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Full Length Research Paper

Molecular characterization, polymorphism of growth differentiation factor 5 gene and association with ultrasound measurement traits in native Chinese cattle breeds

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Growth differentiation factor 5 (*GDF5*), involved in the development and maintenance of bone and cartilage, is an important candidate gene for growth and carcass traits selection through marker-assisted selection (MAS). Genomic structural analysis showed that bovine *GDF5* shares much similarity with human *GDF5*. The latest findings demonstrate that the single nucleotide polymorphism (SNP) T586C in exon 1 is significantly associated with ultrasound marbling score (UMAR) and ultrasound backfat thickness (UBF). Furthermore, the analysis of T586C SNP marker shows there are significant effects on the UBF (P = 0.0498) and on the UMAR (P = 0.0058) in 465 individuals. These results clearly suggest that the *GDF5* gene is among target genes for carcass traits in bovine reproduction and breeding.

Key words: Cattle, GDF5 gene, ultrasound measurement, polymorphism, association analysis.

INTRODUCTION

Growth differentiation factor 5 (*GDF5*), known as cartilage-derived morphogenetic protein 1, (*CDMP1*), is a member of the transforming growth factor-b (*TGF-b*) super family and also closely related to the subfamily of bone morphogenetic proteins (*BMPs*) (Miyamoto et al., 2007). *GDF5* was identified as a growth factor involved in skeletal development and joint morphogenesis in humans and mice playing a crucial role in the morphogenesis of tendons, ligaments and bones (Chabra et al., 2003; Coleman and Tuan, 2003; Nakamura et al., 2003; Chen et al., 2006). The expression of *GDF5* was observed in the regions between skeletal elements where joints later form (Storm and Kingsley, 1996; Thomas et al., 1996). It plays a crucial role in the morphogenesis of tendon, ligament and bone. A null mutation of *GDF5* could cause developmental failure of skeletal structure and intra-articular ligaments in mice (Harada et al., 2007; Masuya et al., 2007). Besides, *GDF5* can express in the regions of future joints during early development, which indicates that it could be involved in joint formation (Francis-West et al., 1999; Merinoa et al., 1999). Previous reports have demonstrated that the mutants of *GDF5* in both mouse and human *GDF5* led to abnormal joint development (Storm and Kingsley, 1996; Thomas et al., 1996). There are observations of thick and enlarged cartilage compo-

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Abbreviations: ***GDF5*,** Growth differentiation factor 5; **MAS,** marker-assisted selection; **SNP,** single nucleotide polymorphism; **UMAR,** ultrasound marbling score; **UBF,** ultrasound backfat thickness; ***CDMP1*,** cartilage-derived morphogenetic protein 1; ***TGF-b*,** transforming growth factor-b; ***BMPs*,** bone morphogenetic proteins; **ULMA,** ultrasound longissimus muscle area; **NCBI,** national center for biotechnology information; **PCR,** polymerase chain reaction; **RFLP,** restriction fragment length polymorphism; **CDS,** coding sequence.

#These Authors contributed equally to this paper.

nents of the appendicular skeleton in *GDF5* transgenic mice (Kellgren and Moore, 1952; Oliveria et al., 1995), which further provides the evidence of the chondrogenic possibility of *GDF5 in vivo* (Erlacher et al., 1998; Wolfman et al., 1997). In humans, *GDF5* is present in adult articular cartilage and able to stimulate proteoglycan synthesis in articular cartilage explants (Erlacher et al., 1998).

Especially, we found a single nucleotide polymorphism (SNP) in exon 1 that is significantly associated with body length, hip height and heart girth in Chinese cattle (Liu et al., 2009). Based on the important roles of the *GDF5* in chondrogenesis, differentiation and proliferation of bone as determined in mouse and human *GDF5* could be an attractive candidate gene for growth and carcass traits in bovine. Therefore, the objective of this study is to analyze molecular characterization of bovine *GDF5* gene and to explore their possible association with ultrasound measurement traits in native Chinese cattle breeds.

