

Study of the correlation between *GH* gene polymorphism and growth traits in sheep

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ABSTRACT. The growth hormone gene plays an important role in the physiological function of an organism. The current study aimed to investigate the correlation between polymorphisms in the 5' regulatory region, exon 4, and 3' untranslated region (UTR) of the sheep GH gene and sheep growth traits. The DNA from 510 adult sheep was analyzed by DNA sequencing and polymerase chain reaction single-strand conformation polymorphism. Two alleles (A and B) and 3 genotypes (AA, AB, and BB), 2 alleles (A and B) and 3 genotypes (AA, AB, and BB), and 3 alleles (A, B, and C) and 4 genotypes (AA, AB, BB, and AC) were found within the 5' regulatory region, exon 4, and 3' UTR, respectively. In Tibetan sheep, the association analysis indicated that there were statistically significant differences in the scores of weight, length, and heart girth within the 5' regulatory region; weight, length, wither height, and heart girth within exon 4; and weight, length, wither height, and heart girth within the 3' UTR among the different genotypes. For exon 4, Poll Dorset sheep individuals with genotype AA showed a lower score than those of genotypes BB and AB (P < 0.05). With regard

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to the 3' UTR, Poll Dorset sheep with genotype AC showed higher scores than those of genotypes AA and AB (P < 0.05).

Key words: *GH* gene; Growth traits; Polymorphism; DNA sequencing; Polymerase chain reaction single-strand conformation polymorphism; Sheep

INTRODUCTION

Growth hormone (GH) is single polypeptide chain protein hormone that is synthesized and secreted by the anterior pituitary eosinophils cells in vertebrates (Chen et al., 1989). The GH can accelerate metabolism and promote growth of many organs and tissues, especially the bone, muscle, and visceral organs (Ikonen et al., 2001). The *GH* gene has a direct effect on the synthesis and secretion of GH and plays an important role in animal growth (Gadelha et al., 2012). Therefore, studies investigating the structure and function of the *GH* gene have received increasing interest in recent years.

China is rich in sheep and goats, and the varieties in this region have many unique traits. For instance, Small Tail Han sheep have high reproductive performance and Tibetan sheep have good adaptability. These varieties are rare throughout the world. Meanwhile, China has also introduced some foreign varieties with faster growth rates and better heterosis, including the German Merino and Polled Dorset sheep. German Merino or Polled Dorset sheep make male parent and hybrid with Small Tail Han or Tibetan sheep, thus, improving sheep production in China.

Consequently, in the present study, we characterized the genetic variation of sheep GH gene complete sequences and investigated the correlation between genotypes and growth traits to identify suitable candidate markers that may be related to animal resistance. The results presented herein could potentially lead to the use of genetic strategies to improve sheep breeding in the future.

MATERIAL AND METHODS

Animal care and use in this study was consistent with the animal care and use requirements of Gansu Agricultural University.

Samples and data collection

The animals used in this experiment were obtained from the Huajia Sheep Breeding Farm (Dingxi, Gansu, China). We examined 510 adult sheep, including Tibetan (N = 126, ZY), Small Tail Han (N = 128, XW), German Merino (N = 118, DM), and Polled Dorset (N = 138, WT) sheep.

Growth traits (i.e., weight, length, heart girth, and wither height) of experimental sheep were measured by Animal Breeding. Genomic DNA was extracted from sheep blood (jugular vein samples) by the standard phenol-chloroform extraction procedure (He et al., 2012) and quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA).

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Genotyping by polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP)

Primers were designed according to the published GH sequence for Tibetan sheep (GenBank accession No.: EF077162) (Ma et al., 2008) (Table 1). PCR analysis was conducted using a 25- μ L total volume reaction with 100 ng/ μ L genomic DNA, 10 pmol/ μ L of each primer, 2 mM dNTPs, 5 U Taq DNA polymerase (TaKaRa, Dalian, China), and 10X PCR Buffer (15 mM Mg²⁺). The following reaction was performed using a Robocycler Gradient 96 thermocycler (Table 2). PCR products were detected via 2% agarose gel electrophoresis (Figures 1-3).

