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Associations between polymorphisms of the *GFI1B* gene and growth traits of indigenous Chinese goats

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ABSTRACT. This study aimed to investigate polymorphisms of the eighth exon in the *GFI1B* gene among three indigenous Chinese goat breeds (QianBei Ma goats, GuiZhou white goats, and GuiZhou black goats). Furthermore, association analysis was conducted between these polymorphisms and growth traits. Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP), direct DNA sequencing, and PCR-restricted fragment length polymorphism (RFLP) were applied to detect polymorphism sites, and a general linear model was used to analyze their association with growth traits. We found two consistent single nucleotide polymorphism (SNP) sites in the eighth exon of the *GFI1B* gene among the three breeds: 263 bp G→T and 340 bp G→A. The fixed effects model used to analyze growth traits revealed significant differences in body weight, body length, chest depth, and chest breadth between genotypes CD, CC, and DD ($P < 0.01$). The 340(G/C) polymorphic sites identified here will provide a basis to further study associations between the *GFI1B* gene and growth

traits, as well as establish a theoretical foundation to develop better feeding and genetic resources of indigenous goats.

Key words: *GFI1B* gene; Indigenous Chinese goats; PCR-SSCP; PCR-RFLP; Growth trait

INTRODUCTION

The growth factor independent 1B gene (*GRI1B*) was the second to be found in the GRI1 gene family. In 1933, Gilks first identified the *GFI1* gene in the integration sites of rat T cell lymphoma, which is located in chromosome 1p22 of the human genome (Gilks et al., 1993, Rödel et al., 1998). In 1998, Rödel (Elmaagacli et al., 2007) found that the *GFI1B* gene was located in chromosome 9q34.13 of the human genome. Several studies have shown that *GRI1B* is a nuclear transcriptional repressor encoding 329 amino acids, which contains an N-terminal SNAG domain composed of 20 amino acids, and a C2H2-like zinc finger domain containing six carboxyl ends. The area between these two domains contains 144 amino acids (Robeas and Cowell, 1997; Karsunky et al., 2002). GRI1B is predominantly expressed in fetal and adult erythroid and megakaryocytic cells, and therefore, it plays a very important role in the formation and differentiation of human erythroid cells.

The QianBei Ma goat variety is one of the three highest quality goat breeds in GuiZhou Province, China, and is widely consumed locally. It has a highly adaptive capacity, crude feed tolerance, multi-reproductive rate, produces more mutton of good taste, and has excellent skin quality (Luo et al., 2010; Cai et al., 2011). The GuiZhou white goat breed also has many advantages, including medium size, sound constitution, high fertility, good taste, and good skin quality. This goat breed is also suitable for hill grazing (Ran et al., 2009). The GuiZhou black goat breed has been listed as an excellent local variety since 1984. It has a strong physical build and good taste (Tian et al., 2010).

The objective of this investigation was to determine genetic variations in the *GFI1B* gene and their association with growth traits in three Chinese goat breeds (QianBei Ma goat, GuiZhou white goat, and GuiZhou black goat). Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP), direct DNA sequencing, and PCR-restricted fragment length polymorphism (RFLP) methods were applied to scan nucleotide substitutions. The polymorphism reported here may contribute to applications in goat breeding and genetics.

MATERIAL AND METHODS

Animals

QianBei Ma goats (N = 322), aged 36-48 months, were obtained from the breeding center of QianBei Ma goats in XiShui county, GuiZhou Province, China, in 2011. GuiZhou white goats (N = 100), aged 36-48 months, were obtained from the GuiZhou white goat origin center, China, in 2011. GuiZhou black goats (N =100), aged 36-48 months, were obtained from the Animal and Veterinary Research Institute of GuiZhou Province, China, in 2011.

All experimental animals were fed under the same conditions, and seven growth indices (body weight, body height, body length, heart girth, chest depth, chest breadth, and cannon circumference) were measured (Table 1).

Table 1. Growth traits recorded for three goat varieties.

