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The Polymorphism of a Novel Mutation of *KAP13.1* Gene and its Associations with Cashmere Traits on Xinjiang Local Goat Breed in China

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Abstract: Genetic variations of *KAP13.1* gene have been studied in 816 animals of Xinjiang goat breeds in China. The genotypes and allele frequencies of *KAP13.1* gene were detected by PCR-RFLP techniquies. At the same time, parts of our samples were sequenced and analyzed with cashmere production traits data. The results showed that TT genotype significantly higher of body weight after combed trait than GT genotype ($p<0.05$) and down cashmere thickness trait showed genotype GT with significant higher than GG ($p<0.05$) in Nanjiang cashmere goat. In Xinjiang goat, the fiber diameter showed significant differences ($p<0.05$) among the age two, three and four years old. The fiber diameter trait in one year old cashmere goat had significant differences ($p<0.05$) compared with two, three and four years old. The frequencies of the *KAP13.1*-T allele in Xinjiang goat (n = 220), Nanjiang cashmere goat (n = 310) and Bogeda cashmere goat breeds (n = 286) were 0.996, 0.568 and 0.969, respectively. The χ^2-test showed that the genotype distributions in these three cashmere goat breeds were not in agreement with Hardy-Weinberg equilibrium. According to the classification of PIC, Nanjiang cashmere goat was more polymorphic at this locus. The mutation in our animals at *KAP13.1* locus was recorded for the first time. In conclusion, The results possibly revealed that the polymorphism existed in the three Xinjiang local goat breed and a missense mutation was possibly caused by variations in the number of the decapeptide repeat structures. Further analysis of results leads us to believe that the polymorphism of *KAP13.1* gene might be relevant to fiber diameter and other cashmere traits. Thus, molecular genetic study of *KAP13.1* gene represented valuable results for genetic conservation purposes and economic production of cashmere traits.

Key words: Cashmere goat, *KAP13.1* gene, cashmere traits, missense mutation

INTRODUCTION

Mammalian skin consists of three major compartments: epithelium, dermis and hypodermis. It is important that epidermis is a derivative of the surface ectoderm, as a protective barrier and specific appendages including hair, nails and different eccrine glands

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(Jonker *et al*., 2004; Bond *et al*., 1996). The cuticle, the cortex and the medulla are the main structure of all hairs and wool (Hawkins and Ragnarsdottir, 2009; Feughelman, 2002). The internal cortical cells are long polyhedral spindle-shaped structures (Jones, 2001), which mechanically, are the most important component of any α-keratin fiber (Feughelman, 2002). The flattened overlapping, cuticle cells surround the cortex and forms the external layer of the fiber (Marshall *et al*., 1991). The structure of the cashmere fiber largely involves the expression of hair keratins and their keratins-associated proteins (Langbein *et al*., 1999, 2001). The keratins and Keratin-Associated Proteins (*KAPs*) are a large heterogeneous group of proteins that make up about 90% of the wool fiber (Powell and Rogers, 1994). Human hair is very resistant to external stimuli and high stability due to keratins (Barba *et al*., 2009). So, the keratins and Keratin-Associated Proteins (*KAPs*) play an essential role in hairs and wool.

The keratins and Keratin-Associated Proteins (*KAPs*) are one of the largest gene families in mammalian genomes encode, which is a heterogeneous group of proteins that make up about 90% of the cashmere fiber (Rogers *et al*., 2002). The *KAPs* are encoded by a large number of multigene families. Furthermore, the *KAP* genes are small in size less than 1 kb, generally contain a single exon. The *KAPs* have been divided into three categories, the high sulfur *KAPs* (<30 mol% cysteine content), the ultra-high sulfur *KAPs* (>30 mol% cysteine content) and the high tyrosine/glycine *KAPs* (Powell and Rogers, 1996). The hair keratins represent the type I (acidic) and type II (basic) two multigene families. They form the 8-10 nm intermediate filaments (KIF) of trichocytes by co-polymerization of type I and II members, which are differentially expressed during hair fiber development (Langbein *et al*., 1999, 2001). Previous studies have introduced these *KAPs* are using the abbreviations *KAP1.n* through *KAP23.n* for these members known at that time with n referring to a number identifying individual members, also subdivided into 23 distinct families (Rogers and Powell, 1993) and more than 100 *KAP* genes have been isolated from human and other mammalian species. Genetic markers for the keratin and keratin-associated protein genes have been associated with variation in fiber diameter and staple strength (McLaren *et al*., 1997).

