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Polymorphisms of the Osteocrin gene and its association with meat quality traits in Qinchuan cattle

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ABSTRACT. Here, we detected 2 SNPs, A85C and T335C, that were located on the 3rd exon and the 3 untranslated regions of the bovine Osteocrin gene, respectively, using 413 Qinchuan cattle DNA samples. PCR-SSCP and DNA sequencing methods were specifically used. Three genotypes (AA, AC, and CC) were found at A85C; yet, only 2 genotypes (TC and CC) were found at T335C. Association analysis showed that both loci were associated with certain meat quality traits, including back fat thickness and loin muscle area. At the A85C locus, individuals with the CC genotype had greater back fat thickness. In comparison, at the T335C locus, individuals with the TC genotype had greater back fat thickness and a larger loin muscle area. Therefore, these 2 SNPs could be used as genetic markers to enhance Qinchuan cattle breeding programs.

Key words: Polymorphisms; Qinchuan cattle; Meat quality traits

INTRODUCTION

Skeletal muscle is a major organ involved in energy expenditure, contributing toward maintaining the homeostasis of glucose, lipid, and protein metabolism. Studies demonstrated that skeletal muscle also acts as an endocrine organ, producing bioactive molecules termed myokines, including interleukin (IL)-6, IL-15, insulin-like growth factor-binding protein-5 (IGFBP-5), and insulin-like growth factor-1 (IGF-1) (Musarò et al., 2001; Salih et al., 2004; Sell et al., 2006). Nishizawa et al. (2004) identified a novel skeletal muscle-derived secretory factor named musclin from skeletal muscles. The same protein was described as Osteocrin cloned from developing bones in another study (Thomas et al., 2003). In mice, Osteocrin mRNA is expressed almost exclusively in the skeletal muscle. The expression of this gene markedly decreases during fasting and increases upon feeding (Nishizawa et al., 2004). Furthermore, recombinant Osteocrin protein significantly attenuates insulin-stimulated glucose uptake and glycogen synthesis in C_2 $C1_2$ myocytes. In addition, Yusui et al. (2007) reported that Osteocrin expression is tightly regulated by nutritional status, and that its role might be linked to glucose metabolism. In general, the function of glucose metabolism is to provide fuel to most tissues and muscles inside the body, including the heart muscles, which logically need a continual source of energy to function normally. When glucose supply exceeds the needs of the body, it is often stored inside the liver and muscles in the form of glycogen, for future use. Excess glucose is also often converted to fatty acids, and mostly stored as body fat. Therefore, here, we studied the polymorphisms of the Osteocrin gene in association with the meat quality traits of Qinchuan cattle to provide baseline information to improve breeding programs.

MATERIAL AND METHODS

Animals, genomic DNA, and data collection

A total of 413 blood samples were obtained from female Qinchuan cattle originally from Shaanxi Province, China. The animals were aged from 12 months to 36 months. The blood samples were treated with 2% heparin, then stored at -80°C. Genomic DNA was extracted from the blood samples using standard procedures (Sambrook and Russell, 2002). Three traits were measured; specifically, loin muscle area (LMA), back fat thickness (BFT), and intra-muscular fat (IMF) (Gilbert et al., 1993; Rincon et al., 2009).

Primer design, PCR amplification, and DNA sequencing

Based on the bovine Osteocrin gene (GenBank accession No. AC_000158), 2 pairs of PCR primers were designed by the Primer Premier 5.0 software to amplify the DNA sequence from Osteocrin exon 3 and 3UTR (Table 1). Polymerase chain reaction amplifications were performed in 15 µL volume of PCR product containing 50 ng genomic DNA, 7.5 µL of 2X reaction mixture (including 200 µM dNTP, 10 mM Tris-HCl, 50 mM KCl, and 2 µL $MgCl_2$), 0.2 µM of each primer, and 0.5 U of Taq DNA polymerase. The PCR protocol was 95°C for 5 min, followed by 35 cycles of denaturing at 94°C for 30 s, annealing at ~45°C (Table 1) for 30 s, which was extended to 72°C for 30 s, with a final extension at 72°C for 10 min.

Table 1. Primers used in the experiments.