Breed (N)/trait	Minimum	Maximum	Mean	SE	CV (%)
QianBei Ma goats (322)					
Body weight (kg)	32.00	63.00	43.7171	5.55683	12.71
Bithers height (cm)	53.00	72.80	61.2295	3.51656	5.740
Body length (cm)	54.80	78.90	68.1357	3.87098	5.680
Heart girth (cm)	72.00	92.50	81.7416	3.76769	4.61
Chest depth (cm)	20.90	35.00	30.6578	1.68149	5.48
Chest breadth (cm)	13.40	22.70	18.7901	1.92322	10.24
Cannon circumference (cm)	7.00	10.50	8.0736	0.56998	7.06
GuiZhou white goats (100)					
Body weight (kg)	21.50	39.00	30.0000	3.71236	12.37
Bithers height (cm)	44.50	63.00	53.2750	3.72961	7.00
Body length (cm)	50.00	79.00	61.9460	4.80258	7.75
Heart girth (cm)	55.00	85.00	69.3920	5.78901	8.34
Chest depth (cm)	22.80	30.50	26.7640	1.53731	5.74
Chest breadth (cm)	13.00	21.00	16.5440	1.69153	10.22
Cannon circumference (cm)	6.80	9.00	7.8210	0.53698	6.87
GuiZhou black goats (100)					
Body weight (kg)	22.85	58.00	41.5635	6.01275	14.47
Bithers height (cm)	52.50	70.60	60.7090	3.54857	5.85
Body length (cm)	54.80	74.50	67.2980	3.89488	5.79
Heart girth (cm)	65.50	90.00	80.2700	4.38251	5.46
Chest depth (cm)	25.10	34.00	30.2220	1.70066	5.63
Chest breadth (cm)	12.10	22.00	18.3790	2.09474	11.40
Cannon circumference (cm)	7.00	9.50	7.9270	0.51871	6.54

Genomic DNA extraction

Genomic DNA was extracted from blood samples by the phenol-chloroform method, and then dissolved in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0) and stored at -40°C.

Polymorphism detection

According to the cow GFI1B sequence (AC173824), the specific primers (GFI1B-1-GFI1B-5) were designed using Prime5, which amplified the eighth exon of the *GFI1B* gene specifically (Table 2). PCR amplification was conducted in a total 20 μL volume, containing 2.5 UL 10X PCR buffer, 2 μL Mg^{2+}, 2.5 mmol/L 2.0 UL dNTPs, 1 μL each forward and reverse primer, 5 U/μL 0.4 UL Taq enzyme, and ultra-pure water. The PCR was run under the following cycling program: denaturation at 95°C for 5 min; denaturation at 94°C for 30 s, 72°C extension for 60 s, for 35 cycles, and a final extension at 72°C for 10 min, and then saved at 4°C, and subjected to 1% agarose gel electrophoresis (Fang et al., 2010; Jin et al., 2010; Chu et al., 2011).

For polymorphism detection by PCR-SSCP and direct DNA sequencing, 1.5 μL PCR product was transferred to an Eppendorf tube, mixed with 6 μL gel loading solution, which contained 98% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol, 20 mmol/L EDTA, pH 8.0, and 10% glycerol. The mixture was centrifuged and denatured at 95°C for 10 min, chilled on ice for 5 min, and loaded on neutral polyacrylamide gels. After electrophoresis, the DNA fragments in the gels were visualized by silver staining, photographed, and analyzed. PCR products of different genotypes were then directly sequenced (Chu et al., 2011).

Table 2. Primer sequences of PCR amplification.

Primers	Sequences of primers 5'→3'	Amplified length (bp)	Annealing temperature (°C)
GFI1B-1	F: AAGTCCCAGAGAACAAATGCAG R: GCCTCACTTGAGGTTGTGCT	251	60.5
GFI1B-2	F: CACAGGCTTCAAACCTTTCAG R: CAAGTTTGATGAGTCCTGTCCA	229	60.0
GFI1B-3	F: CCTGGACAGGACTCATCAAACT R: GCAAATAGGGGAGGATTTCTCT	225	59.0
GFI1B-4	F: CCCAGAGAAATCCTCCCCTAT R: GGTACATTGGCTGAAGCAGAG	146	58.5
GFI1B-5	F: GAAATCCTCCCCTATTTGCTG R: CACTGAACAAATGCTGGTCAA	273	61.0
P1	F: AACCTCATCACCCACAGCC R: GCACCATCCCTCCCCAAAG	757	59

After screening by PCR-SSCP and direct DNA sequencing, we applied PCR-RFLP methods to detect nucleotide substitutions in the GFI1B gene, for which the other primer (P1) was designed. Twenty microliter aliquots PCR products of the *GFI1B* gene were digested with 10 U *Mfl*I or *Taq*1 (MBI Fermentas) for 5 h at 37°C following manufacturer instructions. The digested products were detected by 2% agarose gel electrophoresis stained with ethidium bromide (Ganai et al., 2012).

