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Polymorphism Analysis of *LHβ* Gene in Sheep

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ABSTRACT

In this study, *LHβ* gene polymorphism was analyzed by the sequencing technique, SNP mutation sites of *LHβ* gene were detected in five sheep breeds including Lanzhou large-tailed sheep, small-tailed han sheep, Mongolian sheep, large-tailed han sheep and Yuxi fat-tailed sheep, in order to further provide reference to investigate the genetic mechanism of reproductive performance in sheep. The results showed that 3 SNP sites of *LHβ* gene were detected in Lanzhou large-tailed sheep, small-tailed han sheep, Mongolian sheep, large-tailed han sheep and Yuxi fat-tailed sheep, they were C551T C/T, G391A G/A and G394A G/A, respectively. C and T allele frequencies in C551T site were 0.594/0.406, 0.811/0.189, 0.840/0.160, 0.927/0.073 and 0.868/0.132, respectively. G and A allele frequencies in G391A site were 1.000/0.000, 0.705/0.295, 1.000/0.000, 0.711/0.289 and 0.780/0.220, respectively. G and A allele frequencies in G394A site were 1.000/0.000, 0.668/0.332, 1.000/0.000, 0.656/0.344 and 0.698/0.302, respectively. The phylogenetic trees of partial sequences were constructed for partial *LHβ* gene sequences of different species using DNAStar software. The results showed that sheep, goat, water buffalo, yak clustered firstly. Hedgehog, bat and whale were far from sheep. So they were alone. Finally they clustered with sheep.

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Authors' Contribution
JYB conceived and designed the study, collected samples, analyzed the data and wrote the article. YZP helped in sampling. YB Y and XHZ helped in analysis of data. XPJ and YXQ helped in writing of article.

Key words
LHβ gene, SNP, phylogenetic tree

INTRODUCTION

Lutropin (LH) is a glycoprotein synthesized and secreted by basophil leucocytes of pituitary gland. It promotes follicle to mature, secretes estrogen, triggers ovulation, promotes luteinization, secretes progesterone, which plays an important role in animal reproductive process (Bonneau *et al*., 1994). LH comprises subunits α and β. α subunit is common in the same species or even in all mammals, whereas β subunit is species and hormone-specific. ß subunit, therefore, became the focus of investigation (Pierce and Parsons, 1981). In recent years, the treatment of diseases in human, pig, horse, cattle, sheep, fish and other animals with LH has been reported. Li *et al*. (2006a) reported *LHβ* sequence of Nanjiang yellow goat. Ren *et al*. (2010) analyzed the correlation between *LHβ* gene polymorphism and litter size of Qianbei-Pockmarked goat. Huang *et al*. (2010) proved that *LHβ* gene polymorphism in Guizhou black goat might be correlated with the litter size. Liu *et al*. (2009) reported that the seminal fluid quality of Simmental and Shylock cattle was controlled by *LHβ* gene. In this study, SNP mutation sites of *LHβ* gene were detected in Lanzhou large-tailed sheep, large-tailed han sheep, Yuxi fat-tailed sheep, small-tailed han sheep, Mongolian sheep and other sheep breeds in order to provide reference to verify the genetic mechanism of reproductive performance in sheep.

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MATERIALS AND METHODS

Experimental materials

Large-tailed han sheep, small-tailed han sheep, Mongolian sheep, Lanzhou large-tailed sheep and Yuxi fat-tailed sheep were selected, thirty each. Blood samples (2 ml) were collected from each sheep, mixed with ACD anticoagulant, rapidly transported to the laboratory at low temperature and preserved at -20°C for further analysis.

Genomic DNA extraction

DNA was extracted with blood genomic DNA extraction kit whole blood DNA kit SK1262, Sangon Biotech, Shanghai Co. Ltd**).** DNA was pooled according to Li *et al*. (2011) and Sun *et al*. (2015), for which DNA with the same characteristics was quantified, diluted and pooled.

PCR amplification and nucleotide sequencing of LHß *gene*

PCR amplification of *LHß* gene was done by using primer sequence used by Ren *et al*. (2010).
F: 5' GGGGCCTGAGGTGTTGGGGTGTCT 3'
R: 5' ATGGGCATGGGAGGTTGAAGTG 3'

The primers were synthesized by Beijing Dingguo Changsheng Biotechnology Co., Ltd. The total volume of PCR amplification reaction mixture was 15μL, which

comprised 1.5µL 10×buffer, 1.2µL of dNTP (4×2.5mol/L), 0.3µL each of forward and reverse primers, 1.0µL template DNA (100ng/µL), 0.3µL Taq polymerase (Tiangen, Beijing, China) and 10.4µL double distilled water. PCR thermal cycles were denaturation at 94°C for 5 min followed by 30 cycles each of 94°C for 30s, annealing at 56-60°C for 30s and extention at 72°C for 30s end then at 72°C for 7min; stored at 4°C.