Name	Function	Primer sequence (5'-3')	Tm (°C)
H3	PCR	GGACTCACCTTTCTTGC	46.6
		GTGACCCAACTCTTTTGT	45
H5	PCR	GGTTGAACCGAATTGAC	45.7
		CAGTTAAGCCAAGTTGG	47.4

Single-strand conformation polymorphism (SSCP) and sequencing

PCR products were analyzed by SSCP. Aliquots of 4 μL of the above PCR products were mixed with 8 μL of the denaturing solution (95% formamide, 25mM EDTA, 0.025% xylene-cyanole, and 0.025% bromophenol blue), which were incubated at 98°C for 10 min, and then chilled on ice. Denatured DNA was loaded on an 8% PAGE in 1X TBE buffer, and then a constant voltage of 121V was applied for 14 h. The gel was stained with 0.1% silver nitrate, and visualized with 2% N_A OH solution (containing 0.1% formaldehyde), following the method described by Zhang et al. (2007). To confirm the results based on the PCR-SSCP technique, the PCR products from the reaction mixture with template DNA were sequenced in both directions. The DNAMAN (version 6.0) software was used to analyze the sequences.

Statistical analysis

The genotypic frequencies, allelic frequencies, heterozygosity (H_E), polymorphism information content (PIC), effective number of alleles (N_E), and Hardy-Weinberg equilibrium were calculated according to Nei and Roychoudhury (1974; Nei and Li, 1979) (Table 2). The SPSS software (13.0 Version) was used to analyze the relationship between different genotypes and measured traits in Qinchuan cattle. The statistical linear model used was $Y_{ijk} = \mu + A_i + G_j + MK + E_{ijk}$, where Y_{ijk} was the observation for the trait, μ was the overall population mean, A_i was the fixed effect of the age, G_j was the fixed effect of genotype, MK was the effect of breed, and E_{ijk} was the random error.

Table 2. Genotypic and allelic frequencies and genetic diversity of bovine Osteocrin A85C and T335C single nucleotide polymorphisms (SNPs).

SNP	Genotype	Frequencies	Allelic frequencies	χ^2	H_O	H_E	N_E	PIC
A85C	AA	0.3027 (138)	A (0.6331)	7.2136	0.5124	0.4876	1.8676	0.3687
	AC	0.5521 (201)	C (0.3669)					
	CC	0.1453 (73)						
T335C	CC	0.6804 (281)	C (0.8402)	14.9410	0.7315	0.2685	1.3671	0.2325
	TC	0.3196 (132)	T (0.1598)					

RESULTS AND DISCUSSION

In the current study, sequencing analysis revealed the presence of 2 SNPs, A85C and T335C, on exon3 and 3UTR of bovine Osteocrin. SSCP identified 3 genotypes (AA, AC, and CC) at A85C, but only 2 genotypes (TC and CC) at T335C (Figures 1-3). The TT genotype might have been absent from T335C either because 1) this genotype does not exist in this pop-

ulation or 2) the number of samples used was too limited. According to PIC classification (low polymorphism if PIC value <0.25, medium polymorphism if 0.25 < PIC value < 0.5, and high polymorphism if PIC value > 0.5), A85C exhibited medium polymorphism, whereas T335C exhibited low polymorphism. The genotype distributions of the experimental population did not agree with the Hardy-Weinberg equilibrium at any of the loci (P > 0.05).

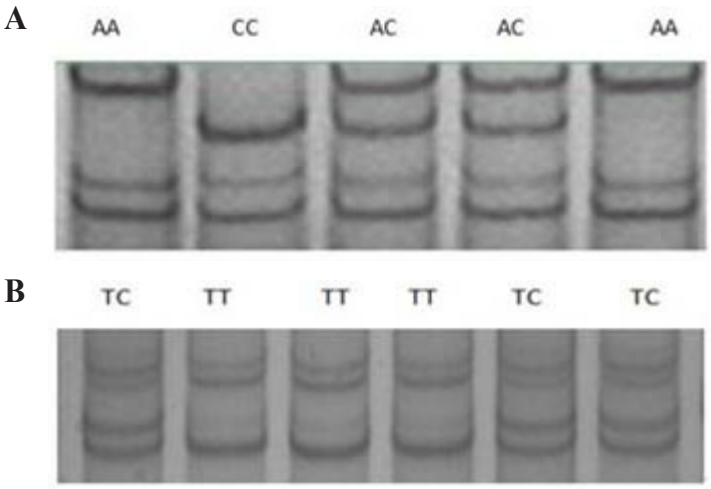

Figure 1. SSCP result showing the different genotypes of A85C (**A**) and T335C (**B**) single nucleotide polymorphisms.