Statistical analysis

A fixed effects model was employed for the analysis of goat growth traits, and least squares means were used for multiple comparisons of growth traits among different genotypes. Age and sex were ignored in the statistical analysis since all tested goats were ewes of the same age. The following model was used: $Y_{ijk} = u + marker_k + e_{ijk}$; where Y_{ijk} is the individual phenotype value, u is the group average, $marker_k$ is the effect of marker gene type, and e_{ijk} is the random error (Song et al., 2011; Liu et al., 2011; Wu et al., 2012).

RESULTS

Detection of genetic polymorphisms by PCR-SSCP

PCR-SSCP was used to identify the exact mutation. Results showed that the PCR products amplified by primers GFI1B-2 and GFI1B-3 displayed polymorphisms (Figure 1).

Detection of polymorphisms by DNA sequencing

PCR products of different genotypes were purified and subject to directional sequencing. The results showed two mutation sites in the eighth exon, which were 263 and 340 bp in length, respectively. For primer GFI1B-2, sequencing revealed a nucleotide mutation (263 bp G→T) between genotype AA and genotype BB. Additionally, a nucleotide mutation (340 bp G→A) was revealed between genotype CC and genotype DD with GFI1B-3 (Figure 2).

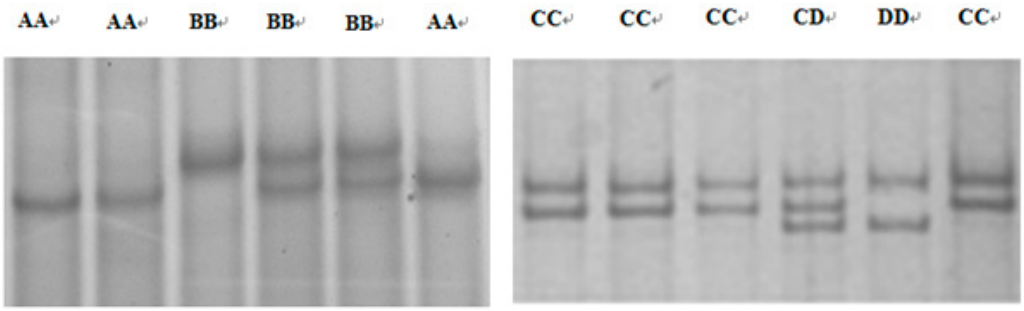

Figure 1. Single strand conformation polymorphism analysis of PCR amplification.

