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Short Communication

Polymorphism Analysis of GHRL Gene in Goat

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ABSTRACT

This study is to search candidate genes that control goat growth and explore SNP loci of GHRL genes, thus laying foundations for genetic marker of growth traits of goats. In this study, GHRL genes of black goat and Yaoshan goat were collected for PCR amplification. Polymorphism of goat species was tested by sequencing technology. Results demonstrated that there are two mutation sites (SNP loci) of the primer GHRL-2 in black goat and Yaoshan goat, which are G447C site and T498C site. At G447C site, gene frequencies of G and C in black goat and Yaoshan goat were detected 0.621/0.379 and 0.793/0.207, respectively. In black goat and Yaoshan goat, G is the dominant allele in G447C. At T498C site, gene frequencies of T and C in black goat and Yaoshan goat were tested 0.675/0.325 and 0.847/0.153, respectively. At this site, T is the dominant allele in Yaoshan goat. In black goat, GHRL-6 has polymorphism and its mutation site is C105T. The gene frequencies of C and T at C105T in black goat were detected 0.698 and 0.302, respectively. T is the dominant allele at C105T in black goat. These research results lay a theoretical foundation for further correlation analysis with growth indexes of goats.

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Authors' Contributions
JYB conceived and designed the study, conducted the lab work, analyzed the data and wrote the article. YGZ helped in sampling. YQW helped in analysis of data.

Key words
Black goat, GHRL gene, SNP loci, Phylogenetic tree, Yaoshan goat.

GHRL is the endogenous ligand of growth hormone secretagogue (GHSR) discovered by (Kojima *et al*., 1999). It contains 28 peptides of compounds which mainly distribute in peripheral organs and nervous system of human being, rats and animals. It is a kind of Ocacylated growth hormone releasing peptidein stomach and possesses many important functions, such as adjusting stomach digestion, evacuation and dynamiting, adjusting fat, *etc*. (Gualillo *et al*., 2003). GHRL has been widely studied in livestock. It is located in 12# chromosome of chicken, including 5 exons and 4 introns (Li *et al*., 2010). Li *et al*. (2009) discovered three mutation sites of GHRL in 8 duck species: 9 bp missing at 157 bp, mutation of T-C at 431 bp, and mutation of A-G at 909 bp. In goat, GHRL is located onto the 22# chromosome and has 4 exons and 3 introns (Zhang *et al*., 2009). Luo *et al*. (2014) made a polymorphism study in goat by PCR-SSCP, finding a mutation site (C345T) at GHRL exon 4 where has two genotypes CC and CT. Body weight, body height and chest width of CT gene individuals are significantly higher than those of CC gene individuals. It was speculated that this mutation is a sense mutation. Song *et al*. (2015) discovered mutations of PCR products of GHRL genes at T78C, G141A and C14T in goat, which lie in exon 2, intron 2 and exon 4. Among them, body weight, body height and chest width of CT gene individuals at C14T are significantly higher than those of CC gene individuals ($P<0.05$), but no significant difference was observed in rest groups and trait indexes ($P>0.05$). This indicated that GHRL can be potential molecular genetic marker as growth trait of goat. Currently, many beneficial explorations of GHRL gene in animals like chicken, duck and rats have been made, which achieved many fruits. However, there are few researchers concerning GHRL in sheep and goat. Considering its importance in goat growth, GHRL genes are used as candidate genes of goat growth traits to search possible SNP loci, laying foundations for genetic marker of goat growth traits.

Materials and methods

Yaoshan goat (40) and black goat (40) were collected from Mutton Sheep Test Station in Luoyang City of Henan Province. Blood samples (10mL) were collected from neck veins of each goat, processed by ACD anti-freezing (1:6) and stored under -20°C. Genome DNA was extracted by whole blood DNA kit provided by Beijing Dingguo.

The primer sequences of GHRL-2 and GHRL-6 gene (Table I) was from Song *et al*. (2015). The primers were synthesized by Beijing Dingguo Changsheng Biotechnology Co., Ltd.

GHRL-2 and GHRL-6 amplification products of mixed DNA of different goat species were sent to Beijing Qinke Xinye Biotech Co., Ltd for sequencing. Assembly analysis of sequencing results was carried out by DNAStar and SeqMan Program.

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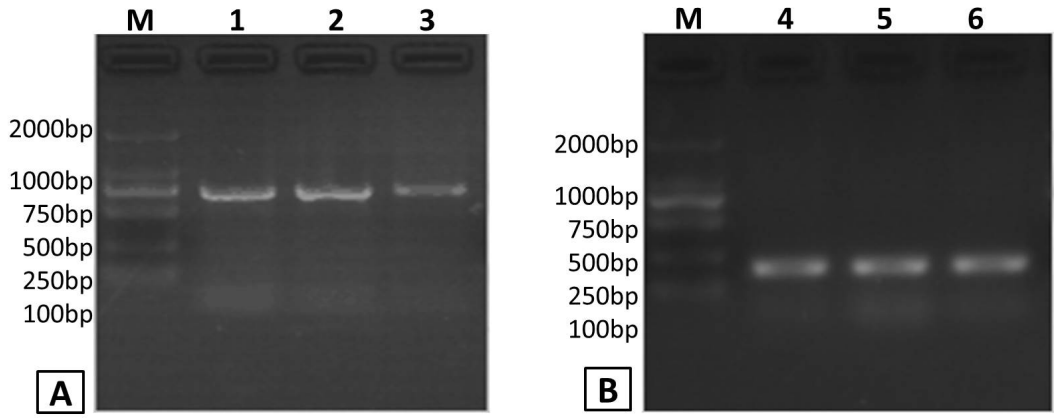

