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Full Length Research Paper

Genetic variation of calsarcin-1 gene and association with carcass traits in 3 Chinese indigenous cattle

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This study aimed to investigate polymorphisms of Calsarcin-1 gene and evaluate its effect on carcass traits in 429 samples of 3 Chinese indigenous cattle breeds, namely, Luxi (LX), Nanyang (NY) and Jiaxianred (JXR) breeds, PCR products with a 320 bp fragment of the Calsarcin-1 gene spanning over a part of intron 2, complete exon 3 and a part of intron 4 were amplified and sequenced. A synonymous alteration (NW_001495138:g.16718C>A) in the exon 3 region of the calsarcin-1 gene was detected and PCR-SSCP method was then developed to genotype all of the individuals. The results showed that the allele frequencies of A/C in LX, NY and IXR breeds were 0.1116/0.8884, 0.1591/0.8409 and 0.0947/0.9053, respectively. Least squares analysis revealed a significant effect (P<0.05) of genotypes on backfat thickness and tenderness of the animals, with the AC and AA genotype being favourable compared to the CC genotype.

Key words: Calsarcin-1 gene, carcass traits, cattle, SNP.

INTRODUCTION

Calsarcins comprise a novel family of muscle-specific calcineurin-interacting proteins and play an important role in modulating both the function and substrate specificity of calcineurin in muscle cells (Wang et al., 2006). Several groups independently and simultaneously identified the calsarcin family and termed it calsarcin (Frey et al., 2000; Frey and Olson, 2002), FATZ (Faulkner et al., 2000), myozenin (Takada et al., 2001) and c4 or f5 (Ahmad et al., 2000). The calsarcin family consists of 3 members, with CS-1 being expressed in the adult heart and in slow-twitch fibers of skeletal muscle, while CS-2 and CS-3 are exclusively expressed in fast-twitch fibers of skeletal muscle tissue (Frey et al., 2000; Frey and Olson, 2002; Wang et al., 2007; Frey et al., 2008). Calsarcins are hallmarked by a multitude of Z-disc interaction partners that, in addition t°calcineurin, include α-actinin, LIM domain-binding 3 (LDB3, also known as Cypher, ZASP and Oracle), Telethonin/T-cap, γ-filamin (Frey et al., 2000; Frey and Olson, 2002) and myotilin (Gontier et al., 2005).

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In slow-twitch skeletal muscle, the lack of CS-1 led to an increase in calcineurin activity, as a consequence, mice with a null mutation in CS-1 show an excess of slow skeletal muscle fibers (Frey et al., 2004). CS-1 affected the formation of Z-line and the calcium binding by combination with these identified proteins and participated in the calcium ion regulation and maintaining the cytoskeleton stabilization. By this way, the calcium ion concentration also affect other factors and signaling pathways related to the slow and fast muscle fiber formation and transformation (Yang et al., 2009).

The fast-twitch and slow-twitch fibers express different isoforms and frequently different concentrations (Pette and Staron, 1997) and they exhibit characteristic differences in functional properties due to differences in the isoforms and quantities of expression of most muscle proteins (Liu et al., 2005). Muscle fibre types play an important role in bovine meat tenderness and it is generally regarded as the single most important component of meat quality for the consumer (Strydom et al., 2000), with meat quality determined by the proportions of muscle fiber type (Fonseca et al., 2003). Meat tenderness has been inconsistently correlated to muscle fibre frequency and size (Cross et al., 1972; Calkins et al.,

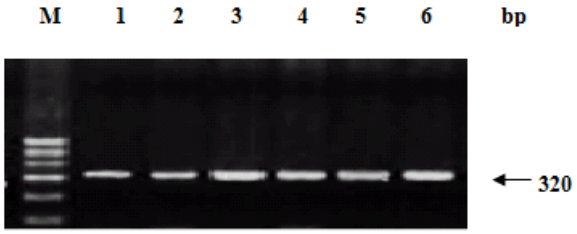

Figure 1. The PCR product of *CS-1* gene exon 3 and its flanking region. M: Marker; Lanes 1-6: PCR products of the *CS-1* gene exon 3 and its flanking region.

1981; Crouse et al., 1991; Thornberg, 1996; Maltin et al., 1998; Krausgrill et al., 1999; Sazili et al., 2005), thus any effects on muscle structure and morphology might have an impact on ultimate meat tenderness. Therefore, the knowledge of calsarcins and their relationship with carcass traits will contribute to the understanding and improvement of bovine meat quality.

