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Short Communication

Analysis of Polymorphism of Growth Hormone Secretagogue Receptor in Sheep

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ABSTRACT

The purpose of this study was to find candidate genes regulating the growth and development of sheep. The polymorphism of GHSR gene in five sheep populations was analyzed by PCR and sequencing techniques. The results showed that large tailed han sheep, small tailed han sheep, yuxi fatty tailed sheep, dorper sheep, hu sheep all have two mutation sites (C155T and C624T). For locus C155T, allele frequencies of C in large tailed han sheep, small tailed han sheep, yuxi fatty tailed sheep, dorper sheep and hu sheep were 0.73, 0.83, 0.54, 0.69 and 0.68 respectively, which indicated that C was the dominant allele in five sheep populations. For locus C624T, allele frequencies of C in large tailed han sheep, small tailed han sheep, yuxi fatty tailed sheep, dorper sheep and hu sheep were 0.70, 0.87, 0.52, 0.70 and 0.76 respectively, which indicated that C was the dominant allele in five sheep populations. The C155T mutation site of GHSR-3 gene led to a codon change from GCC to GCT, both of which coding the same aa, alanine, indicating C155T was a synonymous mutation site. The C624T mutation site of GHSR-4 gene led to a code change from CTT to CCT, and the corresponding aa changed from leucine to proline, which was a missense mutation site.

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Authors' Contribution
JYB conceived and designed the study, collected samples, analyzed the data and wrote the article. YBY and YQW helped in sampling. HDF and XW helped in analysis of data. HC and XYF helped in writing of article.

Key words
Small tailed han sheep, Large tailed han sheep, GHSR gene, SNP, Polymorphism

GHRL is an endogenous ligand with growth hormone secretagogue rceptor (GHSR) or ghrelin receptor found in mammals in recent years. The binding of GHRL to receptor GHSR can specifically stimulate GH release and increase animal appetite, thus regulating body weight, energy metabolism and fat accumulation. In livestock production, *GHRL* gene and *GHSR* gene have been reported in ducks (Nie *et al*., 2009; Li *et al*., 2009; Li *et al*., 2010), cattle (Zhang *et al*., 2011), goats (Bai *et al*., 2019), which reveal that they are important candidate genes for body growth and development. Therefore, exploring the combination effect of *GHRL* gene and *GHSR* gene has guiding significance for livestock breeding and production.

Considering importance of GHSR on sheep growth, *GHSR* gene was used as the candidate gene of sheep growth traits to search possible SNP sites. Research results lay foundations for genetic marker of sheep growth traits and provide scientific theoretical references for breeding and quality identification of other sheep species.

Materials and methods

Blood samples (10mL) were collected from venous in wings of large tailed han sheep (50), small tailed han sheep (50), yuxi fatty tailed sheep (50), dorper sheep (50), hu sheep (50) and processed by ACD anti-freezing (1:6). Genomic DNA was extracted by whole blood DNA kit provided by Beijing Dingguo.

The primer sequences of *GHSR* gene were from Song *et al*. (2015) (Table I). The primers were synthesized by Beijing Dingguo Changsheng Biotechnology Co., ltd. The total size of the PCR reaction system was 12.5μL, including 8.65μL of ddH_2O, 1.25μL of 10×buffer, 0.75μL of Mg^{2+}(25 mmol/L), 0.5μL of DNA template, 0.5μL (10 mmol/L) of upstream and downstream primers, 0.25μL of dNTPs, and 0.1μL of Taq enzyme. The PCR amplification process was as follows: denaturation for 3 min at 95℃; denaturation for 45 s at 94℃, annealing for 60s at 55℃ or, extension for 60s at 72℃ and 30 cycles, extension for 12 min at 72℃, and preserving at 4℃. GHSR-3and GHSR-4 amplification products of mixed DNA were sent to Beijing Qinke Xinye Biotech Co., Ltd for sequencing. Assembly analysis of sequencing results was carried out by DNAStar and SeqMan program.

Sequencing peak diagram read by SeqMan program in DNAStar software and Chromas software for calibration and sequencing comparison of sequencing results. Scaleplate in Mwsnap software was

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Table I. The information of primer sequences.

