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Allelic Variation in the Intron 6 of Yak and Cattle *CAPNS1* Gene

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Abstract: CAPNS1 also known as CAPN4, encodes the small subunit of CAPN1 and CAPN2 which is required to maintain stability and activity of both calpains. But to date polymorphism of yak CAPNS1 has not been reported. In this study, variation in the exon 6-intron 6 region of CAPNS1 was investigated in 1059 yaks and Chinese cattle by Polymerase Chain Reaction-Single Strand Conrmational Polymorphism (PCR-SSCP). Five PCR-SSCP patterns representing five allelic variations and containing four Single Nucleotide Polymorphisms (SNPs) in intron 6 were observed. Allele B was the most common allele with a frequency of 48.12% in yak and 93.29% in Chinese cattle whereas allele A and C were only in yak as well as allele D and E were rare (0.42 and 0.16%, respectively) and only in Chinese cattle. These results indicate that yak and cattle CAPNS1 is polymorphic and suggest further analysis is required to see if the variation detected affects their meat quality.

Key words: *CAPNS1* gene, variation, PCR-SSCP, yak, Chinese cattle

INTRODUCTION

The calpains as Ca^{2+}-dependent intracellular cysteine proteases with broad functions in cell spreading, migration, proliferation and apoptosis (Gandolfi *et al*., 2011), affect the post mortem muscle proteolysis and the meat tenderization processes (Melody *et al*., 2004) as well as the muscle development and muscle fiber determination (Goll *et al*., 1992; Sultan *et al*., 2000).

CAPNS1 also known as CAPN4 is small 28 kDa regulatory subunit of ubiquitous μ and m-calpains (Goll *et al*., 2003), plays a pivotal role in proteolytic processes of muscle, degrading quite a large number of myofibrillar proteins but not actin, myosin and actinin under maintaining stability and activity of μ and m-calpains (Zhang *et al*., 2010). Sequences of the cDNA for the *CAPNS1* gene have been reported for several species including rat, rabbit, cattle, pig (Elce *et al*., 1997; Emori *et al*., 1986; McCelland *et al*., 1989; Sakihama *et al*., 1985) and the structure of the human gene has been described (Ohno *et al*., 1986). The bovine *CAPNS1* gene contains 11 exons and has been located on chromosome 18 (Juszczuk-Kubiak *et al*., 2010). Variation in CAPNS1 has been revealed for cattle (Juszczuk-Kubiak *et al*., 2010; Zhang *et al*., 1996) and associations between variation in CAPNS1 and beef tenderness in Chinese cattle has been analyzed (Li, 2006). However, polymorphism of *CAPNS1* gene in yak (*Bos grunniens*) has not been described earlier.

In this study, researchers used Polymerase Chain Reaction-Single Stranded Conrmational Polymorphism (PCR-SSCP) to analyze genetic variation in key region (exon 6-intron 6) of CAPNS1 in yak and Chinese cattle (*Bos taurus*) which two well widespread and characterized species in Northwest China and researchers report five novel sequences in this region of gene.

MATERIALS AND METHODS

Animals and DNA extraction: Total 1059 yak and cattle blood samples were collected and investigated from three yak populations and one cross cattle population distributing in different area of Northwest China. There were 977 yaks including Gannan yak (n = 722), Tianzhu white yak (n = 200) from Gansu Province and Datong yak (n = 55) from Qinghai Province and 82 Chinese cattle (Qinchuan cattle and their crosses) from Gansu Province. The Qinchuan cross cattle were crossed with European breeds including Simmental, South Devon and Limousin. The blood samples were stored below -70°C and genomic DNA was extracted using phenol-chlororm procedure (Kramvis *et al*., 1996) for Polymerase Chain Reaction (PCR) amplification.

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Primer and PCR amplification: Two PCR primers, up (5'accttcgacctgtatccca3') and down (5'-aaagctacaccctgactgc-3') were designed based on published bovine CAPNS1 sequence (GenBank accession No.: EF139087) and synthesized (Sangon, Shanghai, China) and to amplify a fragment (approximately 227 bp) including entire exon 6 and partial intron 6 of CAPNS1 in yak and Chinese cattle.