KAP13.1 is one of high sulfur *KAPs* which are important for the hair structure, localized in the hair cortex, showed matrix, cuticular expression and might play an important role in the hair forming compartment (Rogers *et al*., 2002). Apparent molecular weights of the high sulfur proteins were 26.5-43.0 kd, but it is probably higher than the real values 75-150% (Marshall, 1983). The high sulfur proteins predominantly found in the cuticle and some also found in the cortex (Irvine, 2005).

China has a centuries-old history of breeding cashmere goat and abundant cashmere breeds resources and Xinjiang is one of the biggest cashmere-producer in China. The Xinjiang goat is a native indigenous breed. The breed of Nanjiang cashmere goat is hybrid offspring of Liaoning cashmere goat, which is famous for its high cashmere yield, strong adaptability and stable heritage. Nanjiang goat is hybrid offspring of Liaoning cashmere goat breed and Xinjiang indigenous goat and was named by Ministry of livestock of the People's Republic of Xinjiang (Xinjiang Autonomous Region) in 1997 of China.

To date, no polymorphisms of *KAP13.1* gene have been reported for Xinjiang local cashmere goat. Therefore, the aim of our study is analyzed the genetic variations of *KAP13.1* gene in 816 individuals of Xinjiang goat breeds in China by PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) and DNA sequencing methods.

MATERIALS AND METHODS

Animal Source

Eight hundred and sixteen blood samples from unrelated animals (Xinjiang goat, n = 220; Nanjiang cashmere goat, n = 310; BoGeDa cashmere goat, n = 286) were collected and used

in this study in June of 2008. The Xinjiang goat were from the breeding centre of KuErLe of XinJiang in China, the Nanjiang cashmere goat were from AkeSu Goat Research Center of XinJiang and Nanjiang goat were from Urumuqi of XinJiang in China. Many records of cashmere traits and body weight were used for statistical analysis.

DNA Preparation and Primer Design

Genomic DNA of 816 cashmere goat were isolated from 2% heparin-treated blood samples and stored at -80°C, following standard procedures (Sambrook and Russell, 2001). According to the sequence of *KAP13.1* (GenBank accession number AY510115), one pair of PCR primers was designed with Primer5.0, as follow:

Forward: 5'- TCCCATCGTAACTCACAT -3',
Reverse: 5'- AGGTCGGTTGGAAAGAAA -3'

They were used to amplify 501 bp PCR products, containing the CDS region of *KAP13.1* gene.

PCR Amplification

One pair of PCR primers was designed using Primer 5.0 software to amplify the whole CDS region of Capra *KAP13.1* gene (high sulfur *KAPs*) (GenBank accession number AY510115), the size of the PCR products was 501 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 followed by 33 cycles of 94°C for 40 sec, annealing for 35 sec and 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 *KAP13.1* were incubated with 6 units restriction enzyme PvuII (Promega) for 6 hours at 37°C, then electrophoresed on 2% agarose gels with 1×TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA), containing 200 ng mL^{-1} ethidium bromide. A 7 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 (100v) for 0.4-0.6 h.

The PCR fragments from different patterns in the three breeds were amplified by the pair of primers were sequenced in both directions by ABI PRIZM 501bp DNA sequencer (PerkinElmer) and the sequences were analyzed with BioXM software (version 2.6).