Primer name	Sequences	Amplification fragment	Production size (bp)	Annealing temperature (℃)
GHSR-3	F:CGTTCTCTTTCTCATTGTCTTTTCA R:TCCCAAGTTCTGCTGTGCTAT	E2-3'UTR	413	55.0
GHSR-4	F:TCACTCATTATTCTACACCAGAAGC R: ACACCCAATGTCCAAATTAAGG	3'UTR-E2	549	57.3

Table II. Estimation of SNP allele frequency of *GHSR* gene in sheep.

Loci	Large tailed han sheep	Small tailed han sheep	Yuxi fatty tailed sheep	Dorper sheep	Hu sheep
C155T	C (0.73)	C (0.83)	C (0.54)	C (0.69)	C (0.68)
	T (0.27)	T (0.17)	T (0.46)	T (0.31)	T (0.32)
C624T	C (0.70)	C (0.87)	C (0.52)	C (0.70)	C (0.76)
	T (0.30)	T (0.13)	T (0.48)	T (0.30)	T (0.24)

used to measure peak height corresponding to different SNP alleles. Gene frequency was estimated according to the following formula (Bai *et al*., 2016a, 2016b, 2017): $F_1 = H_i/(H_1+H_2)$ (i=1, 2), where F_1 is frequency of an allele at SNP site, H_1 and H_2 are heights of peak 1 and peak 2 of this SNP allele on the sequencing diagram.

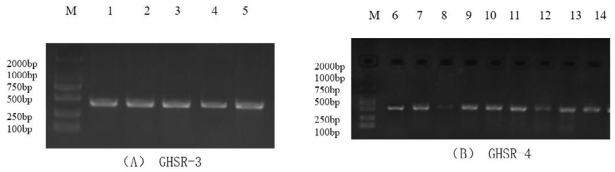

Fig. 1. Agarose electrophoresis detection of GHSR-3 and GHSR-4; Note: M: marker DL2000,1、6、7: Large tailed han sheep, 2、8、9、Small tailed han sheep, 3、10、11、Yuxi fatty tailed sheep, 4、12、13、Dorper sheep, 5、14、Hu sheep.

Results and discussion

Figure 1 shows agarose gel electrophoresis (2%) results of PCR amplification products of *GHSR* gene in sheep. PCR products of *GHSR*-3 and *GHSR*-4 have single band (Fig. 1).

Based on comparison observation of sequencing results, it found that large tailed han sheep, small tailed han sheep, yuxi fatty tailed sheep, dorper sheep, hu sheep all have two mutation sites (SNP sites). C155T and C624T were detected in PCR products of GHSR-3 and GHSR-4. Sequencing maps of these two mutation sites are shown in Figure 2.

For locus C155T, allele frequencies of C in large tailed han sheep, small tailed han sheep, yuxi fatty tailed sheep, dorper sheep and hu sheep were 0.73,0.83,0.54,0.69,0.68,

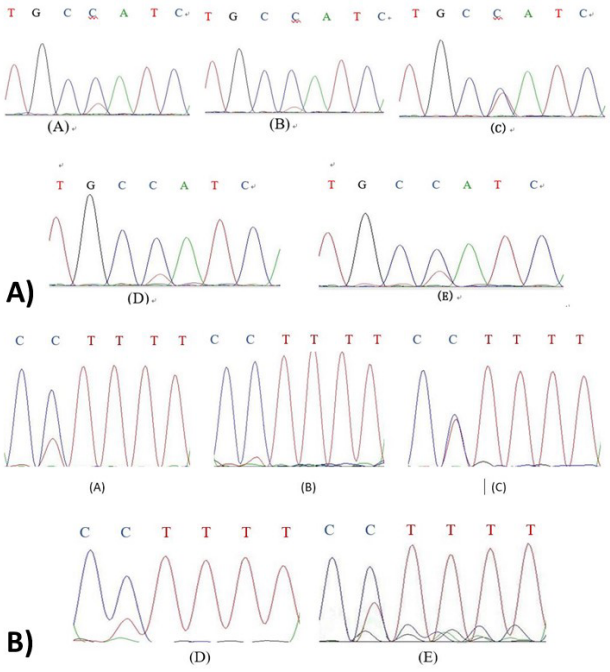

Fig. 2. Polymorphism detection of C155T Site and C624T Site; Note: (A) Large tailed han sheep; (B) Small tailed han sheep; (C) Yuxi fatty tailed sheep; (D) Dorper sheep; (E) Hu sheep.

respectively, which indicated that C was the dominant allele in five sheep populations. For locus C624T, allele frequencies of C in large tailed han sheep, small tailed han sheep, yuxi fatty tailed sheep, dorper sheep and hu sheep were 0.70,0.87,0.52,0.70,0.76, respectively, which indicated that C was the dominant allele in five sheep

populations (Table Ⅱ).

