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A novel polymorphism of the GDF_{10} gene and its association with body measurement traits in Chinese indigenous cattle

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ABSTRACT. Body measurement traits are known to play numerous important roles in the assessment of productivity and economic value. They are influenced by several factors, among which genetic factors are predominant. The gene GDF_{10} is involved in skeletal morphogenesis and is associated with body measurement traits. It may be an important candidate gene for marker-assisted selection. We used the PCR-SSCP technology to examine a possible association of the single nucleotide polymorphism (SNP) (G142A) of the bovine GDF_{10} gene with body measurement traits in 417 animals belonging to six different Chinese cattle populations: Xue long (Xl), Luxi (Lx), Qinchuan (Qc), Jiaxian red (Jx), Xianang (Xn), and Nanyang (Ny). In the Jx population, least squares analysis revealed significant effects on hip width, chest depth and chest circumference. The animals with the GG genotype had higher mean values than those

with the GA genotype for all three traits. We conclude that the SNP of the GDF_{10} gene could be a very useful genetic marker for body traits in Jx cattle reproduction and breeding.

Keys words: Cattle; GDF_{10} gene; SNP; Body measurement traits

INTRODUCTION

The economic importance of body measurement trait (BMT) in cattle breeding has prompted several attempts to identify individual factors associated with their variation. Results have generally indicated that genetic factors are predominant, and for many genes, a correlation exists between genotypic and BMT variation. Growth differentiation factor 10 (GDF_{10}) is one of the important members of the transforming growth factor (TGF) superfamily, a group of multifunctional proteins that are regulators of cell growth and differentiation in both embryonic and adult tissues (Hino et al., 2004). Previous studies in mice allowed GDF_{10} mRNA detection in both neonatal and adult bone samples with higher levels being detected in calvaria than in long bone (Cunningham et al., 1995; Takao et al., 1996). These results suggest that the GDF_{10} gene may play multiple roles in regulating cell differentiation events and skeletal morphogenesis. In order to determine the biological function of GDF_{10}, Zhao et al. (1999) carried out a detailed analysis of the expression pattern of GDF_{10}. They found that during embryogenesis GDF_{10} is prominently expressed in developing skeletal structures both in the craniofacial region and in the vertebral column. Besides, Galdones and Hales (2008) suggested that GDF_{10} may have novel uncharacterized roles in transducing teratogenic signals in the limb. Furthermore, they localized GDF_{10} by *in situ* hybridization (ISH) to an area of programmed cell death in the limb. To our knowledge, there has been no research on the bovine GDF_{10} gene until now. Based on the role of GDF_{10} in bone morphogenesis as determined in mice and humans, GDF_{10} could be an attractive candidate gene for BMTs in bovine genetic improvement. Therefore, the objective of this study was to detect single nucleotide polymorphisms (SNPs) of the GDF_{10} gene in different Chinese indigenous cattle populations and explore their possible association with BMTs.

MATERIAL AND METHODS

Animals and DNA isolation

A total of 417 female animals stratified into age categories of 12 to 36 months, comprising Xue long (Xl) cattle (N = 50, Dalian); Luxi (Lx) cattle (N = 62, Shandong); Qinchuan (Qc) cattle (N = 148, Shaanxi); Jiaxian red (Jx) cattle (N = 71, Henan); Xianang (Xn) cattle (N = 38, Henan), and Nanyang (Ny) cattle (N = 48, Henan Province), were randomly selected from breeding populations and used to determine the GDF_{10} allelic frequencies. The traits measured, as described previously (Gilbert et al., 1993), include body length (BL), withers height (WH), hip height (HH), rump length (RL), hip width (HW), chest depth (CD), and chest circumference (CC). In order to minimize systematic error, the same person was assigned to measure one of the seven traits in all animals.

Blood samples were obtained from the 417 animals and treated with 2% heparin and

then stored at -80°C. DNA was extracted from blood samples according to standard procedures (Sambrook and Russell, 2002).

Primers and polymerase chain reaction (PCR) conditions

Based on the bovine GDF_{10} gene (GenBank accession No. NC_007329.3), one pair of PCR primers (forward: 5' CCGCAGGGTCTGTTCTCA 3' and reverse: 5' CCTTCGGCTGTA TTTCTCA 3') was designed to amplify a 358-bp PCR product in exon 1.

PCR amplifications were performed in a 20-μL reaction mixture containing 50 ng template DNA, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM $MgCl_2$, and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The cycling protocol was 5 min at 95°C, 32 cycles of 94°C for 30 s, 56°C annealing for 30 s, and 72°C for 30 s, with a final extension at 72°C for 10 min. PCR products were electrophoresed on 1.5% agarose gels (containing 200 ng/mL ethidium bromide) using 1X TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA).

