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Association of Polymorphisms in the Calpain I Gene with Meat Quality Traits in Yanbian Yellow Cattle of China*

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ABSTRACT : The calpain I (CAPN1) gene is an important marker for meat tenderness and marbling score in the bovine, but there were no studies to determine whether the CAPN1 gene had an association with other meat quality traits. In this study, we examined the relation between genetic polymorphisms of the CAPN1 gene and some meat quality traits in Yanbian Yellow Cattle of China. By PCR-SSCP and gene sequencing in 321 unrelated Yanbian yellow cattle, twenty seven single nucleotide polymorphisms (SNPs) were detected in CAPN1, two existed SNPs in exon 8 and exon 17 resulted in the change of AA at F311S and M599V, respectively, and the other polymorphisms were at intron 7, 8, 14, 16 and 17. There were different preponderant genotypes at the corresponding gene locus and all genotypes were not associated with tenderness but other meat traits. This is the first study of the relationship between CAPN1 and meat quality besides tenderness in Yanbian yellow cattle of China. (**Key Words :** Yanbian Yellow Cattle, CAPNI, Meat Quality, SNPs)

INTRODUCTION

Yanbian yellow cattle are one of the most famous native cattle breeds in the Northeast of China. The breed has a long history of evolution. For a long time, this cattle breed has been used as farm cattle and strong draught animals. In recent decades, the cattle have been found to have high quality meat. Thus, Yanbian cattle are a rich genetic resource. Ten years ago, the blood protein polymorphism technology was first used to study the origins and genetic variability of these cattle, which indicated that Yanbian cattle have a close relationship with Korean yellow cattle breeds and that they contain *Bos taurus* blood. As molecular biology technology developed, DNA polymorphism has been widely used in the study of animal breed resources.

The palatability of beef is the final criterion for evaluating its quality, and meat quality depends on several important characteristics, including appearance, color, taste, fat content, texture and tenderness. Tenderness is one of the important issues in beef cattle production affecting market prices because it has a major impact on consumer satisfaction. Tenderness is also a complex structural and functional process that depends on species, genetic background, metabolic status of the animal ante mortem, the protein complement of the muscle and environmental factors. Amongst these factors, the post-mortem proteolytic change in meat has received much attention in recent years (Koohmaraie et al., 1996a; Roberts et al., 1996; Gollasch and Nelson, 1997).

Two enzymes responsible for beef tenderness are the micromolar calcium-activated neutral protease μ-calpain (CAPN1), which is encoded by the CAPN1 gene, and its inhibitor, calpastatin (CAST), which is encoded by the CAST gene (Koohmaraie et al., 1996b). The calpain/calpastatin system is an endogenous, calcium-dependent proteinase system, theorized to mediate the proteolysis of the key myofibrillar proteins during post-mortem storage of carcass and cuts of meat at refrigerated temperatures (Koohmaraie et al., 1995). Some markers in the CAPN1 gene have been suggested to fill this role; the amino acid (AA) polymorphisms at position 316 (glycine/alanine) and 530 (valine/isoleucine) are the important markers associated with tenderness in many families or crossbred *Bos taurus* cattle (Page et al., 2002). However, in Brahman cattle markers 316 and 530 do not identify variation at CAPN1. The other synonymous

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changes (SNPs) in code sequence or introns also have significant effects on meat tenderness (Page et al., 2004; White et al., 2005; Morris et al., 2006; Rincon and Medrano, 2006). Besides affecting tenderness, polymorphism in the CAPN1 3'UTR (*c.2151*479C>T*) also showed significant association with marbling score (Cheong et al., 2008).

There are many SNPs identified in the CAPN1 gene in different *Bos indicus* or *Bos taurus*, but there is no publicly available evaluation of the association of these SNPs in Yanbian yellow cattle, which were a cross-bred of Korean cattle with local cattle in Yanbian Korean autonomous prefecture. The big body conformation, cold resistance and disease resistance make Yanbian yellow cattle an important breeding material in China. The objective of this study was to evaluate the association between polymorphism of the CAPN1 gene and some meat quality characteristics in Yanbian yellow cattle.

