

Single Nucleotide Polymorphisms of the Calpain 3 (*CAPN3*) Gene in Yak

^{1,2}Li Zhang, ¹Yuzhu Luo, ¹Jiang Hu, ¹Xiu Liu, ¹Shaobin Li and ¹Wei Yan

¹Gansu Key Laboratory of Herbivorous Animal Biotechnology,
Faculty of Animal Science and Technology, Gansu Agricultural University,
730070 Lanzhou, Gansu, P.R. China

²Qinghai Vocational and Technical College of Animal Husbandry and Veterinary Science,
812100 Huangyuan, Qinghai, P.R. China

Abstract: The Calpain3 (*CAPN3*) gene, located within the confidence interval of the Quantitative Trait Loci (QTL) affecting meat tenderness, being regarded as a candidate gene for meat tenderness in cattle. In this study, SNPs in exon 23-intron 23 region of the yak *CAPN3* was investigated from 1032 yak with Polymerase Chain Reaction-Single Strand Conformational Polymorphism (PCR-SSCP). Three Single-Nucleotide Polymorphisms (SNPs), resulting in three novel alleles were detected in this region, respectively. These variations included one synonymous in exon 23 and two SNPs in intron 23. Allele C was the most common allele (63.18%) in all of yak. These SNPs are the first report in yak *CAPN3* and also suggest further analysis is required to explore the relations between variation sites and meat tenderness.

Key words: Yak (*Bos grunniens*), Calpain 3 (*CAPN3*) gene, Single Nucleotide Polymorphism (SNP), PCR-SSCP, China

INTRODUCTION

Calpain 3 (*CAPN3*), also named p94 is a member of the so-called tissue-specific calpains, being predominantly expressed in skeletal muscle (Sorimachi *et al.*, 1989; Kinbara *et al.*, 1998). It has been suggested that *CAPN3* may play a role in sarcomere remodelling and in mitochondrial protein turnover (Cohen *et al.*, 2006). Mutations in *CAPN3* gene are responsible for Limb-Girdle Muscular Dystrophy type 2A (LGMD2A) in humans (Zatz *et al.*, 2003; Richard *et al.*, 1995; Sorimachi *et al.*, 1995). Variations in *CAPN3* have been revealed in cattle (Barendse *et al.*, 2008) and associations between variation in *CAPN3* and beef tenderness in Chinese cattle has been analyzed (Cafe *et al.*, 2010). However, polymorphism of *CAPN3* gene in yak (*Bos grunniens*) has not been described previously. The purpose of this study was therefore, to identify the yak *CAPN3* using Polymerase Chain Reaction-Single Stranded Conformational Polymorphism (PCR-SSCP) and to characterize any variation found in the gene.

MATERIALS AND METHODS

Animals and DNA extraction: Total 1032 yak blood samples were collected and investigated from 3 yak

populations including Gannan yak (n = 722), Tianzhu white yak (n = 200) from Gansu Province and Datong yak (n = 110) from Qinghai Province. Genomic DNA was extracted using phenol-chloroform procedure (Fabio *et al.*, 2011) for Polymerase Chain Reaction (PCR) amplification.

Primer and PCR amplification: Two PCR primers, up (5'-TCTATGCATTCTTCCGAGCA-3') and down (5'-CAGCTTGTTTCAGGCATACA-3') were designed based on published bovine *CAPN3* sequence (GenBank Accession No.: NC_007308) and synthesized (Sangon, Shanghai, China) and to amplify a fragment (approximately 449 bp) in the key region (exon 23-intron 23).

Amplification was performed 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 Mg²⁺, 0.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany) and 1× the 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 (Quantum ST4, Vilber, France) in 1×TBE buffer (89 mM

Corresponding Author: Jiang Hu, Gansu Key Laboratory of Herbivorous Animal Biotechnology,
Faculty of Animal Science and Technology, Gansu Agricultural University, 730070 Lanzhou,
Gansu, P.R. China

Tris, 89 mM boric acid, 2 mM Na₂EDTA) containing 0.1 µL of 4S Green Nucleic Acid/mL at 250 V for about 10 min.

