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A Novel PCR-RFLP (Xba I) Polymorphism Analysis of the Bovine Calpastatin (*CAST*) Gene in Chinese Luxi Cattle

Dongying Yang
College of Life Science, Dezhou University,
Shandong Key Laborotary in Universities of Biotechnology and Utilization of Biological Resources,
Shandong Key Laborotary of Functional Macromolecules and Biophisics,
253023 Dezhou, Shandong, China

Abstract: Calpastatin (CAST) is a specific inhibitor of the ubiquitous calcium-dependent proteases-μ-calpain and m-calpain, found in mammalian tissues. In the coding region of the bovine *CAST* gene (CAST) the new nucleotide sequence polymorphism was found being a substitution G¬C at position 962 nt (consensus sequence-GenBank AF281256). Consensus of bovine CAST sequence with that of human revealed that G¬C substitution was located at position 1460 nt of exon 12. Computer analysis of the mutation showed the Ser¬Thr substitution for CAST protein. The frequency of alleles C and G was 0.41 and 0.59, respectively. The message from these results is that *CAST* gene may probably be considered as a candidate marker for beef quality.

Key words: Genetic polymorphisms, *CAST* gene, haplotype, RFLP, cattle

INTRODUCTION

As an endogenous calpains' inhibitor, Calpastatin (CAST) plays a central role in regulation of calpains activity in the cell (Morris *et al*., 2006) and is considered to be one of the important modulators of protein turnover. So, CAST may affect proteolysis of myofibrils due to regulation of activity of calpains and is responsible for initiation of post mortem degradation of myofibrillar protein (Barendse *et al*., 2007). The bovine *CAST* gene (CAST) has been mapped to BTA7 (Schenkel *et al*., 2006) with relative position of 117.8 cM (Calvo *et al*., 2014). Chung and Davis (2012) found DNA polymorphism in the intron 6 using PCR-RFLP technique and XmnI as the restriction enzyme. DNA marker technology represents a promising means for determining the genetic identity and has been applied in the association study between genetic variations within functional genes and economic traits in cattle breeding. To identify a minimal set of candidate genes with sufficient power for the concerned traits was a challenging approach in the program (Heaton *et al*., 2002). Growth rate and meat quality are the most economically important traits in the beef cattle industry and therefore that of controlling myogenesis and skeletal muscle development may be candidate gene in Marker-Assisted Selection (MAS) System for its growth potential characteristics.

Detection of additional polymorphism is necessary to find the relationship between CAST polymorphic variants and beef quality indicators. This report presents the results of searching for genetic variation in CAST DNA and the PCR-RFLP polymorphism of the Chinese Luxi bovine gene.

MATERIALS AND METHODS

Samples: The 166 Luxi individuals blood samples were collected in Shandong Province and genomic DNA was isolated from leukocytes as recommended by Sambrook and Russell (2001).

Primer design and Polymerase Chain Reaction (PCR) amplification: For sequence variability detection, primer pairs were designed to amplify the bovine *CAST* gene based on the assembly *Bos taurus*_UMD 3.1 sequences retrieved from GenBank using Primer V 5.0 Software (Supplementary Online Resource 1). The forward primer was 5'-GTGCCCAGGACCCCATTGAT-3' and the reverse prime was 5'-AGCAGGCTTCTTGTCTTTGT-3'. PCR was performed in a 25 μL reaction mixture, consisting of 50 ng genomic DNA, 0.5 μM of each primer, 1×Buffer (including 1.5 mM $MgCl_2$), 200 μM dNTPs (dATP, dTTP, dCTP and dGTP) and 0.625 units of Taq DNA polymerase (Takara). The thermal cycling program was 5 min at 95°C, 35 cycles of 94°C for 30 sec, annealing at 56°C for 30 sec, 72°C for 30~90 sec with a final extension at 72°C for 10 min and subsequently cooling to 4°C.

Genomic DNA isolation and SNP genotyping: Genomic DNA from the blood samples was isolated according to

Sambrock and Russell (2001) and its concentration was measured by spectrophotometry. DNA samples were stored at -80°C. Using the DNA as PCR templates, amplifications were carried out and PCR products were sequenced on an automated sequencer (ABI PRISM 3730 DNA analyzer) for DNA polymorphisms detecting.