MATERIAL AND MOTHEDS

Animal

Three hundred and twenty one YanBian Yellow Cattle (steers) obtained from local farms in Yanji (Jilin Province, China) were used in this study from 2004 to 2008. After two years, animals were slaughtered every September 1 to 15 at a commercial facility in Hunchun (Jilin province, China). They were weaned at 3 months of age and fed until they were 6 months old with 30% concentrates and 70% roughage. After 6 months of age, they were fed with concentrates consisting of 15% crude protein (CP) and 71% total digestible nutrients (TDN) until 14 months; 13% CP and 72% TDN until 20 months; and 11% CP and 73% TDN until 24 months. The roughage was offered *ad libitum*, and steers had free access to fresh water during the whole period.

Live weights were measured at the time of slaughter. After a 24 h chill, the striploin from the right side of the carcass was frozen (-18°C) to be used for sensory evaluation. Each striploin from the left side of the carcass was stripped of fat, bone, and superficial muscle tissue, and the *longissimus dorsi* (LD) muscle was prepared for meat quality evaluation. Muscle pH was measured in the LD muscle at the interface between the 12th and 13th ribs on the 6th day postmortem using an Oakton Instruments Model pH 100 Series pH meter. Roasts were cooked in a convection oven at 75°C to an internal temperature of 72°C. Cooking loss was determined by the difference between the fresh roast weight at the time placed in the oven and its cooked weight 30 min after removal from the oven. Cooked roasts were covered and placed in a 1 to 4°C cooler for 48 h before Warner-Bratzler shear values were determined. Three cores were cut parallel to the muscle fibers of the LD muscle, and the average of the shear values was recorded.

Meat colors (brightness, redness, yellowness, chroma, and hue angle) were measured by Brontes Colorimeter (Ocean Optic, USA) and double beam laboratory spectrophotometer (SPM, Shanghai China) at 0, 1, 3, 5, and 7 days postmortem. Meat tenderness was measured as Warner-Bratzler shear force (WBSF), tenderness score, juiciness, and flavor intensity. Warner-Bratzler shear force data were collected on LD samples from steers on d14 postmortem. Wheeler et al. (2005) and Riley et al. (2003) describe the method for obtaining tenderness scores, juiciness, and flavor. In brief, frozen samples (2.54 cm thick) were thawed between 4 and 5°C over 18 to 24 h. Samples were trimmed to 200-250 g of LD and placed in a water bath at 100°C until the centre of the sample reached 82°C. Samples were left to cool to 7°C then tested using a MIRINZ Tenderometer machine (AgResearch, Hamilton, New Zealand). The panel members evaluated the samples for tenderness, juiciness, and beef flavor on scales of 1 through 8 (1 = extremely tough, extremely dry, extremely bland; 8 = extremely tender, extremely juicy, extremely intense).

Fat content was measured by Soxtec extraction (Soxtec System HT6, Perstorp Analytical/Tecator Inc., Herndon, VA) with ethanol and dichloromethane as solvents. For the analysis of fatty acids, lipid extraction was carried out according to a modification of the procedure of Lepage et al. (1986). Methyl esters were prepared from extracted fat by base-catalyzed transmethylation, according to the method of Chouinard et al. (1997). Composition analyses of the fatty acids were carried out with a gas chromatograph (HP 5890A Series II, Hewlett Packard, Palo Alto, CA) equipped with a 100-m CP-Sil 88 capillary column (i.d., 0.25 mm; film thickness, 0.20 mm; Chrompack, Middelburg, The Netherlands) and a flame ionization detector. At the time of the sample injection, the column temperature was 80°C for 1 min and then was increased at 2°C/min to 215°C and maintained for 30 min. Inlet and detector temperatures were 220 and 230°C, respectively. The split ratio was 100:1. The flow rate for hydrogen carrier gas was 1 ml/min. Each fatty acid peak was identified and quantified using pure methyl ester standards (Nu Chek Prep., Elysian, MN).