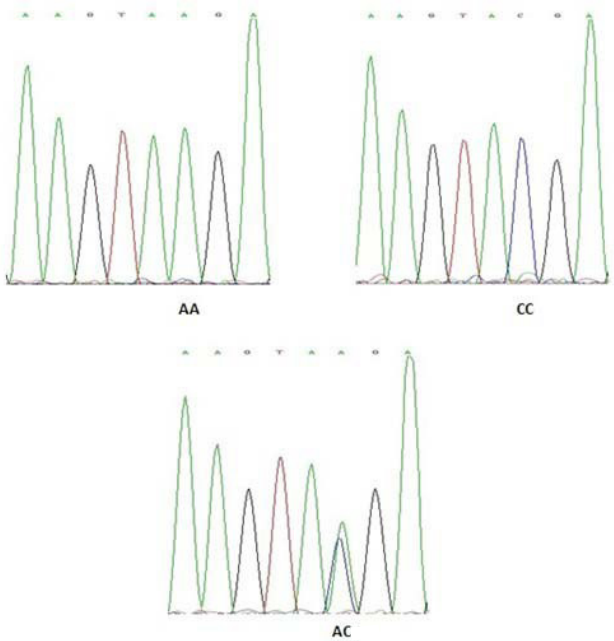

Figure 2. Sequencing map of the novel A85C single nucleotide polymorphism.

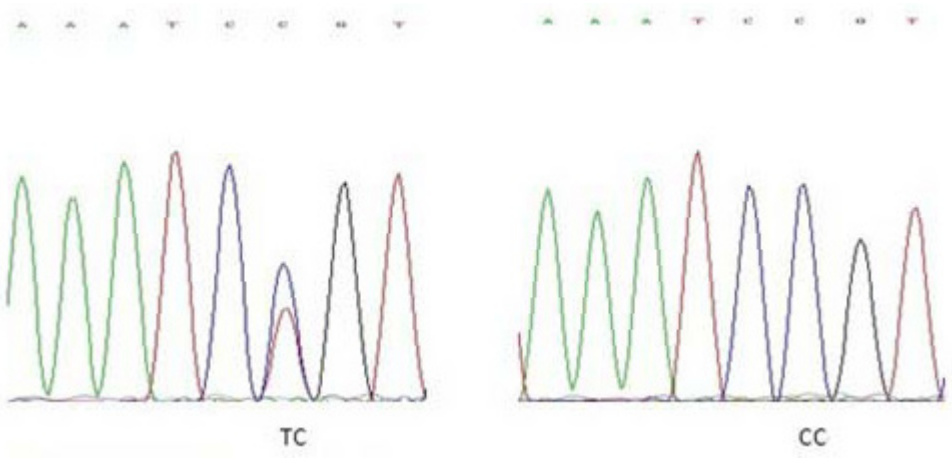

Figure 3. T335C single nucleotide polymorphism sequencing map.

Meat quality and quantity traits play an important role in the assessment of productivity and economic value of farm animals. However, the cattle are influenced by many factors, including genes. The Osteocrin gene might represent an important factor, as it is involved in the conversion of glucose to fatty acids via the regulation of glucose metabolism. The association of polymorphism with production traits has been reported by several studies previously published (Adoligbe et al., 2011; Ujan et al., 2011; Fu et al., 2013). The results of the current study showed that A85C SNP was associated with back fat thickness; whereas T335C was associated with both back fat thickness and loin muscle area (Table 3). A85C SNP is a synonymous mutation such that the produced amino acid sequence is not modified. However it was reported that the degeneracy of the genetic code enables the same amino acid sequences to be encoded and translated in many different ways (Kurland, 1991; Komar, 2007). Also it is well-established that the genome is highly redundant in terms of tRNA species for each amino acid; yet, it clearly under-represents a number of specific codons (Shah et al., 2008). Thus, the rate of Osteocrin gene protein expression might change during its synthesis if the change at the base of the third position of the codon is not represented by a corresponding anti-codon within the nuclear tRNA. In conclusion, the current study identified 2 novels SNPs related to cattle meat quality traits. However, further investigation is required before using these markers in marker assisted breeding programs.

