Growth hormone polymorphisms and growth traits in Chinese Tibetan sheep *Ovis aries*

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ABSTRACT. Growth hormone (GH) plays an important role in promoting growth, protein and muscle accretion, and fat catabolism, suggesting that GH is a potential candidate gene affecting growth traits in vertebrates. In this paper, polymorphisms in GH were investigated in 632 Chinese Tibetan sheep, by using DNA sequencing. Three single nucleotide polymorphisms were identified, including two mutations (g.616G>A and g.624G>A) in intron 2 and one synonymous mutation (g.498G>C) in exon 2. Association analyses showed that both g.498G>C and g.616G>A were significantly associated with several growth traits (at $P < 0.01$ or $P < 0.05$) in three investigated breeds. Our results demonstrate that GH variation may be used as a molecular marker for growth traits in Chinese Tibetan sheep.

Key words: Chinese Tibetan sheep; Growth hormone; Growth traits; SNP

INTRODUCTION

The Qinghai-Tibetan plateau is the highest and largest plateau in the world, known for its extremely harsh environment, including high altitude, severe cold, strong UV radiation, and short-growing season (Wiener et al., 2003). Tibetan sheep were the first artificially bred sheep in the natural ecosystem of the Qinghai-Tibetan plateau to adapt well to these conditions (Xin et al., 2011). Undoubtedly, they are an important species of grazing livestock with great economic value and high tolerance to the extreme environments, such as extreme cold, low oxygen concentrations, and low air pressure (Zhou et al., 2015).

Growth hormone (GH), a polypeptide hormone, is synthesized and secreted by the anterior pituitary eosinophil cells in mammals (Nørrelund et al., 2003). Together with other hormones of the somatotropic axis, GH can accelerate metabolism and promote the growth of many organs and tissues (Butler and Le Roith, 2001) that are directly involved in promoting growth, protein and muscle accretion, and fat catabolism (Chen et al., 2015). Zhang et al. (2007) identified two mutations in the third intron of the GH that were associated with abdominal fat in chickens. Jia et al. (2014) identified three novel SNPs in the ovine GH, which can affect growth traits (body weight, body length, body height, and heart girth) in sheep. Two SNPs (C253T and C303T) were identified in the GH promoter region that could affect carcass traits in Japanese Black cattle (Sugita et al., 2014). Similarly, SNPs in bovine GH (bGH), at bGH codons 127 and 172, were shown to affect carcass traits and fatty acid composition in Japanese Black cattle (Ardiyanti et al., 2009). Additionally, in goat, two active SNPs (A781G and A1575G) were associated with litter size and superovulation response (Zhang et al., 2011). Taken together, these findings lend credence to the hypothesis that GH is an excellent candidate gene for growth-related traits in livestock.

Thus far, no study has examined the association between SNPs and growth traits in general. Therefore, the present study was performed to identify SNPs in GH of Chinese Tibetan sheep and to evaluate the association of these polymorphisms with growth traits.

MATERIAL AND METHODS

This study was approved by the Ethics Committee of the Qinghai University (Qinghai, China), and all efforts were made to minimize suffering.

Animal and genomic DNA isolation

Blood samples were collected from 632 Chinese Tibetan sheep representing three breeds: Black Tibetan sheep (BT, N = 226), Gaoyuan Tibetan sheep (GT, N = 191), and Oula Tibetan sheep (OT, N = 215). These three groups represent the main Chinese breeds that are reared in the Provinces of Qinghai, Gansu, and Henan, respectively. Their growth traits (body weight, body height, body length, and chest circumference) were recorded at 3 years of age.

Genomic DNA was extracted from sheep blood (jugular vein samples) by the standard phenol-chloroform extraction procedure (He et al., 2012). DNA quantity and purity (A_{260}/A_{280} ratio) for each sample was assessed using a Nano-Drop™ 1000 Spectrometer (Thermo Scientific, Waltam, MA, USA).

Primer design and polymerase chain reaction (PCR) conditions

Primers to amplify the ovine GH gene were designed based on NCBI database sequences (GenBank accession No. NC_019468.1) using the Primer v. 5.0 software (PREMIER Biosoft, Palo Alto, CA, USA). Forward primer 3'-Region-F (5'-CTCCTGGTCTCTCCCTAG-3') and reverse primer 3'-Region-R (5'-GCCACTCACTGATTTCTG-3') were used to amplify a fragment of 532 bp in exons 2 and 3 of the GH gene. A PCR was conducted in 20-μL reactions containing 50 ng DNA, 10 pM each primer, 0.20 mM dNTPs, 2.5 mM $MgCl_2$, and 0.5 U Taq DNA polymerase (TaKaRa, Shiga, Japan). The following PCR conditions were used: 5 min at 95°C; 35 cycles of 30 s at 94°C, 35 s at 62.8°C, 40 s at 72°C; and a final extension at 72°C for 10 min. Digested products were detected by electrophoresis on 1.5% agarose gels.