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, 25 mM EDTA, 0.025% xylene-cyanole, and 0.025% bromophenol blue), incubated at 98℃ for 10 min and then chilled on ice. Denatured DNA was loaded on an 8% PAGE in 1X TBE buffer, and a constant voltage of 121 V was applied for 14 h. The gel was stained with 0.1% silver nitrate and visualized with 2% NaOH solution (containing 0.1% formaldehyde) according to 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 analyses

The parameters genotypic frequencies, allelic frequencies, Hardy-Weinberg equilibrium, gene homozygosity (Ho), gene heterozygosity (He), effective allele numbers (Ne), and polymorphism information content (PIC) were statistically analyzed according to the previous approaches of Nei and Roychoudhury (1974) and Nei and Li (1979). The association between SNP marker genotypes of the GDF_{10} gene and records of BMTs (BL, WH, HH, RL, HW, CD, CC) was analyzed by the SPSS software (version 17.0) according to the following statistical linear model:

$$Y_{ijk} = \mu + G_j + A_i + E_{ijk}, \quad \text{(Equation 1)}$$

where Y_{ijk} is the observation for the BMTs, μ is the overall mean for each trait, G_j is the genotype effect, A_i is the fixed effect of age, and E_{ijk} is the random error.

RESULTS

PCR-SSCP analysis of the GDF_{10} gene

Using the PCR-SSCP method, the entire exon 1 of the GDF_{10} gene, 358 bp long (Fig-

ure 1), was amplified in all the experimental animals and exhibited two different patterns. We labeled the pattern with two bands GG and the pattern with three bands GA (Figure 2). The sequencing map of the novel SNP of the bovine GDF_{10} exon 1 region revealed a G > A synonymous mutation at 142 bp (Figure 3).

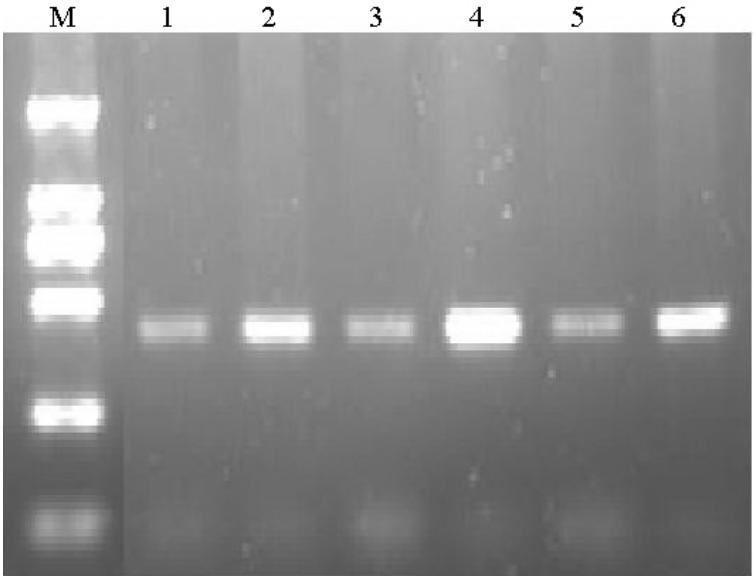

Figure 1. The PCR product of GDF_{10} gene exon 1. M = DNA marker; *lanes 1-6* = PCR products of GDF_{10} gene exon 1.

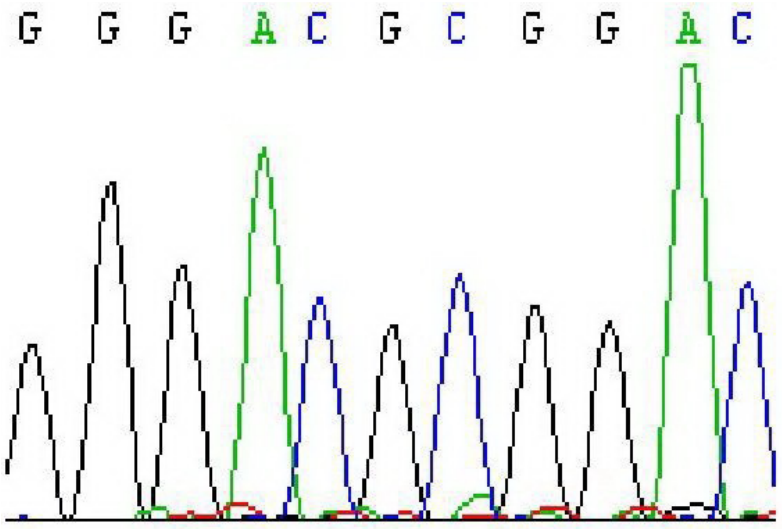

Figure 2. Electrophoretic patterns of PCR-SSCP exon 1 of bovine GDF_{10}. *Lanes 2*, *3*, *4*, and *5* = GG genotype; *lanes 1* and *6* = GA genotype.

