



## Short Communication

# Association Analysis between Exon II of *GH* Gene and Growth Traits in Sheep

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### ABSTRACT

SSCP were employed to investigate the genetic polymorphism of sheep *GH* gene and to analyze the correlation of genetic polymorphic sites with growth traits. The results showed that AA, AB and BB genotypes were detected in exon II of *GH* gene in the six sheep populations. The highest frequencies of AB genotype detected in Mongolia sheep, Small-tailed han sheep, Tong sheep, Lanzhou large-tailed sheep and Henan large-tailed Han sheep were 0.375, 0.531, 0.545, 0.350 and 0.596, respectively, and the highest frequency of BB genotype in Yuxi fatty-tailed sheep was 0.378. Exon II of *GH* gene was significantly correlated with body weight, carcass weight, chest width and hip height ( $P < 0.05$ ). Moreover body weight and carcass weight of AB genotype were significantly higher than those of AA and BB genotypes ( $P < 0.05$ ) and chest width and hip height of AB genotype were remarkably higher than those of AA genotype ( $P < 0.05$ ).

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### Authors' Contribution

JB conceived and designed the study and conducted the lab work. ZD and YC analyzed the data and wrote the article. YL and YY helped in sampling. JL helped in analysis of data.

### Key words

Sheep, *GH* gene, Growth traits, SSCP, Association analysis

As the major gene influencing animal growth traits, *GH* gene has functions of improving feed conversion and promoting protein synthesis in muscle. Warwick *et al.* (1984) cloned *GH* gene of sheep for the first time and positioned it on chromosome 11. The *GH* gene of sheep consists of five exons (the lengths of which were 13, 161, 117, 162 and 198 bp, respectively) and four introns (the lengths of which were 246, 231, 227 and 273 bp, respectively). Goat *GH* gene was positioned on chromosome 12, and its length is largely identical to that of sheep *GH* gene. Afifi *et al.* (2019) found that intron II, intron III and exon IV of *GH* gene had significant correlations with milk yield of Najdi sheep. Henan large-tailed han sheep, small-tailed han sheep and Yuxi fatty-tailed sheep are excellent local sheep breeds in Henan Province, especially Henan large-tailed han sheep and small-tailed han sheep have the characteristics of multiple lambs (Bai *et al.*, 2014, 2016, 2017, 2020). Hence, the polymorphisms of *GH* gene in six sheep populations were detected in this study, and further association analysis between *GH* gene polymorphisms and growth traits were performed, aiming at providing a

theoretical basis for marker assisted selection and further variety breeding of sheep.

### Materials and methods

Fifty sheep were sampled for each breed, 10 mL of jugular venous blood was collected in the presence of anticoagulant ACD from each sheep and preserved at  $-20^{\circ}\text{C}$ . Genomic DNA was extracted by the whole-blood DNA kit (Shanghai Bioengineering Co., Ltd.). The primer sequences of exon II of *GH* gene are as follows: F: TCTAGGACACATCTCTGGGG, R: CTCTCCCTAGGCCCCGGAC (Hu *et al.*, 2007) and the annealing temperature is  $57^{\circ}\text{C}$ .

Seven  $\mu\text{L}$  of each PCR product was mixed with 13  $\mu\text{L}$  of loading buffer containing. After denaturation at  $94^{\circ}\text{C}$  for 5 min and snap-cooling on a freeze-block ( $-20^{\circ}\text{C}$ ), 20  $\mu\text{L}$  of each sample was loaded onto a  $0.5 \times \text{MDE}$  gel and subjected to electrophoresis in a Mini-Protean 3 Cell (Bio-Rad, USA) at 120 V and  $20^{\circ}\text{C}$  for 5 h using  $0.5 \times \text{TBE}$  as the buffer. After electrophoresis, gels were stained with ethidium bromide for 1 h and photographed using ultraviolet transillumination.

SPSS17.0 statistical software was used to analyze the association between different genotypes and growth traits, and Duncan multiple comparison method was used to make multiple comparison. The final results were expressed in the form of mean value  $\pm$  standard error.