Statistical Methods and Analysis

Based on the genotypes of *KAP13.1* locus in Xinjiang local goat breeds, the genotypic frequencies, the allelic frequencies and Hardy-Weinberg equilibriums were directly calculated. Differences in genotypic frequencies at *KAP13.1* locus among Xinjiang indigenous goat and cashmere goat populations in China were analyzed using a χ^2-test, which were performed by SPSS software (version 16.0) (Norusis, 2008). Population genetic indexes, such as He (gene heterozygosity), Ho (gene homozygosity), Ne (effective allele numbers) and PIC (Polymorphism Information Content) were calculated according to Nei and Roychoudhury (1974) and Nei and Li (1979), respectively.

$$H_o = \sum_{i=1}^{n} P_i^2, \quad H_e = 1 - \sum_{i=1}^{n} P_i^2, \quad Ne = 1 \Big/ \sum_{i=1}^{n} P_i^2, \quad PIC = 1 - \sum_{i=1}^{m} P_i^2 - \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} 2P_i^2 P_j^2$$

Furthermore, statistical analysis was performed for the records of cashmere traits in Xinjiang goat (XJG, n = 220) and Nanjiang cashmere goat (NJG, n = 310). All analyses were done in two steps, first using a full animal model and then using a reduced animal model. The full animal model included fixed effects of marker genotype, age of ram, ewe, sex, farm, body weight, after combed cashmere fineness, down cashmere thickness, cashmere yield and random effects of animal. The reduced model was used in the final analysis (Boldman *et al*., 1993; Zhao *et al*., 2004). The software SPSS (version 16.0) was used to analyze the relationship between the genotypes and cashmere traits in goat. The reduced linear model with fixed effects was established and included effects of ewe, ram within ewe, age and genotype, as well as interaction between ram and genotype was involved. Reduced linear model:

$$Y_{ijklm} = u+S_i+D_{ij}+A_k+Gl+(SG)\ il+E_{ijklm}$$

where, Y_{ijklm} was the trait measured on each of the ijklmth animal, u was the overall population mean, S_i was the fixed effect associated with the ith ram, D_{ij} was the fixed effect associated with jth ewe with ram i, A_k was fixed effect due to the kth age, Gl was the fixed effect associated with lth genotype (*KAP13.1*/TT, GTand GG genotype), (SG)il was interaction between the ith ewe and the lth genotype and E_{ijklm} was the random error.

An effect associated with farm, sex were not matched in the linear model, as the preliminary statistical analyses indicated that these effect did not have a significant influence on variability of traits in analyzed populations. The Least Square Means Estimates (LSM) with standard errors for three genotypes of *KAP13.1* gene and growth traits (Zhao *et al*., 2004).

RESULTS

In this study, the CDS region of *KAP13.1* gene demonstrated polymorphic patterns in three breeds populations by PCR-RFLP method and DNA sequencing (Fig. 1), the result revealled a mutation T>G (CAGCTG>CAGCGG) and three genotypes (named genotype TT, GT and GG (Fig. 2). The mutation was confirmed by restriction digestion (PvuII-CAG/CTG), which resulted an important missense mutation in *KAP13.1* protein, namely, CTG (Leu) > CGG (Arg). In detail, the T>G mutation was located in (g.186 T>G) nucleotide position of GenBank Accession No. AY510115 at *KAP13.1* gene locus.

The result showed that one mutation located in the CDS region of *KAP13.1* gene in Xinjiang local goat breeds, which leads to an important deletion restriction-reaction polymerase locus. Three genotypes (TT, GT and GG) were firstly detected by restriction enzyme PuvII. Genotypic and allele frequencies of *KAP13.1* gene in the three breeds were showed in Table 1 and 2. The frequencies of alleles T and G were 0.568, 0.432 in Nanjiang

Table 1: Genotype distribution and allelic frequencies at the *KAP13.1* gene locus

	Observed genotypes				Allelic frequencies		
Breeds	TT	GT	GG	N	T	G	χ^2 (HWE)
NJG	166	20	124				
	0.5355	**0.0645**	**0.4**	310	0.5677	0.4323	233.860
BGDG	271	12	3				
	0.9476	0.0419	0.0105	286	0.9685	0.0315	27.782
XJG	**219**	**0**	**1**				
	0.9955	0	0.0045	220	0.9955	0.0045	220.000

Bold values: Genotype frequencies at the *KAP13.1* gene locus. χ^2 (HWE): Hardy-Weinberg equilibrium χ^2 value, NJG: NanJiang cashmere goat, BGDG: BoGeDa cashmere goat, XJG: XinJiang cashmere goat

