yorkshire and Large White pigs. This was confirmed by the present study regarding Stamboek fatteners

Table 1. Frequency of genotypes at the *loci CAST/HinfI*, *CAST/MspI* and *CAST/RsaI* in several pig breeds and lines bred in Poland

Breed/line	n	Frequency of genotypes at *locus*								
		CAST/HinfI			*CAST/MspI*			*CAST/RsaI*		
		AA	*AB*	*BB*	*CC*	*CD*	*DD*	*EE*	*EF*	*FF*
PL I	30	0	4	26	0	8	22	30	0	0
	%	0.0	13.3	86.7	0.0	26.7	73.3	100.0	0.0	0.0
PL II	20	0	3	17	0	5	15	17	3	0
	%	0.0	15.0	85.0	0.0	25.0	75.0	85.0	15.0	0.0
P	30	0	0	30	0	0	30	30	0	0
	%	0.0	0.0	100.0	0.0	0.0	100.0	100.0	0.0	0.0
ZS	30	2	10	18	3	9	18	18	9	3
	%	6.7	33.3	60.0	10.0	30.0	60.0	60.0	30.0	10.0
Torhyb I	106	0	51	55	0	53	53	56	50	0
	%	0.0	48.1	51.9	0.0	50.0	50.0	52.8	47.2	0.0
Torhyb II	39	0	16	23	0	20	19	21	18	0
	%	0.0	41.0	59.0	0.0	51.3	48.7	53.8	46.2	0.0
Stamboek	39	7	25	7	9	24	6	10	18	11
	%	17.9	64.2	17.9	23.1	61.5	15.4	25.6	46.2	28.2
Total	294	9	109	176	12	119	163	182	98	14
	%	3.1	37.1	59.8	4.1	40.5	55.4	61.9	33.3	4.8

PL – Polish Landrace, P – Pietrain; ZS – Złotnicka Spotted, Torhyb – P × (Polish Large White × PL), Stamboek – Dutch Large White × Dutch Landrace.
n – number of animals.

(Large White × Landrace) crosses.

A relationship between the *CAST* genotypes and carcass traits

Associations between *CAST* gene variants and carcass traits were analysed in 39 Stamboek fatteners. Earlier studies by Leach *et al.* [1996] and Cieślak *et al.* [2002] showed that sex and *RYR1* genotype both affected significantly the quality of carcass. All Stamboek castrated males (n = 39) analysed in this study appeared to be of *CC* genotype at the *RYR1 locus* as shown earlier by Kurył *et al*. [2002]. It allowed to exclude an effect of sex and genotype at the *RYR1 locus* on carcass traits analysed in the present study. Moreover, all possible genotypes at the *CAST loci* considered here were found in Stamboek pigs. Thus, the line has been chosen as an experimental material for evaluation of an effect of *CAST* genotype on carcass quality traits.

Table 2. Significance of effect of genotype at the *locus CAST* on carcass traits in Stamboek fatteners (Dutch Large White × Dutch Landrace)

Trait	Effect of the genotype at the *locus CAST*		
	CAST/*Hin*fI	*CAST*/*Msp*I	*CAST*/*Rsa*I
Backfat thickness (cm)			
over the shoulder	ns	ns	**
at the last rib	ns	ns	**
at *sacrum* point I	ns	ns	ns
at *sacrum* point II	ns	ns	ns
at *sacrum* point III	ns	*	ns
mean from the 5 measurements	ns	ns	*
Ham			
meat weight (kg)	ns	ns	ns
meat content (%)	ns	ns	ns
subcutaneous fat with skin (%)	ns	ns	ns
Loin			
meat weight (kg)	ns	ns	ns
meat content (%)	ns	ns	ns
subcutaneous fat with skin (%)	ns	ns	*
eye area (cm^2)	ns	**	*
Meat content of carcass (%)	ns	ns	ns
Meat content of carcass UFOM (%)	ns	ns	ns
Weight of meat in carcass-side (kg)	ns	ns	*

*P≤0.05; **P≤0.01; ns – not significant.

