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Full Length Research Paper

Association analysis of *CAPN1* gene variants with carcass and meat quality traits in Chinese native cattle

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The *CAPN1* gene plays an important role in post mortem tenderization of meat and it is a main candidate gene for assessing the meat quality characteristics of beef. In this study, two single nucleotide polymorphisms (SNPs) A3717G and A3854G of bovine *CAPN1* gene were predicted and identified by bioinformatics and DNA sequencing methods. Furthermore, the association between the genetic polymorphisms of SNPs and meat quality characteristics of beef was analyzed. Statistical analysis revealed that these two SNPs were completely linked, and they were significantly associated with Warner-Bratzler shear force (WBSF) (P<0.05), but had no significant association with the other six traits in the whole populations. These results suggested that both markers may be effective for the marker-assisted selection of meat quality traits in Chinese native cattle, and also added new evidence that *CAPN1* gene was an important candidate gene for the selection of carcass and meat quality traits in the cattle industry.

Key words: Bovine, *CAPN1* gene, Carcass and meat quality traits, SNPs.

INTRODUCTION

Carcass and meat quality traits, which are influenced by multiple genes, play economically important roles in the value assessment of beef cattle industry. Selection of animals with better carcass composition and higher meat quality is of great significance in the competitiveness of beef cattle production. Genetic improvement at present is to identify, map and analyze quantitative trait loci (QTL) affecting production traits and to use genetic markers for marker-assisted selection (MAS) to increase frequency of favorable QTL alleles in target population (Stone et al., 2005). Identification of genetic marker associated with economic trait would be the key to the application of MAS.The micromolar calcium-activated neutral protease 1 gene, also known as Calpain1 gene (*CAPN1)* encoding the cysteine protease μ-calpain, is a member of the calpain gene family. The role of the cysteine protease μ-calpain in degrading myofibrillar proteins under post-mortem conditions was thought to be the primary enzyme in the post mortem tenderization of meat (Belhran et al., 1997; Koohmaraie, 1992, 1994, 1996). The bovine *CAPN1 gene* was mapped to the telomeric end of the BTA29 linkage group (Smith et al., 2000), which coincides with the position of a quantitative trait locus influencing meat tenderness (Casas et al., 2003). This demonstrates that *CAPN1* is a positional candidate gene potentially affecting meat tenderness in a bovine-resource population.

Furthermore, some single nucleotide polymorphisms (SNPs) in *CAPN1* gene associated with meat tenderness

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Table 1. The characteristic of the primers.

SNPs	The primer Sequences (5' - 3')	Amplified fragments (bp)	Annealing temperature (℃)	Region
A3717G	L 5'GCCTTACAAGTGTTGCCTGC-3' R 5'- TTTGGGAACCAGGAGGAAGCC-3'	827	66.5	Intron 4 – Intron 6 3354 – 4180 bp
A3854G	L 5'-GAAGTCCTCAAAGCCCTCACA-3' R 5'-GTGGCCTAGAATCAGGTATC-3'	396	58.6	Intron 4 – Exon 6 3480 – 3875 bp

have been reported in previous studies (Casas et al., 2006; White et al., 2005; Van et al., 2007; Page et al., 2002; Jennifer et al., 2009; Yun et al., 2008), but there was no publicly available evaluation of the association of SNPs markers in Chinese native cattle. Hence, the objective of this study was to verify the two SNPs (A3717G and A3854G) in Luxi, Jinnan and Qinchuan cattle. We also evaluated the association between these SNPs, meat tenderness and other important carcass and meat quality traits in the three cattle breeds. The result of this study could provide new important evidences that *CAPN1* is an important candidate gene to be used for selection of meat traits in the beef cattle industry.

MATERIALS AND METHODS

Animals and carcass data

In total, 264 animals including Luxi (N = 91), Jinnan (N = 65) and Qinchuan (N = 108), were randomly selected from commercial populations and used in the association analysis. All experimental animals were steers. The diets for the animals were mainly composed of 50% corn grain, 18 to 20% cotton seed cake, 10 to 11% distiller's grains, 11% wheat bran, and 4 to 5% vitamin and mineral supplements. The animals were slaughtered at the age of 30 ± 2 months and at the average weight of 456 ± 50.5 kg. Carcass and meat quality traits were measured according to the criterion GB/T 17238-1998 cutting standard of fresh and chilled beef in China (China Standard Publishing House). Slaughter body weight (SBW) was measured just before slaughter after a 24 h period of fasting. Carcass weight (CW) was measured just after slaughter.

Other carcass traits (dressing percentage (DP), marbling score (MBS) and back fat thickness (BFT)) measurements were carried out at 4 days post-mortem. Rib area (REA) and backfat thickness (BF) were measured between the 12th and 13th rib. Meat samples for measuring Warner-Bratzler shear force (WBSF) were taken from the interface between the 12th and 13th rib and were measured at seven days postmortem. WBSF is the internationally accepted standard for the determination of tenderness and was used as our quantitative measurement (Hackelford et al., 1997, 1999; Heeler et al., 1999). The evaluation of MBS for quality grade in China consists of a six-point regime with values 1 to 6 corresponding to traces, slight, small, modest, moderate and abundant, respectively.