The samples were freeze dried (model, company, comnfry), then the AA composition was detected by HPLC (HP1100 LC, USA) and automatic biochemical analyzer (L-8900, HITACHI, Japan).

Genomic DNA extraction and primer design

Genomic DNA was prepared from blood samples of Yanbian Yellow cattle using a standard phenol: chloroform extraction method and dissolved in TE solution. The DNA samples were stored at -20°C and/or at 4°C. Five pairs of primers for PCR-SSCP analysis were designed using the

Oligo 6.0 program to amplify 300-400 bp fragments of the CAPN1 gene according the cattle genomic sequence in GenBank (Accession number: AF252504S1 and AF252504S2). The sequences of primers are shown in Table 1.

Genotyping and sequencing

Primers were selected to amplify the regions from exon 7 to exon 19, including all introns and exons in between, of the *CAPNI* gene and screened for polymorphisms (Table 1). The PCR was performed in a 25 μl reaction mixture containing 10 pmol of forward primer and the same amount of reverse primer, 200 μ*M* dNTP (deoxyribonucleotide triphosphate), and 10×reaction buffer, which contained 2.0 m*M* $MgCl_2$, 1.5 unit of Taq-DNA polymerase, and 50 ng of genomic DNA as template. For all the reactions, PCR conditions were 95°C for 4 min, followed by 35 cycles of 95°C for 35 s, annealing for 45 s (Table 1), and 72°C for 45 s. After 35 cycles, reactions were finished by an extension of 7 min at 72°C.

The single-strand conformation polymorphism (SSCP) method was used to identify the mutations within the amplified region. The PCR products were half diluted in denaturing loading dye (95% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol). The mixture was denatured at 95°C for 5 min, and placed in ice for 10 min. Then the samples were loaded on 8 to 12% nondenaturing polyacrylamide gels, with 10% formamide to improve the resolution of the DNA bands on the gel. The samples were run in 1 TBE (Tris-EDTA) buffer at 180 V for 14 to 16 h at a constant temperature of 4°C. Gels were stained by silver nitrate. The PCR fragments harboring various electrophoresis profiles were sequenced and analyzed for nucleotide changes. SSCP was used to genotype the animals.

Statistical analysis

Genotype and allele frequencies were calculated for each polymorphism as previously described (Weir et al., 1990). The phenotype data of meat quality traits and AA content were analyzed by least square analysis of variance (p = 0.05) using the General Linear Model (GLM) procedure of the SAS program (Cary, NC). The following linear model was employed to analyze associations:

$$Y_{ijkl} = \mu + Farm_i + Year_j + Genotype_k + bX_{jkl} + e_{ijkl}$$

In which Y_{ijkl} is the observation of the trait; μ is the mean of the population, $Farm_i$ is the effect of i-th farm(i = 1 for Tianyi Farm, 2 for Yilan Farm, 3 for Jixing Farm), $Year_j$ is the effect of j-th year (j = 2006, 2007 and 2008), $Genotype_k$ is the effect of k-th genotype (k = AA, AB, BB, CC and CD), *b* is the regression coefficient of the slaughter day and e_{ijkl} is the random residue.

RESULT AND DISCUSSION

SNP analysis is a well-established tool for the identification of genes associated with traits of economic interest in livestock populations. More recently, haplotype analysis has become an area of intense research for complex genetic phenotypes (Stone et al., 2005). A haplotype can be defined as a set of SNPs on a single chromosome that are closely linked and inherited as a unit. The knowledge of haplotype for several SNPs in one gene could provide more information about genotype-phenotype associations than individual underlying SNPs.

