ASSOCIATION OF NEW CALPASTATIN ALLELES WITH MEAT QUALITY TRAITS OF COMMERCIAL PIGS[1]

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Background

The calpain system, a Ca^{2+}-activated protease family, and indirectly calpastatin (*CAST*), a calpain inhibitor, plays an important role in postmortem tenderization of skeletal muscle due to its involvement in the degradation of important myofibrillar and structural proteins (Koohmaraie, 1992; Sensky et al., 2001; Ciobanu et al., 2002). Two new *CAST* polymorphisms, *CAST Arg249Lys* and *Ser638Arg*, are located in or close to subdomain C of their respective domains. This subdomain potentiates the inhibitory activity of CAST. In order to investigate whether the alleles/haplotypes of the *CAST* gene are associated with meat quality traits such as ultimate pH, Minolta L*, cooking loss, tenderness and juiciness, a large number of pigs was harvested at commercial processing plants and screened for these two markers.

Objectives

Estimate the association of the *CAST* alleles with meat quality traits using a large number of commercial pigs harvested at commercial processing plants.

Materials and methods

Tissue Sampling and DNA Isolation. Tail and muscle samples and phenotypic data were collected from four different groups of commercial pigs. Genomic DNA was isolated using the DNeasy tissue kit (Qiagen, Valencia, CA).

Phenotypic measurements. Four separate experiments were conducted on four different commercial pig crosses: Group A: N=205; Group B: N=161; Group C: N=691; Group D: N=1829. All test pigs were harvested at two different commercial processing plants. *Longissimus dorsi* and *Gluteus medius* (ham) muscle pH was measured 24 hours post-mortem directly on the carcass using a Start Probe pH meter. Additional meat quality measurements such as Minolta L*, a*, a, b* (both muscles), instrumental tenderness (shear force, star-probe), cooking loss, sensory measurements (juiciness and tenderness), and pH (Longissimus muscle) were evaluated after 14 days of ageing. In experiment A, shear force was measured with an Instron 1222 Universal Testing Machine (Instron, Canton, MS) fitted with a Warner-Bratzler shear attachment. In experiment B, two chops were evaluated for instrumental texture using a circular, five-pointed star-probe attached to a texture analyzer (Texture Technologies, Scarsdale, NY). The star probe attachment was used to determine the amount of force needed to puncture and compress the chop 80% of the sample height. Subjective juiciness and tenderness were scored from 1 to 10 with higher values indicating more tender/juicier meat. For cooking loss measurements, a chop was cut from the muscle after ageing, weighed, cooked to 70C^{o} in a Faberware open hearth electric grill, refrigerated until cool, and re-weighed. During the time of cooking, temperature in each chop was monitored in its center with thermocouples. Cooking loss was calculated from weights taken before and after cooking and was expressed as a percentage.

Genotyping and PCR-RFLP Analysis. The region flanking each missense polymoprhism (*Arg249Lys* and *Ser638Arg*) was amplified by PCR and then digested with *Hpy*188I (*Arg249Lys*) and *Pvu*II (*Ser638Arg*).

Statistical Analysis. Haplotypes defined by the *CAST Arg249Lys* and *CAST Ser638Arg* polymorphisms were assigned based on inferences derived from the genotypes of homozygous individuals: haplotype 1, *249Lys – 638Arg*; haplotype 2, *249Arg – 638Arg* and haplotype 3, *249Arg – 638Ser.* All the predicted haplotypes were

present in all four groups of commercial pigs analyzed. The substitution effects of the haplotypes were estimated using a mixed model (SAS procedure MIXED, SAS Institute Inc., Cary, NC) and were considered as deviations from the effect of haplotype 3, which was arbitrarily set to zero. Analysis of haplotype associations was based on a model with genetic background of the four commercial pig groups included as a fixed effect.

