A Mutation at Exon 3 of KIF-1 Gene of Three Goat Breeds in China

1Liu Wu-Jun, 1Wang Qiong, 1Huang Xi-Xia, 1Yu Shi-Gang and 2Tian Ke-Chuan
1College of Animal Science, Xinjiang Agricultural University, Urumqi, 830052 Xinjiang, China
2Xinjiang Academy of Animal Science, Urumqi, 830000 Xinjiang, China

Abstract: Keratin Intermediate-Filament (KIF) proteins form the main structural elements of a wool fibre. In this study, PCR-RFLP and DNA sequencing methods were used to detect polymorphisms of KIF-1 gene in 786 samples of 3 Xinjiang local goat breeds (Xinjiang, Nanjiang Cashmere and Bogeda Cashmere goat). The results showed that KIF-1-P3 locus has polymorphisms. The frequencies of genotype AA were 0.675, 0.577 and 0.766, genotype AB were 0.296, 0.400 and 0.234, genotype BB were 0.030, 0.023 and 0.000 for Xinjiang, Nanjiang Cashmere and Bogeda Cashmere goat breeds, respectively. The genotypic distributions were in disagreement with Hardy-Weinberg equilibrium in Nanjiang Cashmere goat breed. In addition, the mutation was detected at KIF-1-P3 locus by DNA sequencing method, a novel of SNP was revealed in exon 3 (Genebank M23912: c.3212 C>T) and it belongs to synonymous mutation. However, there is no significant difference in Xinjiang goat breed between polymorphisms of the KIF-1 gene and cashmere production traits (fineness, thickness, yield and body weight after combing). Moreover, analysis of variance indicated that various genotypes in the 3 Xinjiang goat breeds were highly significant (p<0.01).

Keywords: KIF-1 gene, PCR-RFLP, polymorphism, SNP, genotype, frequencies

INTRODUCTION

The wool fibre has a complex morphology consisting of an outer layer of cuticle scales surrounding an inner cortex (Hoeker, 2002; Leeder, 1986; Rippon, 1992). The proteins of wool are products of several gene families each having a number of closely related members (Powell and Rogers, 1996). Keratins, a family of Intermediate Filament (IF) proteins are primarily involved in the mechanical and structural functions of epithelial tissue (Hatzfeld and Franke, 1985). The content of keratin in wool fibre structure can account for 99%. So keratins are most likely playing an important role in the control of wool traits.

It is known that keratins can be classified as Keratin Intermediate-Filament (KIF) proteins and Keratin-Associated Proteins (KAP). Keratin intermediate-filament is low-sulfur proteins classified into type I keratins (acidic) and type II keratins (non-acidic) (Schweizer et al., 2006; Powell, 1996) with >50 genes encoding these proteins in vertebrates (Prueff et al., 2004). To date, a wide variety of keratin and keratin-related genes have been reported (Rogers et al., 2004, 2005, 2006).

However, the reports about the polymorphism of KAP are much more than that of KIF. In the present study, the polymorphism of KIF-1 gene was analyzed as a genetic marker candidate for growth traits in 3 Xinjiang local goat breeds (Xinjiang, Nanjiang Cashmere and Bogeda Cashmere goat). It can provide reference in cashmere production and other performance for animal breeding and genetics.

MATERIALS AND METHODS

Animal source: In this study, data on cashmere traits and genomic DNA samples were obtained from 786 individuals belonging to 3 Xinjiang local goat breeds (Xinjiang goat, n = 203; Nanjiang Cashmere goat, n = 305; Bogeda Cashmere goat, n = 278). Xinjiang goat were from the breeding centre of Luntai of Xinjiang in China; Nanjiang cashmere goat were from AkeSu goat Research Center of Xinjiang and Bogeda cashmere goat were from Breeding Farm of Bogeda goat in Urumqi.

DNA preparation: Genomic DNA of 786 cashmere goat were isolated from 2% heparin-treated blood samples and stored at -80°C following standard procedures (Sambrock and Russell, 2001).

 Primer design and PCR amplification: According to the sequence of KIF-1 (GenBank accession number M23912), one pair of PCR primers was designed with Primer 5.0 as follow:

Forward: 5'-TCAAACCAAACTAGATGGAG-3,
Reverse: 5'-CAGATTCTTTGACAGTTGAGC-3,
They were used to amplify 298 bp PCR products containing for Capra KIF-1 gene partial of intron 2, exon 3 and partial of intron 3 locus (P3 locus). One pair of PCR primers was designed using primer 5.0 software to amplify the coding and flanking region of Capra KIF-1 gene, the size of PCR products was 298 bp. The 25 µL volume contained 50 ng genomic DNA, 0.5 µM of each primer, 1×Buffer (including 1.5 mM MgCl₂), 200 µM dNTPs and 0.625 units of Taq DNA polymerase (MBI). The PCR was performed using the following program: 94°C for 5 min, 35 cycles of 94°C for 40 sec, annealing 61°C for 35 sec, 72°C for 35 sec and a final extension at 72°C for 10 min.