No associations between *CAST*/*Hin*fI genotype and carcass quality traits were found in this study, whereas *CAST*/*Msp*I and *CAST*/*Rsa*I affected some of the meat and fat deposition traits in Stamboek pigs (Tab. 2). Genotype *DD* at *locus CAST*/*Msp*I and *EF* at the *locus CAST*/*Rsa*I proved less fatty, *i.e*. thinner backfat and lower weight of backfat with skin in loin) than two remaining genotypes at each of these *loci*. Moreover, genotype *DD* at the *locus CAST*/*Msp*I and *EE* at the *locus CAST*/*Rsa*I were the most advantageous for eye-muscle area when compared to the remaining genotypes at these *loci* (Tab. 3). A similar effect of *CAST*/*Rsa*I genotype was observed on weight of meat in carcass side. It should be mentioned that all P pigs (high meat deposition in the carcass) were of *DD* and *EE* genotype at the *loci CAST*/*Msp*I and *CAST*/*Rsa*I, respectively (Tab. 1).

Due to role of calpain-calpastatin system in protein turnover in muscle tissue the significant difference in fat deposition traits observed between *CAST* genotypes is rather astonishing. It may be assumed that this was a secondary effect resulting from difference in the muscle maturation rate between animals of various *CAST* genotypes. In a maturing muscle the proliferative potential of satellite cells decreases and the further growth of body mass results from the connective tissue growth, including adipose tissue [Grant and Gerrard 1998].

Kretchmar *et al*. [1994] examined calpain and calpastatin activities in lean and

Table 3. A relation between *CAST* genotypes and carcass traits in Stamboek fatteners (Dutch Large White × Dutch Landrace)

Trait	Effect of the genotype at the *locus CAST*					
	CAST/*Msp*I			*CAST*/*Rsa*I		
	CC (n=9)	*CD* (n=24)	*DD* (n=6)	*EE* (n=10)	*EF* (n=18)	*FF* (n=11)
Backfat thickness (cm)						
over the shoulder						
LSM	3.42	3.23	3.38	**3.58**^{A}	**3.09**^{B}	**3.39**^{a,B}
SE	0.18	0.10	0.20	**0.15**	**0.11**	**0.14**
at the last rib						
LSM	1.97	1.81	2.04	**2.13**^{A}	**1.71**^{B}	**1.94**^{a,B}
SE	0.14	0.10	0.17	**0.12**	**0.09**	**0.12**
at *sacrum* point III						
LSM	**2.56**^{a}	**2.24**^{ab}	**2.05**^{b}	2.27	2.16	2.30
SE	**0.16**	**0.09**	**0.19**	0.15	0.11	0.14
mean from the 5 measurements						
LSM	2.41	2.19	2.27	**2.39**^{a}	**2.11**^{b}	**2.36**^{ab}
SE	0.12	0.07	0.14	**0.10**	**0.08**	**0.10**
Loin						
subcutaneous fat with skin (%)						
LSM	31.5	29.5	32.5	**32.3**^{a}	**29.0**^{b}	**31.1**^{ab}
SE	1.4	0.8	1.6	**1.2**	**0.9**	**1.2**
eye area (cm^2)						
LSM	**40.4**^{A}	**42.3**^{a,B}	**44.8**^{B}	**44.5**^{a}	**42.1**^{ab}	**40.6**^{b}
SE	**1.5**	**0.87**	**1.7**	**1.3**	**1.0**	**1.3**
Carcass-side meat weight (kg)						
LSM	19.4	19.8	19.6	**19.6**^{ab}	**20.0**^{a}	**19.0**^{b}
SE	0.13	0.12	0.15	**0.14**	**0.12**	**0.13**

^{A,B}Within rows and *loci* means bearing different superscripts differ significantly: small letters – $P \leq 0.05$; capitals – $P \leq 0.01$.

obese lines of pigs at the age of 2.5 and 7 months and showed that the latter decreased with age in both lines, but it was significantly higher in obese pigs at both ages.