Fig. 1. Agarose detection of GHRL-2 (A) and GHRL-6 (B) genes in goats. Lane M, makers D2000; Lanes, 1, 2 and 3, GHRL-2 genes; Lanes 4, 5 and 6, GHRL-6 genes in Yaoshan goat, Black goat and Black goat, respectively.

Allele frequency is to display gene diversity in one population of one species. Sequencing peak diagram read by SeqMan Program in DNAStar Software and Chromas Software for calibration and sequencing comparison of sequencing results. Scale plate in Mwsnap Software was used to measure peak height corresponding to different SNP alleles (Cui *et al*., 2005). Gene frequency (Bai *et al*., 2016a, 2016b, 2017) was estimated according to the following formula:

$$F_1 = H_1 / (H_1+H_2) \ (i=1, 2)$$

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.

Results

Agarose gel electrophoresis (2%) results of PCR amplification products of GHRL gene in goat are shown in Figure 1. PCR products of all primers have single strip and high specificity. They are applicable for next sequencing test.

Table I.- Relational information for GHRL gene.

Gene (Size)	Primer sequence	T_A (°C)
GHRL-2 (839bp)	F:ATTCCTGGGTTCCTCCTAGTTC R:CCTGTGGTCTCGGAAGTGTC	61.5
GHRL-6 (227bp)	F:CCAGACACAGCTTTTTGACTTG R:CCGTTTGAGTATTTATTCACCTCC	59.5

PCR amplification products with clear strips were chosen and sent to Shanghai Sangon for sequencing. According to BLAST comparison analysis of two GHRL gene sequences on NCBI, they show 99% similarity with GHRL mRNA of *Capra hircus*. Therefore, the amplified GHRL genes indeed are target gene fragments. Based on sequencing results, PCR products of GHRL-2 and GHRL-6 in Yaoshan goat and black goat detected three sites: G447C, T498C and C105T. Sequencing maps of these three mutation sites are shown in Figures 2 and 3.

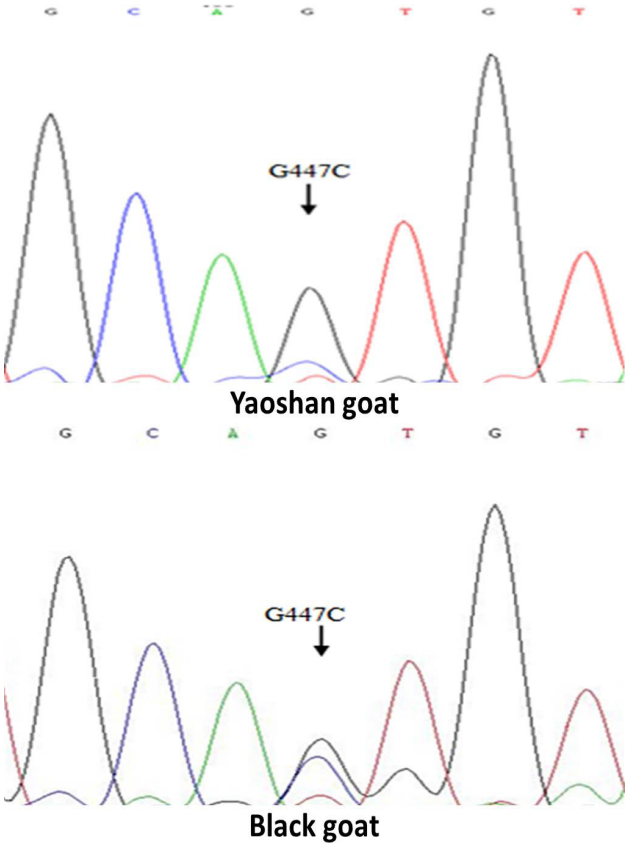

Fig. 2. G447C loci of the GHRL-2 gene of the same goat.