Determination of candidate SNP loci for the *CAPN1* gene and PCR reaction

The NCBI website and the Blast program were searched for the *CAPN1* gene sequences, including those for nucleic acid, sequence tagged sites (STSs), expressed sequence tags (ESTs) and the genome sequence. The retrieved sequences were downloaded, then redundant and non-homologous sequences were deleted. The resulting sequences were assembled by DNAStar software. Candidate SNPs were found by sequence alignment and two candidate SNPs loci were selected at 3717 and 3854 bp. Based on the standard sequence of AF252504, specific polymerase chain reaction (PCR) primers were designed to verify the presence of this SNPs. The primers, amplified fragments (bp), annealing temperature (℃) and region were listed in Table 1.

PCR amplifications were performed in a 20 µL volume containing 50 ng of DNA template, 10 pM each primer, 0.25 mM dNTP, 2.5 mM $MgCl_2$ and 0.5 U of Taq DNA polymerase. The PCR protocol was 94℃ for 5 min, followed by 35 cycles of 94℃ for 30 s, 64℃ for 30 s and 72℃ for 40 s, and a final extension at 72℃ for 10 min. The final products were then purified and sequenced (Beijing Aolaibo Biotechnology Co., Ltd. P. R. China).

Statistical analysis

The genetic characters of the cattle *CAPN1* gene, including genotypic frequencies, allelic frequencies, Hardy–Weinberg equilibriums, gene homozygosity (Ho), gene heterozygosity (He), effective allele numbers (Ne) and polymorphism information content (PIC) were statistically analyzed according to the previous approaches. The linkage disequilibrium (LD) was performed by SHEsis software (Shi and He, 2005; Li et al., 2009). In addition, the association between a single SNP marker and the haplotype genotypes of the *CAPN1* gene, and carcass and meat quality traits were analyzed by the least-squares method as applied in the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). The association analysis was calculated according to the following statistical linear model

$$Y_{ijkl} = \mu + BF_i + Month_j + G_k + e_{ijkl},$$

Where, Y_{ijkl} is the observed value; µ is the overall mean for each trait; BF_i is the fixed effect of i_{th} breed and farm; $Month_j$ is the fixed effect of j_{th} month of slaughtering; G_k is the fixed effect of k_{th} single SNP marker genotype and e_{ijkl} is the random error.

RESULTS AND DISCUSSION

Identification and genotyping of SNPs

In this study, two SNPs (A3717G and A3854G) of bovine *CAPN1* gene were predicted with the bioinformatics methods based on the nr database in GenBank, and they were identified by directly DNA sequencing methods. The experimental results verified the two SNPs and demonstrated the feasibility of these SNPs screening

Table 2. Genotype and allelic frequencies of *CAPN1* gene SNPs in populations.

SNPs	Genotype	Breed (number)			Total (N = 264)
		Luxi (N = 91)	Jinnan (N = 65)	Qinchuan (N = 108)	
A3717G	GG	0.1978(18)	0.3846(25)	0.2130(23)	0.2500(66)
	GA	0.1176(38)	0.4308(28)	0.4815(52)	0.4470(118)
	AA	0.3846(35)	0.1846(12)	0.3055(33)	0.3030(80)
Frequency of allele A		0.5934	0.4000	0.5463	0.5265
A3854G	GG	0.1978(18)	0.3846(25)	0.2130(23)	0.2500(66)
	GA	0.1176(38)	0.4308(28)	0.4815(52)	0.4470(118)
	AA	0.3846(35)	0.1846(12)	0.3055(33)	0.3030(80)
Frequency of allele A		0.5934	0.4000	0.5463	0.5265

Table 3. Genetic indexes of *CAPN1* locus in populations.

Breed	Homozygosity (Ho)	Heterozygosity (He)	Effective number of alleles (Ne)	Polymorphism information content (PIC)	$\chi 2$	P value
Luxi	0.5174	0.4826	1.9326	0.3661	1.65	0.4383
Jinnan	0.5200	0.4800	1.9231	0.3648	0.68	0.7104
Qinchuan	0.5043	0.4957	1.9830	0.3695	0.09	0.9565
All	0.5265	0.4735	1.9945	0.3743	2.83	0.2429

method.