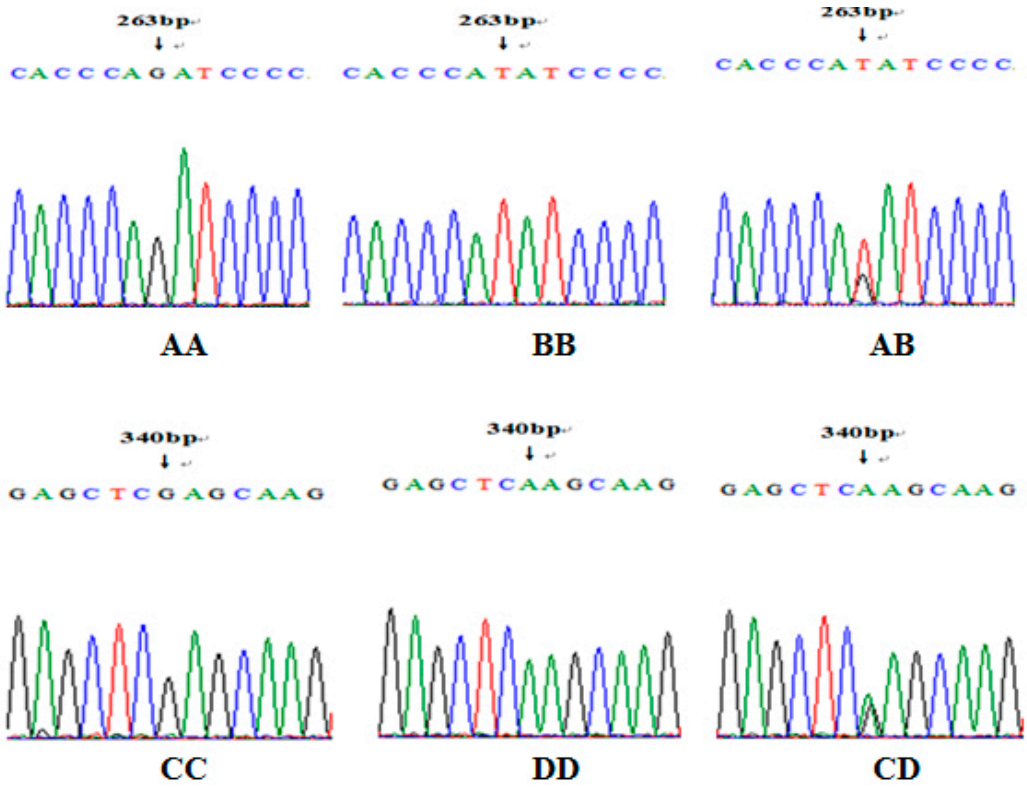

Figure 2. Sequence comparison of genotype.

PCR-RFLP analysis

According to the mutation position, a point mutation was deliberately introduced into one of the newly designed primers so that the new PCR product would contain a forced restriction endonuclease recognition site. Finally, the forced-RFLP method was used to check the frequencies of mutation.

Results showed that when the 757-bp PCR products were digested with restriction enzyme *Mfl*I, the homozygous mutant (AA) animals produced two bands of 208 and 549 bp. The heterozygous (AB) animals produced three bands of 757, 208, and 549 bp, and the other homozygous (BB) animals produced a single band of 757 bp. When the 757-bp PCR products were digested with restriction enzyme *Taq*1, the homozygous mutant (CC) animals produced bands of 285 and 472 bp. The heterozygous (CD) animals produced three bands of 757, 285, and 472 bp, and the other homozygous (DD) animals produced a single band of 757 bp (Figure 3).

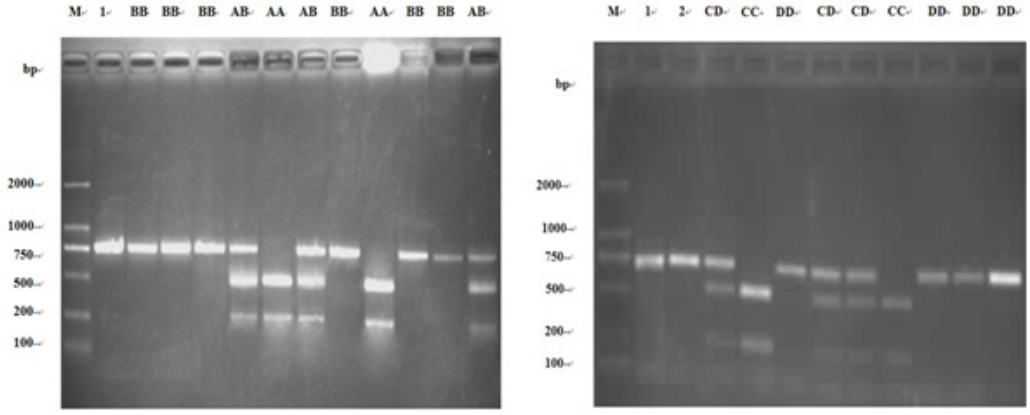

Figure 3. Restricted fragment length polymorphism analysis of PCR amplification.

Genetic characteristics of SNP sites in the GFI1B gene

We determined genetic characteristics of four mutation sites in the three goat varieties. The results showed that the mutation contained alleles A and C, which are advantageous alleles at 263 (G/T) and 340 (G/A). The frequency of this genotype was high (> 0.5) (Table 3).