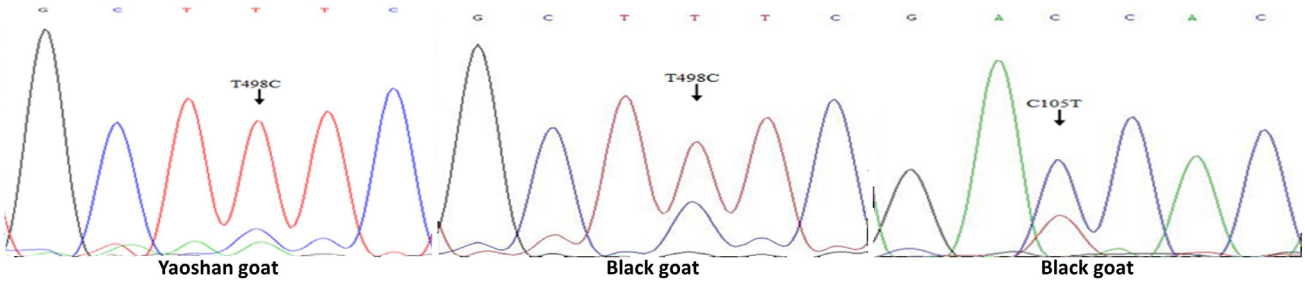

Fig. 3. T498C loci and C105T loci of the GHRL-6 gene of the same goat.

Peak height of SNP alleles in the sequencing map and allele frequencies which are estimated by MWsnap are shown in Table II. The allele frequencies of G and C at G447C in Yaoshan goat are 0.793 and 0.207, respectively. The allele frequencies of G and C at G447C in black goat are 0.621 and 0.379, respectively. Hence, it is concluded that G is dominant allele at G447C in two goat species. Allele frequencies of T and C at T498C in black goat are 0.675 and 0.325, while allele frequencies at T and C at T498C in Yaoshan goat are 0.847 and 0.153, respectively. Therefore, T is the dominant allele at T498C in above two goat species. At C105T in black goat, allele frequencies of C and T are 0.698 and 0.302, indicating that C is the dominant allele at C105T in black goat.

Table II.- GHRL gene SNPs allele frequency.

Loci	Black goat		Yaoshan goat	
G447C	G (0.621)	C (0.379)	G (0.793)	C (0.207)
T498C	T (0.675)	C (0.325)	T (0.847)	C (0.153)
C105T	C (0.698)	T (0.302)	-	-

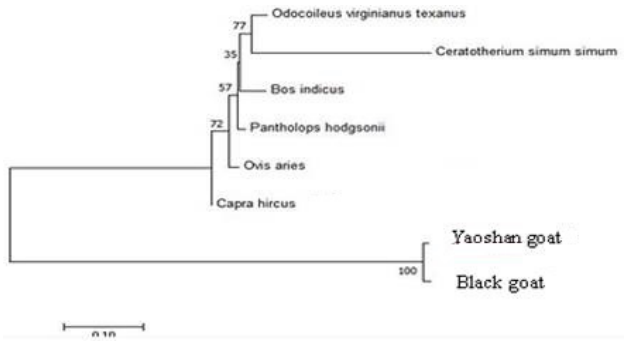

Fig. 4. Phylogenetic tree of the GHRL gene of different species.

GHRL nucleotide sequences of different species (goat XM_018038228.1, deer XM_020872598.1, Tibetan antelope XM_005956675.1, sheep NM_001009721.3, zebu XM_019984068.1 and White Rhinoceros XM_004443598.2) were downloaded from NCBI using MEGA7 for comparison analysis. The evolutionary tree was constructed by DNAstar (Fig. 4). The results demonstrated that Odocoileus virginianus texanus and Ceratotherium simum simum clustered firstly, giving closest relationship, then clustered with Bos indicus, Pantholops hedgsoni, Ovis aries and Capra hircus, all of the six species formed one group. Two goat species, Black goat and Yaoshan goat, formed the other group, which showed a distant genetic relationship with the six species in group one.

Discussion

In this experiment, GHRL gene has three mutation sites in Yaoshan goat and black goat by using PCR product direct sequencing of DNA pool. Studies concerning polymorphism of GHRL gene in the world (Luo *et al*., 2014) discovered that in these two goat species, there's one mutation site at GHRL gene exon 4 (C345T), where covers two genotypes (CC and CT). Frequency of CC in black goat is higher than that in white goat, while polymorphism information content (PIC) show low polymorphism in all three species (PIC<0.25). According to correlation analysis with body weight and body size trait, C345T site is significantly related with body weight, body height and chest width. Specifically, body weight, body height and chest width of CT genotype individuals are significantly higher than those of CC genotype individuals. Therefore, it is speculated that C345T is sense mutation. In 2012, Song *et al*. (2015) discovered one same sense mutation site (C345T) at GHRL exon 4, which was manifested by two genotypes named as CC and CT. another two same sense mutation sites (T78C and C14T) were detected at GHRL exon 2 and exon 4. Mutation at C14T was correlated with growth trait of goat. In this experiment, three mutation sites were discovered in GHRL gene of Yaoshan goat and black goat. It also detected two mutation sites (G447C and T498C) in GHRL-2, one mutation site (C105T) in GHRL-6, these were slightly different with previous research conclusion. Ali *et al*. (2018) reported

that the "W" heterozygous genotype of Leptin Gene showed higher daily gain than other genotypes in Lohi Sheep. we will further study the function of GHRL gene, in particular, make clear that whether 3 SNP loci (T498C, G447C, C105T) were associated with goat growth and development performance or not.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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