SNP discovery and genotyping

Using single-strand conformation polymorphism to detect the three mutations would be time-consuming as well as complicated. Additionally, there are no suitable restriction endonucleases for restriction fragment length polymorphism, which is commonly used to detect mutation genotypes. Thus, the DNA from the 632 Chinese Tibetan sheep was sequenced to distinguish the genotypes of the three mutations in GH.

Statistical analysis

Genotype and allele frequencies, gene heterozygosity (H_E), effective allele numbers (N_E), polymorphism information content (PIC), and tests for deviation from Hardy-Weinberg equilibrium (HWE) were calculated by POPGENE v. 1.32 (Yeh et al., 1997).

The effects of genotype on Chinese Tibetan sheep growth traits were analyzed by the least-square method as applied in the general linear model procedure in SPSS 21 (IBM, Armonk, NY, USA). The following statistical linear model was used: $Y_{ij} = u + G_i + S_i + E_{ij}$, where Y_{ij} is the trait measured on each of the individual cattle, u is the overall population mean for the trait, G_i is the fixed effect associated with the genotype, S_i is the fixed effect due to sex, and E_{ij} is the standard error.

RESULTS

Polymorphisms and genetic diversity

Sequence analysis of the GH gene revealed three mutations: G>C mutation at 498 bp, G>A mutation at 616 bp, and G>A mutation at 624 bp, named g.498G>C, g.616G>A, and g.624G>A, respectively (Figure 1). The g.616G>A and g.624G>A mutations were located in intron 2, whereas the g.498G>C mutation was located in exon 2 and was a synonymous mutation (Pro87Pro) resulting in no change in the structure of the encoded protein.

The allelic and genotypic frequencies, and the genetic diversity parameters (H_E, N_E, and PIC) for the SNPs are summarized in Table 1. In three breeds, GG (g.498G>C), GG (g.616G>A), and AA (g.624G>A) were the most prevalent genotypes. A chi-square test showed that g.616G>A and g.624G>A were in HWE ($\chi^2 < \chi_{0.05}^2$) in OT sheep, whereas individual genotypic frequencies were severely out of HWE for other SNPs ($\chi^2 > \chi_{0.05}^2$). According to the

conventions for PIC classification (PIC < 0.25 is considered low, 0.25-0.50 is intermediate, and >0.50 is high polymorphism), our data showed that all SNPs fell in the intermediate polymorphism level.

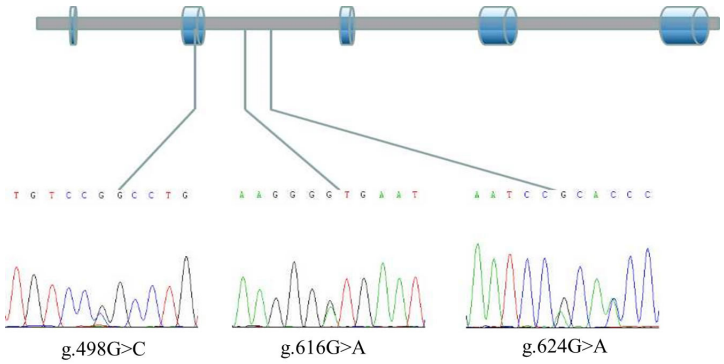

Figure 1. Schematic representation of the GH gene with the localization of the three identified SNPs.

Table 1. Genotype frequencies (%) of the GH for the SNPs in Chinese Tibetan sheep.

Site	Breed	Genotypic frequency			Allele frequency		H_E	N_E	PIC	χ^2 (HWE*)
g.498G>C	e	GG	GC	CC	G	C				
	BT	0.5885	0.2566	0.1549	0.7168	0.2832	0.4060	1.6835	0.3236	30.5830
	GT	0.4869	0.3141	0.1990	0.6440	0.3560	0.4585	1.8469	0.3534	18.9425
	OT	0.4744	0.3628	0.1628	0.6558	0.3442	0.4514	1.8230	0.3495	8.2913
g.616G>A		GG	GA	AA	G	A				
	BT	0.5664	0.2965	0.1372	0.7146	0.2854	0.4079	1.6889	0.3247	16.8670
	GT	0.5759	0.3089	0.1152	0.7304	0.2696	0.3939	1.6498	0.3163	8.8878
	OT	0.5163	0.4047	0.0791	0.7186	0.2814	0.4044	1.6790	0.3226	0.0001
g.624G>A		GG	GA	AA	G	A				
	BT	0.4867	0.2965	0.2168	0.6350	0.3650	0.4636	1.8642	0.3561	29.3694
	GT	0.4712	0.3194	0.2094	0.6309	0.3691	0.3573	0.4657	0.3573	18.8635
	OT	0.4698	0.3814	0.1488	0.6605	0.3395	0.4485	1.8132	0.3479	4.8133

HWE, Hardy-Weinberg equilibrium; $\chi_{0.05}^2 = 5.991$, $\chi_{0.01}^2 = 9.210$.