The results indicated that some genotypes were present as preponderant genotype and allele in different locus of the CAPN1 gene by PCR-SSCP genotyping (Table 2). As the preponderant genotype, AB genotype had high frequency in E7-1 and E14-4; AA was in E7-2, and BB is in E14-5 and E14-6. The genotype CC only existed in E7-1 and E14-4 with very low frequencies and genotype DD was not found in any individual. The frequency displayed by the A allele was high in E7-2 and E14-4, but the B allele had high frequency in E14-5 and E14-6. So differences of genotype and allele frequencies ($p<0.05$) indicated that the CAPN1

Table 1. Primer sequences for *CAPN1* genotyping

Primer name	Sequence (5'-3')	Position	Anneal temperature (°C)
E7-1	CAGCATTCTGGACATGGAG TAACACCCCTTCCCACCAC	Exon7-intron 7	58
E7-2	GAGGATGAGGTGGTGGGAAG CCACAAGGGCAAGTCCAGAG	Intron 7- intron8	62
E14-4	CCATGTGCTGCAAGGAGGA GACAGAAGGGAAGGAAGTG	Intron 16	58
E14-5	TTCTACTGCACTTCCTTCC CCTATGCTGACAACCCAAC	Intron16-exon17	56
E14-6	GATGTATCCTCCTGAGCA CCACAGCGTAAACCAGAC	Exon17-exon18	54

Table 2. The genotype frequency and allele frequency in different loci of CAPN1

Loci	Genotype	Genotype frequency	Allele	Allele frequency
E7-1	AA	0.052	A	0.339
	AB	0.572	B	0.297
	BB	0.010	C	0.364
	CC	0.366		
E7-2	AA	0.896	A	0.948
	AB	0.104	B	0.052
E14-4	AA	0.313	A	0.630
	AB	0.646	B	0.328
	BB	0.010	C	0.026
	CC	0.010	D	0.016
	CD	0.021		
E14-5	AA	0.292	A	0.411
	AB	0.239	B	0.589
	BB	0.469		
E14-6	AA	0.198	A	0.378
	AB	0.360	B	0.622
	BB	0.442		

gene was under selection pressure.

By DNA sequencing, 27 polymorphisms were identified within exons and introns of *CAPN1*: 2 in exon 8 and 17, 25 in intron 7, 8, 14, 16, and 17. The locations of the polymorphisms are shown in Table 3. Both of the polymorphisms in exons 8 and 17 lead to amino acid mutation: F331S and M599V. It is known that F331, located on the front of domain III of CAPN1, is involved in the functional regulation on protease, and M599, located on domain IV of CAPN1, which is the Ca^{2+} binding domain. These amino acid substitutions may have a key role in the enzyme function. Further, the two novel mutants in Yanbian yellow cattle are close to two distinct markers 316 and 530. Previous work has shown that the two non-synonymous SNPs (G316A and V530I) have significant effects on meat quality (Page et al., 2004).

Because Yanbian Yellow cattle have the same ancestor as Korean Yellow cattle, comparing the concrete mutant site between the two breeds of cattle in detail, we found that both had the same mutant sites (c843+269, c843+323, c843+330) in the CAPN1 gene (Table 3). However, Korean Yellow cattle have T>C, T>A and T>C mutants in c843+96, c843+161 and c843+195 (Cheong et al., 2008), and Yanbian Yellow cattle have a contrary mutant to Korean Yellow cattle. These data confirmed both cattle breeds have a blood relationship in evolution.

As one of the most important nutritional components, the amino acid composition determines the nutritive value of beef. We tested the main AA content in Yanbian Yellow cattle and analyzed the association between genotypes of CAPN1 gene and AA contents. The results indicate that there were only two gene loci associated with AA contents, E7-2 and E14-5. The E14-5 had a correlation to histamine and lysine contents ($p<0.05$), and E7-2 had significant correlation to aspartic acid, glutamic acid, arginine, tyrosine, phenylalanine, isoleucine and leucine content (Table 4). Thompson et al. reported a significant association between arginine or histidine and the panel scores for flavor (Thompson et al., 1961), and Fuke et al. reported that glutamic acid was the taste-active chemical that often affected the flavor. So the genotype of E14-5 and E7-2 might be markers for beef flavor (Fuke et al., 1991).