SSCP analysis and DNA sequencing: A 3 µL aliquot of each amplicon was mixed with 7 µL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue and 0.025% xylene-cyanol) and after denaturation at 95°C for 5 min, samples were cooled rapidly on wet ice and then loaded onto 12% acrylamide:bisacrylamide (39:1) gels. Electrophoresis was performed using Protean II xi cells (Bio-Rad) at 350V for 20 h in 0.5×TBE buffer with circulating water coolant at controlled temperature in 18°C and followed by silver-staining (Byun *et al.*, 2009).

Amplicons that were identified as homozygous by PCR-SSCP were directly sequenced in both directions at Bgitech Solutions (BGI), Beijing, China. Those sequences only found in a heterozygous yak were sequenced using a rapid sequencing approach that has been described previously (Hu *et al.*, 2010). The allelic sequences alignment was carried out using DNAMAN (Version 5.2.10, Lynnon, BioSoft, Canada).

RESULTS AND DISCUSSION

Three unique SSCP banding patterns (Fig. 1) representing three unique alleles (designated A-C) were detected in exon 23-intron 23 of CAPN3 in yak with PCR-SSCP. Either one or a combination of different two SSCP patterns observed for individual yak which was consistent with them being either homozygous genotype (named AA, BB and CC) or heterozygous genotype (named AB and BC) in three yak populations, respectively at CAPN3. All of sequences showed high homology to the published bovine CAPN3 sequences (GenBank Accession No. NC_007308) with blast search in GenBank. This suggests that these sequences represent allelic variants of CAPN3 gene in yak are not derived from other loci including other CAPN genes.

The frequency of novel alleles that detected in CAPN3 was different between yak populations (Table 1). The allele C was the most common allele with the frequency from 57.48-79.09% in all yak populations investigated and those results suggested strong selection pressure has been applied to maintain this allele in the population.

Three nucleotide substitutions, including c.56233T>A, c.56242+250T>G and c. C>T 56242+265 were detected in allele A-C of yak CAPN3, respectively (Fig. 2). The nucleotide substitutions at exon 23 were synonymous and the amino acid sequence did not vary in this region. In human genes, roughly 99.8% of DNA

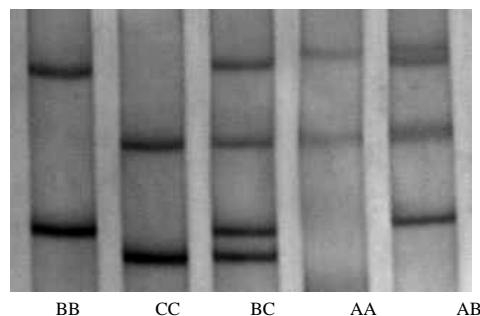


Fig. 1: PCR-single-strand conformational polymorphism of the yak CAPN3 gene. Representative genotypes for the three unique SSCP patterns corresponding to three allelic sequences, A-C are shown

Table 1: Allele frequency of CAPN3 gene in 3 yak breeds

Population	Sample size	Allelic frequency		
		A	B	C
Gannan yak	722	0.1676	0.2576	0.5748
Tianzhu white yak	200	0.0300	0.2200	0.7500
Datong yak	110	0.0818	0.1273	0.7909
Total	1032	0.1318	0.2364	0.6318

sequence variations do not alter the primary sequences of proteins (Venter *et al.*, 2001) and synonymous SNPs show a higher frequency than both missense SNPs and the SNPs in the non-coding regions (Venter *et al.*, 2001; Zwick *et al.*, 2000). Synonymous mutations appear to affect protein folding, possibly by causing translational pausing while rare tRNAs are recruited, this in turn affects the activity of the protein (Parmley and Hurst, 2007). Considering CAPN3 is regarded as a candidate gene for meat tenderness and variations at exon 6 of CAPN3 affected the meat tenderness in Zebu cattle (Barendse *et al.*, 2008), this mutation in exon 23 of yak CAPN3 may be linked to sequence variation elsewhere in the gene which may impact on gene expression.