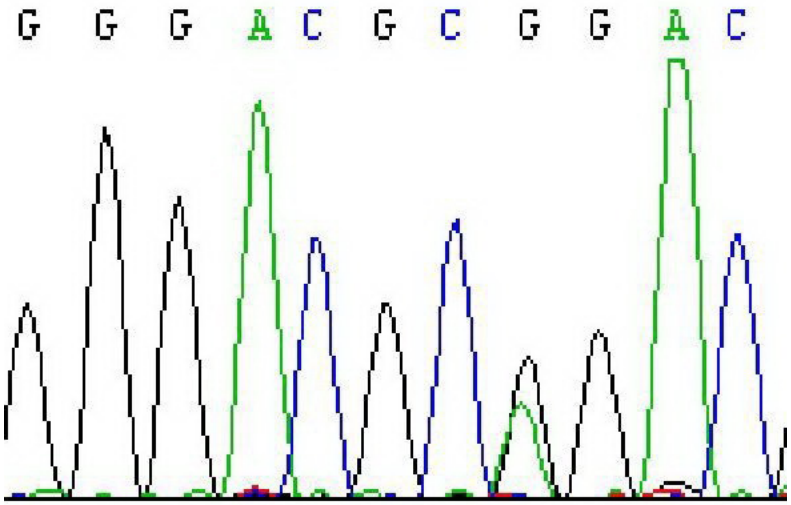

Figure 3. The sequencing map of the novel SNP of bovine GDF_{10} exon 1 region.

Genetic polymorphism of bovine GDF_{10} gene and χ^2 test

Sequence analysis of the GDF_{10} exon 1 revealed a G > A synonymous mutation at the 142-bp position of the amplified product. The genetic diversity of the locus was then calculated (Tables 1 and 2). The results suggested that the mutant allele A is present in five breeds and had lower frequency compared with the wild allele G in these populations. GG genotypic frequency ranged from 0.5968 (Lx) to 1.0000 (Ny), and the GA genotypic frequency, absent in the Ny population, ranged from 0.1200 (Xl) to 0.4032 (Lx). He, Ne and PIC of the Lx and Jx cattle populations in the locus were higher than in the other populations, which implies that the polymorphism and genetic variation of Lx and Jx cattle were higher than that of the others. According to the classification of PIC (low polymorphism if PIC value <0.25, medium polymorphism if 0.25 < PIC value < 0.5, and high polymorphism if PIC value >0.5), Lx and Jx showed a medium polymorphism level, whereas Xl, Qc and Xn showed a low polymorphism level. The χ^2 test showed that genotype distributions in all the population examined agreed with Hardy-Weinberg equilibrium (P > 0.05) (Table 1).

Table 1. Genotypic and allelic frequencies (%) in the GDF_{10} gene exon 1 region in different bovine populations.

Population	Genotypic frequencies		Total	Allelic frequencies		χ^2 (HWE)
	GG	GA		G	A	
Xl	0.8800 (44)	0.1200 (06)	50	0.9400	0.0600	0.5747
Lx	0.5968 (37)	0.4032 (25)	62	0.7984	0.2016	2.7554
Qc	0.7905 (117)	0.2095 (31)	148	0.8953	0.1047	1.0598
Jx	0.6056 (43)	0.3944 (28)	71	0.8028	0.1972	3.0844
Xn	0.7105 (27)	0.2895 (11)	38	0.8553	0.1447	0.2400
Ny	1.0000 (48)	0.0000 (00)	48	1.0000	0.0000	0

HWE = Hardy-Weinberg equilibrium; Xl = Xue long; Lx = Luxi; Qc = Qinchuan; Jx = Jiaxian red; Xn = Xianang; Ny = Nanyang.

Table 2. Population genetic indices in the GDF_{10} exon 1 region.

Population	Gene homozygosity	Gene heterozygosity	Effective allele numbers	Polymorphic information content
Xl	0.8872	0.1128	1.1271	0.1064
Lx	0.6781	0.3219	1.4748	0.2701
Qc	0.8125	0.1875	1.2308	0.1699
Jx	0.6834	0.3166	1.4633	0.2665
Xn	0.7524	0.2476	1.3290	0.2169
Ny	1.0000	0.0000	1.0000	0.0000

For population abbreviations, see legend to Table 1.