The PCR products was sent to Beijing Qingke Xinye Biotechnology Co., Ltd. For sequencing.

Allele frequency estimation

The peak height of each SNP allele in the DNA sequence analysis was measured, using the software Chromas.exe and software Mwsnap (Cui *et al.*, 2005). The gene frequency was estimated according to the following formula:

$$f_i = h_i/(h_1+h_2), \qquad (i=1,2)$$

Where f_i represents the frequency of i allele at the SNP locus, h_1 and h_2 , respectively, expressed the height of the peak of the SNP allele 1 and allele 2 on the sequencing map.

Bioinformatics analysis

The homologous comparison of *LHβ* gene sequence in sheep was performed using blast module in NCBI. The phylogenetic tree of *LHβ* gene sequences of different species were drawn using MegAlign module in DNAStar software.

RESULTS

Characteristics of LHβ gene

Figure 1 shows 750 bp amplified band of *LHß* of large-ailed han sheep, small tail han sheep, Yuxi fat-tailed sheep, Lanzhou large-tailed sheep and Mongolian sheep, which coincided with 800 bp in the target band.

LHβ gene sequence in small-tailed han sheep was compared with that of sheep using BLAST in NCBI. The results showed 100% similarity, proving that the amplified *LHβ* gene was indeed the target gene fragment. The homologous alignment of *LHβ* gene sequences were conducted in small-tailed han sheep on NCBI website. The result showed that *LHβ* gene sequence in small-tailed han sheep had 100% similarity with that of sheep and had very high similarity with goat, buffalo, yak, Tibetan antelope, horse and other livestock.

SNP sites of LHβ gene

Three SNP sites of *LHβ* gene were detected in Lanzhou large-tailed sheep, small-tailed han sheep, Mongolian sheep, large-tailed han sheep and Yuxi fat-tailed sheep. These were C551T site (C/T), G391A site (G/A) and G394A site (G/A), respectively. Figures 2-4 show the specific mutations.

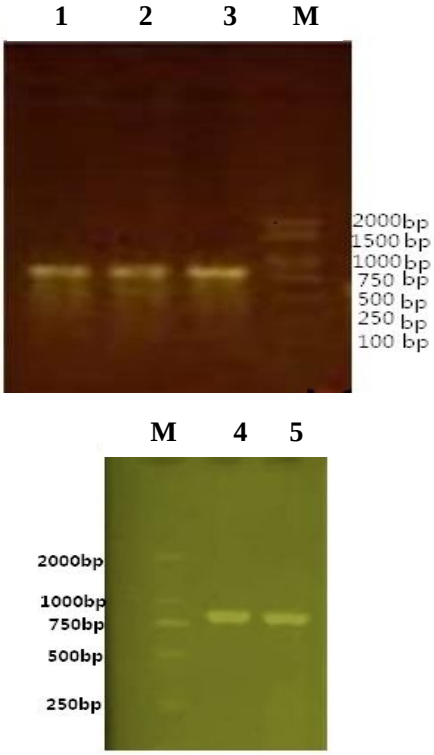

Fig. 1. Amplified *LHβ* gene in Yuxi fat-tailed sheep (lane # 1), small-tailed han sheep (lane # 2), large-tailed han sheep, (lane #3) Lanzhou large tailed sheep (lane # 4) and Mongolian sheep (lane # 5). M is Marker D2000

C allele frequency in C551T site was 0.927 in large-tailed han sheep and T allele frequency was 0.073. Two allele frequencies were 0.811/0.189, 0.868/0.132, 0.594/0.406 and 0.840/0.160, respectively, in small-tailed han sheep, Yuxi fat-tailed sheep, Lanzhou large-tailed sheep and Mongolian sheep. C in C551T site was the dominant allele in the above five sheep populations. G and A frequencies in G391A site were 0.711/0.289, 0.705/0.295, 0.780/0.220, 1.000/0.000 and 1.000/0.000, respectively, in large-tailed han sheep, small-tailed han sheep, Yuxi fat-tailed sheep, Lanzhou large-tailed sheep and Mongolian sheep. G in G391A site was the dominant allele in the above five sheep populations. G and A frequencies in G394A site were 0.656/0.344, 0.668/0.332, 0.698/0.302, 1.000/0.000 and 1.000/0.000, respectively in large-tailed han sheep, small-tailed han sheep, Yuxi

fat-tailed sheep, Lanzhou large-tailed sheep and Mongolian sheep. G in G394A site was the dominant allele in the above five sheep populations (Table I).