Some research have speculated that complex traits result more often from non-coding regulatory variants than from coding sequence variants (Mackay, 2001; King and Wilson, 1975; Korstanje and Paigen, 2002). An increasing amount of evidence also 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 non-coding sequence of *Calpain* genes that have been detected affected the meat quality such as SNPs in intron 14 and 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). Variations in non-coding region including intron 10-11

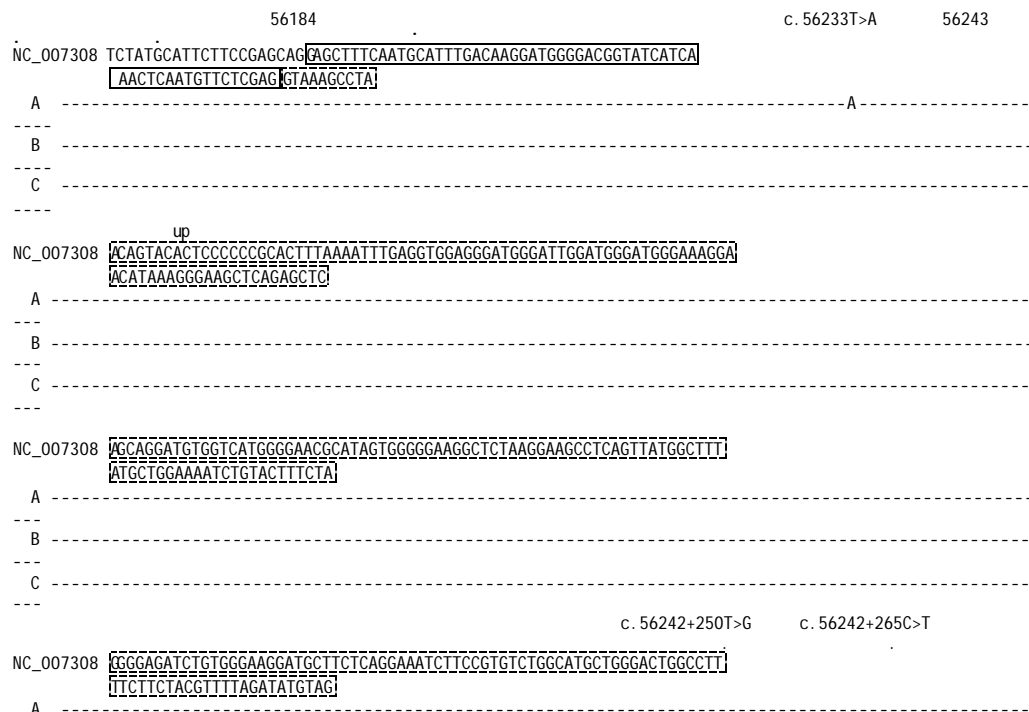


Fig. 2: Alignment of the yak CAPN3 alleles together with the published bovine sequence (Genbank No.:NC_007308); Bars represent nucleotides identical to the top sequence; Exon 23 is shown in a solid box and intron 23 is in a dashed box; The PCR primer binding regions are indicated by horizontal bars together with the primer names; The SNPs positions refer to the bovine CAPN3 sequence NC_007308 in GenBank

and 3'-UTR (Barendse *et al.*, 2008) of bovine CAPN3 have been detected. The same as SNPs in exon 23, the function of these SNPs in intron 23 of CAPN3 in yak were unclear up to now for lack of phenotypic data of relevance to meat quality.

CONCLUSION

In this study, three SNPs in exon 23-intron 23 of yak CAPN3, corresponding to three novel alleles was identified and the allele C was the most common allele with the frequency from 57.48-79.09% in 3 yak populations. Further research will be carried out to investigate the effects of the novel mutations on function of CAPN3 and the meat quality traits of yak.

ACKNOWLEDGEMENTS

The financial support from the National Natural Science Foundation of China (31160451), the Gansu Agricultural Biotechnology Research and Application Development Program (GNSW-2010-02) and Gansu Science Fund for Distinguished Young Scholars (1210RJDA008) are gratefully acknowledged. The

researchers thank Jian Cao, Xinian Wang, Mingming Zhang, Mingna Li, Haiqing Chen and Ningning Shi for technical assistance.

