

Short communication

Polymorphisms of the *CAST* gene in the Meishan and five other pig populations in China

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Abstract

The aim of the study was to characterize the polymorphism of the Calpastatin (*CAST*) gene identified with three restriction enzymes (*TaqI*, *HinfI*, *MspI*) in Meishan and five other pig populations, and to provide information on their potential in marker-assisted selection and conservation. Meishan pigs appeared to be monomorphic at loci *CAST/HinfI* and *CAST/MspI*. A high frequency of the favoured genotype, FF, in terms of meat quality was detected in Meishan pigs, a breed well known for high quality meat. However, the frequency of the genotype, FF, was very low in Sutai pigs, a breed developed from a Duroc (50%) × Meishan (50%) cross. This is probably partially due to the fact that genetic improvement in this breed was achieved through the use of traditional quantitative genetics. It is suggested that traditional selection techniques combined with the use of the polymorphisms discovered have an important potential to improve overall meat quality.

Keywords: Polymorphism, *CAST*, Meishan, Sutai

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Calpastatin (*CAST*), which is an endogenous inhibitor (Ca²⁺ dependent cysteine proteinase), plays a central role in the regulation of calpain activity in cells (Murachi *et al.*, 1981; Murachi, 1983; Forsberg *et al.*, 1989) and is considered to be one of the major modulators of the calpains. There is evidence that the activity of *CAST* from the skeletal muscle is highly related to the rate of meat tenderization and protein turnover after slaughter (Koochmarai *et al.*, 1991; Goll *et al.*, 1998; Sensky *et al.*, 1998; Parr *et al.*, 1999; Hao *et al.*, 2000). Therefore, the *CAST* gene represents an excellent candidate gene for studying variation in pork quality (Ernst *et al.*, 1998).

Ernst *et al.* (1998) amplified the porcine *CAST* gene fragment encompassing intron 6 and identified RFLP polymorphism with restriction endonuclease, *HinfI* and *MspI*. Before these markers could be used efficiently in breeding and management decisions, studies with different populations would be necessary. The Meishan breed is famous for its prolificacy and good meat quality. However, only a small population is presently being conserved and the status of favoured genes is not known. Sutai is a breed developed by the Breeding Centre of Taihu Pig, China from the cross between the Duroc (50%) and the Meishan (50%). It is important to know whether the traditional selection method can concentrate favoured genes as successfully as marker-assisted selection (MAS). Li *et al.* (2006) have reported the present genetic status by analyzing the polymorphism of H-FABP, MC4R and ADD1 genes in Meishan and four other pig populations (Sutai, Landrace × Sutai, Yorkshire × Sutai, Duroc × Landrace × Yorkshire). The aim of this study was to detect new polymorphisms of the *CAST* gene and compare all the tested loci in the six populations to provide further information for MAS and their conservation.

The studies included 235 unrelated fatteners of the following breeds/populations: 28 Meishan (M), 27 Sutai (S), 15 Yorkshire × Sutai (YS), 15 Landrace × Sutai (LS), 25 Duroc × Landrace × Yorkshire (DLY) and 125 PIC hybrid pigs (L402×Cambrough).

Genomic DNA was isolated according to Kanai *et al.* (1994). One pair of primer was designed in the porcine *CAST* cDNA sequence (M20160) to find new SNPs by sequencing. The primer sequences were: forward primer, 5'-GTGATGACAAAAAAGTTGACG-3'; and reverse primer,

5'-TCATCCTTATCCAAGA GATGTC-3'. The *CAST* genotype was identified with the restriction enzymes *HinfI* and *MspI*, according to the method used by Ernst *et al.* (1998). Data were analyzed using the *FREQ* procedures of SAS 6.12 (SAS, 1998).

In this study, we amplified the porcine *CAST* gene fragment encompassing part of exon 24, intron 24 and part of exon 25. After sequencing the 1991 bp DNA fragment, a C/T transition was determined in intron 24. A novel RFLP polymorphism with restriction endonuclease *TaqI* was developed on this position. After digestion with the endonuclease, three genotypes were identified. The AA genotype has three fragments, 963 bp, 806 bp and 222 bp; the AB genotype has four fragments, 1028 bp, 963 bp, 806 bp and 222 bp; and the BB genotype has two fragments, 1028 bp and 963 bp. Experiments are in progress to evaluate their influence on meat traits in pigs.

The frequencies of genotypes at loci *CAST/TaqI*, *CAST/HinfI* and *CAST/MspI* in the tested populations (235 pigs) are shown in Table 1. The present study shows that Meishan pigs appeared to be monomorphic at loci *CAST/HinfI* and *CAST/MspI*. Only the DD genotype at the locus *CAST/HinfI* and the FF genotype at the locus *CAST/MspI* were found in Meishan. A similar distribution of *CAST* genotypes in the Meishan breed was reported by Ernst *et al.* (1998). Both the earlier studies by Li *et al.* (2006) and current results indicated that the Meishan breed is a conserved population with a special genetic background, and should be conserved as a valuable genetic resource.

