



Association analysis of GHR gene polymorphism with growth traits in sheep

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GHR gene codes single-stranded transmembrane structural protein of target cell, and the mature sheep GHR protein consists of 616 amino acids, of which 350, 24 and 242 are in intracellular region, transmembrane region and extracellular region, respectively. It is found that as GH could not directly cross membrane, it must bind to GHR on target cell so as to transfer signal into intracellular region. Therefore, the quantity of GHR is directly related to the exertion of GH biological functions. GH binds to GHR to regulate many kinds of physiological activities in animals, and relative researches have made certain progress, but only few studies regarding the correlation of sheep growth traits were reported. 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.* 2015, 2016, 2017, 2019, 2020). Hence, the polymorphisms of GHR genes in three sheep populations were detected in this study, and further association analysis between GHR genes polymorphisms and growth traits were performed, aimed at providing a theoretical basis for marker assisted selection and further variety breeding of sheep.

Sheep(50) were sampled for each breed, and 10 mL of jugular venous blood was collected from each sheep, anticoagulated via ACD and preserved at -20°C. Genomic DNA was extracted by the whole-blood DNA kit (Shanghai Bioengineering Co., Ltd.). The primer sequences of GHR gene were referred from Shi A (2014). The primer sequence is as follows: F: ATTAGGACCATCCATTACC, R: TATTTCCATTCCCACCA. Annealing temperature was kept as 52°C. The primers were synthesized by Zhengzhou Dingguo Biotech Co., Ltd.

Amplified product of GHR was digested by restriction endonuclease Hpa II, and the enzyme digestion reaction mix (total volume 20 µL) consisted of 7.4 µL ddH₂O, 10 µL PCR product, 2 µL 10× Buffer, 0.6 µL restriction enzyme Hpa II. After digesting for 4 h, polymorphisms of enzyme

digestion products were detected through 3% agarose gel electrophoresis.

It could be seen from Fig. 1 that amplified fragment of sheep GHR gene was identical with the target fragment band, which was clear, so the follow-up experiment could be implemented. Two genotypes CD and DD were detected by GHR gene in sheep populations. Yan *et al.* (2019) detected II, ID and DD genotypes from intron I of GHR gene in white cashmere goat of northern Shaanxi. Shi A (2014) found cutting site of Hpa II in exon IV of GHR gene and detected CD and DD genotypes in Tan sheep. This study indicated that CC, CD and DD genotypes were only detected in Henan large-tailed han sheep population, CD and DD genotypes were detected in other sheep populations, which was similar to the study of Shi A (2014).

Table 1 shows that three genotypes CC, CD and DD were detected by GHR gene in Henan large-tailed han sheep, while only DD and CD were detected in Small-tailed han sheep, Yuxi fatty-tailed sheep, Hu sheep and Dorper sheep. GHR gene had the highest DD genotype frequencies in Yuxi fatty-tailed sheep (0.889), Dorper sheep (0.784), Henan large-tailed han sheep (0.785), Hu sheep (0.529) and Small-tailed han sheep (0.750). GHR gene had the highest genetic heterozygosity and polymorphic information content in Hu sheep, being 0.360 and 0.292, respectively, so the genetic polymorphism of Hu sheep was higher than other sheep populations.

Table 2 shows that chest circumference and abdomen circumference of CD genotype were notably higher than those of DD genotype ($P < 0.05$). The determined growth traits of CD genotype like body weight, body height, body length, back height, carcass weight and rump height were higher than those of DD genotype, but the differences were insignificant ($P > 0.05$). The study by Sun *et al.* (2012) indicated that GHR expression was in direct proportion of meat quality of Hu sheep. Taking Gannan Oula sheep as study object, Yang *et al.* (2015) obtained two alleles and three genotypes on exon X of GHR gene, where body weight and body height of AA type Oula sheep were significantly higher than those of AB and BB genotypes. Yan *et al.* (2019) showed that there were three genotypes of

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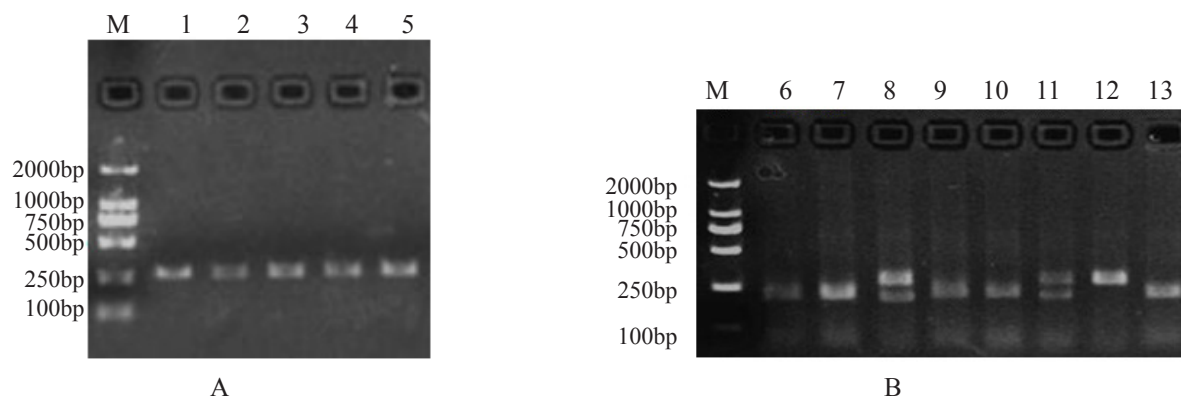