 Table 1. Sequences of primers used for sequencing, polymerase chain reaction single-strand conformation

 polymorphism (PCR-SSCP), and quantitative PCR analysis.

Primer	Primer sequence (5'-3')	Region	Length of products (bp)
GH-P1	FAACAGGATGAGTGAGAGGAGGT	5' regulatory region	210
	R CCATCATAGCTGGTGAGCTG		
GH-P2	F CCAGTTCACCAGACGACTCA	Exon 1, Exon 2	450
	R CTGGGTGTTCTGGATGGAGTA		
GH-P3	F GCACAGCACCTGCATCAACT	Exon 3	340
	R GATGGTTTCGGAGAAGCAGA		
GH-E4	F GGACTTGGAGCTGCTTCGCAT	Exon 4	191
	R GGAAGGGACCCAACAATGCCA		
GH-P5	FAGCAGAGTCTTCACCAACAGC	Exon 5	480
	R TAGTTCTTGAGCAGCGCATC		
GH-P6	F GGCAGGAGCTGGAAGATGTC	3' UTR	320
	R CCTACTCAGACAATGTGATGCAA		

Table 2	Table 2. Polymerase chain reaction program.						
Primer	Pre-denaturalization	Denaturalization	Annealing	Extension	Cycles	Extension	
GH-P1	94.0°C, 5 min	94.0°C, 30 s	62.5°C, 30 s	72.0°C, 30 s	30	72.0°C, 7 min	
GH-P2	94.0°C, 5 min	94.0°C, 30 s	62.4°C, 30 s	72.0°C, 45 s	32	72.0°C, 10 min	
GH-P3	94.0°C, 5 min	94.0°C, 30 s	59.5°C, 30 s	72.0°C, 45 s	30	72.0°C, 7 min	
GH-P4	94.0°C, 5 min	94.0°C, 30 s	60.0°C, 30 s	72.0°C, 30 s	30	72.0°C, 7 min	
GH-P5	94.0°C, 5 min	94.0°C, 30 s	62.0°C, 30 s	72.0°C, 45 s	35	72.0°C, 7 min	
GH-P6	94.0°C, 5 min	94.0°C, 30 s	59.5°C, 30 s	72.0°C, 30 s	30	72.0°C, 7 min	



Figure 1. Agarose gel electrophoresis of GH-P1 PCR-SSCP. *Lanes 1, 2, 4, 5*, and 7 = genotype AB; *lanes 3* and 6 = genotype BB; *lanes 8, 9*, and 10 = genotype AA.

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Figure 2. Agarose gel electrophoresis of GH-E4 PCR-SSCP. *Lane 1* = genotype AA; *lanes 2* and 4 = genotype BB; *lanes 3* and 5 = genotype AB.



Figure 3. Agarose gel electrophoresis of GH-P6 PCR-SSCP. *Lanes 1, 2* and *5* = genotype AA; *lane 3* = genotype AC; *lane 4* = genotype BB; *lane 6* = genotype AB.

The amplified region polymorphism of all samples was screened for by SSCP. A 2- μ L volume PCR product was mixed with 8 μ L denaturing solution (98% formamide, 0.025% bromophenol blue, 0.025% xylene cyanole, and 10 mM EDTA). Denatured DNA samples were placed on 7 x 7 cm, 14% acrylamide:bisacrylamide (29:1) gels. Electrophoresis was run at 15 h (180 V) with constant incubation. Gels were silver-stained according to the method of Byun et al. (2009).

The polymorphic regions were sequenced by Shanghai Biological Engineering Co. (Shanghai, China; Figures 4-6).



Figure 4. Sequencing map GH-P1.

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Figure 5. Sequencing map of GH-E4.



Figure 6. Sequencing map of GH-P6.