Effect of the polymorphism locus on growth traits in BT sheep

The association analysis between each marker and the growth traits in BT sheep is shown in Table 2. For g.498G>C, individuals with genotype GG had increased body weight and body length compared to the CC genotype ($P < 0.05$). For g.616G>A, individuals with genotype AA had higher values than those with GG and GA on body weight ($P < 0.05$). Unlike the other mutations, g.624G>A genotypes did not show any significant correlations with any of the measured growth traits ($P > 0.05$).

Effect of the polymorphism locus on growth traits in GT sheep

Table 3 shows the effects of the SNPs on growth traits in GT sheep. For g.498G>C, individuals with genotype GG had higher values than those with CC for body weight, body height, body length, and chest circumference ($P < 0.01$). Additionally, the body height and body length of individuals with genotype GC were higher than those with genotype CC (P <

0.05). For g.616G>A, individuals with genotype AA had higher values than those with GG on body weight ($P < 0.05$), while genotype AA had higher mean values for body length than those with genotype GG ($P < 0.01$). As observed in the BT sheep, there were no significant correlations between g.624G>A and any of the growth traits.

Table 2. Association of different genotypes of SNPs in GH with growth traits in BT sheep.

Site	Genotypes	Body weight (cm)	Body height (cm)	Body length (cm)	Chest circumference (cm)
g.498G>C	GG	43.52 ± 3.18^{a}	64.85 ± 5.29	69.30 ± 6.12^{a}	89.09 ± 8.21
	GC	41.33 ± 3.59	65.41 ± 5.37	67.24 ± 6.52	88.92 ± 8.59
	CC	40.75 ± 4.82^{b}	64.38 ± 6.92	66.39 ± 6.39^{b}	88.03 ± 8.22
g.616G>A	GG	41.88 ± 3.58^{b}	64.53 ± 7.25	68.12 ± 7.69	88.84 ± 7.60
	GA	41.98 ± 3.27^{b}	65.22 ± 6.39	68.16 ± 7.30	88.83 ± 7.38
	AA	44.75 ± 3.97^{a}	65.38 ± 6.77	68.99 ± 6.52	89.03 ± 8.26
g.624G>A	GG	42.04 ± 3.89	64.41 ± 5.92	67.92 ± 5.55	88.52 ± 7.21
	GA	42.29 ± 4.11	65.37 ± 5.40	68.33 ± 5.96	88.97 ± 7.52
	AA	43.96 ± 4.54	65.44 ± 6.29	69.20 ± 6.71	89.57 ± 7.70

Means with different superscript lower case letters are significantly different at $P < 0.05$.

Table 3. Association of different genotypes of SNPs in GH with growth traits in GT sheep.

Site	Genotypes	Body weight (cm)	Body height (cm)	Body length (cm)	Chest circumference (cm)
g.498G>C	GG	46.71 ± 3.92^{A}	67.28 ± 5.62^{A}	70.34 ± 6.36^{A}	93.32 ± 8.69^{A}
	GC	45.42 ± 3.59^{A}	66.67 ± 5.28^{a}	69.98 ± 6.55^{a}	90.42 ± 9.11
	CC	40.18 ± 4.29^{B}	62.50 ± 6.36^{Bb}	65.72 ± 7.21^{Bb}	88.68 ± 7.20^{B}
g.616G>A	GG	44.31 ± 3.28	65.77 ± 5.88	68.32 ± 5.59^{B}	90.92 ± 8.52
	GA	45.80 ± 5.44	66.48 ± 5.30	69.98 ± 6.21	91.78 ± 7.22
	AA	46.35 ± 4.26	67.06 ± 6.88	72.44 ± 6.67^{A}	93.48 ± 7.89
g.624G>A	GG	44.22 ± 3.47	65.32 ± 6.25	68.68 ± 5.77	90.85 ± 7.92
	GA	45.62 ± 3.95	66.86 ± 6.65	69.36 ± 5.10	91.63 ± 7.88
	AA	45.83 ± 4.62	66.88 ± 6.74	70.64 ± 6.82	92.67 ± 9.32

Means with different superscript lower and upper case letters are significantly different at $P < 0.05$ and $P < 0.01$, respectively.