Identification of sequence variations in the bovine *CAST* gene was carried out *in silico* using BLASTN and sequencing maps results. In this study, amplification created Restriction Site-restriction Fragment Length Polymorphism (PCR-RFLP) was established to genotype the detected SNPs and primers used for introducing specific restriction sites. Briefly, 10 μL of PCR products obtained for the detected the polymorphisms in bovine *CAST* gene was digested overnight at 37°C with 10 U XbaI (TaKaRa, Dalian, China) restriction enzyme in a final volume of 20 μL containing 10×enzyme reaction buffer. The PCR-RFLP products were resolved on 2.0~3.0% agarose gels stained with ethidium bromide.

Statistical analysis: Genotypic and allelic frequencies were calculated for each polymorphism. Hardy-Weinberg equilibrium was estimated through χ^2-test performed by PopGene Software V 3.2. Heterozygosity (H) and Polymorphism Information Content (PIC) were determined by the HET Program (Ott, 1997).

RESULTS AND DISCUSSION

Identifying SNPs and mapping on the cattle QTLdb: In this study, sequencing and PCR-RFLP Methods were applied to identify the polymorphisms in the *CAST* gene. The sequencing results revealed a novel SNPs C-960-G. Moreover, the bovine *CAST* gene and the SNPs was mapped on the cattle QTLdb.

Genetic diversity and linkage disequilibrium analysis: The genotypic, allelic frequencies and genetic parameters were estimated in the cattle breeds and considerable differences were discerned in Table 1. The χ^2-test revealed that the genotypes distribution were not at the Hardy-Weinberg equilibrium, The values of genetic Heterozygosity (H) was 0.483 and the discrepancy between observed and expected heterozygosity might be due to different continued selection pressure in the studied populations. According to the classification of Polymorphism Information Content (PIC) whereas the SNPs was low Polymorphism (PIC<0.5) in Luxi cattle. Alleles of the CAST single nucleotide polymorphism identified in this study were segregating in the beef population with an overall greater frequency for allele C than G. Allele C was associated with more tender LM across days of postmortem aging (Chung and Davis, 2012) but tended to reduce LM area and lean yield and increase fat yield (Gandolfi *et al*., 2012). Importantly for the beef industry, the difference in the tenderness at 2 and 7 days of postmortem aging is expected to substantially reduce the percentage of steaks rated unacceptably tough by consumers based on an assumed threshold level. Thus, it is expected that single and double C cattle would produce more tender steaks and that this would result in significantly fewer unsatisfactory eating experiences.

Table 1: Distribution of gene and genotype frequencies in the *CAST* gene in cattle

AA	AB	BB	N	PAA	PBB	Jk	Hk	Ne	PIC	χ^2
56	25	74	166	0.409	0.591	0.517	0.483	1.936	0.367	4.18

$\chi^2_{\alpha = 0.05}(2) = 5.991$; $\chi^2_{\alpha = 0.01}(2) = 9.210$; value with ** and ** differ significantly at $p<0.01$ and $p<0.05$, respectively

In cattle breeding industry, the links between genetic variability and phenotypic traits are well established and considerable research effort is therefore underway to unravel the genetic control of these traits and finally to identify DNA markers for MAS Programs (Hayes *et al*., 2007; Goddard and Hayes, 2009; Hayes and Goddard, 2010). The genes located in the QTL region influencing the growth and production traits could be used as candidates in selection project (Nishimura *et al*., 2012).

CONCLUSION

Three novel SNPs and the corresponding haplotypes were identified in five Chinese cattle populations. Substantial differences in haplotype frequencies were observed among different cattle breeds. The subsequent correlation analyses of single SNP and haplotype combinations with growth traits revealed that all three SNPs were significant associated with body sizes traits in early stages which provided evidence in supporting the three polymorphisms as markers for improvement of growth traits for early breeding selection project. Furthermore, developing the high performing culture breeds is a complex program and further investigation and validation will be necessary to confirm many pivotal details before the SNPs can contribute to the MAS program in future.

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