Effect of the GDF_{10} gene genotypes on BMT

Seven BMTs were analyzed by comparison between genotypes of 417 individuals and their phenotypic data. The results of association analysis of the gene-specific marker are shown in Table 3. There were significant effects on HW and CD ($P < 0.01$) and CC ($P < 0.05$) in the Jx cattle population.

Table 3. Least square means and standard errors (mean ± SE) of the body measurement traits obtained for the genotypes of the GDF_{10} gene polymorphism in Jx cattle population.

Genotype	Traits (cm, mean ± SE)						
	BL	WH	HH	RL	HW	CD	CC
GG	137.605 ± 3.568	125.843 ± 2.324	126.433 ± 2.699	43.910 ± 1.653	41.345 ± 1.537^{A}	64.900 ± 2.134^{A}	175.119 ± 2.948^{a}
GA	135.186 ± 3.463	124.090 ± 2.256	123.938 ± 2.619	42.271 ± 1.604	38.488 ± 1.492^{B}	60.443 ± 2.071^{B}	171.071 ± 2.861^{b}
P	0.325	0.274	0.181	0.152	0.003	0.003	0.049

BL = body length; WH = withers height; HH = hip height; RL = rump length; HW = hip width; CD = chest depth; CC = chest circumference. a,b = means with different superscripts were significantly different ($P < 0.05$); A,B = means with different superscripts were significantly different ($P < 0.01$).

DISCUSSION

The TGF superfamily is a group of multifunctional proteins that are regulators of cell growth and differentiation in both embryonic and adult tissues (Cunningham et al., 1995; Hino et al., 1999).

GDF_{10}, also known as BMP-3b and closely related to bone morphogenetic protein 3 (BMP3), is one of the important members of this superfamily of proteins. It can induce endochondral bone formation (Wozney et al., 1988). The results of Cunningham et al. (1995) in previous studies in mice suggest that GDF_{10} may play multiple roles in regulating cell differentiation events, including those involved in skeletal morphogenesis. GDF_{10} has been discovered in rat and human femur tissue (Hino et al., 1996, 1999). Moreover, GDF_{10} has been localized by ISH to an area of programmed cell death in the limb (Galdones and Hales, 2008). Therefore, GDF_{10} may influence BMT.

In this study, the possible relationship between GDF_{10} polymorphism and BMTs was evaluated by using blood samples from 417 cattle belonging to seven different cattle populations. The data revealed that the mutant heterozygous genotype GA is present at low frequencies in five populations. This may be an observation of the occurrence of low frequency of allele A due to allelic drift. In addition, in the Ny population, no GA genotype was found. There are two possible explanations, either GA genotype does not exist in this population or

the number of samples used was limited. Among the seven populations included in the study, comparison of BMT between individuals with genotype GG and GA revealed significant effects on HW and CD ($P < 0.01$) and CC ($P < 0.05$) only in the Jx cattle population. Similar results were found with bovine growth differentiation factor 5 (GDF_5), a gene belonging to the same family (Liu et al., 2010). The fact that an association of GDF_{10} SNP (G142A) with BMT was revealed only in the Jx cattle population suggests that it may be correlated with other direct or indirect factors specific to this cattle population. Individuals with genotype GG have superior BMT, which indicates that allele G may be the beneficial allele for BMT in the Jx cattle population. Allele A could be a recent mutation, where the presence of the A allele in the heterozygous individuals significantly decreases their BMT. The novel SNP (G142A) could result in a synonymous mutation in GDF_{10} protein. 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). We know that the genome is highly redundant in terms of tRNA species for each amino acid but enigmatically under-represents a number of specific codons (Shah et al., 2008). Thus, in the synthesis of GDF_{10} 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 GDF_{10} protein could change.

In the TGF-β superfamily, GDF_5 and BMP4 were reported to be associated with body measurement traits in cattle by Liu et al. (2010) and Zhong et al. (2010), respectively.

Many studies have been conducted on the GDF_{10} gene in humans and mice, but few of them were related to its association with body measurement traits. Until now, no research in cattle or other livestock has been reported. Therefore, considering the evolutionary conservation between cattle and humans, considering the relationship with regard to the BMP/GDF family gene, we applied the results of the research cited above to determine the polymorphism and genetic effect of cattle GDF_{10} gene exon 1.

In conclusion, the present study revealed a novel SNP in GDF_{10} gene exon 1. The SNP is significantly associated with HW, CD, and CC in the Jx cattle population, with the GG genotype being favorable compared to the GA genotype. Our results provide evidence that the GDF_{10} gene may have potential effects on body measurement traits in the Jx cattle population. Therefore, further study will be necessary to use the SNP for marker-assisted selection in a larger population.

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