Fig. 1. Polymorphism detection of GHR gene in sheep. A. Detection of PCR products. Lanes 1,2,3,4,5 are Henan large-tailed han sheep, Small-tailed han sheep, Yuxi fatty-tailed sheep, Hu sheep and Dorper sheep respectively. B. Detection of PCR-RFLP products. Lanes 6, 7, 9, 10, 13, DD genotype; Lanes 8 and 11, CD genotype; Lane 12, CC genotype; M, Marker DL2000.

GHR gene in Northern Shaanxi White Cashmere Goat: II, ID and DD gene, in terms of body weight, hip width, body height, recommendation height and chest depth, genotype ID and DD individuals had more significant advantages than type II individuals ($P<0.05$). Shi A (2014) found Hpa II incision enzyme site on exon IV of GHR gene in Tan sheep, and pointed out that body weight of CD genotype was higher than that of DD genotype. This study verified that GHR gene of sheep had significant correlations with chest circumference and abdomen circumference, and CD genotype was better than other genotypes, and this result was similar to the study result of Shi A (2014).

SUMMARY

In this study, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was employed to investigate the polymorphism of GHR gene and the correlations of polymorphic sites with growth traits. The results showed that GHR detected CC, CD and DD genotypes in the five sheep populations. GHR gene had the highest DD genotype frequencies in Yuxi fatty-tailed sheep, Dorper sheep, Henan large-tailed han sheep, Hu sheep and small-tailed han sheep, being 0.889, 0.784, 0.785, 0.529 and 0.750, respectively. The correlation analysis indicated that GHR gene had significant correlations with chest circumference and abdomen circumference of sheep

Table 1. Population genetic polymorphism of GHR gene in sheep

Population	Genotype frequency			Gene frequency		Heterozygosity	Polymorphism information content	Number of effective alleles
	DD	CD	CC	C	D			
Yuxi fatty-tailed sheep	0.889	0.111	0	0.056	0.944	0.106	0.100	1.118
Dorper sheep	0.784	0.216	0	0.108	0.892	0.193	0.174	1.239
Henan large-tailed han sheep	0.785	0.196	0.019	0.116	0.884	0.205	0.184	1.258
Hu sheep	0.529	0.471	0	0.235	0.765	0.360	0.295	1.561
Small-tailed han sheep	0.750	0.250	0	0.125	0.875	0.219	0.195	1.280

Table 2. Association analysis of GHR gene and growth traits in sheep

Growth traits	CD genotype	DD genotype	Growth traits	CD genotype	DD genotype
Body weight (kg)	50.21±6.79a	50.18±7.86a	Back height (cm)	72.42±1.45a	69.98±0.84a
Body height (cm)	68.08±10.34a	67.38±10.28a	Carcass weight (kg)	23.66±1.43a	22.75±1.01a
Body length (cm)	68.92±8.26a	67.80±7.09a	Rump height (cm)	73.81±1.64a	67.67±2.77a
Chest circumference (cm)	96.42±7.12a	87.58±8.83b	Rump length (cm)	21.90±0.90a	23.65±0.85a
Chest width (cm)	28.58±5.58a	27.45±4.61a	Head length (cm)	20.27±1.24a	20.40±0.55a
Chest depth (cm)	33.33±4.59a	33.60±3.87a	Head depth (cm)	17.72±0.98a	16.77±0.63a
Hip width (cm)	26.50±5.44a	29.00±13.67a	Neck length (cm)	34.27±4.29a	29.17±2.03a
Buttock tip width (cm)	20.32±3.73a	20.15±3.44a	Frontal width (cm)	13.00±0.82a	12.97±0.44a
Buttock tip height (cm)	65.00±3.25a	66.85±8.93a	Abdomen circumference (cm)	104.09±2.69a	93.60±2.65b
Circumference of cannon bone (cm)	9.58±1.65a	9.80±1.61a	Hip circumference (cm)	92.36±3.67a	91.95±2.25a

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

($P<0.05$), namely chest breadth and abdominal girth of CD genotype were notably higher than those of DD genotype ($P<0.05$). In addition, determined values of growth traits of CD genotype, e.g. weight, body height, body length, back height, carcass weight and rump height were higher than those of DD genotype, but the differences were insignificant ($P>0.05$).

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