MATERIALS AND METHODS

DNA samples and data collections

For the gene variants identified, purebred and crossbred individuals representing 7 breeds including Qinchuan (QC, n = 93, Shaanxi province of China), Qinchuan improvement steers (QI, n = 96, Shaanxi province of China), Nanyang (NY, n = 38, Henan province of China), Jiaxian red (JR, n = 71, Henan province of China), Xia'nan (XN, n = 52, Henan province of China), Luxi (LX, n = 67, Shandong province of China), Simmental and Luxi crossbred steers (SL, n = 48, Shandong province of China) were randomly selected. Meanwhile, the following traits, ultrasound marbling score (UMAR), ultrasound longissimus muscle area (ULMA) and ultrasound backfat thickness (UBF) were measured (Sherman et al., 2008; Nkrumah et al., 2007). For each of the ultrasound measurement traits, we measured the same one trait with the same people to minimize error.

DNA samples were extracted from leukocytes and tissue samples using standard phenol–chloroform protocol (Mullenbach et al., 1989).

Sequence analysis

The different species of *GDF5* gene amino acid sequences were acquired from the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov), including *Bos taurus* (GenBank Accession No. XP_588072.1), *Homo sapiens* (GenBank Accession No. NP_000548.1), *Mus musculus* (GenBank Accession No. NP_032135.1), *Rattus norvegicus* (GenBank Accession No. XP_001066344.1), *Pan troglodytes* (GenBank Accession No. XP_001164592.1), *Macaca mulatta* (GenBank Accession No. XP_001099702.1), *Canis familiaris* (GenBank Accession No. XP_542974.1), *Equus caballus* (GenBank Accession No. NP_001075989.1), *Gallus gallus* (GenBank Accession No. NP_989669.1), *Xenopus laevis* (GenBank Accession No. NP_001086466.1). Bovine *GDF5* gene was compared with the corresponding protein of other species. The translated polypeptide sequences were aligned using ClustalX software with the default parameters. Phylogenetic trees were constructed using the MEGA program (http://www.megasoftware.net/).

Polymerase chain reaction (PCR) amplification and genotyping of MvaI *GDF5* allele by PCR-restriction fragment length polymorphism (RFLP)

According to the sequence of bovine *GDF5* gene (GenBank accession No. NC_007311), one pair of primers (5'- TGT CCG ATG CTG ACA GAA AGG -3' and 5'- GAG TGA GGT TAA TCC CAG ATA CCA -3') was designed to amplify a 235 bp product of the *GDF5* exon 1 and its intron region. PCR amplifications were performed in 20 µl reaction mixture containing 50 ng mixed DNA template (DNA template was mixed 9 DNA samples from 9 different breeds with the same volume, respectively), 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 PCR protocol was 95°C for 5 min followed by 32 cycles of 94°C for 30 s, 60°C annealing for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. The products were purified by using a Wizard Prep PCR purification kit (Shanghai Bioasia Biotechnology, P. R. China) and sequenced (Beijing Aolaibo Biotechnology, P. R. China; Applied Biosystems 3730 xl DNA sequencer, Foster city, CA, USA).

Aliquots of 20 µl PCR products were digested with 10 U MvaI (MBI, Fermentas) at 37°C for 5 h following the supplier's manual. The digested products were detected by electrophoresis in 2.5% agarose gel stained with ethidium bromide. To confirm the results based on the PCR-RFLP technique, the PCR products from the mix DNA template were sequenced in both directions (Beijing Aolaibo Biotechnology, P. R. China; Applied Biosystems 3730 xl DNA sequencer, Foster city, CA, USA).

Statistical analysis

The association between SNP marker genotypes of the *GDF5* gene and records of ultrasound measurement traits (UMAR, ULMA and UBF) were analyzed by the least-squares method as applied in the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC, USA), and according to the following statistical linear model:

$$Y_{ijkl} = \mu + G_i + S_j + BF_k + Ma_l + e_{ijkl},$$

Where, Y_{ijkl} is the observed ultrasound measurement trait; µ is overall mean for each trait; G_i the genotype effect; S_j the fixed effect of sex; BF_k the fixed effect of breed and farm; Ma_l the regression variable for measure age; ε_{ijkl}, the random environment effect.