The objectives of this study were to detect the single nucleotide polymorphism (SNP) of CS-1 gene and to investigate their associations with carcass traits in 429 samples representing 3 Chinese indigenous cattle breeds, namely, Luxi (LX), Nanyang (NY) and Jiaxianred (JXR) breeds. These breeds have been a great source of genetic diversity in China, it is expected that the results of this study will provide basic data for marker-assisted selection related to the economic traits and theory base for the improvement of genetic characters of the Chinese indigenous cattle.

MATERIALS AND METHODS

Sample collection and DNA isolation

Three breeds of 2-year Chinese indigenous cattle [Luxi (LX), Nanyang (NY) and Jiaxianred (JXR) were used in this study. The Luxi animals (n = 224) were from the reserved farm (Hezhe city, Shan-dong Province, P. R. China], the Nanyang animals (n = 110) were from the breeding centre of Nanyang cattle (Nanyang city, Henan Province, P. R. China), the Jiaxian animals (n = 95) were from the breeding farm of Jiaxian cattle (Jiaxian county, Henan Province, P. R. China). Performance records of the 2-year animals' carcass traits (Slaughter weight, Carcass weight, backfat thickness, loin-eye area, intramuscular fatty content, marbling score, tenderness and water holding capacity) were collected for statistical analysis. Genomic DNA of all the animals were isolated from 2% heparin-treated blood samples and stored at -80°C, following standard procedures (Sambrook et al., 2002).

SNP identification and genotyping

PCR was performed to amplify a 320 bp fragment of CS-1 gene comprising part of intron 2, complete exon 3 and part of intron 4 using forward (5'- AAG CCG TAT CAT TCA GAC -3') and reverse (5'- ATA CAA ATG GAG TCT CGC -3') primers designed with primer 5.0 based upon bovine CS-1 gene sequence (GenBank accession No. NW_001495138).

The 25 µl PCR reaction mixture contained 50 ng genomic DNA, 0.5 µM of each primer, 1 × Buffer (including 1.5 mM $MgCl_2$), 200 µM dNTPs (dATP, dTTP, dCTP and dGTP) and 0.625 units of Taq DNA polymerase. The cycling protocol was 5 min at 95°C, 35 cycles of 94°C for 35 s, 57.0°C annealing for 35 s, 72°C for 35 s, with a final extension at 72°C for 10 min. PCR products were electrophoresed on 1% agarose gels using 1 × TBE buffer (89mM Tris, 89 mM boric acid, 2 mM Na_2 EDTA), containing 200 ng/ml ethidium bromide.

Aliquots of 8 µl PCR products were mixed with 4 µl denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice. Denatured DNA was subjected to PAGE (200 × 175 × 1 mm) in 1 × TBE buffer and constant voltage (200 V) for 0.5 h, then 120 V for 12 h. Then the gel was stained with 0.1% silver nitrate and visualized with 2% NaOH solution (supplied with 0.1% formaldehyde). After the polymorphism was detected, the PCR products of different electrophoresis patterns were sent to sequence in both directions in ABI PRIZM 377 DNA sequencer (Perkin-Elmer) and analyze the sequences with BioXM software (version 2.6).

Statistical analysis

Genotype, allele frequencies, the Hardy-Weinberg equilibriums and population genetic indexes: Het (gene heterozygosity), Hom (gene homozygosity), Ne (effective allele numbers) and PIC (Polymorphism information content) were calculated using the POPGENE Version 1.31 software. The analysis of associations between genotypes of CS-1 and carcass traits of the 2 yr animals was conducted using the GLM procedure (SPSS 13.0, SPSS Inc.). The following model was used

$$Yijklm = \mu + Gi + Bj + Sk + Dkl + (GB)ij + eijklm$$

where Yijklm was the trait measured on each of the ijklmth animal, µ was the overall population mean, Gi was the fixed effect associated with ith genotype, Bj was fixed effect due to jth breed, Sk was the fixed effect associated with the kth sire, Dlk was the fixed effect associated with lth dam with sire k, (GB)ij was interaction between the genotype and breed effects of the model, eijklm was the random error.

RESULTS

PCR-SSCP analysis of the *CS-1* gene

A 320 bp of amplified product was obtained by PCR amplification in all the animals studied in this study (Figure 1). In the present investigation, the entire exon 3 and its flanking region of CS-1 gene demonstrate polymorphism (named genotype CC, AC, and AA) by PCR-SSCP method (Figures 2 and 3).

Genetic polymorphism of bovine CS-1 gene and χ^2 test

Sequence analysis of C and A allele of this study revealed a C>A mutation at 191-bp position of the amplified product, this mutation was a synonymous SNP, namely, Ser(TCC) >Ser(TCA) at position 160 of the CS-1 protein.