### Results and discussion

The PCR amplification results of exon II of *GH* gene

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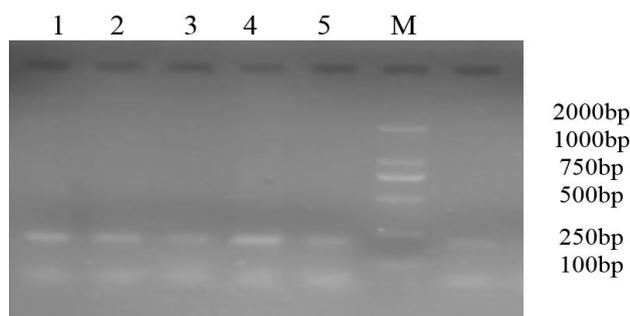
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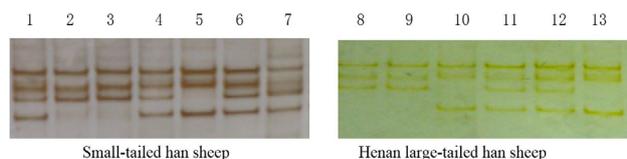
Table I. Genetic polymorphism of exon II of *GH* gene in sheep.

Population	Genotype frequency			Gene frequency		Population genetic polymorphism		
	AA	AB	BB	A	B	Heterozygosity	Polymorphism information content	Number of effective alleles
Mongolia sheep	0.313	0.375	0.312	0.500	0.500	0.500	0.375	2.000
Small-tailed han sheep	0.281	0.531	0.188	0.547	0.453	0.496	0.373	1.982
Tong sheep	0.364	0.545	0.091	0.636	0.364	0.463	0.356	1.862
Yuxi fatty-tailed sheep	0.311	0.311	0.378	0.467	0.533	0.498	0.374	1.991
Lanzhou large-tailed sheep	0.350	0.350	0.300	0.525	0.475	0.499	0.374	1.995
Henan large-tailed han sheep	0.213	0.596	0.191	0.511	0.489	0.500	0.375	1.999

in five sheep populations are shown in Figure 1. PCR product was subjected to SSCP. According to Figure 2 three genotypes were detected by exon II of *GH* gene in sheep, being AA, AB and BB, respectively.

Fig. 1. PCR product detection of exon II of *GH* gene.

Note: 1, 2, 3, 4 and 5 are Mongolia sheep, Small-tailed Han sheep, Tong sheep, Lanzhou large-tailed sheep and Henan large-tailed Han sheep, respectively.

Fig. 2. Detection of exon II polymorphism of *GH* gene in sheep.

Note: Lanes 1, 4, 6, 7, 11, 12 are AB genotype, 2,3,8,9 are BB genotype and 5,10,13 are AA genotype.

Table I shows that the highest AB genotype frequencies in Mongolia sheep, small-tailed han sheep, Tong sheep, Lanzhou large-tailed sheep and Henan large-tailed han sheep were 0.375, 0.531, 0.545, 0.350 and 0.596, respectively, and the BB genotype frequency was the highest in Yuxi fatty-tailed sheep (0.378). The genetic heterozygosity values were the same in Mongolia sheep

and Henan large-tailed han sheep, both being 0.500, and the polymorphic information contents in the two sheep populations were also the highest (0.375), namely genetic diversity of Mongolia sheep and Henan large-tailed han sheep was quite abundant.

As an important candidate gene, *GH* gene has been reported largely. Zhou *et al.* (2009) found that G→C mutation occurred at the 11<sup>th</sup> site of exon II of *GH* gene in Hainan black goat, which led to change of coding amino acid, three genotypes including AA, AB and BB were detected in exon II of *GH* gene. Li (2007) found that AA and AB genotypes of exon II of *GH* gene could be detected in three goat populations. Marques *et al.* (2003) found that A/B genotype was detected from exon II of *GH* gene in Jarlnelistae goat population. Hu *et al.* (2007) detected AB and BB genotypes from exon II of *GH* gene in five goat populations. Han (2016) detected three genotypes from exon II of *GH* gene in China Tibetan sheep, and found a g.498G>C mutation site. In this study, AA, AB and BB genotypes were detected from exon II of *GH* gene in the three sheep populations, and the sequencing results indicated that exon II of *GH* gene experienced G→C mutation, which was according to the study result of Han (2016).