RESULTS

Sequence alignments and inferred phylogenetic tree

A GenBank database search using basic local alignment search tool (BLAST) revealed that bovine *GDF5* amino acid sequence shared high similarity with other species *GDF5* protein sequences, with 95% identity with human and 91% identity with mouse, respectively. Hitherto, the phylogenic relationship among all the characterized members of the *GDF5* gene was illustrated according to the phylogenetic distance calculated by the MEGA4.0.1 program (Figure 1). The phylogenetic tree analysis was employed to find the positions of bovine *GDF5* in relation to a selection of other animals; *GDF5* of bovine, horse, dog, human, troglodyte and macaque, fell into one evolutionarily related group, except for mouse, rat, chicken and

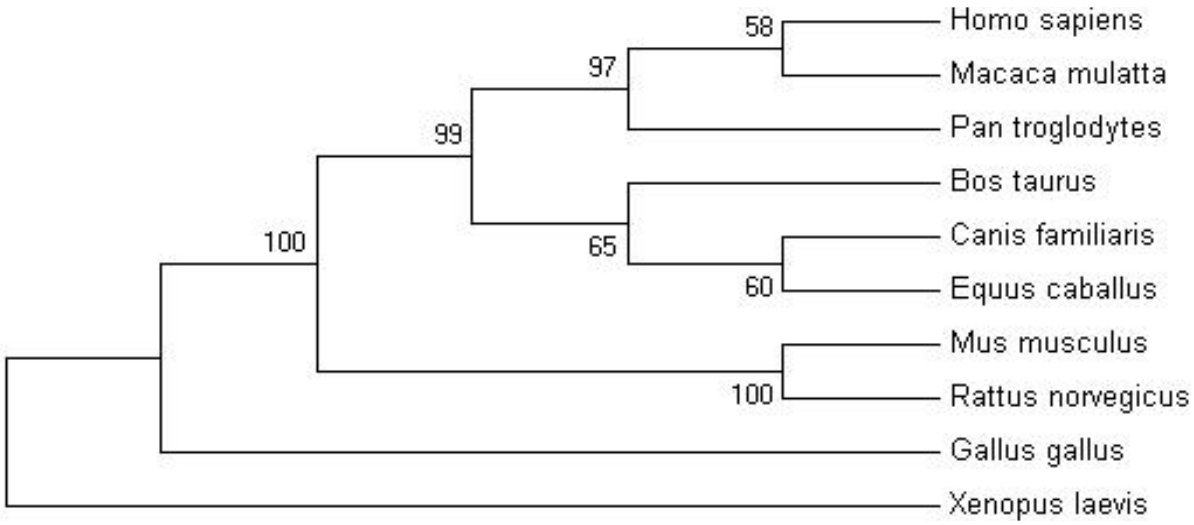

Figure 1. Phylogenetic tree of *GDF5* gene in different species. The bootstrap confidence values are shown at the nodes of the tree. The horizontal branch lengths are proportional to the estimated divergence of the sequence from the branch point.

toad. Like their orthologous genes in horse, dog, human, troglodyte, macaque, mouse, rat, chicken and toad, bovine *GDF5* gene shows the highest homologies in their carboxyl termini, whereas the amino termini are less conserved. These conserved amino acid residues are indicated with asterisks in Figure 2.

Genetic variation and association analysis

We further analyzed the association of T586C SNP of bovine *GDF5* with genotype of unrelated animals from 7 different bovine breeds, including Qinchuan, Qinchuan improvement steers, Nanyang, Jiaxian red, Xia'nan, Luxi and Simmental and Luxi crossbred steers. Three ultrasound measurement traits were analyzed by comparison of the genotypes of 465 individuals and their phenotypic data. The results of association analysis of the gene-specific SNP marker are shown in Table 1. At the T586C SNP marker, there are significant effects on the UBF ($P = 0.0498$) and on the UMAR ($P = 0.0058$) in the total population. Animals with the genotype TC had higher mean values for UBF than those with the TT genotype ($P < 0.05$); and animals with the genotype TC had higher mean values for UMAR than those with the CC genotype ($P < 0.01$). In the seven populations, there are significant effects on some body measurement traits, but the data are not shown because the individuals are fewer.