The genetic diversity of the locus was then calculated (Tables 1 and 2). The results suggested that the mutant

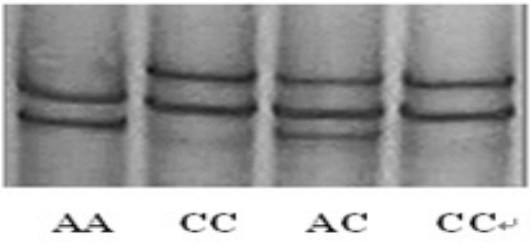

Figure 2. The PCR-SSCP patterns of CS-1 gene exon 3 and its flanking region, three patterns (AA, AC, CC) were observed in 3 Chinese indigenous cattle.

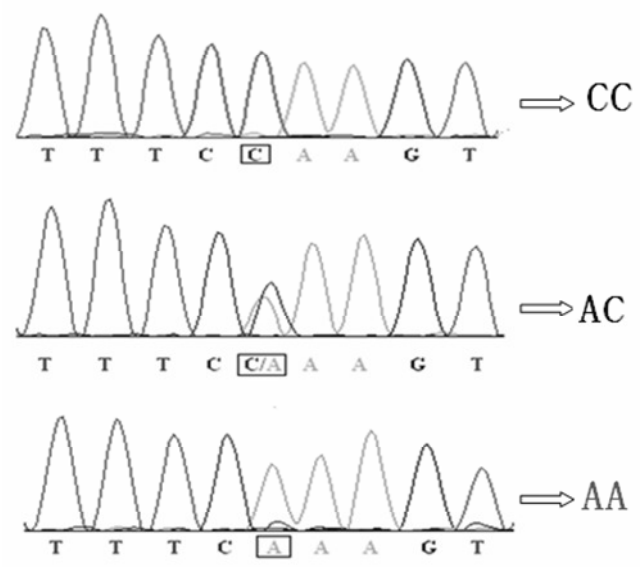

Figure 3. The sequencing map of the novel SNP for exon 3 and its flanking region in the bovine CS-1 gene. Sequencing map revealed a C>A mutation at 191-bp.

allele A had lower frequency compared with the wild allele C in all the experimental populations. The locus of the 3 populations showed low polymorphism. The values of PIC, He and Ne of Nanyang breed in the locus were higher than the other 2 populations, which implied that the polymorphism and genetic variation of Nanyang breed were higher than that of other populations. Gene homozygosity varied from 0.7324 (NY) to 0.8285 (JXR) and the effective allele numbers (Ne) ranged from 1.2070 (JXR) to 1.3653 (NY). The minimum and maximum PIC values were 0.1568 and 0.2318, respectively. The $\chi 2$-test showed that populations of LX, NY and JXR at CS-1 gene locus were all at Hardy-Weinberg equilibrium ($P > 0.05$), which might indicated that the CS-1 gene locus was under homeostasis accompanied by the effect of artificial selection, migration, and genetic drift and that the artificial selection had put little pressure on this gene locus. Hence, the artificial selection must be strengthened in the process of the improvement for Chinese cattle.

Effect of the CS-1 genotypes on carcass traits

Allele frequencies of the polymorphic site were studied in 429 Chinese indigenous cattle. Table 3 showed the comparison of the least square means and respective standard errors of carcass traits, involving the genotypes of the CS-1 polymorphism.

The results showed that individuals with genotype AC has higher backfat thickness ($P < 0.05$) than individuals with genotype CC, animals with genotype AC and AA have better tenderness than animals with genotype CC. However, no significant association of different genotypes with other traits was detected ($P > 0.05$). The result showed animals with genotype AA and AC had better performance than animals with genotype CC in most of the carcass traits. In other words, allele A might be the beneficial allele for carcass traits.

DISCUSSION

It has been known that calcineurin is a calmodulin dependent protein which functions as a regulator of muscle cell growth and function. Several studies have shown that calcineurin controls the skeletal muscle fibre type by stimulating slow muscle gene promoters and slow fibre differentiation both in cultured cells and *in vivo* (Chin et al., 1998; Schulz, 2004). Calsarcins appear to function as bridges between calcineurin and α-actinin at the Z-disc of cardiac and skeletal muscles (Schulz and Yutzey, 2004). Frey et al. (2004) show that CS-1 knockout mice are sensitized to calcineurin signaling, as a consequence of inappropriate calcineurin activation, mice with a null mutation in CS-1 show an excess of slow skeletal muscle fibers. Therefore, CS-1 gene may play a role in muscle differentiation and the formation of different fibre types. Some reports pointed out tenderness of meat has been associated with changes in muscle fibre type characteristics (Seideman and Koohmaraie, 1987; Crouse et al., 1991; Maltin et al., 1998). Thereby CS-1 gene may play a very important part in affecting bovine meat quality. However, no report is published on the effects of calsarcins gene polymorphism on bovine meat quality.