Table 3. Association analysis of bovine Osteocrin A85C and T335C SNP locus with meat quality traits in Qingchuan cattle.

Locus	Genotypes	Lma (cm^2)	Bft (cm)	Imf (cm)
A85C	AA	48.1469 ± 4.37051	0.9069 ± 0.06602[b]	7.6654 ± 0.18168
	AC	49.7213 ± 4.60931	0.9125 ± 0.05833[b]	7.1329 ± 0.30701
T335C	CC	47.5867 ± 3.40275	0.9600 ± 0.10520[a]	7.7500 ± 0.30602
	CC	48.5925 ± 2.80076[b]	0.8850 ± 0.5372[b]	7.3019 ± 0.24264
	TC	53.9525 ± 5.64745[a]	0.9575 ± 0.6377[a]	7.6525 ± 0.17567

[a,b]Different superscripts were significantly different ($P < 0.05$).

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REFERENCES

Adoligbe C, Zan LS, Farogou S, Wang HB, et al. (2011). A novel polymorphism of the GDF_{10} gene and its association with body measurement traits in Chinese indigenous cattle. *Genet. Mol. Res.* 10: 988-995.

Fu CZ, Wang H, Mei CG, Wang JL, et al. (2013). SNPs at 3'-UTR of the bovine CDIPT gene associated with Qinchuan cattle meat quality traits. *Genet. Mol. Res.* 12: 775-782.

Gilbert RP, Bailey DR and Shannon NH (1993). Linear body measurements of cattle before and after 20 years of selection for postweaning gain when fed two different diets. *J. Anim. Sci.* 71: 1712-1720.

Komar AA (2007). Silent SNPs: impact on gene function and phenotype. *Pharmacogenomics* 8: 1075-1080.

Kurland CG (1991). Codon bias and gene expression. *FEBS Lett.* 285: 165-169.

Musarò A, McCullagh K, Paul A, Houghton L, et al. (2001). Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat. Genet.* 27: 195-200.

Nei M and Roychoudhury AK (1974). Sampling variances of heterozygosity and genetic distance. *Genetics* 76: 379-390.

Nei M and Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. U. S. A.* 76: 5269-5273.

Nishizawa H, Matsuda M, Yamada Y, Kawai K, et al. (2004). Musclin, a novel skeletal muscle-derived secretory factor. *J. Biol. Chem.* 279: 19391-19395.

Rincon G, Farber EA, Farber CR, Nkrumah JD, et al. (2009). Polymorphisms in the STAT6 gene and their association with carcass traits in feedlot cattle. *Anim. Genet.* 40: 878-882.

Salih DA, Tripathi G, Holding C, Szestak TA, et al. (2004). Insulin-like growth factor-binding protein 5 (Igfbp5) compromises survival, growth, muscle development, and fertility in mice. *Proc. Natl. Acad. Sci. U. S. A.* 101: 4314-4319.

Sambrook J and Russell DW (2002). Molecular Cloning. A Laboratory Manual. 3rd edn. Science Press, Beijing.

Sell H, Dietze-Schroeder D and Eckel J (2006). The adipocyte-myocyte axis in insulin resistance. *Trends Endocrinol. Metab.* 17: 416-422.

Shah JH, Maguire DJ, Munce TB and Cotterill A (2008). Alanine in HI: a silent mutation cries out! *Adv. Exp. Med. Biol.* 614: 145-150.

Thomas G, Moffatt P, Salois P, Gaumond MH, et al. (2003). Osteocrin, a novel bone-specific secreted protein that modulates the osteoblast phenotype. *J. Biol. Chem.* 278: 50563-50571.

Ujan JA, Zan LS, Wei SJ, Adoligbe C, et al. (2011). Meat tenderness and water holding capacity are associated with a 959AG mutation in the MyoG gene of Chinese indigenous cattle. *Afr. J. Biotechnol.* 10: 5654-5660.

Yusui A, Nishizawa H, Okuno Y, Morita K, et al. (2007). Foxo1 represses expression of musclin, a skeletal muscle-derived secretory factor. *Biochem. Biophys. Res. Comm.* 2: 358-365.

Zhang C, Wang Y, Chen H, Lan X, et al. (2007). Enhance the efficiency of single-strand conformation polymorphism analysis by short polyacrylamide gel and modified silver staining. *Anal. Biochem.* 365: 286-287.