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Polymorphism of KIFI Gene Associated with Cashmere Traits in Xinjiang Goat Breeds

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Abstract: The wool fiber is structurally composed of low-sulfur proteins, for which the Keratin Intermediate-Filaments (KIF) gene is responsible along with Keratin-Associated Proteins (KAPs). In this study, PCR-RFLP and DNA sequencing were used to detect polymorphisms of the Keratin Intermediate-Filaments type I (KIFI) gene in three Chinese cashmere goat breeds (Xinjiang, Nanjiang and Bogeda White), to estimate gene and genotype frequencies and Polymorphism Information Content (PIC) and to determine impacts of genotype for KIFI on expression of cashmere traits. Results showed a novel A-C (GGCA-to-GGCC) mutation in intron 1, which forms a HaeIII endonuclease restriction site. Three unique PCR-RFLP banding patterns (genotypes AA, AC and CC) were found. The frequencies of the A allele in the samples from the goat breeds varied from 0.700-0.747. The genotypic distributions in three cashmere goat breeds were in Hardy-Weinberg equilibrium ($p>0.05$). According to the classification by PIC, the Xinjiang cashmere goat breed was more polymorphic at this locus than the other breeds. Furthermore, analysis of the impact of KIFI gene polymorphism on cashmere traits (cashmere fineness, down cashmere thickness, cashmere yield, body weight after combing) in goats from the Xinjiang breed indicated greater cashmere fineness in genotype AA compared to genotype CC ($p<0.05$), suggesting that this mutation may have significant influence on the cashmere fineness.

Key words: Polymorphism, cashmere goat, KIFI gene, PCR-RFLP, cashmere traits, cashmere fineness

INTRODUCTION

Goats account for a large proportion of total small ruminants in China and cashmere production is an important component of the Chinese goat industry. While gene migration through importation of foreign goat genetics has potential to improve wool quality and yield, adaptation of these imports to austere conditions of western China is questionable. Consequently, genetic improvement of native breeds in wool traits is desirable because of the adaptation and fitness of these breeds in their production environment. However, breed improvement through traditional genetics and breeding methods is difficult because of the demographics of the goat herds and extensive production systems in western China (Subramanian *et al*., 2005). Consequently, efforts in genetic improvement of goats might be accomplished more efficiently through the use of DNA markers using Marker-Assisted Selection (MAS) (Lan *et al*., 2009).

The physical properties of the wool fiber can be attributed to proteins from the keratin family which are the primary constituents of the fiber (Itenge-Mwezaa *et al*., 2007). Keratins and Keratin-Associated Proteins (KAPs) are a large heterogeneous group of proteins comprising about 90% of the wool fiber. Heteropolymers of the type I and type II keratins form microfibrils of the wool fiber (Powell and Rogers, 1997). Microfibrils are embedded in a matrix of KAPs and consist of Keratin Intermediate-Filaments (KIF) (Schweizer *et al*., 2006; Powell, 1996). Consequently, variation in family of genes plays an important role in determining different properties of cashmere quality and production.

Keratin intermediate-filaments are also known as hard a-keratins as might be associated with hair, nails, or hooves. They are 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). Sheep type I and type II genes are at 11q25~q29 and 3q14~q22 (Dolling and Brooker, 1966) with the type I gene about 4~5 kb in length and containing 6 introns and the Type II gene about 7~9 kb in length and containing 8 introns (Powell and Rogers, 1997).

Xinjiang Cashmere Goat (XJG) is a native indigenous goat breed that has not been subject to artificial selection or gene introgression from imported breeds. The Nanjiang Cashmere Goat (NJG) is a composite breed, from crosses of Liaoning Cashmere Goat with Xinjiang local goat

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(Xiao and Xu, 2002). Bogeda Cashmere Goat (BG) is a composite of Liaoning Cashmere Goat, *Capra ibex* and Xinjiang local goat, named as a breed by the Autonomous Regional Committee of Livestock Species in 1998. Few molecular studies have been done in these breeds (YeErXiaTi and Ye, 2002).

The objective of this study was to detect polymorphisms within Keratin Intermediate-Filaments type I (KIFI) gene in three Chinese goat breeds (XJG, NJG, BG) and assess the effect of these polymorphisms on cashmere traits, with the intent of identifying markers useful in marker-assisted selection.

MATERIALS AND METHODS

Animal source: Data on cashmere traits and DNA from Xinjiang cashmere goat (n = 208) were obtained from two different households in Kurbanmuye Village, Cedaya Township, Luntai County, Bayinguoleng Prefecture, Xinjiang Autonomous Region; DNA from Nanjiang Cashmere Goat (n = 253) were obtained from two flocks, reared in BaiHuTai Breeding Farm, Wensu County, Aksu Prefecture, Xinjiang Autonomous Region; and data on cashmere traits and DNA from Bogeda Cashmere Goat (n = 251) were collected from Banfanggou Farm, Urumqi City, Xinjiang Autonomous Region.