The data revealed that the mutant homozygotes (AA) are present at low frequencies, accordingly, the mutant allele A had lower frequency compared with the wild allele C in all the experimental populations. Comparisons of carcass traits among individuals with genotype CC, AC and AA, individuals with genotype AC and AA have some superior economic traits, which indicates that allele A might be the beneficial allele for some economic traits of Chinese indigenous cattle. Allele A might be a recent mutation, the presence of the A allele in the heterozyous

Table 1. Genotype distribution and haplotype frequencies at the bovine CS-1 exon 3 and its flanking region locus.

Breed	Observed genotypes			Total	Haplotype frequencies	
	CC	AC	AA		C	A
Luxi (LX)	178	42	4	224	0.8884	0.1116
Nanyang (NY)	79	27	4	110	0.8409	0.1591
Jiaxianred (JXR)	77	18	0	95	0.9053	0.0947

Table 2. Genetic diversity at CS-1 exon 3 and its flanking region locus in Chinese indigenous bovine breeds.

Breeds	Gene homozygosity (Ho)	Gene heterozygosity (He)	Effective allele numbers (Ne)	Polymorphic information content (PIC)	χ^2 test
Luxi	0.8017	0.1983	1.2474	0.1786	0.266
Nanyang	0.7324	0.2676	1.3653	0.2318	0.318
Jiaxianred	0.8285	0.1725	1.2070	0.1568	0.237

Table 3. Least square means and standard errors of the carcass traits obtained for the genotypes of the CS-1 polymorphism in Chinese indigenous cattle.

Carcass traits	Genotype (LSM±SE)		
	CC	AC	AA
Slaughter weight (Kg)	378.51±2.56	383.54±8.37	381.67±14.61
Carcass weight (Kg)	176.94±3.00	183.13±3.78	185.60±13.91
Backfat Thickness (cm)	1.19±0.03 [a]	1.23±0.04 [b]	1.21±0.12 [ab]
Loin-eye Area (cm^2)	75.20±0.75	77.56±1.24	77.21±4.46
Intramuscular Fatty Content (%)	6.56±0.20	6.89±0.30	7.01±0.78
Marbling Score (No.)	2.13±0.07	2.17±0.11	1.95±0.50
Tenderness (No.)	2.74±0.02 [a]	2.36±0.04 [b]	2.28±0.13 [b]
Water holding capacity (%)	76.01±0.35	76.72±0.41	77.45±2.08

Marbling score: scored from 1 (abundant) to 5 (poor).
Tenderness: scored from 1 (extremely tender) to more than 11 (extremely tough).
[a,b]Different letters indicate significant difference in a row ($p < 0.05$).

individuals significantly improves their production traits. These results could be explained that the polymorphism of CS-1 gene exon 3 might be relevant to bovine carcass traits. The novel SNP at CS-1 gene could result in a synonymous mutation in CS-1 protein, which maybe lead to protein with the same amino acid sequence but different structural and functional properties (Komar, 2007). The degeneracy of the genetic code enables the same amino acid sequences to be encoded and translated in many different ways (Kurland, 1991). We know that the genome is highly redundant in terms of tRNA species for each amino acids but enigmatically under represents a number of specific codons (Shah et al., 2008), so in the synthesis of CS-1 protein, if the change of base at the third position of codon is not represented by a corresponding anti-codon within the nuclear tRNA, the rate of expression of the CS-1 protein could change, which may affect muscle differentiation and the formation of different fibre types. As the frequency of AA and AC genotypes together is not exceeding than that of the CC genotype, there is a good possibility of improvement by selecting animals possessing A allele either in homozygous or in heterozyous condition.

In conclusion, the present study revealed a novel SNP in exon 3 of the CS-1 gene. The SNP is association with backfat thickness and tenderness significantly at the 2-year cattle, with the AC and AA genotype being favourable compared to the CC genotype. The presence of the A allele in the heterozygous individuals significantly improves their preformance, there is a good possibility of improvement by selecting animals possessing A allele either in homozygous or in heterozyous condition. Al-

though confirmation of our data needs further analysis on other herds, the association of genotypes with better performance is a very interesting finding, which could be used for the genetic improvement of Chinese indigenous bovine carcass traits.

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