Results and discussion

CAST Arg249Lys and *Ser638Arg*, are located in or close to subdomain C of their respective domains. This subdomain potentiates the inhibitory activity of CAST (Takano and Maki, 1999). Both substitutions (*Arg249Lys* and *Ser638Arg*) are outside the most conserved area of subdomain C: KPxxEDDxIDALSxDF (reviewed by Takano and Maki, 1999), but *Ser638Arg* is separated by just one amino acid from this sequence. An additional substitution, the *Ser66Asn,* is situated in Domain L. The function of this domain is not clear even though its sequence is well conserved among mammalian species. Recently, a role of the L domain was identified in regulation of L-type Ca^{2+} channels in guinea pig cardiac myocytes, suggesting the involvement of CAST in restoring Ca^{2+} channel activity, which facilitates Ca^{2+} influx and subsequently activation of calpain (Hao et al., 2000).

Detailed results representing Group A test pigs (N=205) are provided in Table 1. For all traits measured in this group of pigs, haplotype 1 was the favorable one and it was associated with higher pH, better tenderness and juiciness, lower shear force and cooking loss (Table 1). For instance, the effect of haplotype 1 was significantly different from the effect of haplotype 2 for shear force ($P < 0.04$), and for percentage of cooking loss ($P < 0.001$). The effect of haplotype 1 was also significantly different from that of haplotype 3 for muscle pH ($P < 0.03$), cooking loss ($P < 0.002$), juiciness ($P < 0.008$), and tenderness ($P < 0.09$) in this test group.

The results were not so clear for Group B test pigs (N=161), although some associations were detected. For example, for meat firmness, haplotype 2 had a higher substitution effect than that of haplotype 1 ($P<0.05$), and haplotype 3 ($P<0.01$). A suggestive association was also discovered for star probe with haplotype 1 being associated with a better meat quality than haplotype 2 (as for Group A). No significant differences were found for meat pH at 24hr and cooking loss.

Data from Group C pigs (N=691) revealed that haplotype 1 and haplotype 3 were again associated with higher quality pork. These haplotypes were significantly different from haplotype 2 for loin pH at 24 hr ($P<0.01$), and ham Minolta L* ($P<0.05$).

Commercial pigs from Group D (N=1829) were characterized by a significant association of Loin Minolta a* with haplotype 1 ($P < 0.0001$) and haplotype 3 ($P < 0.05$). Haplotype 1 and 3 were associated with lower value for this component of color and were significantly different from haplotype 2.

Conclusions

The results indicate that the porcine calpastatin variants have significant effects on meat ultimate pH, tenderness, cooking loss, and other economically important pork quality traits. It remains to be further demonstrated if the observed effects were caused by these polymorphisms alone or by their linkage disequilibrium with the causative mutations. Genetic background may be responsible for the differences in effects observed in the different commercial populations analyzed. The polymorphisms, or DNA markers, can potentially be incorporated into breeding programs to improve overall meat quality and hence the economic value for the pork supply chain and quality products for consumers.

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Table 1. Haplotype substitution effects for *Longissimus dorsi* meat quality traits

Trait	Mean (s.e.)	s.d.	Estimate[A]			Contrast P values		
			Haplotype			Haplotypes		
			1	2	3	1 vs 2	1 vs 3	2 vs 3
pH	5.73 (0.02)	0.18	0.055	0.026	0	0.34	0.03	0.37
Shear Force	1.92 (0.04)	0.55	-0.074	0.097	0	0.04	0.28	0.22
Cooking loss %	24.23 (0.36)	5.01	-1.916	0.727	0	0.001	0.002	0.30
Tenderness score[B]	7.33 (0.10)	1.32	0.276	0.199	0	0.69	0.09	0.29
Juiciness score[B]	8.02 (0.08)	1.14	0.381	0.253	0	0.45	0.008	0.12

[A] haplotype 1: *249Lys – 638Arg* (frequency = 0.48); haplotype 2: *249Arg – 638Arg* (0.21); haplotype 3: *249Arg – 638Ser* (0.31);

[B] Tenderness and Juiciness scores are assigned using a subjective method;

Number of observations = 158 – 205 depending on the trait measured