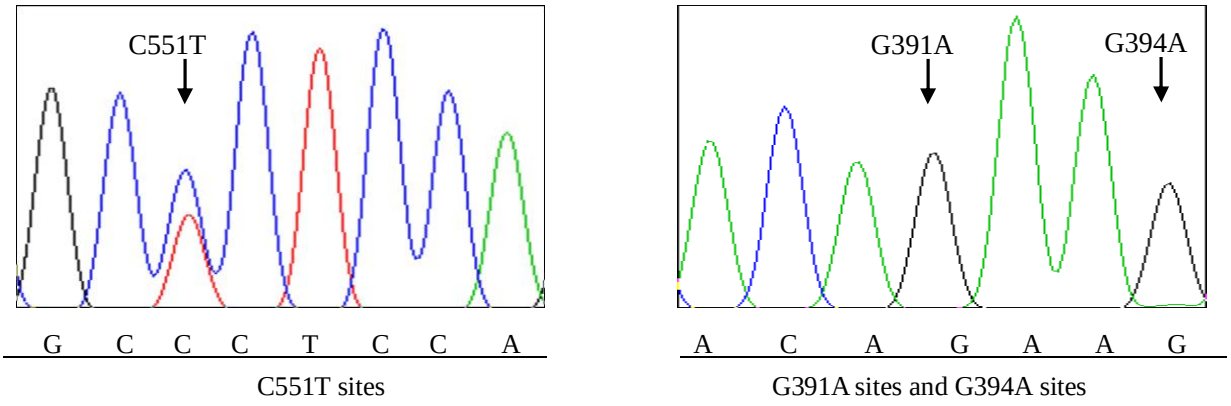

Fig.2. C551T sites, G391A sites and G394A sites of *LHβ* gene in Lanzhou large-tailed sheep

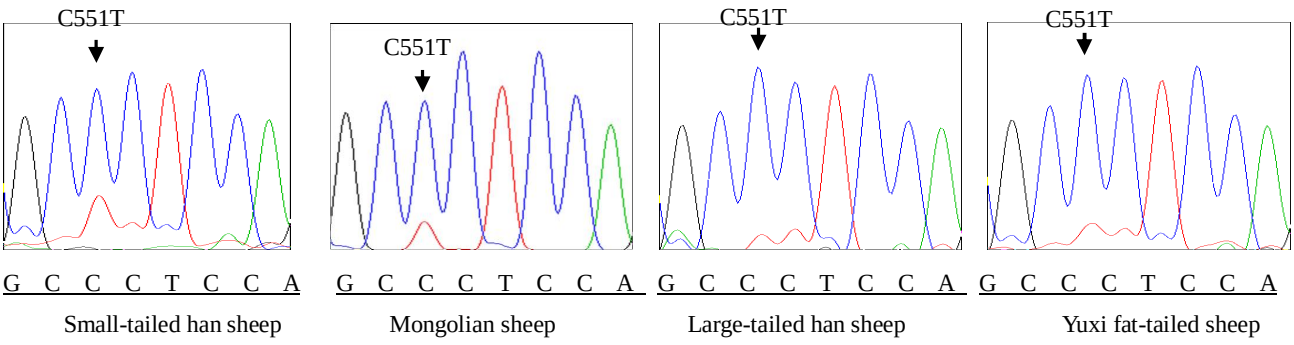

Fig.3. C551T sites of *LHβ* gene in sheep

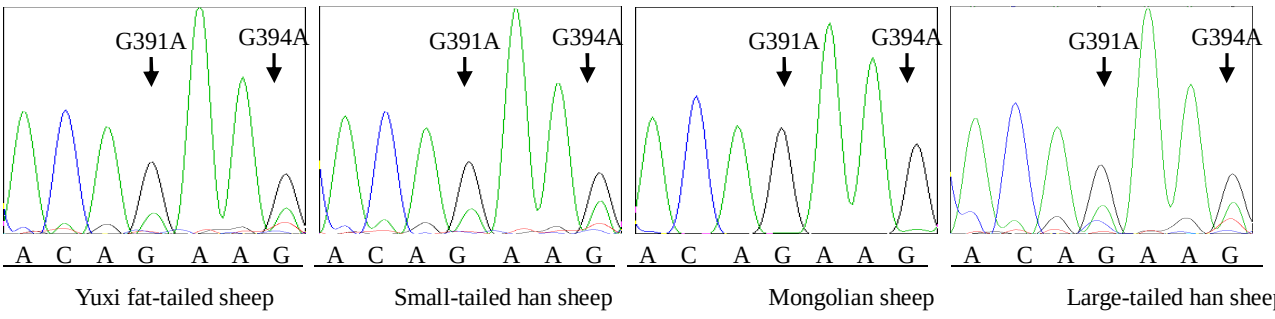

Fig. 4. G391A sites and G394A sites of LHß gene in sheep.