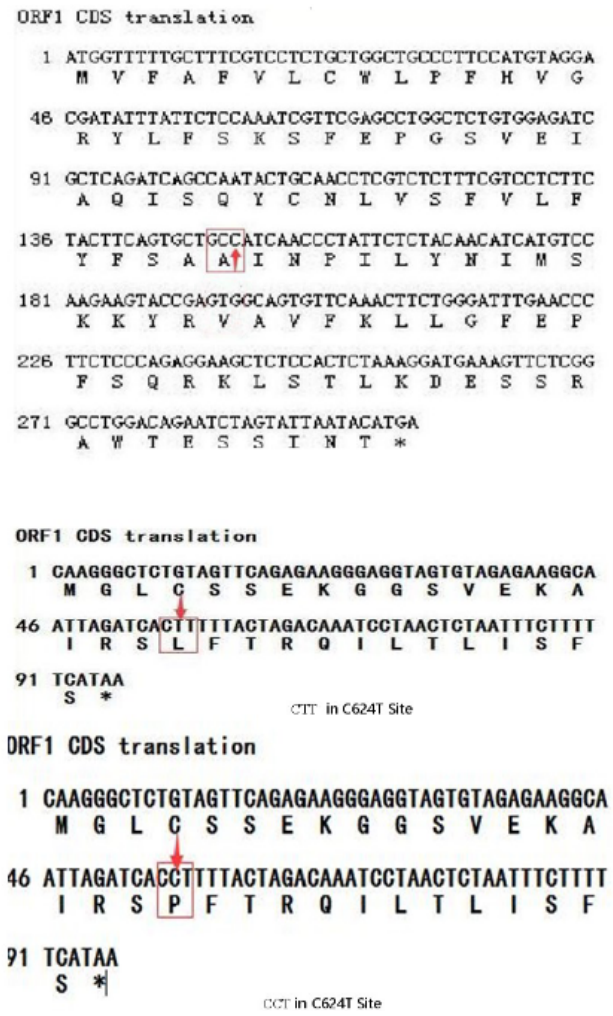

Fig. 3. Amino acid alignment of C155T and C624T mutation in sheep.

At present, there are few studies on SNPs of *GHSR* and *GHRL* genes. Liu *et al.* (2013) found exons 2 and 3'UTR of GHSR in Qianbei Ma sheep and found G996A locus, which is closely related to body weight. Song *et al.* (2015) detected G200A mutation in exon 2 of GHSR to exon 3 of Guizhou white goat and Guizhou black goat, and C14T mutation in exon 4 of *GHRL*. Luo *et al.* (2014) found C345T mutation in *GHRL* gene exon of Guizhou goats, which was significantly associated with body weight, height and chest circumference. In this study, C155T and C624T mutation sites were found in *GHSR* exons 2 to 3' -UTR in five Henan local sheep. Sequence comparison confirmed that there were two mutation sites in sheep.

By comparing the amino acid of C155T mutation site of *GHSR*-3 gene, it was found that the mutation site changed from GCC to GCT, and both before and after mutation were alanine, which was synonymous mutation site (Fig. 3). Amino acid comparison of C624T mutation site of GHSR-4 gene showed that the mutation site changed from CTT to CCT, from leucine to proline, and was a missense mutation site (Fig. 3).

Prediction of amino acid structure showed that the fat index of CTT of *GHSR*-4 gene was higher than that of CCT, which belonged to hydrophobic structure, had good stability and was conducive to fat deposition. The prediction of secondary structure of GHSR-4 showed that the structure of GHSR-4 was mainly alpha-helix, which was consistent with the prediction of secondary protein of Song *et al.* (2015), The transformation of amino acid CTT into CCT resulted in the transformation of protein 17-22 beta into beta folding. The results of gene localization and functional prediction showed that CCT protein encoded more in nucleus and mitochondria, and increased fatty acid metabolism, which was consistent with the results of long-term evolution of sheep.

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Statement of conflict of interest

The authors declare no conflict of interest.

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