Journal of Animal and Veterinary Advances 10 (5): 610-613, 2011
ISSN: 1680-5593

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

[1]Liu Wu-Jun, [1]Wang Qiong, [1]Huang Xi-Xia, [1]Yu Shi-Gang and [2]Tian Ke-Chuan
[1]College of Animal Science, Xinjiang Agricultural University, Urumqi, 830052 Xinjiang, China
[2]Xinjiang 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 ($p<0.01$).

Key words: *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 (Hocker, 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 (Pruett *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 Urumuqi.

DNA preparation: Genomic DNA of 786 cashmere goat were isolated from 2% heparin-treated blood samples and stored at -80°C following standard procedures (Sambrook 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'-TCAAACCAAACTAGAGTCAAGG-3,
Reverse: 5'-CAGATTCTTTGACTAGTTGAGC-3,

Corresponding Author: Tian Ke-Chuan, Xinjiang Academy of Animal Science, Urumqi, 830000, Xinjiang, China

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 $MgCl_2$), 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 Haeβ (MBI fermentas) for 8 h at 37°C then electorphoresed on 2% agarose gel stained with 1×TBE buffer (89 mM Tris, 89 mM boric acid and 2 mM Na_2EDTA) 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-I-P3 locus were analyzed with SPSS software (version1 6.0) (Norusis, 2008). He (gene heterozygosity), Ho (gene homozygosity), Ne (effective allele numbers) and PIC (Polymorphism Information Content) were calculated according to Nei and Roychoudhury (1974), Nei and Li (1979), respectively.

$$H_o = \sum_{i=1}^{n} P_i^2,\ H_e = 1 - \sum_{i=1}^{n} P_i^2$$

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).

$$Y_{ijklm} = \mu + S_i + D_{ij} + A_k + G_l + (SG)_{il} + E_{ijklm}$$

Where:

Y_{ijklm} = The trait measured on each of the ijklmth animal
μ = The overall population mean
S_i = The fixed effect associated with the ith ram
D_{ij} = The fixed effect associated with jth ewe with ram i
A_k = Fixed effect due to the kth age
G_l = The fixed effect associated with lth genotype (IGF-1/AA, AB and BB genotype)
$(SG)_{il}$ = Interaction between the ith ewe and the lth 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 Xinjing local goat breeds. Gene Homozygosity (Ho), gene Heterozygosity (He), effective

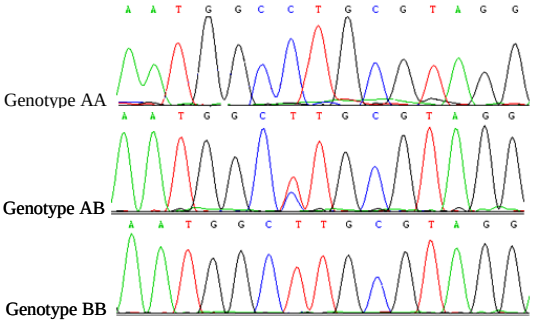

Fig. 1: DNA sequencing results of different genotypes at KIF-1-P3 locus

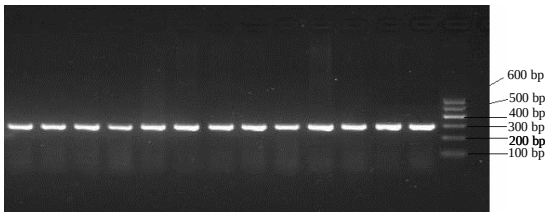

Fig. 2: Detection of PCR product of the KIF-1-P3 locus

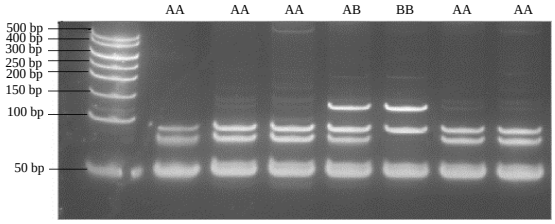

Fig. 3: PCR-RFLP patterns of HaeIII digesting KIF-1-P3 locus

Table 1: Genotype distribution and allelic frequencies at the KIF-1-P3 locus

	Genotype and genotype frequencies				Allele frequencies	
Breeds	AA	AB	BB	No	A	B
Xinjiang goat	137 (0.675)	60 (0.2960)	6 (0.030)	203	0.823	0.177
Nanjiang cashmere goat	176 (0.577)	122 (0.4000)	7 (0.023)	305	0.777	0.223
Bogeda cashmere goat	213 (0.766)	65 (0.2340)	0 (0.000)	278	0.883	0.117

Table 2: He, Ho, PIC and Ne of KIF-1-P3 locus in goat breeds

Breeds	Ho	He	Ne	PIC
Xinjiang goat	0.7082	0.2918	1.4120	0.2492
Nanjiang cashmere goat	0.6535	0.3465	1.5302	0.2865
Bogeda cashmere goat	0.7935	0.2065	1.2602	0.1852

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

Table 3: Chi-Square analysis of genotype distribution at KIF-1-P3 locus

Breeds	Xinjiang goat	Nanjiang cashmere goat	Bogeda cashmere goat
Xinjiang goat	-	13.220* (0.001)	11.283* (0.004)
Nanjiang cashmere goat		-	26.70** (0.000)
Bogeda cashmere goat			-

Above diagonal data showed p-value of genotype distribution below diagonal data showed χ^2 of genotype; Value with * and ** differ significantly at $p<0.05$ and $p<0.01$, respectively

Table 4: Least square means for genotype of KIF-1-P3 locus in Xinjiang cashmere goat

	Genotypes (Mean±SE)		
Cashmere traits	AA	AB	AC
Cashmere fineness (μm)	16.057±0.108	16.229±0.167	15.995±0.6590
Cashmere thickness (cm)	3.604±0.101	3.377±0.157	2.800±0.6190
Cashmere yield (g)	151.747±3.990	146.903±6.207	130.000±24.435
Body weight after combed (kg)	30.213±0.673	27.581±1.047	28.000±4.1230

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 valu e<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 χ^2-test showed that the genotype distributions of KIF-1-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 *KIF* 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 *KIF* 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 KIF-1-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 KIF-1-P3 region may not be a causal mutation in KIF-1 protein. Moreover, the association between the polymorphism of KIF-1-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 KIF-1-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 KIF-1-P3 locus, there was a mutation (Genebank M23912: c.3212 C>T) and further analysis showst that the mutation belongs to synonymous mutation. The SNP at KIF-1-P3 region may not be a causal mutation in KIF-1 protein. In addition, KIF-1-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: XJEDU2009I13), GEF Applied Research Project (Grant No: GEF052456 CHA)., 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. nycytx-40).

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