Statistical analysis

Sequence alignment, translation, and comparisons were performed using MEGA5 (Tamura et al., 2011). Genotype and allele frequencies, gene heterozygosity (H_E), polymorphism information content (PIC), and testing for deviation from Hardy-Weinberg equilibrium were calculated by POPGENE version 1.32 (Yeh et al., 1997).

The effects of genotype on sheep growth traits (i.e., weight, length, height, and girth) were estimated using the general linear model (GLM) procedure of the SAS software (SAS, 1999) according to the following statistical model:

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$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk},$$

where $i = 1, \dots, a; j = 1, \dots, b; k = 1, \dots, n; \gamma_{ij}$ was interaction effect, and ε_{ijk} was the random residual effect.

Our model was $Y = \mu + B + G + G \times B + e$ where Y was the value of the trait measured, μ was the population mean, G was the genotype effect, B was the group effect, G x B was the interaction effect for genotypes and groups, and e was the random residual effect. Preliminary analysis also included the fixed interaction effects of breed, genotype, and growth traits; however, these interaction effects were subsequently removed because they did not have significant effects.

RESULTS

Polymorphism analysis of the GH gene

Polymorphisms in the *GH* gene were identified within the 5' regulatory region (GH-P1), exon 4 (GH-E4), and the 3' UTR (GH-P6). Based on the PCR products, SSCP was used for initial screening of the samples. Three unique SSCP patterns (i.e., AA, BB, and AB) and 2 mutations in BB genotype individuals (i.e., $T \rightarrow C$ at 84 bp and $G \rightarrow A$ at 85 bp) were detected in GH-P1 (Figure 4). Three unique SSCP patterns (i.e., AA, BB, and AB) and one mutation in BB genotype individuals (i.e., $T \rightarrow C$ at 88 bp) were detected in GH-E4 (Figure 5). Four unique SSCP patterns (i.e., AA, BB, AB, and AC) and 3 mutations were detected in GH-P6 (B allele when compared to the A allele had a T nucleotide insertion at 234 bp and a $G \rightarrow T$ mutation at 257 bp). In comparison to the A allele, the C allele had a C \rightarrow T mutation at 294 bp (Figure 6).

In GH-P1, AA was the dominant genotype and A was the dominant allele. The GH-P1 polymorphism was not found in the German Merino or Poll Dorset sheep (Table 3). For GH-E4, BB was the dominant genotype and B was the dominant allele in Tibetan and Small Tail Han Sheep; AA was the dominant genotype and A was the dominant allele in German Merino and Poll Dorset sheep (Table 4). For GH-P6, AA was the dominant genotype and A was the dominant allele in the 4 breeds. The BB genotype was not found in German Merino or Poll Dorset sheep (Table 5).

Table 3.	Table 3. Genotype and allele frequencies of GH-P1.							
Breed	Genotypes frequency			Allele frequency				
	AA	BB	AB	A	В			
ZY	0.8175 (103)	0.0714 (9)	0.1111 (14)	0.8532	0.1468			
XW	0.6071 (17)	0.1071 (3)	0.2858 (8)	0.6607	0.3393			
DM	1.0000 (18)	0.0000 (0)	0.0000 (0)	1.0000	0.0000			
WT	1.0000 (38)	0.0000 (0)	0.0000 (0)	1.0000	0.0000			

Breed	Genotype frequency			Allele frequency	
	AA	BB	AB	А	В
ZY	0.0872 (11)	0.6667 (84)	0.2461 (31)	0.4206	0.5794
XW	0.1429 (4)	0.6071 (17)	0.2500 (7)	0.4464	0.5536
DM	0.6111 (11)	0.2222 (4)	0.1667 (3)	0.7222	0.2778
WT	0.5263 (20)	0.2632 (10)	0.2105 (8)	0.6579	0.3421

