



## VARIATION IN THE YAK CALPASTATIN GENE (CAST)

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**Abstract-** Calpastatin (CAST) is a specific inhibitor of calpain (calcium-dependent cysteine protease). This study investigated the potential for variation in yak (*Bos grunniens*) CAST. PCR-SSCP analysis of exon 6 of yak CAST revealed three unique patterns (named A-C). Sequencing of the amplicons revealed two nucleotide substitutions. One substitution (c.398G/C) would nominally change the amino acid sequence (p.S133T) of yak calpastatin. The variant sequence A which carried c.398C was the most common in the yaks tested (95.1%). This is the first report that found yak CAST is variable, and as in pigs, sheep and cattle, this variation may affect animal production traits.

**Key words-** CAST, genetic variation, Yak (*Bos grunniens*), PCR- single strand conformational polymorphism (PCR-SSCP).

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### Introduction

Calpastatin (CAST) is a specific inhibitor of calpain (the calcium-dependent cysteine protease). It affects meat tenderisation by binding with calpain, which in turn inactivates proteolysis. An increased level of calpastatin in lamb meat was found to be associated with decreased calpain activity, decreased proteolysis and increased toughness [1].

Calpastatin is composed of an L domain and four repetitive calpain inhibitory domains (domain 1-4) [2]. In cattle (*Bos taurus*), CAST is a multi-promoter gene, and encodes a series of calpastatin isoforms using different alternative splicing processes [3]. Variation in CAST has been revealed for cattle, sheep and pigs [4-6] and associations between variation in CAST and meat tenderness have been found in pig and cattle [7, 8] and with birth weight and growth until weaning in sheep [9]. Genetic testing based on detecting variation in CAST has been commercialized as a gene-marker for improving meat tenderness in cattle [2].

Although a cDNA sequence for yak CAST has been identified [10], variation in the yak gene has not been described previously. In this study, the potential for variation in exon 6 of yak CAST which encodes the L-domain of calpastatin was investigated.

### Materials and Methods

#### DNA sample collection

Blood samples from 216 yaks from nine different farms in three different regions of the Peoples Republic of China (Qinghai Province, Gansu-Tianzhu Region and Gansu-Tibet Region) were collected onto FTA cards (Whatman, Middlesex, UK). A rapid two-step DNA extraction method [11] was used to get purified genomic DNA from these samples.

#### PCR amplification and SSCP analysis

The yak CAST exon 6 was amplified using PCR primers (5'-gttatgaattgctttctactc-3' and 5'-atcagattgagagacttcac-3') as described previously [6]. Amplicons were subject to SSCP analysis using conditions described in Zhou *et al.* [6].

#### Sequencing and sequence analysis

PCR amplicons representative of different SSCP patterns from yaks that were homozygous for CAST were sequenced at the Lincoln University DNA Sequencing Facility.

Sequence alignments, translations and comparisons were carried out using DNAMAN (version 5.2.10, Canada).

## Results

Three unique SSCP banding patterns were detected for the amplicon from the yak *CAST* exon 6 region (Fig.1). Either one or two of these patterns were observed in individual yaks, which is consistent with them having homozygous or heterozygous genotypes, respectively. Sequencing confirmed that each pattern represented a novel *CAST* sequence variant. These sequences were named as A-C and deposited onto GenBank with accession nos. HM466969-HM466971, respectively.

Two nucleotide substitutions (c.398G/C and c.468C/T) were detected in the exon 6 of *CAST* (Table 1). The substitution c.398G/C would potentially change the amino acid sequence of yak *CAST* (p.S133T).

Allele frequencies of *CAST* were determined for 216 yaks from nine different farms in three different regions of the Peoples Republic of China (Qinghai Province, Gansu-Tianzhu Region and the Gansu-Tibet Province). Genotypes AA (93.5%), AB (2.8%), AC (0.4%), BB (1.4%) and CC (1.9%) were observed, with individual variant frequencies of 95.1% (A), 2.8% (B) and 2.1% (C) (Table 2).

Table 1- Nucleotide substitutions detected in exon 6 of yak *CAST*

Substitution <sup>1</sup>	Variant A	Variant B	Variant C
c.398C/G <sup>2</sup>	C	G	G
c.468C/T	C	T	C

<sup>1</sup>The positions of the nucleotide substitutions refer to sequence EU009141.

<sup>2</sup>This nucleotide substitution would result in an amino acid change of p.S133T

Table 2- Frequencies of the genotypes and variants of yak *CAST*

Genotype/Aallele	Frequency (%) (n=216)
AA	93.5
AB	2.8
AC	0.4
BB	1.4
BC	0
CC	1.9
A	95.1
B	2.8
C	2.1

## Discussion

Exon 6 encodes the L-domain of the calpastatin which is known to interact with the inactive form of calpain. It plays an important role in the calpain/calpastatin system [12]. Given the crucial role that exon 6 may play in the calpastatin/calpain system, it is therefore seems likely that the variation revealed here in exon 6 of yak *CAST* could have both structural and functional effects on the calpastatin system and potentially affect the tenderisation of yak meat.

In the 216 yaks investigated, variant A (frequency 95.1%; Table 2) encodes a putative threonine at residue position 133 as opposed to the serine encoded in alleles B and C. This nucleotide and amino acid variation has not been recorded to date in sheep or cattle (Fig.2).

Yaks are slow growing due to the extreme environment in which they are found [13]. Given the association found between variation in the *CAST* and birth weight and growth rate (to weaning) in

sheep [9], it is also possible that the low growth performance of yak could be partially explained by having unique variation in *CAST*. In cattle, Žgur *et al.* [14] have associated low growth rates with increased tenderness which is, in consistent with the more tender meat and low growth rate found in yak although as discussed by Žgur *et al.* [14] the influence of growth rate on meat tenderness seems to depend mainly on changes in muscle protein turnover. How this manifests in yak is also yet to be understood, but the variation reported here in *CAST* suggests are possible deserve for further investigation.

This is the first report of polymorphic variation in yak *CAST* and we accept that it is limited by the lack of phenotype data from yaks for meat tenderness or growth rate. Future work is now required to investigate more samples and for which these phenotypic data are available.

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