In addition to market requirements for fat cover and marbling, softer fat is more quickly processed by boning room workers, as well as potentially having some flavor advantages (Melton et al., 1982). The melting point (hardness) of long-chain fatty acids and their esters is related to the proportion of unsaturated fatty acids present. Melting point decreases as the proportion of unsaturated fatty acids increases. Cohort differences were also highly significant for fatty acid composition and melting point. The fatty acid composition of tissues was affected by some factors such as animal sex (Clemens et al., 1973), diet (Mandell et al., 1998), and genetic factors (Perry et al.,

Table 3. The SNPs at detected loci of CAPN1 gene

Position	Intron 7								
SNPs	*c843+96 C>T*	*c843+141 A>G*	*c843+161 A>T*	*c843+167 A>G*	*c843+195 C>T*	*c843+265 T>C*	*c843+269 T>C*	*c843+323 C>T*	*c843+330 G>A*

Position	Exon 8	Intron 8			Intron 14	Intron 16			
SNPs	*c 862 T>C*	*c928+37 A>G*	*c928+129 T>C*	*c928+147 T>C*	*c1604+410 A>G*	*c1735+375 A>G*	*c1735+470 C>G*	*c1735+492 C>T*	*c1735+507 G>A*
AA change	F311S								

Position	Intron 16			Exon 17	Intron 17				
SNPs	*c1735+794 CTC insdel*	*c1735+920 A>G*	*c1735+951 T>C*	*c1795 A>G*	*c1800+117 A>G*	*c1800+120 C>T*	*c1800+146 G>A*	*c1800+216 T>C*	*c1800+660 C>T*
AA change				M599V					

Table 4. Association between CAPNI genotypes and amino acid contents

Amino acid \ Genotyp	E7-2			E14-5		
	AA	BB	AB	AA	BB	AB
Aspartic (D)	1.44±0.08[a]	1.41±0.10[ab]	1.37±0.10[b]	NS	NS	NS
Glutamic (E)	2.46±0.11[a]	2.42±0.13[ab]	2.37±0.13[b]	NS	NS	NS
Histidine (H)	NS	NS	NS	0.71±0.10[ab]	0.73±0.11[a]	0.67±0.10[b]
Arginine (R)	1.09±0.04[a]	1.08±0.04[ab]	1.06±0.04[b]	NS	NS	NS
Tyrosine (Y)	0.51±0.03[a]	0.50±0.03[ab]	0.49±0.03[b]	0.50±0.03[ab]	0.51±0.03[a]	0.49±0.03[b]
Phenylalanine (F)	0.59±0.03[a]	0.58±0.04[ab]	0.57±0.04[b]	NS	NS	NS
Isoleucine (I)	0.65±0.04[a]	0.64±0.05[ab]	0.62±0.05[b]	NS	NS	NS
Leucine (L)	1.22±0.06[a]	1.20±0.08[ab]	1.17±0.08[b]	NS	NS	NS
Lysine (K)	NS	NS	NS	1.27±0.08[ab]	1.28±0.10[a]	1.22±0.09[b]

[a, b] Within breed groups means for individual traits bearing different superscripts differ significantly at p≤0.05.
NS = Not significant.

1998). In this study, we found that E7-1 and E14-5 were associated with fatty acid content. E14-5 was associated with stearate ($p<0.05$), and E7-1 had an association with myristic, palmitic, linoleic, and linolenic acids (Table 5). These data indicated that E7-1 had a correlation ($p<0.05$) to the content of unsaturated fatty acid. With the health benefits of unsaturated fatty acids being well recognized, consumers pay more attention to the unsaturated fatty acid composition, so E7-1 may be used as a marker of unsaturated fatty acid composition in breeding.