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Table 5	Table 5. Genotype and allele frequencies of P6.						
Breed	Genotypes frequency				Allele frequency		
	AA	AB	BB	AC	А	В	С
ZY	0.4286 (54)	0.3095 (39)	0.1032 (13)	0.1578 (20)	0.6623	0.2589	0.0788
XW	0.5357 (15)	0.1429 (4)	0.1786 (5)	0.1428 (4)	0.6786	0.2501	0.0713
DM WT	0.2222 (4) 0.5000 (19)	0.6111 (11) 0.2895 (11)	0.0000 (0) 0.0000 (0)	0.1667 (3) 0.2105 (8)	0.6111 0.6053	0.3056 0.1447	0.0833 0.2500

For GH-P1, GH-E4, and GH-P6, significant deviations from Hardy-Weinberg equilibrium were tested for genotype distributions in the 4 sheep breeds (P < 0.01). The PIC for the studied populations ranged from 0.2691 to 0.4864. According to the protocol of Vaiman et al. (1994), this locus was moderately polymorphic in the 3 populations (0.25 < PIC < 0.50; Table 6).

Locus	Breed	H_{0}	$H_{\rm E}$	$N_{\rm E}$	PIC
GH-P1	ZY	0.7495	0.2505	1.3342	0.3691
	XW	0.5516	0.4484	1.8127	0.3478
	DM	1.0000	0.0000	1.0000	0.0000
	WT	1.0000	0.0000	1.0000	0.0000
GH-E4	ZY	0.5126	0.4874	1.9508	0.3686
	XW	0.5057	0.4943	1.9773	0.3721
	DM	0.5987	0.4013	1.6702	0.3208
	WT	0.5499	0.4501	1.8186	0.3488
GH-P6	ZY	0.5119	0.4881	1.9536	0.4230
	XW	0.5281	0.4719	1.8935	0.4089
	DM	0.7438	0.5262	2.1107	0.4499
	WT	0.4498	0.5502	2.2231	0.4864

The nucleotide characterizations of these polymorphisms were revealed by DNA sequencing. A comparison with previously identified alleles indicated that new mutations (c. 1268 T \rightarrow C) were found for the first time in sheep. The new allele sequences have been deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/nuccore/) under accession No. EF077162.

Correlation between the GH gene polymorphisms and growth traits

With regard to the 5' regulatory region, the statistical results showed that a significantly different locus was observed between the effects of genotype and growth traits in Tibetan sheep (P < 0.05). The least square means along with the standard errors for growth trait scores among the different genotypes are given in Table 7. The genotypic linear contrasts suggest that growth trait scores for the AA genotype were significantly lower than those for genotypes AB and BB (P < 0.05). There were no significant differences in genotype effect and growth traits in Small Tail Han sheep (P > 0.05). Polymorphisms were not detected for the German Merino or Poll Dorset sheep. The results were the same as Komisarek et al. (2011), Wang et al. (2012), Min et al. (2005), and Gao (2004).

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Table	Table 7. Correlation analysis for the GH gene and growth traits in GH-P1.						
Breed	Genotype	Weight	Length	Height	Heart girth		
ZY	AA (N = 103) AB (N = 14)	55.1760 ± 1.2712^{B} 69.8210 ± 1.7994^{A}	$\begin{array}{l} 71.0100 \pm 0.4334^{\rm B} \\ 75.5000 \pm 1.1180^{\rm A} \end{array}$	$\begin{array}{c} 73.1840 \pm 0.5850^{\rm B} \\ 77.7860 \pm 1.4188^{\rm A} \end{array}$	$\begin{array}{c} 97.7570 \pm 0.9987^{\text{B}} \\ 106.2100 \pm 1.0227^{\text{A}} \\ \end{array}$		
XW	BB (N = 9) AA (N = 17) AB (N = 8) BB (N = 3)	70.8330 ± 2.7821^{A} 60.9410 ± 2.5516^{A} 61.8750 ± 3.6520^{A} 66.6670 ± 1.6667^{A}	$74.6670 \pm 0.9574^{\text{A}}$ $78.8240 \pm 0.9125^{\text{A}}$ $77.1250 \pm 1.3016^{\text{A}}$ $81.6670 \pm 1.7638^{\text{A}}$	81.4440 ± 1.1194^{A} 77.3530 ± 1.4576^{A} 74.7500 ± 2.0938^{A} 80.6670 ± 3.4801^{A}	$106.3300 \pm 2.1344^{\text{A}} \\ 84.6470 \pm 1.4968^{\text{A}} \\ 85.3750 \pm 2.4996^{\text{A}} \\ 88.6670 \pm 1.7638^{\text{A}} \\ \end{cases}$		