Table 3. Analysis of genetic diversity of GFI1B gene.

Site	Breeds	Genotype frequencies			Allele frequencies		H_E	H_O	N_E	PIC
		AA	AB	BB	A	B				
GFI1B-1	Q	0.475	0.242	0.283	0.696	0.404	0.352	0.648	1.543	0.194
263 (G/T)	W	0.65	0.17	0.18	0.735	0.265	0.390	0.610	1.639	0.314
	B	0. 52	0.14	0.34	0.59	0.41	0.484	0.516	1.938	0.367
Site	Breeds	Genotype frequencies			Allele frequencies		H_E	H_O	N_E	PIC
		CC	CD	DD	C	D				
GFI1B-2	Q	0.553	0.270	0.177	0.688	0.312	0.429	0.571	1.751	0.336
340 (G/A)	W	0.51	0.21	0.28	0.615	0.385	0.474	0.526	1.901	0.362
	B	0.48	0.29	0.23	0.625	0.375	0.468	0.531	1.883	0.385

PIC > 0.5 high diversity; 0.25 < PIC < 0.5 moderate; PIC < 0.25 low diversity

Influence of fixed effects on growth traits

A fixed effects model was employed for the analysis of growth traits in QianBei Ma

goats. Results revealed that the 263(G/T) site did not significantly affect body weight. In contrast, body heights and chest depths of individuals of genotype AB were significantly higher than those of individuals with genotypes AA or BB ($P < 0.01$). Body weight, body length, chest depth, and chest breadth significantly differed between genotypes CD compared to genotypes CC and DD ($P < 0.01$) (Table 4).

Table 4. Association of four loci with growth traits in 322 QianBei Ma goats.

Trait	Genotype		
	AA (N = 153)	BB (N = 78)	AB (N = 91)
Body weight (kg)	43.80 ± 4.92	42.82 ± 5.07	44.35 ± 6.80
Bithers height (cm)	61.44 ± 3.40^{a}	60.24 ± 3.15^{aA}	61.72 ± 3.86^{bB}
Body length (cm)	68.03 ± 4.04	67.69 ± 3.07	68.69 ± 4.17
Heart girth (cm)	81.92 ± 3.64	80.91 ± 3.18^{a}	82.15 ± 4.34^{b}
Chest depth (cm)	30.68 ± 1.50^{a}	30.16 ± 1.65^{aA}	31.06 ± 1.89^{bB}
Chest breadth (cm)	18.94 ± 1.77	18.77 ± 1.70	18.56 ± 2.31
Cannon circumference (cm)	8.04 ± 0.52	8.07 ± 0.59	8.12 ± 0.63
Trait	Genotype		
	CC (N = 178)	DD (N = 87)	CD (N = 57)
Body weight (kg)	43.38 ± 4.95^{aA}	42.65 ± 6.37^{aA}	46.39 ± 5.27^{bB}
Bithers height (cm)	61.42 ± 3.51	60.53 ± 3.50	61.69 ± 3.48
Body length (cm)	67.84 ± 3.95^{aA}	67.59 ± 3.70^{aA}	69.89 ± 3.40^{bB}
Heart girth (cm)	81.79 ± 3.71^{a}	80.85 ± 3.92^{aA}	82.96 ± 3.40^{bB}
Chest depth (cm)	30.71 ± 1.56^{aA}	30.07 ± 1.83^{bB}	31.4 ± 1.51^{abAB}
Chest breadth (cm)	18.69 ± 1.87^{aA}	18.48 ± 2.04^{aA}	19.58 ± 1.71^{bB}
Cannon circumference (cm)	8.06 ± 0.56	8.08 ± 0.65	8.11 ± 0.45

Data marked with different superscripts within the same line differ significantly, Capital letters, $P < 0.01$, small letters $P < 0.05$.

