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Short Communication

Genetic variants in *BMP8B* gene are associated with growth traits in Chinese native cattle

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ABSTRACT

As a signaling molecule, bone morphogenetic protein 8B (BMP8B) plays an essential role in bone metabolism and is able to regulate thermogenesis and energy balance, which suggests that *BMP8B* gene may be a new candidate for growth traits. Here, to characterize the effects of *BMP8B* gene on growth traits, we first used three Chinese indigenous cattle breeds (n = 845) to detect single nucleotide polymorphisms (SNPs). Five novel SNPs of *BMP8B* gene (g.−242C>T, g.2164C>T, g.2639T>C, g.2900C>G and g.10817C>T) were identified by DNA pool sequencing and forced PCR–RFLP. And then we associated the five SNPs with four growth traits (body weight, body length, heart girth, and hucklebone width). Results from association analysis showed that the SNPs 1, 2, and 3 affected growth trait(s) markedly ($P < 0.05$). Further, 6 combined haplotypes were constructed to guarantee the reliability of analysis results. There were also significant differences in body length, heart girth and body weight between the 6 combined haplotypes ($P < 0.05$), but not in hucklebone width ($P > 0.05$). Collectively, our results suggest a modulatory role of *BMP8B* gene in cattle growth and development, and 3 SNPs could be used as molecular markers in early marker assisted selection (MAS) in beef cattle breeding program.

1. Introduction

Bone morphogenetic proteins (BMPs) are multifunctional growth factors which belong to the transforming growth factor β (TGF-β) superfamily. The activity of BMPs was first identified by their ability to induce ectopic bone formation when implanted under the skin of rodents (Urist, 1965). To date, more than 30 BMP family members have been identified and characterized, accounting for almost one third of the TGF-β superfamily (Ducy and Karsenty, 2000). Although originally named for their osteogenic function, these secreted molecules have been revealed to regulate process as diverse as cell fate determination, proliferation, apoptosis, and differentiation during both embryogenesis and adulthood in extensive studies, so the historical term of BMPs may be inaccurate to depict this subfamily (David et al., 2009; Huang et al., 2013).

As an important member of BMPs, BMP8B regulates many biological processes. A new study in mouse shows that BMP8B is highly expressed in hypothalamus and brown adipose tissue (BAT), and it increases BAT thermogenesis through both central and peripheral actions (Whittle et al., 2012). And Whittle et al. found that *Bmp8*$^{-}$/$^{-}$ mouse exhibits impaired thermogenesis, reduced metabolic rate, and increased body weight despite hyperphagia. Studies in animals and humans indicate that mammal BAT, which consumes incredible energy to control body temperature, plays an important role in regulation of body weight (Cypess et al., 2009; Rosen and MacDougald, 2006; Saito et al., 2009; Van Marken Lichtenbelt et al., 2009). Thus it is reasonable to think that BMP8B can regulate body weight by BAT indirectly according to these findings. Besides that, BMP8B regulates cell proliferation, survival and/or differentiation in the process of placentation and is required for primordial germ cell (PGC) generation (Ying et al., 2000; Zhao and Hogan, 1996). In human, BMP8B, also called BMP8, plays a protective role in the glucocorticoid-induced apoptosis on bone cells, suggesting that it may be an essential player in bone metabolism (Kosa et al., 2011). Furthermore, BMP4 and BMP7, which could induce lipid accumulation in brown preadipocytes, were demonstrated to be involved in regulation of cattle growth traits as well (Huang et al., 2013; Tseng et al., 2008; Zhong et al., 2010). Consequently, we propose the hypothesis that the *BMP8B* gene is relevant to cattle growth traits based on this emerging evidence.

Abbreviations: Asp, aspartic acid; BAT, brown adipose tissue; bp, base pair(s); BMPs, bone morphogenetic proteins; GLM, General Linear Model; Hap, haplotype; *He*, observed heterozygosity; *Ho*, observed homozygosity; HWE, Hardy–Weinberg equilibrium; JX, Jiaxian cattle; LD, linkage disequilibrium; LE, linkage equilibrium; MAS, marker assisted selection; *Ne*, effective allele numbers; NY, Nanyang cattle; PCR–RFLP, polymerase chain reaction–restricted fragment length polymorphisms; PGC, primordial germ cell; *PIC*, polymorphism information content; QC, Qinchuan cattle; SE, standard error; SNP(s), single nucleotide polymorphism(s); TBE, a buffer solution containing a mixture of Tris base, boric acid and EDTA; TGF-β, transforming growth factor-beta.

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Table 1
Primers information for PCR amplification of bovine *BMP8B* gene.

Primer	Sequence	Tm	Position (ref. NC_007301)	Amplicon
P1	F:5′-GTCCATTCCTGGTTAGTTCAG-3′ F:5′-CGTGTTTGGCAAGGAGAT-3′	63.2 °C	112393648-112394694	1047 bp/exon 1, promoter
P2	F:5′-GTCAGCTACACGCCCAATC-3′ F:5′-GGTGCCTTCCAGACCTTATC-3′	66.8 °C	112395662-112396600	939 bp/exon 2, partial intron 1, 2
P3	F:5′-ATCAGTCCTGGGTTCAAGTCC-3′ F:5′-TCTATCAACCTCCTCACAGTCG-3′	66.3 °C	112396451-112397251	801 bp/exon 3, partial intron 2, 3
P4	F:5′-CTTCAGAGGAGGAAACCCA-3′ F:5′-CCATTAAGGTCAAGCAAAGG-3′	61.5 °C	112404545-112405230 112404545-112405230	686 bp/exon 4, partial intron 3, 4

Here we identified single nucleotide polymorphisms (SNPs) of *BMP8B* gene in Chinese indigenous breeds and carried out haplotype construction and association analysis, so as to contribute to the understanding of the role of BMP8B in cattle growth and development, which possibly provided some useful information on cattle breeding and genetics.