Amplification was perrmed in a 20 μL reaction containing 50-100 ng genomic DNA, 0.25 μM of each primer, 150 μM each dNTP (Eppendorf, Hamburg, Germany), 2.5 mM of Mg^{2+}, 0.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany) and 1 ×reaction buffer supplied. The thermal profile consisted of 2 min at 94°C, followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec and the final extension step was at 72°C for 5 min. Amplification was carried out in an ABI-9902 thermocycler (Applied Biosystems, USA).

The PCR amplicons were checked on 1% agarose gel in 1×TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA) containing 200 ng mL^{-1} of ethidium bromide at 150 V for about 25 min. The gel was visualized under UV light of Quantum ST4 (Vilber, France).

SSCP analysis and DNA sequencing: Variation in the amplicons of CAPNS1 was screened for using SSCP. An aliquot of 3 μL of each amplicon was mixed with 7 μL of loading dye (98% rmamide, 10 mM EDTA, 0.025% bromophenol blue and 0.025% xylene-cyanol). After denaturation at 95°C for 5 min, samples were cooled rapidly on wet ice and then loaded onto 16×18 cm, 14% acrylamide:bisacrylamide (39:1) gels. Electrophoresis was carried out using Protean II xi cells (Bio-Rad) at 250 V for 18 h in 0.5×TBE buffer with circulating water coolant at controlled temperature in 10°C. The gels were silver-stained by the method of Byun *et al.* (2009).

Amplicons that were identified as homozygous by PCR-SSCP were directly sequenced in both directions at BGI in Beijing, China. For those alleles that were only found in heterozygous, they were cloned and sequenced using a rapid approach described by Yang *et al.* (2011). The allelic sequences alignment was carried out using DNAMAN (Version 5.2.10, Lynnon, BioSoft, Canada). The blast algorithm was used to search the NCBI GenBank (http://www.ncbi.nlm.nih.gov/) databases for homologous sequences.

RESULTS AND DISCUSSION

The primers designed for amplifying the exon 6-intron 6 region of CAPNS1 worked well on all of yak and Chinese cattle genomic DNA under the conditions established.

Amplicons of the expected size were obtained for PCR primers. These amplicons exhibited polymorphism upon SSCP analysis with five unique SSCP patterns representing five alleles named as A-E observed in all of yak and Chinese cattle samples (Fig. 1). Either one or a combination of different two SSCP patterns observed for individual yak and cattle which was consistent with them being either homozygous or heterozygous genotype in CAPNS1, respectively.

Sequencing of the PCR amplicons representative of the unique SSCP patterns revealed five sequences for exon 6-intron 6 region of CAPNS1 in yak and cattle. All of sequences showed high homology to the published ovine and caprine CAPNS1 sequences (GenBank accession Nos. AF309634 and AY935995, respectively) with blast search in GenBank. This suggests that these sequences represent variant rms of *CAPNS1* gene in yak and cattle are not derived from other loci including other *CAPN* genes.

The frequencies of the five alleles in 1059 yak and Chinese cattle investigated were shown in Table 1. Allele B was the most common allele with a frequency of 48.12%

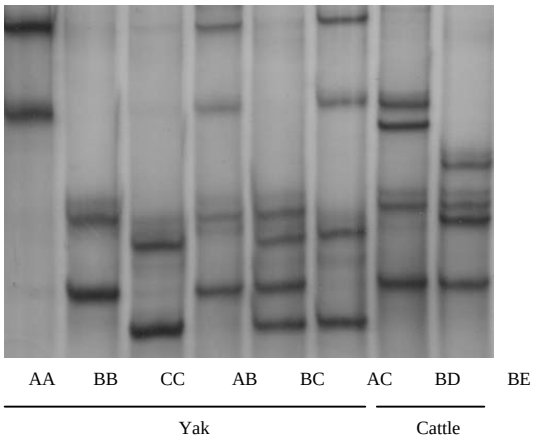

Fig. 1: PCR-single-strand conformational polymorphism of the *CAPNS1* gene in yak and Chinese cattle. Yak and cattle representative of the five unique PCR-SSCP patterns corresponding to five allelic variants A-E are shown