DISCUSSION

The *GFI1B* gene plays important roles in the human erythropoiesis and differentiation process. Recent studies have found that GFI1B has growth inhibition properties (Jegalian and Wu, 2002; Osawa et al., 2002). In view of this, in our previous work, we designed a specific primer based on cow (ruminant animal) *GFI1B* gene sequences. The *RERG* gene of goat was cloned successfully, which proved that the sequence had high homology, and the designed primer was feasible for PCR gene cloning. The sequence contained a coding sequence, including the start and stop codons ATG and TGA, and was submitted to GenBank (Chen et al., 2012). We also revealed polymorphisms of exon1, exon2, and part of exon8 of GFI1B in QianBei Ma goat and evaluated its relationship with growth traits. The SNPs were detected by directional sequencing, and one new mutation site of C347A was identified in the sequence. Body weight differences between genotypes AA and AB were significant ($P < 0.05$).

In the present study, however, we adopted the PCR-SSCP and PCR-RFLP detection techniques to identify polymorphisms, as these methods allow for rapid mutation detection, high sensitivity, robustness, and scalability. PCR-SSCP, PCR-RFLP, and direct DNA sequencing methods have become popular methods for detecting mutations in the field of animal genetic resources (Chen et al., 2011). Because we also included more breeds of indigenous Chinese goats in this study, adopting this combined strategy was particularly useful, as they can scan mutations in large sample sizes, and overcome common difficulties in polymorphism

detection related to inaccuracy, high expense, complicated technical demands, slow speeds, and unstable reproducibility (Lan et al., 2010).

The present study aimed to evaluate polymorphisms of the *GFI1B* gene exons and their relationship with growth traits in indigenous Chinese goats. We detected polymorphisms in the eighth exon among three breeds of indigenous Chinese goats (QianBei Ma goats, GuiZhou white goats, and GuiZhou black goats) by PCR-SSCP, PCR-RFLP, and direct DNA sequencing; two new mutations were identified in the three breeds. The genetic characteristic analysis suggested that alleles A and C are advantageous alleles and are present in higher frequencies. The polymorphism could be classified as moderate. This is due to practices of the local breeding center that input external blood of the same species with good quality and high value characteristics for high quality cultivation, which will enrich the genetic variation in the population.

Due to several factors, such as the large sample number, we only conducted the association analysis of SNPs with growth traits for QianBei Ma goats. Results showed that body height and chest depth of genotype AB individuals differed highly significantly from those of genotypes AA and BB ($P < 0.01$). Furthermore, body weights, body lengths, chest depths, and chest breadths of genotype CD individuals differed highly significantly from those of genotypes CC and DD ($P < 0.01$). The specific relationship of SNPs with growth traits suggested that genotypes AB and CD were superior to other genotypes in several growth traits. These results will provide a basis for further studies of the effect of GFI1B genes on growth traits, and contribute to a theoretical foundation for developing better feeding and genetic resources of indigenous Chinese goats. The 263(G/T) site did not appear to affect body weight in the QianBei Ma goat; therefore, further research is required to determine whether it can be used as an advantageous genotype for breeding.

CONCLUSION

This study screened polymorphisms of the eighth exon of the *GFI1B* gene in three indigenous Chinese goats, and identified two SNP sites. We also conducted an association analysis with growth traits in QianBei Ma goats. These results suggested that the 340(G/A) molecular marker could be used for improving growth characteristics of indigenous Chinese goats. Therefore, this marker can be treated as a candidate type gene to facilitate breeding practices of indigenous Chinese goats. However, results also showed that the *GFI1B* gene could be treated as a candidate gene to facilitate goat breeding. Therefore, results in the present study are preliminary and further investigations are required.

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REFERENCES

Cai HF, Chen Z, Luo WX and Zhang YY (2011). Polymorphism and Bio-information of TGFB3 gene in goat. *Guizhou Agric. Sci.* 39: 144-146.

Chen Z, Luo WX, Liu RY, Shi ZY, et al. (2012). Cloning and bioninformatics analysis of GFI1B gene cDNA in the GuiZhou black goat. *Anim. Husbandry Vet. Med.* 44: 27-32.

Chen Z, Sun J, Li Z, Lan X, et al. (2011). Novel SNPs in the caprine stearoyl-CoA desaturase (SCD) and decorin (DCN) genes that are associated with growth traits in Chinese goat breeds. *Mol. Biol. Rep.* 38: 3121-3127.