DNA sample preparation: Genomic DNA of 712 animals was isolated from 2% heparin-treated blood samples and stored at -80°C, following procedures of Sambrook and Russell (2002).

PCR amplification: Based on a published gene sequence for sheep (GenBank accession No.M23912), one pair of PCR primers(F:5'-GGCTTGGTGAACTTGGCTCTG-3' and R: 5'-TCGGATTCCCTCCCTACAAC-3') was designed using Primer 5.0 software to amplify cashmere goat KIFI gene. The size of the PCR products was 459 bp, containing partial 5' Untranslated Regions (UTR), exon 1 and part of intron 1.

A 25 μL PCR solution containing 50 ng DNA template, 10 umol of each primer, 0.20 mM dNTP, 2.5 mM $MgCl_2$ and 0.5 U Taq DNA polymerase (Dingguo, Beijing and China) was used. The PCR was performed using the following program: 95°C for 5 min followed by 34 cycles of 94°C for 30 sec, annealing for 40 sec and 72°C for 45 sec and a final extension at 72°C for 10 min. PCR products were electrophoresed on 1.5% agarose gels using 1×TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA), containing 200 ng mL^{-1} ethidium bromide.

Genotyping of HaeIII KIFI allele by PCR-RFLP: PCR products (10 μL) were digested with HaeIII (5 U, With BSA) at 37°C, overnight. The digested products were then electrophoresed in 2.0% agarose gel using 1×TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2 EDTA), containing 200 ng mL^{-1} ethidium bromide. Genotypes were assigned according to PCR-RLFP banding patterns (Fig. 1) for each individual.

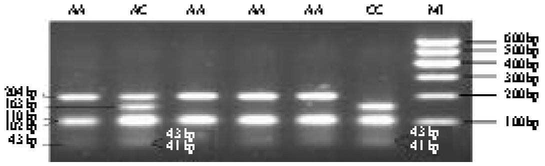

Fig. 1: PCR-RFLP patterns of the KIFI gene in goats (Genotype AA, Genotype AC, Genotype CC)

Statistical analysis: Based on results of genotyping for KIFI, genotypic frequencies, allelic frequencies and Hardy-Weinberg equilibriums were estimated. Differences among Chinese cashmere goat populations in genotypic and allelic frequencies of the KIFI gene were tested using a χ^2-test (SPSS, Inc., Shanghai, PRC, version 15.0). Population genetic indexes (gene heterozygosity, gene homozygosity and effective allele numbers) were calculated by methods of Nei and Li (1979). The Polymorphism Information Content (PIC) was calculated according to Botstein *et al*. (1980).

The linear model used in analyses of the impact of KIFI genotype on cashmere traits in Xinjiang cashmere goat (n = 208) included effects of age of goat, origin and KIFI genotype with random residual used as error (SPSS, Inc., Shanghai, PRC, version 15.0).

RESULTS AND DISCUSSION

The KIFI gene has been located on ovine chromosome 11 and was identified as a QTL for wool staple strength (Powell and Rogers, 1994; Purvis and Franklin, 2005). Polymorphism associated with the candidate gene for wool keratin has been described in several sheep breeds (Rogers *et al*., 1993). There are few reports in the literature on polymorphisms of KIFI in cashmere goat.

In this research, we detected the partial 5'UTR, the entirety of exon 1 and part of intron 1 of the cashmere goat KIFI gene. The mutation A to C (GGCA-to-GGCC) located at intron 1 formed a HaeIII endonuclease restriction site. Three unique PCR-RFLP banding patterns (genotype AA, AC and CC) were detected (Fig. 1). The PCR-RFLP banding patterns were sequenced in both directions and were published in the GenBank database (GenBank accession FJ429182-FJ429184) (Fig. 2).

The frequencies of allele A in Xinjiang cashmere goat (n = 208), Nanjiang Cashmere Goat (n = 253) and Bogeda Cashmere Goat (n = 251) were 0.700, 0.729 and 0.747, respectively and frequencies of allele C was 0.300, 0.271 and 0.253 (Table 1). Population genetic indexes (homozygosity, heterozygosity, effective allele Numbers (Ne) and Polymorphism Information Content (PIC)) were calculated according to Nei and Li (1979) (Table 2). All breeds showed moderate polymorphism for KIFI and therefore moderate genetic diversity of this gene in Chinese cashmere goat populations. However, PIC in the Xinjiang cashmere goat breed was greater than those of the other two breeds, implying that the polymorphism and genetic variation in the Xinjiang cashmere goat breed were higher than those of the Nanjiang Cashmere Goat and Bogeda Cashmere Goat. Tests of Hardy-Weinberg equilibrium confirmed that all three breeds were in equilibrium ($p>0.05$) (Table 2).