Table 1: Allelic frequency of bovine CAPNS1

		Allelic frequency (%)				
Breeds or population	n	A	B	C	D	E
Gannan yak	722	0.2458	0.4785	0.2756	-	-
Tianzhu White yak	200	0.2800	0.5000	0.2200	-	-
Datong yak	55	0.2273	0.4818	0.2909	-	-
Chinese cattle[a]	82	-	0.9329	-	0.0488	0.0183
Total	1059	0.2273	0.5198	0.2471	0.0042	0.0016

[a]Chinese cattle were predominantly a cross-bred population based on the Qinchuan breed

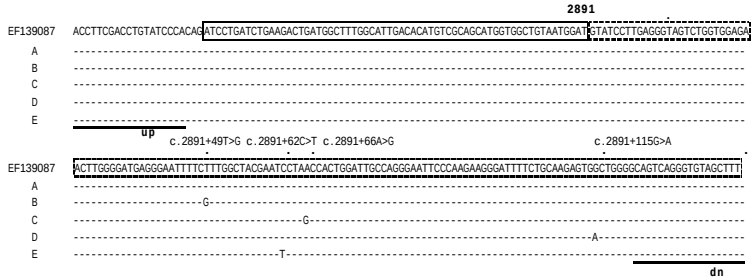

Fig. 2: Alignment of the yak and Chinese cattle CAPNS1 alleles together with the published bovine sequence (Genbank No. EF139087). Bars represent nucleotides identical to the top sequence. Exon 6 is shown in a solid box and intron 6 is in a dashed box. The PCR primer binding regions are indicated by horizontal bars together with the primer names. The SNPs positions refer to the bovine CAPNS1 sequence EF139087 in GenBank

in all of yak and 93.29% in Chinese cattle, followed by allele C and A that were only presented in yak. Alleles D and E were rare (the frequencies of 0.42 and 0.16%, respectively) and were detected only in Chinese cattle. Investigated Chinese cattle were the Qinchuan cattle and their cross cattle sourced from commercial farms in northwest China. The Qinchuan breed is thought to have been derived from a mixed *Bos taurus* and *Bos indicus* background based on mitochondrial gene analysis (Barendse *et al*., 2008; Cai *et al*., 2007). The occurrence of alleles D and E of CAPNS1 in only these Chinese cattle at lower frequencies suggests that these alleles may be derived from *Bos indicus* originally. But this deduction need to be confirmed in future as polymorphic variation has not been reported in zebus CAPNS1 to this day. The most common occurrence of allele B was also notable in Chinese cattle suggesting strong selection pressure has been applied to maintain this allele in the population.

The nucleotide sequence of allele A in yak and Chinese cattle was same as bovine CAPNS1 (GenBank No. EF139087) when the sequences were aligned (Fig. 2). Four nucleotide substitutions including c.2891+49T>G, c.2891+66A>G, c.2891+115G>A and c.2891+62C>T were observed at intron 6 in alleles B-E of yak and cattle CAPNS1, respectively. It has been speculated that complex traits result more often from noncoding regulatory variants than from coding sequence variants (Mackay, 2001; King and Wilson, 1975; Korstanje and Paigen, 2002). An increasing amount of evidence indicates that genomic variants in both coding and non-coding sequences can have unexpected deleterious effects on the splicing of the gene transcript (Pagani and Baralle, 2004). Some variations in noncoding sequence of *CAPN* genes that have been detected affected the meat quality such as SNPs in intron 14 and intron 17 of bovine CAPN1 were associated with the lean share in valuable cuts and red/yellow intensities of the meat, respectively (Juszczuk-Kubiak *et al*., 2004; Pinto *et al*., 2011). The CAPNS1 that encoded a common 28 kDa regulatory subunit as being essential for proteolytic activity of CAPN1 can be regarded as a candidate gene for meat tenderness (Goll *et al*., 2003). Novel variations in noncoding region including intron 6 (Zhang *et al*., 1996) and 3'UTR (Juszczuk-Kubiak *et al*., 2010) of bovine CAPNS1 have been detected but the same as SNPs in intron 6 of CAPNS1 in yak and Chinese cattle, the function of these SNPs were unclear up to now for lack of phenotypic data of relevance to meat quality.

CONCLUSION

This is the first report of sequence variation in intron 6 of CAPNS1 in yak and Chinese cattle. Further research will be needed to ascertain whether SNPs in the intron of CAPNS1 may be used in future as genetics marker to identify genomic regions with QTLs at yak and cattle for meat quality traits.

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