Table 1 Genotype frequencies in Meishan (M) and five other populations

Loci	Genotype	Breeds (number of individuals)					
		M (28)	S (27)	LS (15)	YS (15)	DLY (25)	PIC (125)
<i>TaqI</i>	AA	-	0.11	0.40	0.13	0.12	0.12
	AB	0.43	0.15	0.60	0.60	0.36	0.16
	BB	0.57	0.74	-	0.27	0.52	0.72
	CC	-	0.26	-	-	0.64	0.08
<i>HinfI</i>	CD	-	0.56	0.33	0.47	0.36	0.32
	DD	1.00	0.18	0.67	0.53	-	0.60
	EE	-	0.26	-	-	0.24	0.24
<i>MspI</i>	EF	-	0.56	0.33	0.47	0.32	0.28
	FF	1.00	0.18	0.67	0.53	0.44	0.48

S - Suta; LS - Landrace × Suta; YS - Yorkshire × Suta; DLY - Duroc × Landrace × Yorkshire
PIC - PIC hybrid pigs (L402×Cambrough)

Animals with genotype FF at locus *CAST/MspI* appeared to be less fatty (thinnest backfat and lower weight of backfat with skin in login) compared with the other two genotypes. Moreover, genotype FF at locus of *CAST/MspI* was the most advantageous for eye-muscle area when compared to the remaining genotypes (Kuryl *et al.*, 2003, Krzeczio *et al.*, 2005). The high frequency of the favoured genotype, FF, in Meishan pigs could be responsible for their good meat quality. The Suta breed was developed from the cross between the Duroc (50%) and Meishan (50%) breeds. Presently, Suta is used mainly as a maternal line to cross with Landrace or Yorkshire boars to produce commercial pigs that compete mainly with Duroc × Landrace × Yorkshire in the pork market. However, data from the current study showed that the Suta has the lowest frequency of the beneficial FF genotype among the six pig populations. This is probably partially due to selection for trait improvement using traditional quantitative genetics, which resulted in selection into one direction. The pig industry should launch a new breeding project to increase the frequency of beneficial genotypes. Moreover, the current study stated that the beneficial FF genotype was present in all the breeds and populations tested, which may provide significant improvements for the pig industry as it can be used in MAS to produce naturally tender and juicy pork.

The present finding demonstrated that all possible genotypes at the loci of the *CAST* gene considered here were found in the Suta and the PIC hybrid pigs. Thus, the two breeds could be chosen as experimental

material for evaluation of the effect of *CAST* genotype on meat and carcass quality traits. In addition, further research will be performed on the relationship of the polymorphism of *CAST* gene with meat and carcass quality traits in the six populations.

Highly significant differences in allele frequencies of the loci *CAST/TaqI* and *CAST/MspI* were observed between the Meishan and the other five populations (Table 2). The loci *CAST/HinfI* and *CAST/MspI* are in linkage disequilibrium in the M, S, LS, YS breeds with a Meishan background while the extra haplotype occurs in the European DLY and PIC breeds. It appears that some breed differences exist for allele frequencies at all tested loci. The compared result of the *HinfI* alleles among the six populations was almost the same as the *MspI* alleles which are not shown in the Table 2.

Table 2 Significant differences of allele frequencies between breeds

Breeds	Breeds					
	M	S	LS	YS	DLY	PIC
	<i>TaqI</i> allele frequencies					
M	■	*	**	*	ns	*
S	**	■	**	*	ns	ns
LS	**	**	■	*	**	**
YS	**	**	**	■	ns	*
DLY	**	ns	ns	ns	■	ns
PIC	**	ns	ns	ns	ns	■
	<i>MspI</i> allele frequencies					

M – Meishan; S - Sutai; LS - Landrace × Sutai; YS - Yorkshire × Sutai; DLY - Duroc × Landrace × Yorkshire; PIC - PIC hybrid pigs (L402×Cambrough)
 ns – P > 0.05; * P ≤ 0.05; ** P ≥ 0.01

Highly significant differences in alleles frequencies at all the loci considered, existed between the six populations. The traditional selection method in the development of the Meishan and Sutai is based on quantitative genetics and not on molecular genetics. The results obtained indicate that selection based on quantitative genetics cannot concentrate effectively on favoured genes. Therefore, this combined with the use of the polymorphisms discovered would increase the accuracy of selection and improve rates of genetic progress for meat traits.

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