Trait values followed by different superscript letters indicate significance (P < 0.05).

At Exon 4, the statistical results showed that a significantly different locus was observed between the effects of genotype and growth traits in Tibetan and Poll Dorset sheep (P < 0.05). The least square means along with the standard errors for growth trait scores among the different genotypes are given in Table 8. The genotypic linear contrasts suggest that the growth trait scores of genotypes BB were significantly greater than those of genotypes AA and AB in Tibetan sheep, and growth trait scores of genotype AA were significantly lower than those of genotypes AB and BB in Poll Dorset sheep (P < 0.05). There were no significant differences in genotype effect and growth traits in Small Tail Han or German Merino sheep (P > 0.05).

Breed	Genotype	Weight	Length	Height	Heart girth
ZY	AA(N = 11)	$40.9670\pm2.3081^{\rm B}$	$69.4670 \pm 1.7344^{\rm B}$	$70.8670 \pm 1.1583^{\rm B}$	$90.6000 \pm 2.3275^{\rm B}$
	AB(N = 31)	44.6750 ± 2.5689^{B}	69.0000 ± 1.1562^{B}	70.9000 ± 1.2647^{B}	88.9000 ± 2.6025^{B}
	BB(N = 84)	$63.6270 \pm 0.9636^{\rm A}$	$76.2420 \pm 0.5430^{\rm A}$	$74.3740 \pm 0.3766^{\rm A}$	$103.0300 \pm 0.7075^{\text{A}}$
XW	AA(N=4)	$54.0000 \pm 1.8708^{\text{A}}$	$74.4000 \pm 3.2650^{\text{A}}$	$76.2000 \pm 0.5831^{\text{A}}$	$86.5000 \pm 3.6125^{\text{A}}$
	AB(N=7)	$54.3333 \pm 2.5386^{\rm A}$	$74.8333 \pm 1.4472^{\text{A}}$	$76.5000 \pm 0.4282^{\rm A}$	$83.7500 \pm 1.4592^{\text{A}}$
	BB(N = 17)	$56.7650 \pm 2.1837^{\text{A}}$	$78.4710 \pm 1.4905^{\rm A}$	$80.1180 \pm 1.0142^{\text{A}}$	$88.4710 \pm 1.4706^{\text{A}}$
DM	AA(N = 11)	$55.6400 \pm 3.1739^{\text{A}}$	$66.4000 \pm 0.9713^{\text{A}}$	$65.9000 \pm 0.6658^{\text{A}}$	$93.3333 \pm 2.2964^{\text{A}}$
	AB(N=3)	$46.5333 \pm 1.1392^{\text{A}}$	$63.3333 \pm 0.6667^{\text{A}}$	$64.0000 \pm 0.5774^{\rm A}$	$85.6667 \pm 1.4530^{\text{A}}$
	BB(N=4)	$50.2400 \pm 2.3112^{\text{A}}$	$68.2000 \pm 1.4714^{\text{A}}$	$66.5000 \pm 1.1937^{\text{A}}$	$89.3568 \pm 1.8138^{\text{A}}$
WT	AA(N = 20)	$102.2600 \pm 2.3073^{\rm B}$	72.7890 ± 1.2741^{B}	66.4210 ± 0.8696^{B}	$91.4210 \pm 1.3665^{\text{B}}$
	AB(N=8)	$140.1111 \pm 6.1293^{\text{A}}$	$80.6667 \pm 3.0596^{\text{A}}$	$70.5000 \pm 1.1547^{\text{A}}$	$115.3900 \pm 3.3414^{\text{A}}$
	BB(N = 10)	$128.8000 \pm 5.0767^{\rm A}$	$80.5000 \pm 1.4776^{\text{A}}$	$70.4000 \pm 0.9911^{\text{A}}$	$113.9000 \pm 3.1392^{\text{A}}$

Same trait values with different superscript letters indicate significance (P < 0.05).