It can be seen from Table II that body weight and carcass weight of AB genotype were significantly higher than those of AA and BB genotypes ( $P<0.05$ ), and AA and BB genotypes had no significant differences in the body weight and carcass weight ( $P>0.05$ ). Chest width and hip height of AB genotype were remarkably higher than those of AA genotype ( $P<0.05$ ), but AB genotype and BB genotype had insignificant differences in the aspect of chest width and hip height ( $P>0.05$ ). Therefore, exon II of *GH* gene had no significant influence on other growth traits ( $P>0.05$ ). Abdelmoneim *et al.* (2017) indicated that intron II, intron IV and exon IV of *GH* gene had significant correlations with 120d body weight and daily weight gain of Harri sheep. The study by Han (2016) manifested

that exon II (g.498G>C) of *GH* gene was significantly correlated with sheep body weight in all of the three sheep populations. The study by Yousefi *et al.* (2012) showed that exon V of *GH* gene had no remarkable influence on one-year-old body weight of Zel sheep. The study by Hu *et al.* (2007) indicated that exon II of *GH* gene had no significant correlation with body weight, body height and chest circumference of Xinong Saanen dairy goat. This study pointed out that exon II of *GH* gene had significant correlations with body weight, carcass weight, chest width and hip height of sheep ( $P<0.05$ ), and AB genotype showed certain advantages, and this study result was similar to that of Han (2016).

**Table II. Correlation analysis between exon II of *GH* gene and growth traits in sheep.**

Growth traits	AA	AB	BB
Body weight (g)	46.25±11.25 <sup>b</sup>	54.003±1.78 <sup>a</sup>	46.769±1.20 <sup>b</sup>
Body height (cm)	69.00±11.00 <sup>a</sup>	69.50±2.08 <sup>a</sup>	65.00±3.21 <sup>a</sup>
Body length (cm)	68.00±14.00 <sup>a</sup>	69.47±1.76 <sup>a</sup>	66.62±1.64 <sup>a</sup>
Chest width (cm)	22.50±3.50 <sup>b</sup>	29.24±1.23 <sup>a</sup>	26.92±1.179 <sup>ab</sup>
Rump height (cm)	71.00±9.00 <sup>a</sup>	71.03±3.24 <sup>a</sup>	68.92±1.87 <sup>a</sup>
Hip height (cm)	57.00±6.00 <sup>b</sup>	69.06±1.76 <sup>a</sup>	64.23±2.27 <sup>ab</sup>
Foreleg height(cm)	22.50±4.50 <sup>a</sup>	26.06±2.33 <sup>a</sup>	26.08±3.01 <sup>a</sup>
Head length (cm)	18.00±2.00 <sup>a</sup>	21.35±0.69 <sup>a</sup>	19.31±0.83 <sup>a</sup>
Chest circumfer- ence (cm)	88.50±16.50 <sup>a</sup>	90.68±2.14 <sup>a</sup>	91.54±2.29 <sup>a</sup>
Circumference of cannon bone (cm)	9.50±1.50 <sup>a</sup>	10.12±0.36 <sup>a</sup>	9.23±0.34 <sup>a</sup>
Rump length (cm)	22.00±1.00 <sup>a</sup>	23.94±1.02 <sup>a</sup>	22.54±0.93 <sup>a</sup>
Neck length (cm)	22.50±3.50 <sup>a</sup>	33.03±3.18 <sup>a</sup>	31.54±3.02 <sup>a</sup>
Waist width (cm)	22.50±5.50 <sup>a</sup>	20.47±3.01 <sup>a</sup>	27.08±2.88 <sup>a</sup>
Hip width (cm)	21.50±2.50 <sup>a</sup>	20.77±0.83 <sup>a</sup>	19.39±1.05 <sup>a</sup>
Chest depth (cm)	32.50±5.50 <sup>a</sup>	33.94±1.11 <sup>a</sup>	33.08±0.85 <sup>a</sup>
Head depth (cm)	18.50±4.50 <sup>a</sup>	17.35±0.69 <sup>a</sup>	16.50±0.75 <sup>a</sup>
Back height (cm)	68.00±8.00 <sup>a</sup>	72.79±1.62 <sup>a</sup>	70.00±1.63 <sup>a</sup>
Carcass weight (cm)	21.00±5.50 <sup>b</sup>	24.41±0.86 <sup>a</sup>	20.64±1.42 <sup>b</sup>
Hip circumference (cm)	92.00±8.00 <sup>a</sup>	93.88±1.91 <sup>a</sup>	89.46±3.74 <sup>a</sup>
Abdomen circum- ference (cm)	98.00±17.00 <sup>a</sup>	97.47±3.08 <sup>a</sup>	97.62±2.72 <sup>a</sup>

Note: different letters showed significant difference ( $P<0.05$ ); the same letter showed no significant difference ( $P>0.05$ ).

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#### Statement of conflict of interest

The authors have declared no conflict of interest.

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