Low post-mortem muscle pH and associated pale meat and poor water holding capacity are particularly important because they affect the processing quality of meat (Dransfield et al., 1999). Variations in pH value and muscle color have been implicated to be associated with various measures of eating quality (Huff-Lonergan et al., 2002). In our research, genotypic frequencies and meat quality were analyzed by least squares analysis of variance ($p = 0.05$). The results showed that E14-4 was associated ($p<0.05$) with pH at an early stage (0, 1, 3 d), and that E14-4 and E14-5 were associated ($p<0.05$) with pH at a late stage (at 7 d). E14-4 had a correlation ($p<0.05$) to lightness at almost all time points except for 7 d, and E14-5 and E14-6 were associated with lightness at 5 and 7 d respectively (Table 6). Redness is an important index for beef classification. In this study, although many genotypes (E7-1, E7-2, E14-4, E14-5, and E14-6) were identified to be associated with redness, this association occurred at individual time points (E14-4 only at d 0, E7-1 at d 1, E14-5, E14-6 at 5 d). E14-4 and E14-5 were associated with yellowness (Table 6). Only E14-4 was associated with chroma, hue angle, and cooking loss. Summarizing the results, CAPN1 may be a candidate gene for pH and color scores.

Because of Ca^{2+} dependent neutral protease being involved in post-mortem meat proteolysis, the CAPN1 gene has been studied in many cattle as an important tenderness

Table 5. Association between CAPNI genotypes and fatty acid contents

Genotype \ Traits		Myristic	Palmitic	Stearic	Linoleic	Linolenic
E7-1	AA	3.64±0.25[a]	28.37±0.44[a]	NS	1.77±0.04[b]	0.33±0.09[b]
	BB	3.33±0.17[ab]	27.15±0.87[ab]	NS	1.97±0.05[ab]	0.48±0.08[a]
	CC	3.40±0.21[ab]	27.12±1.01[b]	NS	1.95±0.18[ab]	0.43±0.09[ab]
	AB	3.36±0.20[b]	27.05±0.94[b]	NS	1.98±0.17[a]	0.45±0.08[a]
E14-4	AA	NS	NS	NS	NS	NS
	BB	NS	NS	NS	NS	NS
	CC	NS	NS	NS	NS	NS
	AB	NS	NS	NS	NS	NS
	CD	NS	NS	NS	NS	NS
E14-5	AA	NS	NS	15.04±1.67[ab]	NS	NS
	BB	NS	NS	14.54±1.73[b]	NS	NS
	AB	NS	NS	15.70±1.82[a]	NS	NS

[a, b] Within breed groups means for individual traits bearing different superscripts differ significantly at p≤0.05.
NS = Not significant.