Genotype and allele frequencies

The three genotypes for A3717G and A3854G were identified in the populations in this study. The genotype and allelic frequencies of SNP3717 and SNP3854 are shown in Table 2; their allele and genotype frequencies were all the same. For SNP A3717G and A3854G in the population, the frequency of allele A were 0.5934, 0.4000 and 0.5463, respectively and allele A was predominated in Luxi and Qinchuan cattle, while allele G was predominated in Jinnan cattle; if $r^2 > 0.33$, then the dis-equilibrium is considered strong. The linkage disequilibrium between SNP3717 and SNP3854 in the population was estimated and the results indicated that two SNP were completely linked ($r^2 = 1$). This region can be inherited as a unit and since they are completely linked, we took A3717G as an example for genetic diversity and character in the cattle populations and association analysis.

Genetic diversity and character in cattle populations

For the three populations, values were determined for gene heterozygosity (He), gene homozygosity (Ho), effective number of alleles (*N*e), polymorphism information content (PIC), and χ^2 (Table 3). Generally, PIC is classified into the following three types: low polymorphism (PIC value < 0.25), median polymorphism (0.25 < PIC value < 0.5) and high polymorphism (PIC value > 0.5). According to these classifications as aforementioned, these three populations which had been studied in this paper belongs to the median polymorphism level. The results of Hardy-Weinberg equilibrium for the two locus also indicated that the polymorphism site in the three popu-lations was in Hardy-Weinberg equilibrium ($P > 0.05$).

Association analysis of the cattle *CAPN1* gene polymorphisms with economic traits

Least squares means (LSM), standard errors (SE) and levels of significance were presented in Table 4. The gene-specific SNP marker correlation analysis indicated that the SNP3717 had significant association with WBSF ($P < 0.05$). As shown in Table 4, animals with AA genotype had higher WBSF than those with AG and GG genotypes ($P < 0.05$). However, no significant association was observed between SNPs and other six traits.

The *CAPN1* gene is known to play a key role in the postmortem tenderization of meat (Casas et al., 2006; White et al., 2005; Schenkel et al., 2006; Page et al., 2004), and many SNPs in the *CAPN1* gene have been associated with tenderness in previous studies (Casas et

Table 4. Association analysis between SNPs and seven traits in beef cattle.

SNPs	Genotype	Number	SBW [1] (kg)	CW[1] (kg)	BF[1] (cm)	REA[1] (cm^2)	DP[1] (kg)	MBS[1] (1 - 5)	WBSF[1] (kg)
	AA	80	556.32 ± 10.21	308.25 ± 7.68	1.08 ± 0.10	72.48 ± 1.90	54.49 ± 0.56	2.01 ± 0.21	4.70 ± 0.28[a]
A3717G	GA	118	566.18 ± 9.20	316.73 ± 6.98	1.18 ± 0.06	71.96 ± 1.70	55.81 ± 0.45	2.05 ± 0.09	4.25 ± 0.12[b]
	GG	66	565.90 ± 7.52	319.04 ± 5.31	1.19 ± 0.07	72.36 ± 1.52	55.36 ± 0.36	2.25 ± 0.21	4.20 ± 0.11[b]
P-value			0.7321	0.4328	0.5638	0.8183	0.7163	0.2515	0.0215

[1]Slaughter body weight (SBW); carcass weight (CW); backfat thickness (BF); rib area (EA); dressing percentage (DP); marbling score (MBS); Warner-Bratzler Shear force (WBSF).
[a,b]Means with different superscripts were significantly different(*P<0.05*).

al., 2006; White et al., 2005; Van et al., 2007; Page et al., 2002; Jennifer et al., 2009; Yun et al., 2008). The four SNPs (CAPN316, CAPN530, CAPN4685 and CAPN4751), have been shown to be significantly related to the tenderness of beef (White et al., 2005; Van et al., 2007; Page et al., 2002, 2004; Morris et al., 2006; Rincon and Medrano, 2006), and Jennifer reported that CAPN316 had significant association with not only meat tenderness but also weight of the hind-quarter, whereas animals inheriting the CC genotype had more tender meat and heavier hindquarters (Jennifer et al., 2009). Although, many researches had been performed on the CAPN1 gene, no study has conducted an association analysis between A3717G and A3854G with beef tenderness. The results of this study showed that the two SNPs (A3717G and A3854G) were completely linked and the shear force for the AA genotype of A3717G (4.70 ± 0.28, kg) is significantly different from those for the AG (4.25 ± 0.12, kg) and GG genotypes (4.20 ± 0.11, kg) ($P < 0.05$). The shear forces for the AA genotypes of the two SNPs were significantly higher than those for the AG and GG genotypes. This indicates that the AA genotype reduces the tenderness of beef.

In conclusion, our results verified the two SNPs A3717G and A3854G in the *CAPN1* gene. We also demonstrated the feasibility of our research method, which screens candidate SNPs using various published sequence resources from public databases. Our results therefore provide evidence that SNPs A3717G and A3854G have significant effects on the tenderness of beef. Moreover, further work will be necessary to use these SNPs for marker-assisted selection (MAS) in a larger population and to investigate whether the *CAPN1* gene plays a role in this trait or is in linkage disequilibrium with other causative mutations.

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