REFERENCES

- Barendse, W., B.E. Harrison, R.J. Bunch and M.B. Thomas, 2008. Variation at the Calpain 3 gene is associated with meat tenderness in zebu and composite breeds of cattle. *BMC Genet.*, Vol. 9. 10.1186/1471-2156-9-41.
- Byun, S.O., Q. Fang, H. Zhou and J.G.H. Hickford, 2009. An effective method for silver-staining DNA in large numbers of polyacrylamide gels. *Anal. Biochem.*, 385: 174-175.
- Cafe, L.M., B.L. McIntyre, D.L. Robinson, G.H. Geesink and W. Barendse *et al.*, 2010. Production and processing studies on calpain-system gene markers for tenderness in Brahman cattle: 2. Objective meat quality. *J. Anim. Sci.*, 88: 3059-3069.
- Cohen, N., E. Kudryashova, I. Kramerova, L.V. Anderson and J.S. Beckmann *et al.*, 2006. Identification of putative in vivo substrates of calpain 3 by comparative proteomics of overexpressing transgenic and nontransgenic mice. *Proteomics*, 6: 6075-6084.

- Fabio, D.P., O. Francesco, T. Martina, C. Fabio and N. Valerio, 2011. Genomic DNA extraction from whole blood stored from 15-30 years at 20°C by rapid phenol-chloroform protocol: A useful tool for genetic epidemiology studies. *Mol. Cell Prob.*, 25: 44-48.
- Hu, J., H. Zhou, A. Smyth, Y. Luo and J.G. Hickford, 2010. Polymorphism of the bovine ADRB3 gene. *Mol. Biol. Rep.*, 37: 3389-3392.
- Juszczuk-Kubiak, E., T. Sakowski, K. Flisikowski, K. Wicinska, J. Oprzadek and S.J. Rosochacki, 2004. Bovine mu-calpain (CAPN1) gene: New SNP within intron 14. *J. Applied Genet.*, 45: 457-460.
- Kinbara, K., H. Sorimachi, S. Ishiura and K. Suzuki, 1998. Skeletal muscle-specific calpain, p49: Structure and physiological function. *Biochem. Pharmacol.*, 56: 415-420.
- King, M.C. and A.C. Wilson, 1975. Evolution at two levels in humans and chimpanzees. *Sci.*, 188: 107-116.
- Korstanje, R. and B. Paigen, 2002. From QTL to gene: The harvest begins. *Nat. Genet.*, 31: 235-236.
- Mackay, T.F., 2001. Quantitative trait loci in drosophila. *Nat. Rev. Genet.*, 2: 11-20.
- Pagani, F. and F.E. Baralle, 2004. Genomic variants in exons and introns: Identifying the splicing spoilers. *Nat. Rev. Genet.*, 5: 389-396.
- Parmley, J.L. and L.D. Hurst, 2007. How do synonymous mutations affect fitness? *BioEssays*, 29: 515-519.
- Pinto, L.F.B., J.B.S. Ferraz, V.B. Pedrosa, J.P. Eler and F.V. Meirelles *et al.*, 2011. Single nucleotide polymorphisms in CAPN and leptin genes associated with meat color and tenderness in Nellore cattle. *Genet. Mol. Res.*, 10: 2057-2064.
- Richard, I., O. Broux, V. Allamand, F. Fougereousse and N. Chiamkulchai *et al.*, 1995. Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A. *Cell*, 81: 27-40.
- Sorimachi, H., S. Imajoh-Ohmi, Y. Emori, H. Kawasaki, S. Ohno, Y. Minami and K. Suzuki, 1989. Molecular cloning of a novel mammalian calcium-dependent protease distinct from both m- and mu-types. Specific expression of the mRNA in skeletal muscle. *J. Biol. Chem.*, 264: 20106-20111.
- Sorimachi, H., K. Kinbara, S. Kimura, M. Takahashi and S. Ishiura *et al.*, 1995. Muscle-specific calpain, p94, responsible for limb girdle muscular dystrophy type 2A, associates with connectin through IS2, a p94-specific sequence. *J. Biol. Chem.*, 270: 31158-31162.
- Venter, I.C., M.D. Adams, E.W. Myers, P.W. Li and R.J. Mural *et al.*, 2001. The sequence of the human genome. *Science*, 291: 1304-1351.
- Zatz, M., F. de Paula, A. Starling and M. Vainzof, 2003. The 10 autosomal recessive limb-girdle muscular dystrophies. *Neuromusc. Disorders*, 13: 532-544.
- Zwack, M.E., D.J. Cutler and A. Chakravarti, 2000. Patterns of genetic variation in Mendelian and complex traits. *Ann. Rev. Genom. Human Genet.*, 1: 387-407.