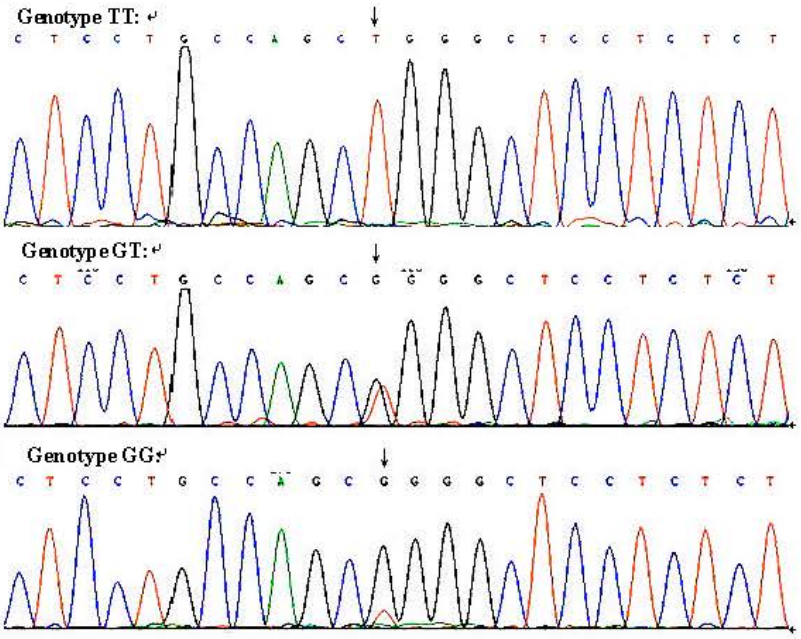

Fig. 1: Sequencing maps from different genotypes in cashmere goat *KAP13.1* gene

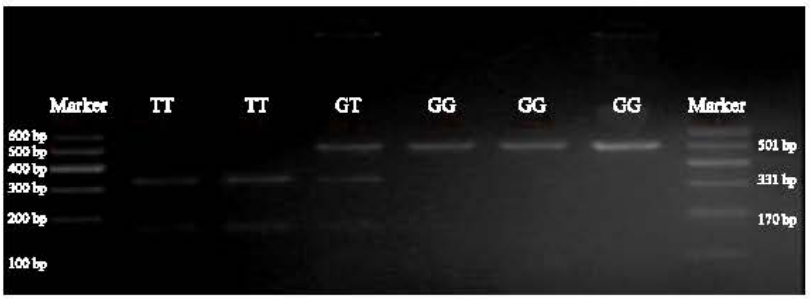

Fig. 2: PCR-RFLP patterns of the *KAP13.1* gene in cashmere goat, M: 100 bp ladder marker, lane 1, 2: Homozygous TT genotype, lane 3: Heterozygous GT genotype, lane 4, 5, 6: Homozygous GG genotype

Table 2: Genetic index in three Xinjiang local goat in China

Breeds	Types	Ho	He	Ne	PIC
NJG	Cashmere	0.509	0.491	1.964	p = 0.370
BGDG	Cashmere	0.939	0.061	1.065	p = 0.059
XJG	Cashmere	0.991	0.009	1.009	p = 0.009

Ho: Gene homozygosities, He: Gene heterozygosities, Ne: Effective allele number, PIC: Polymorphic information content

cashmere goat, 0.969, 0.031 in BoGeDa cashmere goat and they were 0.996, 0.004 in Xinjiang goat, respectively. T allele and TT genotype were predominant in three breeds. The genotypic frequencies at *KAP13.1* locus were significantly different between three breeds. The χ^2-test showed that the genotype distributions of *KAP13.1* gene were not in agreement

with Hardy-Weinberg equilibrium in three breeds (Table 1). Based on χ^2-test, genotypic frequencies of the various polymorphism at *KAP13.1* gene were found to be significantly different in the three breeds ($\chi^2 = 247.337$, df = 4, $p<0.01$).