Clustering analysis based on LHβ gene sequence

The phylogenetic trees of *LHβ* genes sequences were constructed for different species using DNAStar software (Fig. 5). The species, which were relatively close in taxonomic relationship were clustered. For example, sheep, goat, water buffalo and yak firstly

clustered, but Tibetan antelope did not cluster with sheep or goat. Hedgehog, bat and whale were far from sheep. So they were alone. Finally they clustered with sheep.

Table I.- SNPs allele frequency of *LHβ* gene in five sheep breeds.

Sites	Large-tailed han sheep	Small-tailed han sheep	Yuxi fat-tailed sheep	Mongolian sheep	Lanzhou large-tailed sheep	SNPs
C551T	C(0.927) T(0.073)	C(0.811) T(0.189)	C(0.868) T(0.132)	C(0.840) T(0.160)	C(0.594) T(0.406)	C(1.000) T(0.000)
G391A	G(0.711) A (0.289)	G(0.705) A(0.295)	G(0.780) A(0.220)	G(1.000) A(0.000)	G(1.000) A(0.000)	G(1.000) A(0.000)
G394A	G(0.656) A(0.344)	G (0.668) A(0.332)	G (0.698) A(0.302)	G(1.000) A(0.000)	G(1.000) A(0.000)	G(1.000) A(0.000)

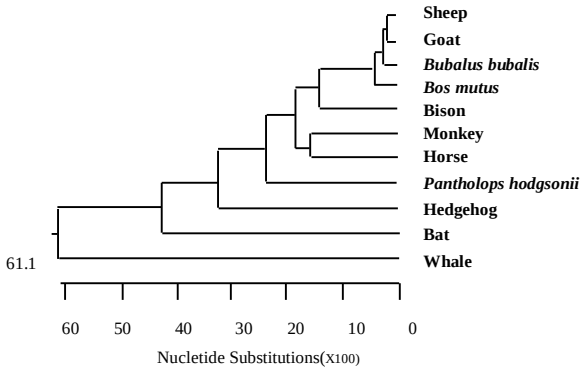

Fig.5. Phylogenetic tree based on sequence of *LHβ* gene of different animal species.

DISCUSSION

Nucleotide composition of LHβ *gene in sheep*

The genomic GC content was about 40~45% in vertebrate. But these GC contents were uniformly distributed in the genome. GC content in some DNA fragments were as high as 60%, and only about 33% in other regions (Sueoka, 1962). The vertebrate genome seems to be a chimera of GC rich regions and GC poor regions (Bernadi *et al*., 1985). The difference of GC contents played an important role in gene expression regulation and gene mutation (Bernadi *et al*.,1988). In this experiment, the important characteristic of *LHβ* gene was high GC content *viz.,* 67.6% (C32.4% and G35.2%). The AT content were only 32.1% (A14.7% and T17.7%), suggesting that *LHβ* gene was located in GC rich homogeneous region. The close connection between the base composition deviation and gene function needs further study.

Polymorphism of LHβ *gene in sheep*

The research on gene mutation is mainly concentrated in human reproductive system diseases. But there are few reports on sheep, cattle, pigs or other species. Di *et al*. (2009) discovered two mutation sites 202(C-A) and 210(C-T) in *LHβ* gene 5'control region in Jining gray goat and detected C-T mutation in 1124bp of exon 2. Li *et al*. (2006) detected 8 polymorphic sites in Nanjiang Yellow Goat, including 1 [322 (G-A)] single information site and 7 simple information sites [39(G-T), 42(G-A), 45(T-A), 359(G-T), 401(G-A-C), 543(T-C) and 877(T-A)]. Re et al.(2010) showed that 2 mutation sites were detected in *LHβ* gene of Qianbei-Pockmarked goat. C-T base mutation occurred in the 414th site in exon 2, resulting in threonine mutation into methionine. 3 mutation sites of *LHβ* gene were detected in Yuxi fat-tailed sheep, large-tailed han sheep, Mongolian sheep, small-tailed han sheep and Lanzhou large-tailed sheep, they were C551T C/T, G391A G/A and G394A G/A, respectively, which was different with the previous research results.

In recent years, the correlation of *LHβ* gene polymorphism has been studied continuously with sheep breeding mechanism. Huang *et al*. (2010) showed that *LH* gene 138 bp site mutation may correlate with the reproductive performance of Guizhou black goat. Ren *et al*. (2010) showed that AA and Aa were detected in *LHβ* gene of Qianbei-Pockmarked goat. There was no significant difference in litter size between the two genotypes. Li *et al*. (2006b) showed that newborn litter weight of first-fifth children and litter size in Nanjiang Yellow Goat showed no significant difference. Three mutation sites of *LHβ* (C551T, G391A and G394A) were detected in sheep populations in the study. The correlation of 3 mutation sites of *LHβ* gene with

reproductive performance in sheep needs further study.

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