Effect of the polymorphism locus on growth traits in OT sheep

The association results of single markers with the four growth traits in OT sheep are shown in Table 4. For g.498G>C, individuals with genotype GG had higher values than those with CC for body weight and body length ($P < 0.01$). In addition, the body weights of individuals with genotype GG were higher than those with genotype GC ($P < 0.05$). For g.616G>A, significant differences in body weight were observed between the AA and GG genotypes ($P < 0.05$). Compared with GG, individuals with the AA genotype showed better performance for body length ($P < 0.01$). Again, no significant correlations were observed in any of the growth traits for g.624G>A.

Table 4. Association of different genotypes of SNPs in GH with growth traits in OT sheep.

Site	Genotypes	Body weight (cm)	Body height (cm)	Body length (cm)	Chest circumference (cm)
g.498G>C	GG	61.01 ± 4.25^{Aa}	73.32 ± 5.24	76.44 ± 6.80^{A}	93.38 ± 9.58
	GC	56.43 ± 5.18^{b}	73.05 ± 5.85	75.29 ± 7.64^{A}	92.37 ± 9.26
	CC	52.71 ± 5.53^{Bc}	73.013 ± 7.43	70.65 ± 6.57^{B}	91.13 ± 7.66
g.616G>A	GG	57.35 ± 3.56^{b}	72.32 ± 6.95^{B}	74.44 ± 6.63^{b}	91.38 ± 7.26^{B}
	GA	57.79 ± 5.21^{b}	73.11 ± 7.36^{b}	75.18 ± 7.19	92.27 ± 7.57^{B}
	AA	63.29 ± 5.22^{a}	79.05 ± 7.62^{Aa}	78.76 ± 7.85^{a}	102.85 ± 9.84^{A}
g.624G>A	GG	57.75 ± 4.89	72.81 ± 7.51	75.01 ± 6.62	92.38 ± 9.25
	GA	57.88 ± 4.28	73.37 ± 5.83	75.12 ± 7.36	92.78 ± 9.81
	AA	59.08 ± 4.15	73.81 ± 5.26	75.21 ± 7.59	93.15 ± 7.50

Means with different superscript lower and upper case letters are significantly different at $P < 0.05$ and $P < 0.01$, respectively.

DISCUSSION

In the process of livestock breeding, growth traits are considered to be a crucial tool to assess the economic value of animals. These growth traits are affected by many factors, such as genotype, sex, age, breed, nutritional level, and other environmental factors (Gui et al., 2014). Accumulating evidence suggests that the candidate gene approach is one of many methods suitable for analysis of the association between gene polymorphism and phenotypes, which is a promising strategy for the genetic improvement of economically important quantitative traits (Edmunds et al., 2009; Periasamy et al., 2014). GH is a single-chain polypeptide hormone mainly secreted by somatotropes of the anterior pituitary gland and is an important regulator of somatic growth in almost all vertebrates (Thomas et al., 2007; Rak and Gregoraszczuk, 2008; Delgadin et al., 2015; Odle et al., 2015). Therefore, we hypothesized that GH might be associated with growth traits in Chinese Tibetan sheep.

In the present study, we detected one SNP (g.498G>C) in an exon and another two SNPs (g.616G>A and g.624G>A) mapped to introns, revealing their associations with growth traits in Chinese Tibetan sheep. Except for g.624G>A, both g.498G>C and g.616G>A were found to affect many growth traits. Specifically, at the g.498G>C locus, individuals with genotype GG had significantly greater body weight and body length in all breeds, compared with genotype CC. This suggests that the G allele might be associated with an increase in body weight and body length. At the g.498G>C locus, individuals with genotype AA had significantly greater body weight in all breeds, compared with genotype GG. Thus, the A allele appeared to be the beneficial genotype for body weight.

We noted that g.498G>C was a synonymous mutation and that g.616G>A was located in the intron region. Neither of the SNPs changed the structure of the encoded proteins, but our results demonstrated that they were still associated with some of the growth traits. There are two main reasons for these results: 1) such associations may be the result of linkage disequilibrium between these SNPs and other genes on the same chromosome that have significant effects on the growth traits studied here (Li et al., 2013). 2) Mutations could also affect both the splice donor site or nearby regions and regulatory motifs (Van Laere et al., 2003; Capon et al., 2004; Nackley et al., 2006; Krawczak et al., 2007). Further verifications are needed for understanding the underlying mechanisms.

In summary, genotyping and association analyses performed on the g.498G>C and g.616G>A demonstrated that these SNPs were significantly associated with growth traits. Hence, our findings suggest that GH could be used as a genetic marker for marker assisted selection (MAS) during animal breeding for excellent growth traits. We recommend that further research be performed by including a larger population size before its application in MAS.

Conflicts of interest

The authors declare no conflict of interest.

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