Analyses of KIFI genotype with cashmere quality traits (cashmere fineness, down cashmere thickness, cashmere yield and body weight after combing) were analyzed in Xinjiang cashmere goat. The mean fiber diameter for genotype AA was smaller than genotype CC ($p<0.05$), demonstrating the influence of this gene on the cashmere fineness. Genotype CC had a trend of numerically thicker down cashmere fleece and greater cashmere yield (Table 3). However, no significant difference. This is consistent with results of other research that show negative relationships of fiber diameter and fleece yield (YeErXiaTi and Ye, 2002).

Since, the A-C mutation is located in one of the intron regions, the SNP studied here may not be a causal mutation. If not, it is possible that it is linked to another mutation in the coding or regulatory regions of another gene, which is a causal mutation for cashmere traits (Lai *et al*., 2009). However, introns (especially intron 1) have been shown to affect transcriptional efficiency of numerous genes in a variety of organisms (Greenwood and Kelsoe, 2003; LeHir *et al*., 2003). For example, Marie-Laure *et al*. (2006) found a splice defect (c.357 +2T-C) in intron 3 of the human Hesx1 gene, which led to the synthesis of truncated proteins partiallly or entirely lacking the homeodomain, with no transcriptional repression, as shown by their inability to inhibit Prop1 activity (Marie-Laure *et al*., 2006).

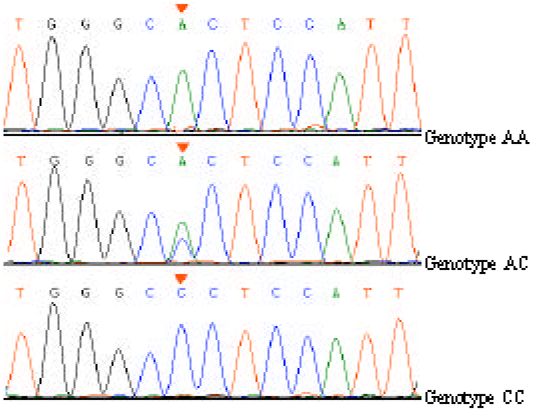

Fig. 2: Sequencing maps from different genotypes in cashmere goat KIFI gene

Table 1: Genotypic distribution and allelic frequencies of cashmere goat KIFI gene

	Observed genotypes and their frequencies				Allele frequencies	
Breeds[1]	AA	AC	CC	Total	A	C
XJG	105(0.505)	81(0.389)	22(0.106)	208	0.700	0.300
NJG	128(0.506)	113(0.447)	12(0.047)	253	0.729	0.271
BG	141(0.562)	93(0.370)	17(0.068)	251	0.747	0.253

[1]XJG: Xinjiang Cashmere Goat; NJG: Nanjiang Cashmere Goat; BG: Bogeda Cashmere Goat

Table 2: Genetic diversity estimates for Chinese cashmere goat gene KIFI

Breeds[1]	Gene Homozygosity (Ho)	Gene Heterozygosity (He)	Effective allele Numbers (Ne)	Polymorphism Information Content (PIC)	X2 (HW**)	p-value (HW*)
XJG	0.5796	0.4204	1.7252	0.3320	1.1282	>0.05
NJG	0.6051	0.3949	1.6526	0.3169	4.3451	>0.05
BG	0.6220	0.3780	1.6076	0.3065	0.0976	>0.05

[1]XJG: Xinjiang Cashmere Goat; NJG: Nanjiang Cashmere Goat; BG: Bogeda Cashmere Goat

Table 3: Means for KIFI genotypes for cashmere traits in Xinjiang cashmere goat

Traits	AA (n = 105)	AC (n = 81)	CC (n = 22)	p-value
Cashmere fineness (μm)	15.763±0.104a	15.976±0.121ab	16.474±0.257bc	<0.05
Down cashmere thickness (mm)	3.387±0.095	3.424 ±0.110	3.571±0.233	>0.05
Cashmere yield (g)	146.576±4.051	147.238±4.706	156.571±9.982	>0.05
Body weight after combed (kg)	29.635± 0.629	30.159±0.731	28.214±1.551	>0.05

Means on the same line with different superscripts differ ($p<0.05$)

CONCLUSION

The present study revealed a novel substitution in the intron 1 of cashmere goat KIFI gene which forms a HaeIII endonuclease restriction site. This mutation appears to be associated with cashmere fineness ($p<0.05$) and may be useful in genetic improvement of cashmere traits of Chinese cashmere goats through marker assisted selection.

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