At the 3' UTR, statistical results showed that a significantly different locus was observed between genotype effect and growth traits in Tibetan and Poll Dorset sheep (P < 0.05). The least square means and the standard errors for growth trait scores among the different genotypes are given in Table 9. The genotypic linear contrasts suggested that growth trait scores of genotypes BB were significantly lower than those of genotypes AA, AB, and AC in Tibetan sheep, and growth trait scores of genotype AC were significantly greater than those of genotypes AA and AB in Poll Dorset sheep (P < 0.05). There were no significant differences in genotype effect and growth traits in Small Tail Han or German Merino sheep (P > 0.05). Genotype BB individuals were not detected for German Merino or Poll Dorset sheep.

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Breed	Genotype	Weight	Length	Height	Heart girth
ZY	AA(N = 54)	$47.4540 \pm 1.6194^{\text{B}}$	70.6110 ± 0.7654^{B}	$61.8520 \pm 0.6515^{\rm B}$	$93.2410 \pm 1.4236^{\mathrm{B}}$
	AB(N = 39)	62.8970 ± 1.1761 ^A	$75.1030 \pm 0.7527^{\text{A}}$	$73.8460 \pm 0.5674^{\rm A}$	$102.44000 \pm 1.0311^{\text{A}}$
	BB(N = 13)	$68.0460 \pm 2.0894^{\text{A}}$	$76.9230 \pm 1.4609^{\text{A}}$	$74.4500 \pm 0.6976^{\text{A}}$	$104.3100 \pm 2.2571^{\text{A}}$
	AC $(N = 20)$	69.9000 ± 1.3507 ^A	$79.6000 \pm 1.1503^{\text{A}}$	$76.9230 \pm 1.0157^{\text{A}}$	$106.3500 \pm 1.1477^{\text{A}}$
XW	AA(N = 15)	$60.3750 \pm 2.6487^{\text{A}}$	$77.1880 \pm 1.5417^{\text{A}}$	$78.3750 \pm 0.8459^{\rm A}$	$87.3750 \pm 1.5669^{\text{A}}$
	AB(N=4)	65.0000 ± 2.2361 ^A	$75.8000 \pm 1.5937^{\text{A}}$	$78.8000 \pm 2.2672^{\text{A}}$	$88.2000 \pm 1.4283^{\text{A}}$
	BB(N=5)	$60.0000 \pm 7.3598^{\text{A}}$	$74.7500 \pm 4.2500^{\text{A}}$	$77.2500 \pm 2.1747^{\text{A}}$	$83.5000 \pm 4.9917^{\text{A}}$
	AC $(N = 4)$	$66.6667 \pm 1.6667^{\text{A}}$	80.6667 ± 3.4801 ^A	$81.6667 \pm 1.7638^{\text{A}}$	$88.6667 \pm 1.7638^{\text{A}}$
DM	AA(N = 4)	58.1600 ± 1.5197 ^A	$64.7000 \pm 0.7348^{\text{A}}$	$65.4000 \pm 0.5788^{\text{A}}$	$95.7000 \pm 0.9165^{\text{A}}$
	AB(N = 11)	$50.9140 \pm 4.5830^{\text{A}}$	$66.6430 \pm 1.4172^{\text{A}}$	$65.7140 \pm 0.9872^{\text{A}}$	89.3750 ± 3.2891 ^A
	AC(N=3)	$50.0000 \pm 1.9023^{\text{A}}$	$67.5000 \pm 1.3904^{\text{A}}$	$66.0830 \pm 1.0600^{\text{A}}$	$88.7500 \pm 1.5798^{\text{A}}$
WT	AA(N=8)	101.5000 ± 2.8752^{B}	72.0000 ± 1.4220^{B}	$68.3000 \pm 1.2207^{\text{B}}$	$91.3000 \pm 1.8682^{\text{B}}$
	AB(N = 11)	112.2900 ± 7.8479^{B}	76.2500 ± 2.9468^{B}	68.0000 ± 1.1871^{B}	$95.2920 \pm 4.0248^{\mathrm{B}}$
	AC $(N = 19)$	$132.3400 \pm 3.1714^{\text{A}}$	$88.7500 \pm 1.2764^{\text{A}}$	$80.7190 \pm 0.8391^{\text{A}}$	$114.310 \pm 2.5261^{\text{A}}$