**PCR-RFLP and DNA sequencing:** Aliquots of 10 µL PCR products of KIF-1 gene were incubated with 6 units restriction enzyme HaeIII (MBI ferments) for 8 h at 37°C then electrophoresed on 2% agarose gel stained with 1×TAE buffer (89 mM Tris, 89 mM boric acid and 2 mM Na₂EDTA) containing 200 ng mL⁻¹ ethidium bromide. A 6 µL aliquot of PCR products was added to 1.5 µL of loading dye (0.025% bromophenol blue, 0.025% xylene cyanol, 40%, w/v sucrose) and the gels were run at a constant voltage (120 V) for 0.2–0.3 h.

The PCR fragments from different patterns in the 3 breeds were amplified by the pair of primers were sequenced with BioXM software (version 2.6).

**Statistical methods and analysis:** About 3 indigenous goat breeds populations and differences genotypic frequencies at KIF-1-P3 locus were analyzed with SPSS software (version 6.0) (Norusis, 2008). He (gene heterozygosity), Ho (gene homozygosity), Ne (effective allele numbers) and PIC (Polymorphism Information Content) were calculated according to Neil and Roychaudhury (1974), Neil and Li (1979), respectively.

\[
H_o = \sum_{i=1}^{r} F_i^2, H_e = 1 - \sum_{i=1}^{r} F_i^2
\]

The linear model was used in analyses of the impact of KIF-1 genotype and included effects of ewe, ram within ewe, age and genotype as well as interaction between ram and genotype was involved (Zhao et al., 2004).

\[
Y_{ijklm} = \mu + S_i + D_j + A_k + (SG)_{ij} + E_{ijklm}
\]

Where:
- \( Y_{ijklm} \) = The trait measured on each of the ijklth animal
- \( \mu \) = The overall population mean
- \( S_i \) = The fixed effect associated with the ith ram
- \( D_j \) = The fixed effect associated with jth ewe with ram
- \( A_k \) = Fixed effect due to the kth age
- \( (SG)_{ij} \) = Interaction between the ith ewe and the ith genotype
- \( E_{ijklm} \) = The random error

**RESULTS**

DNA sequencing analysis showed at KIF-1-P3 locus, there was a mutation (Genebank M23912: c.3212 C>T) in exon 3 region and it belongs to synonymous mutation (Fig. 1). The PCR product was amplified by one pair of PCR primers and its size was 298 bp (Fig. 2). By PCR-RFLP method, there were three genotypes (named as genotype AA, AB and BB) (Fig. 3).

Genotype and allele frequencies of KIF-1-P3 locus in the 3 breeds were showed in Table 1. Table 1 demonstrated A allele and AA genotype were predominant in the 3 Xinjiang local goat breeds. Gene Homozygosity (Ho), gene Heterozygosity (He), effective

![Fig. 1: DNA sequencing results of different genotypes at KIF-1-P3 locus](image)

![Fig. 2: Detection of PCR product of the KIF-1-P3 locus](image)

![Fig. 3: PCR-RFLP patterns of HaeIII digesting KIF-1-P3 locus](image)
Table 1: Genotype distribution and allelic frequencies at the KIF1-P3 locus

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Genotype and genotype frequencies</th>
<th>Allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AB</td>
</tr>
<tr>
<td>Xinjiang goat</td>
<td>137 (0.675)</td>
<td>60 (0.2960)</td>
</tr>
<tr>
<td>Nanjiang cashmere goat</td>
<td>176 (0.577)</td>
<td>122 (0.4000)</td>
</tr>
<tr>
<td>Bogeda cashmere goat</td>
<td>213 (0.706)</td>
<td>65 (0.2340)</td>
</tr>
</tbody>
</table>

Table 2: He, Ho, PIC and Ne of KIF1-P3 locus in goat breeds

<table>
<thead>
<tr>
<th>Breeds</th>
<th>He</th>
<th>Ho</th>
<th>PIC</th>
<th>Ne</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xinjiang goat</td>
<td>0.7082</td>
<td>0.2918</td>
<td>1.4210</td>
<td>0.2492</td>
</tr>
<tr>
<td>Nanjiang cashmere goat</td>
<td>0.6535</td>
<td>0.3465</td>
<td>1.5302</td>
<td>0.2865</td>
</tr>
<tr>
<td>Bogeda cashmere goat</td>
<td>0.7935</td>
<td>0.2065</td>
<td>1.2602</td>
<td>0.1852</td>
</tr>
</tbody>
</table>

Ho: Gene homozygosity; He: Gene heterozygosity; Ne: Effective allele numbers; PIC: Polymorphism Information Content