DISCUSSION

According to bovine *GDF5* coding sequence (CDS) domain, the comparison with the genome structure of human *GDF5* demonstrates a remarkable similarity between the two species. The CDS region and the putative amino acid sequence of bovine *GDF5* share 93 and 95% identity to that of human, respectively. Phylo-genetic trees analysis also indicated that *B. taurus, E. caballus, C. familiaris*, *H. sapiens*, *P. troglodytes* and *M. mulatta*, *GDF5* protein sequences share higher homology than other species.

The ultrasound measurement traits are affected by many factors, such as genotype, sex, age, breed, herd, location and other random environmental factors. However, we have established one new statistical model in which the three factors (breed, herd, and location) were involved and then we employed the least-squares method in GLM procedure of SAS software to do the related analysis, and no significant difference ($P > 0.05$) was found (data not shown).

For the T586C SNP marker at bovine *GDF5* gene (Liu et al., 2009), there are significant effects on the UBF and UMAR in 465 individuals (Table 1). Moreover, the T>C synonymous mutation of leucine, results in the change of the part of phenotypic variation, especially on the UBF and UMAR phenotypes in cattle breeds. Therefore, we assumed that the mutation for T586C could have important influence on many minor genes involve in ultrasound backfat thickness and ultrasound marbling score in bovine. Liu et al. (2009) found that there were significant effects on the body length in the *B. taurus* and *B. indicus* × *B. taurus* populations ($p < 0.05$); and that animals with the genotype TT had lower mean values for body length and hip width than those with the TC and CC genotype ($p < 0.01$) in Chinese cattle breeds (Liu et al., 2009). Although no available data have been reported measurement traits for bovine and other livestock, on association of *GDF5* gene variants with ultrasound proliferation and differentiation of bone and chondrogenesis, as determined in mouse and human (Edwards and Frances, 2001;