Chu MX, Yang J, Feng T, Cao GL, et al. (2011). GDF9 as a candidate gene for prolificacy of Small Tail Han sheep. *Mol. Biol. Rep.* 38: 5199-5204.

Elmaagacli AH, Koldehoff M, Zakrzewski JL, Steckel NK, et al. (2007). Growth factor-independent 1B gene (GFI1B) is overexpressed in erythropoietic and megakaryocytic malignancies and increases their proliferation rate. *Br. J. Haematol.* 136: 212-219.

Fang XT, Xu HX, Zhang CL, Zhang JM, et al. (2010). Polymorphisms in BMP-2 gene and their associations with growth traits in goats. *Genes Genomics* 32: 29-35.

Ganai TA, Misra SS and Shabir M (2012). Polymorphism analysis of BMPR1B gene by forced RFLP and PCR-SSCP techniques and expression of the mutation in introgressed sheep. *Trop. Anim. Health Prod.* 44: 277-283.

Gilks CB, Bear SE, Grimes HL and Tsichlis PN (1993). Progression of interleukin-2 (IL-2)-dependent rat T cell lymphoma lines to IL-2-independent growth following activation of a gene (Gfi-1) encoding a novel zinc finger protein. *Mol. Cell. Biol.* 13: 1759-1768.

Jegalian AG and Wu H (2002). Regulation of Sots gene expression by the proto-oncoprotein GFl-1 B: two routes for STAT5 target gene induction by erythropoietin. *Biol. Chem.* 277: 2345-2352.

Jin Q, Fang XT, Yang L, Zhang CL, et al. (2010). Novel SNPs of the caprine growth hormone secretagogue receptor (GHSR) gene and their association with growth traits in goats. *Biochem. Genet.* 48: 847-856.

Karsunky H, Mende I, Schmidt T and Moroy T (2002). High levels of the onco-protein Gfi-1 accelerate T-cell proliferation and inhibit activation induced T-cell death in Jurkat T-cells. *Oncogene* 21: 1571-1579.

Lan XY, Lai XS, Li ZJ, Wang J, et al. (2010). Effects of genetic variability of the carpine homeobox transcription factor HESX1 gene on performance traits. *Mol. Biol. Rep.* 37: 441-449.

Liu Y, Lan X, Qu Y, Li Z, et al. (2011). Effects of genetic variability of the dairy goat growth hormone releasing hormone receptor (GHRHR) gene on growth traits. *Mol. Biol. Rep.* 38: 539-544.

Luo WX, Zhang QL, Mu L and Li H (2010). Studies on mutton performance and quality of Qianbei Ma goat. *Southwest China J. Agric. Sci.* 23: 1706-1710.

Osawa M, Yamaguchi T, Nakamura Y, Kaneko S, et al. (2002). Erythroid expansion mediated by the Gfi-1B zinc finger protein: role in normal hematopoiesis. *Blood* 100: 2769-2777.

Ran XQ, Lin JB, Du ZY, Qing C, et al. (2009). Diversity of Bmp15 and Gdf9 genes in white goat of guizhou province and evolution of the encoded proteins. *Zool. Res.* 30: 593-602.

Robeas T and Cowell JK (1997). Cloning of the human gfi-I gene and its mapping to chromosome region lp22. *Oncogene* 14: 1003-1005.

Rödel B, Wagner T, Zörnig M, Niessing J, et al. (1998). The human homologue (GFI1B) of the chicken GFI gene maps to chromosome 9q34.13-A locus frequently altered in hematopoietic diseases. *Genomics* 54: 580-582.

Song CY, Zhao Q, Gao B, Wang XY, et al. (2011). Polymorphism analysis of POU1F1 promoter region and it's association with growth traits. *Sci. Agric. Sin.* 44: 5067-5072.

Tian XG, Zhu HG, Zhu X, Pan SB, et al. (2010). Measurement and analysis of biochemical blood indexes of female GuiZhou black goat. *Acta Ecol. Anim. Domast.* 31: 57-60.

Wu JS and Wang JY (2012). Polymorphism of Exon10 of FSHR gene and its relationship with litter size in xiaomeishan pigs. *Sci. Agric. Sin.* 45: 2728-2736.