2. Materials and methods

2.1. DNA samples and data collection

A total of 845 blood samples were procured from three Chinese native cattle breeds, namely, Qinchuan (QC, n = 396), Jiaxian (JX, n = 365), and Nanyang (NY, n = 84). They are the main beef cattle breeds in China because of their superior growth and meat traits (Hua et al., 2011; Yang et al., 2012). These individuals were reared in Henan and Shaanxi province of China, and they were raised on a corn–corn silage diet after weaning at an average of 6 months.

Genomic DNA were extracted from these blood samples according to the standard procedures (Sambrook et al., 2001), and then they were all diluted to 50 ng/μL. Records of four growth traits of two year old QC cows (n = 298), including body weight, body length, heart girth, and hucklebone width, were collected following the method of Gilbert et al. for association analysis (Gilbert et al., 1993).

2.2. SNPs detecting

Three DNA pools were constructed by mixing all genomic DNA samples that from one breed together (Sham et al., 2002). To amplify the *BMP8B* gene from cattle genomic DNA, seven pairs of PCR primers (Table 1) were designed based on the published sequence of *Bos taurus BMP8B* gene (GenBank ID: 100296238).

Each PCR amplification was performed in 25 μL reaction mixture containing 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 U Taq DNA polymerase (MBI, Lithuania). The cycling protocol was as follows: 5 min at 95 °C, 35 cycles at 94 °C for 30 s, annealing at selected temperatures for 30 s, and 72 °C for 45 s, with a final extension at 72 °C for 10 min.

The PCR products were commercially sequenced using ABI 3730 sequencer (ABI, Foster City, CA, USA) to screen the variations within the amplified regions in both directions.

2.3. Genotyping

Five new pairs of primers were redesigned for enzymatic mutation detection depending on the sequencing results. Detailed information about the primers is given in Table 2. The SNPs 1, 2, 3, and 4 were genotyped by a forced PCR–RFLP method, which required mismatches changed in primers for creating restriction sites. And the SNP 5 was genotyped by PCR–RFLP method, which didn't require mismatches.

Aliquots of 10 μL PCR products were digested with endonuclease (MBI Fermentas) following the manuals, respectively. And then the digested PCR products were detected by electrophoresis on a 3.5% agarose gel stained with ethidium bromide in 1× TBE buffer and constant voltage (120 V) for 0.5–1 h. Finally, of each banding pattern, six samples were randomly taken for sequencing validation.

2.4. Data analyses

Software and online platform were routinely exploited in statistical analysis. Hardy–Weinberg equilibrium (HWE) and the population genetic indices, such as effective allele numbers (*Ne*), gene observed heterozygosity (*He*), and observed homozygosity (*Ho*), were calculated with POPGENE software (version 1.31). Genotypic and allelic frequencies, linkage disequilibrium (LD), and haplotypes (Hap) were analyzed by SHEsis online platform (Li et al., 2009).

Polymorphism information content (*PIC*) was directly figured out in the light of Nei's method (Nei and Roychoudhury, 1974). The relationship between the genotypes and growth traits of QC was analyzed utilizing General Linear Model (GLM) of PASW Statistics (version 18). The linear model:

$$Y_{ijk} = \mu + G_j + E_{ijk},$$

Table 2
Genetic variants identified in bovine *BMP8B* gene.

SNPs[a]	Location	Mutation type	Primer sequence[b]	Tm	Endonuclease	PCR–RFLP pattern
SNP 1 g.−242C>T	5′-Flanking regulatory region	Non-coding	F:5′-AATCCAAAGTGGTCTCTCCCTCCCGGC-3′ R:5′-CCGTGCTGTGGGTTTGTGAAAGCGA-3′	68.0 °C	*Hae* III	186 / 160 + 26 bp
SNP 2 g.2164C>T	Intron 1	Non-coding	F:5′-GAGCAGTCCAACAGGTGCCGG-3′ R:5′-AAACTTTCTTCTCTGAGGGT-3′	58.6 °C	*Hae* III	148 / 127 + 21 bp
SNP 3 g.2639T>C	Exon 3	Silent	F:5′-ACGAGGGCTGGCTGGTGGTCGA-3′ R:5′-AAATGAGGGGGCTCCAGCTGTGT-3′	67.7 °C	*Sal* I	189 / 171 + 18 bp
SNP 4 g.2900C>G	Intron 3	Non-coding	F:5′-TTCCTCAGTGCGGGACTGCA-3′ R:5′-TGCAGGGTTTCTAGTCCCACT-3′	66.0 °C	*Pst* I	156 / 136 + 20 bp
SNP 5 g.10817C>T	Intron 4	Non-coding	F:5′-ATCTTCGGTGAGCCTGGGGATGGAGGC-3′ R:5′-AGAGACCAGGGCGAGGCCTGCTGATT-3′	66.0 °C	*Hae* III	161 + 16 + 46 / 145 + 16 + 46 bp

[a] SNPs: single nucleotide polymorphisms.
[b] The underlined bases show mismatches changed for creating restriction sites.