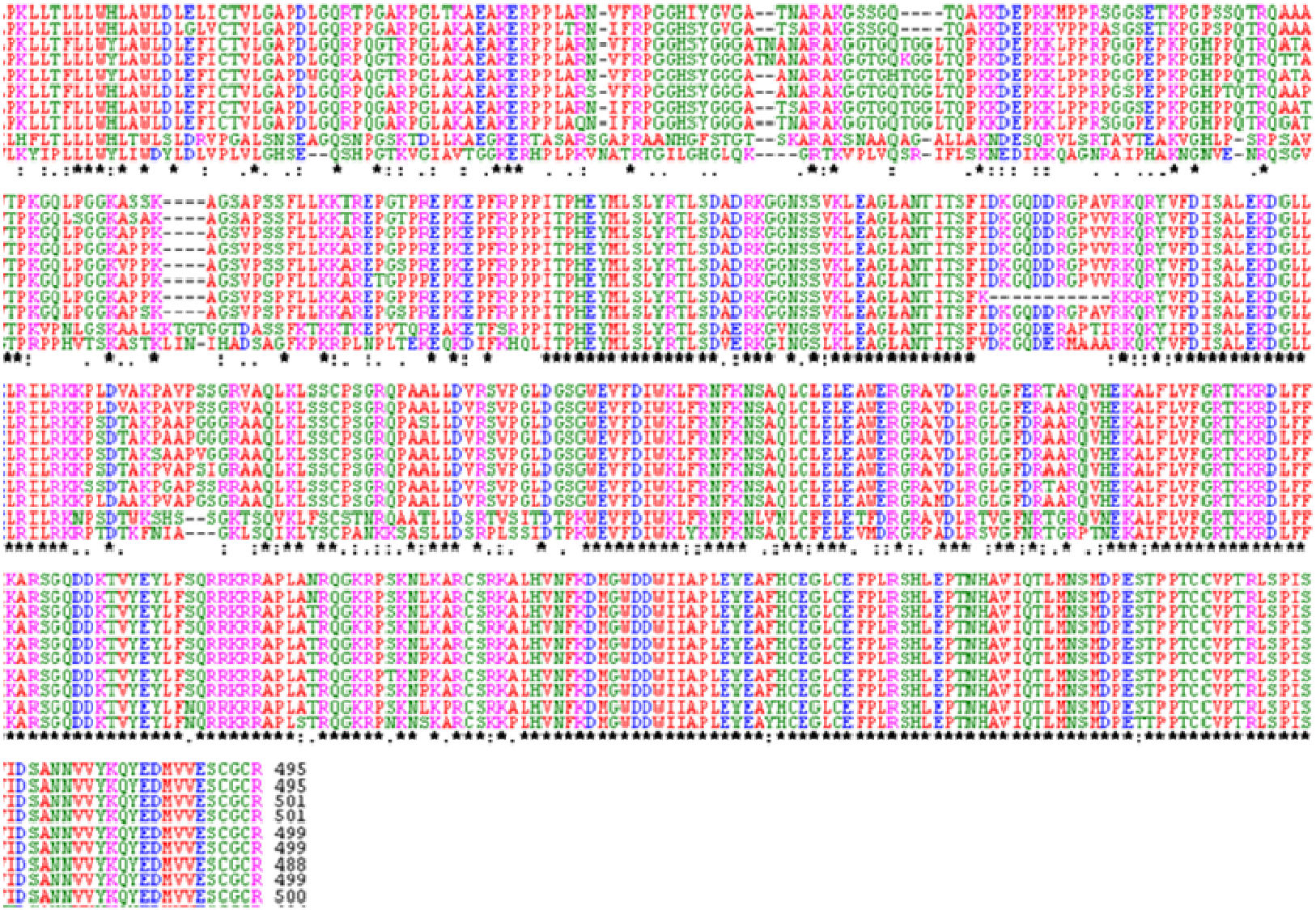

Figure 2. The deduced amino acid sequence of *Bos Taurus GDF5* (GenBank Accession No. XP_588072.1) was aligned to that from *Homo sapiens* (GenBank Accession No. NP_000548.1), *Mus musculus* (GenBank Accession No. NP_032135.1), *Rattus norvegicus* (GenBank Accession No. XP_001066344.1), *Pan troglodytes* (GenBank Accession No. XP_001164592.1), *Macaca mulatta* (GenBank Accession No. XP_001099702.1), *Canis familiaris* (GenBank Accession No. XP_542974.1), *Equus caballus* (GenBank Accession No. NP_001075989.1), *Gallus gallus* (GenBank Accession No. NP_989669.1), *Xenopus laevis* (GenBank Accession No. NP_001086466.1). Conserved amino acid residues among different species are presented with asterisks

Table 1. Associations analysis of T586C SNP genotypes with ultrasound measurement traits at bovine *GDF5* gene.

Genotypes	Traits (Mean ± SE)		
	UBF (cm)	ULMA (cm^2)	UMAR
TT	0.36 ± 0.14^{a}	67.44±2.23	7.63 ± 0.10^{AB}
TC	0.38 ± 0.01^{b}	68.90±0.78	7.72 ± 0.04^{A}
CC	0.37 ± 0.01^{ab}	67.97±1.15	7.42 ± 0.10^{B}
P value	0.0498	0.69	0.0058

^{a,b} Means with different superscripts were significantly different ($P < 0.05$); ^{A,B} means with different superscripts were significantly different ($P < 0.01$).

Mikic, 2004; Nickel et al., 2005), were tightly associated with carcass traits or carcass quality. Therefore, based on our results of bovine *GDF5* gene, we analyzed polymorphism and genetic effect in cattle *GDF5* gene locus. The new findings indicated that the T586C SNP of bovine *GDF5* was significantly associated with ultrasound backfat thickness and ultrasound marbling score.

In summary, we analyzed molecular characterization of bovine *GDF5* gene, and investigated one SNP in the *GDF5* gene and its association in different bovine breed populations. Association analysis performed on the T586C SNP demonstrated that the SNP were significantly associated with ultrasound measurement traits in bovine. Therefore, further work will be necessary to use the SNP for MAS in other breeds and larger population. It is also significant to investigate whether the *GDF5* gene plays a role on development of carcass traits and whether it is involve in linkage disequilibrium with other causative mutations.

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