It is known [Goll *et al*. 1998] that muscle calpastatin activity is highly related to the rate of muscle protein turnover. The relation between *CAST* genotypes and two meat deposition traits (eye-muscle area and meat weight in carcass-side) may result from a different enzymatic activity of various calapastatin isoenzymes involved in protein turnover. On the basis of porcine *CAST* cDNA nucleotide sequence and sequence of primers reported by Ernst *et al*. [1998] one may conclude that gene polymorphism desribed by them is most probably localized in intron 6. Thus, it is rather difficult to conclude about a direct effect of the *CAST* genotypes typed in this study on carcass traits involved. It may be rather suggested a linkage to another mutation being a causal mutation in the coding or regulatory regions of the gene.

Basing on the results presented here one may conclude that porcine *CAST* gene might be considered as a candidate gene for carcass traits of pigs. However, further investigations on this topic are necessary, and Large White breed due to its known rather low frequency of stress sensitivity gene (*RYR1*^{T}) seems to be the most proper experimental material. Moreover, the distribution of *CAST* genotypes within the Stamboek line (Dutch Large White × Dutch Landrace cross) as well as that showed by Ernst *et al*. (1998) for Large White pigs both suggest that the latter may be highly useful as an experimental material for similar studies.

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Jolanta Kurył, Wojciech Kapelański,
Mariusz Pierzchała, Salomea Grajewska, Maria Bocian

Wpływ polimorfizmu genu kalpastatyny (*CAST*) na cechy tuszy świń – badania wstępne

Streszczenie

Celem badań była charakterystyka polimorfizmu genu kalpastatyny (*CAST*) identyfikowanego enzymami restrykcyjnymi *Hinf*I, *Msp*I i *Rsa*I oraz ocena wpływu tego polimorfizmu na cechy jakości tuszy. Polimorfizm genu przebadano u 294 tuczników następujących ras i linii: pbz, pietrain, złotnicka pstra, Torhyb [pietrain × (wbp × pbz)] i Stamboek (holenderska wielka biała × holenderska zwisłoucha) – odpowiednio 50, 30, 30, 145 i 39 zwierząt. Świnie pietrain okazały się monomorficzne względem wszystkich trzech miejsc restrykcyjnych identyfikowanych w genie *CAST*, natomiast wszystkie możliwe genotypy *CAST* zidentyfikowano u świń złotnickich pstrych i w linii Stamboek. Istotność zależności między genotypem *CAST* a cechami jakości tuszy przeanalizowano na 39 tuczonych kastrowanych knurkach Stamboek wolnych od genu podatności na stress (genotyp *CC*). Pozwoliło to pominąć w analizie statystycznej wpływ płci i genotypu *RYR1* na cechy tuszy oraz porównać między sobą wszystkie genotypy *CAST*, które występowały obok siebie właśnie w tej linii. Stwierdzono, że najcieńszą słoninę w niektórych punktach pomiaru oraz najmniejszą zawartość słoniny ze skórą w polędwicy wykazały zwierzęta o genotypie *DD* w *locus CAST*/*Msp*I i *EF* w *locus CAST*/*Rsa*I. Z kolei genotypy *DD* w *locus CAST*/*Msp*I i *FF* w *locus CAST*/*Rsa*I były najbardziej korzystne pod względem powierzchni oka polędwicy. Podobną zależność odnotowano dla masy mięsa w półtuszy. Autorzy wnioskują, że gen *CAST* można by uważać za gen kandydujący o istotnym oddziaływaniu na cechy tuszy. Konieczna jest jednak kontynuacja badań na liczniejszym materiale. Najbardziej dogodną rasą do tych badań byłaby wielka biała. Wynika to z danych pochodzących z piśmiennictwa poświęconego tej rasie, jak również z przedstawionego w niniejszej pracy odpowiednio licznego udziału genotypów *CAST*