Table 3: Chi-Square analysis of genotype distribution at KIF1-P3 locus

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Xinjiang goat</th>
<th>Nanjiang cashmere goat</th>
<th>Bogeda cashmere goat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 229* (0.001)</td>
<td>11 289* (0.004)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>26 379* (0.000)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Above diagonal data showed p-value of genotype distribution below diagonal data showed χ² of genotype: Value with * and ** differ significantly at p<0.05 and p<0.01, respectively

Table 4: Least square means for genotype of KIF1-P3 locus in Xinjiang cashmere goat

<table>
<thead>
<tr>
<th>Genotypes (Mean±SE)</th>
<th>Cashmere traits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
</tr>
<tr>
<td>Cashmere fineness (µm)</td>
<td>16.057±0.108</td>
</tr>
<tr>
<td>Cashmere thickness (cm)</td>
<td>3.604±0.101</td>
</tr>
<tr>
<td>Cashmere yield (g)</td>
<td>151.747±3.990</td>
</tr>
<tr>
<td>Body weight after</td>
<td>30.213±0.673</td>
</tr>
<tr>
<td>combed (kg)</td>
<td></td>
</tr>
</tbody>
</table>

Estimates are given as mean±SE (SE: Standard Error of Means). Data with a different letter (a, b, c) within the same line differ significantly at 0.01<p<0.05

allele Numbers (Ne) and Polymorphism Information Content (PIC) were shown in Table 2. According to the classification of PIC low polymorphism if PIC value <0.25, median polymorphism if 0.25<PIC value <0.5 and high polymorphism if PIC value >0.5, KIF-1 gene in 3 Xinjiang local goats was all at low polymorphic level. He, Ne and PIC of KIF-1 gene in Nanjiang goat population were higher than other two goat breeds.

Moreover, the χ²-test showed that the genotype distributions of KIF1-P3 loci were in disagreement with Hardy-Weinberg equilibrium in Nanjiang goat breeds (Table 1 and 2). Analyses of variance indicated that various genotypes in the three Xinjiang goat breeds were highly significant (p<0.01) (Table 3). However, Table 4 showed that there is no significant difference in Xinjiang goat breed between polymorphisms of the KIF-1 gene and cashmere production traits (fineness, thickness, yield and body weight after combing).

DISCUSSION

Current knowledge of the genetic control of fibre production in sheep and goats is reviewed with the focus at the molecular level (Purvis and Jeffery, 2007). In the past few decades, many fundamental researches are clear indication that sheep type 1 and type 2 in KIF1 genes are at 11q25-q29 and 3q14-q22 (Dolling and Brooker, 1966) with the type 1 gene about 4–5 kb in length and containing 6 introns and the type 2 gene about 7–9 kb in length and containing 8 introns (Powell and Rogers, 1996). However, the study about discussing the association between the polymorphism of KIF1 gene and production traits is little. This research attempted to detect polymorphism at KIF-1 gene in 3 goat breeds of Xinjiang in China. The result showed that there was a mutation (Genebank M23912: c.3212 C>T) at KIF1-P3 locus. The discovery is the first reported in international goat. In addition, further analysis indicates that the mutation belongs to synonymous mutation but the SNP at KIF1-P3 region may not be a causal mutation in KIF1 protein. Moreover, the association between the polymorphism of KIF1-P3 region and cashmere production traits is not significant difference.

He, Ne and PIC of KIF-1 gene in Nanjiang goat population were higher than other 2 goat breeds. Moreover, in Nanjiang cashmere goat breeds, the genotype distributions of KIF1-P3 loci were in disagreement with Hardy-Weinberg equilibrium. This is probably because Nanjiang cashmere goat breed is the variety bred, the breeding of its descendant is artificial selection strictly. Figure 3 showed that there were 3 genotypes (named as genotype AA, AB and BB). However, the Bogeda cashmere goat populations only have genotype AA and AB, genotype BB was disappeared. There may be two reasons, first because the Xinjiang goat populations should only have genotype AA and AB and the other is because the samples of genotype BB could not be collected.

CONCLUSION

At KIF1-P3 locus, there was a mutation (Genebank M23912: c.3212 C>T) and further analysis showed that the mutation belongs to synonymous mutation. The SNP at KIF1-P3 region may not be a causal mutation in KIF1 protein. In addition, KIF1-P3 region is association with cashmere production traits (fineness, thickness, yield and
body weight after combing) not significantly. The KIF-1 gene can not be used as a candidate gene for production traits. A further profound study is needed because the studies in this thesis are preliminary.

ACKNOWLEDGEMENTS

This study was supported by the project of key Science and Research Program of Xinjiang Education Bureau (Grant No: XJEDU2009113), GEF Applied Research Project (Grant No: GEF052456CHA), Xinjiang 11th 5 year plan key project of science and technology (Grant No 200731132-7), The project of National Key Technology R and D Program in the 11th 5 year Plan of China (Grant No 2008BADB2B05) and National Modern Agriculture Industrial System Project (Grant No. nyeytx-40).

REFERENCES