Same trait values with different superscript letters indicate significance (P < 0.05).

DISCUSSION

Genetic diversity of the sheep GH gene

One of the most remarkable features of the sheep GH gene is its extremely high degree of genetic polymorphism within loci. Extensive genetic studies have been conducted on the complete sequence of the GH gene in various species (Ofir and Gootwine, 1997; Jiang and Zhang, 2002). Wang et al. (2002) detected ApaI polymorphism loci in the swine GH gene intron 3 and exon 5 with restriction fragment length polymorphism analysis of PCR-amplified products; these polymorphic loci indicated a correlation between GH gene polymorphisms and growth traits. Gao (2004) detected the bovine GH gene at intron 3, intron 4, 3' UTR, and 5' UTR with PCR-SSCP; the results showed that these polymorphic loci were correlated with growth traits.

 $H_{\rm E}$ and PIC are considered suitable parameters for estimating the genetic variation of populations. High PIC is representative of poor gene consistency, high genetic variability, and great genetic potentialities. The $H_{\rm E}$ and PIC of our study was >0.31 in the 4 sheep breeds; these results indicate that the *GH* gene had intermediate polymorphism (0.25 < PIC < 0.5).

Correlation between the GH gene and growth traits

Previous studies have established that the GH gene is related to sheep meat and goat milk performance (Marques et al., 2003; Ren et al., 2008). The GH gene directs the synthesis and secretion of the GH hormone and regulates body metabolism and growth (Liu, 2001). However, there is currently no research assessing the complete sequence of the sheep GH gene and its effects on growth traits. In the present study, we observed a statistically significant difference in the scores of growth traits between different genotypes within the 5' regulatory region, exon 4, and 3' UTR of the sheep GH gene. In the 5' regulatory region, genotype AA of Tibetan sheep indicated lower occurrence of growth traits than those of genotypes AB and BB. This finding was in accordance with previous observations by Komisarek et al. (2011), Wang et al. (2012), and Min et al. (2005). In exon 4, genotype BB of Tibetan sheep indicated greater

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growth traits than those of genotypes AA and AB; genotype AA of German Merino sheep indicated lower growth traits than those of genotypes AB and BB. These findings support those in previous observations by Bastos et al. (2001), Malveiro et al. (2001), and Zhang et al. (2008). Within the 3' UTR, genotype AA of Tibetan sheep indicated lower growth traits than those of genotypes AB, BB, and AC; genotype AC of German Merino sheep indicated greater growth traits than those of genotypes AB and BB. These findings provide evidence in the support of previous observations by Zhang et al. (1992) and Deng et al. (2008).

To our knowledge, this is the first report describing a significant correlation between the sheep *GH* gene complete sequence and growth traits. The results reported herein provide a theoretical foundation for further research on sheep meat performance. Considering that sheep meat performance is influenced by multiple factors such as birth weight, age, gender, and feeding manners (in particular), further studies (e.g., sample amplification and DNA typing) are required to facilitate the future understanding of the associations between specific alleles and SNPs, which will provide more detailed information with possible applications for meat-type sheep breeding programs in the future.

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