Table 6. Association between CAPNI genotypes and meat quality traits in E14

	E14-4					E14-5			E14-6		
	AA	BB	CC	AB	CD	AA	BB	AB	AA	BB	AB
pH0	5.45± 0.01^{a}	5.45± 0.01^{ab}	5.44± 0.01^{ab}	5.44± 0.01^{b}	5.46± 0.01^{a}	NS	NS	NS	NS	NS	NS
pH1	5.50± 0.01^{a}	5.51± 0.01^{a}	5.48± 0.01^{b}	5.50± 0.01^{b}	5.51± 0.02^{a}	NS	NS	NS	NS	NS	NS
pH3	5.50± 0.01^{b}	5.50± 0.01^{b}	5.51± 0.01^{a}	5.51± 0.01^{a}	5.51± 0.01^{ab}	NS	NS	NS	NS	NS	NS
pH7	NS	NS	NS	NS	NS	NS	5.64± 0.01^{ab}	5.63± 0.01^{b}	5.64± 0.01^{ab}	5.64± 0.01^{a}	5.63± 0.01^{b}
L0	40.60± 1.80^{b}	40.62± 1.67^{ab}	41.47± 1.69^{ab}	41.38± 1.47^{a}	41.42± 1.05^{ab}	NS	NS	NS	NS	NS	NS
L1	44.86± 3.00^{a}	45.00± 3.12^{ab}	41.49± 2.50^{b}	43.02± 2.22^{bB}	44.77± 0.90^{ab}	NS	NS	NS	NS	NS	NS
L3	43.71± 3.88^{ab}	43.64± 4.12^{ab}	44.61± 2.84^{ab}	42.58± 3.52^{b}	47.42± 3.51^{a}	NS	NS	NS	NS	NS	NS
L5	46.51± 1.86^{b}	47.17± 1.93^{ab}	45.91± 0.40^{ab}	47.43± 1.89^{a}	46.33± 0.52^{ab}	46.82± 1.84^{ab}	46.17± 1.39^{b}	47.24± 1.82^{a}			
A0	10.77± 0.61^{ab}	10.91± 0.57^{ab}	10.32± 0.45^{b}	10.74± 0.50^{ab}	11.36± 0.60^{a}	NS	NS	NS	NS	NS	NS
A3	NS	NS	NS	NS	NS	NS	9.91± 0.39^{a}	8.45± 0.76^{b}	8.49± 0.74^{ab}	8.86± 0.61^{a}	8.45± 0.73^{b}
B3	NS	NS	NS	NS	NS	NS	13.05± 0.86^{b}	13.75± 0.98^{a}			
B5	12.02± 1.77^{ab}	11.36± 1.95^{b}	13.96± 0.66^{a}	11.94± 1.80^{ab}	11.75± 0.77^{ab}	NS	NS	NS	NS	NS	NS
C1	16.54± 0.94^{b}	16.71± 1.26^{ab}	16.56± 0.44^{ab}	17.80± 0.71^{a}	17.67± 0.57^{ab}	NS	NS	NS	NS	NS	NS
C3	16.54± 1.81^{b}	16.71± 1.94^{ab}	16.56± 1.03^{ab}	17.80± 2.00^{a}	17.67± 0.49^{ab}	NS	NS	NS	NS	NS	NS
C5	15.40± 1.07^{ab}	15.95± 1.05^{a}	15.02± 0.51^{ab}	15.73± 0.97^{ab}	14.53± 0.91^{b}	NS	NS	NS	NS	NS	NS
C7	14.42± 1.45^{ab}	14.85± 1.61^{ab}	13.44± 0.98^{b}	14.48± 1.27^{ab}	15.99± 1.48^{a}	NS	NS	NS	NS	NS	NS
H0	47.31± 0.92^{ab}	46.74± 0.41^{b}	48.15± 0.53^{a}	47.21± 1.01^{ab}	46.74± 0.54^{ab}	NS	NS	NS	NS	NS	NS
H1	49.87± 0.90^{a}	50.16± 0.96^{a}	49.00± 0.55^{b}	49.56± 0.67^{ab}	49.23± 0.43^{ab}	NS	NS	NS	NS	NS	NS
H5	57.60± 0.97^{b}	57.99± 0.80^{ab}	57.27± 0.59^{ab}	58.16± 1.17^{a}	57.70± 0.12^{ab}	NS	NS	NS	NS	NS	NS
H7	60.26± 1.92^{ab}	60.70± 1.64^{ab}	59.62± 1.06^{ab}	59.96± 1.38^{b}	62.15± 1.95^{a}	NS	NS	NS	NS	NS	NS
Cooking loss	0.38± 0.03^{a}	0.38± 0.03^{ab}	0.38± 0.01^{ab}	0.37± 0.02^{b}	0.40± 0.01^{ab}						

L = Brightness; A = Redness; B = Yellowness; C = Chroma; H = Hue angle.

$^{a, b}$ Within breed groups means for individual traits bearing different superscripts differ significantly at $p \leq 0.05$.

NS = Not significant.

marker to apply in breeding by marker assisted selection (MAS). In this study, whilst some genotypes did not have an association with tenderness but with other meat traits (pH, color scores, fatty acid contents and AA contents), it is important to note that validation is dependent on the specific nature of the population screened and that genetic background may influence the size of the effect of a polymorphism. Validation failure may be due to a lack of

true associations between the trait and marker but could also be caused by differences in SNP frequencies, different marker-causative mutation linkage phases, genotype-by-environment interactions or epistasis as well as sample size effects and the way the trait is measured. Nevertheless, for those associations confirmed here, the additional validation instills confidence in using these markers in selection programmes for improved meat quality.

In conclusion, we found evidence of a large novel variability in the CAPN1 gene in Yanbian Yellow cattle caused by polymorphism to be associated with phenotypic traits related to meat quality. The results of this study will be practical for improvement of breeding native Chinese cattle and for purposes of meat consumption.

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