Gene homozygosity, gene heterozygosity, effective allele numbers and PIC of *KAP13.1* gene in the three breeds were shown in Table 2. According to the classification of *PIC* (high polymorphism if PIC value >0.5, median polymorphism if 0.25<PIC value <0.5 and low polymorphism if PIC value <0.25) (Botstein *et al*., 1980), Xinjiang goat population and Bogeda cashmere goat population were with low polymorphic level, Nanjiang cashmere goat population was with median polymorphic level. Gene heterozygosity, effective allele numbers and PIC of *KAP13.1* gene in Nanjiang cashmere goat population showed higher than that of Xinjiang goat and Bogeda cashmere goat population.

In this study, we also revealed that the polymorphism of *KAP13.1* gene associated with body weight after combed, after combed cashmere fineness (fiber diameter), down cashmere thickness and cashmere yield (cashmere wool fiber weight) (Table 3, 4). Further analysis suggested that the animals with TT genotype significantly higher body weight after combed than GT ($p<0.05$) in Nanjiang cashmere goat. The down cashmere thickness trait showed GT was significantly higher than GG ($p<0.05$) in Nanjiang cashmere goat. No significant differences were observed between the TT, GT and GG genotypes in the Nanjiang cashmere goat related to cashmere production traits ($p>0.05$) (data not show). In Xinjiang goat, the fiber diameter was significant differences ($p<0.05$) among the age of two, three and four years old. The fiber diameter trait in one year old cashmere goat recorded significant differences ($p<0.05$) compared with two, three and four years old cashmere goat (Table 3).

Table 3: Association of genotypes at the *KAP13.1* gene with growth traits in XinJiang goat

		Genotypes at *KAP13.1* gene (Mean±SE)		
Ages	Cashmere traits	TT	GT	GG
1	BWC (kg)	28.618±0.921		
	CD (μm)	15.591±0.158[a]		
	DCT (mm)	3.232±0.145		
	CY (g)	138.100±6.155		
2	BWC (kg)	29.234±0.783		
	CD (μm)	16.229±0.135[a]		
	DCT (mm)	3.589±0.124		
	CY (g)	141.100±5.235		
3	BWC (kg)	29.548±0.565		34.000±1.233
	CD (μm)	16.005±0.096[b]		16.150±0.211
	DCT (mm)	3.495±0.088		3.000±0.192
	CY (g)	162.000±3.742		205.000±8.166
4	BWC (kg)	29.891±0.792		
	CD (μm)	16.799±0.136[c]		
	DCT (mm)	4.267±0.125		
	CY (g)	178.800±5.292		

BWC: Body weight after combed, CD: Cashmere fineness, DCT: Down cashmere thickness, CY: Cashmere yield. *Values with different superscripts within the same line differ significantly at $p<0.05$ (a, b, c). SE: Standard error of means

Table 4: Association of genotypes at the *KAP13.1* gene with growth traits in XinJiang cashmere goat

	Genotypes at *KAP13.1* gene			
Cashmere traits	TT	GT	GG	p-value
BWC (kg)	21.506±0.207[a]	20.250±0.597[b]	21.419±0.240	>0.05
CD (μm)	15.806±0.066	15.517±0.191	15.662±0.077	>0.05
DCT (mm)	4.725±0.054	4.980±0.156[a]	4.587±0.630[b]	<0.05
CY (g)	472.950±8.020	478.000±23.106	455.890±9.279	>0.05

Data are expressed as Mean±SE. BWC: Body weight after combed, CD: Cashmere fineness; DCT: Down cashmere thickness, CY: Cashmere yield

DISCUSSION

Many studies reported genetic polymorphisms in the human high sulfur hair keratin-associated proteins, which also play an important role in determining various wool traits as well as the variation in fiber diameter (McLaren *et al*., 1997; Parsons *et al*., 1994). It is known that the fiber diameter, cashmere yeild and down cashmere thickness were important cashmere production traits in cashmere goat. Present study is considered as the firstly detected novel mutation in the coding region of *KAP13.1* gene in three cashmere goat breeds in China.

The study of SNP in our results was not a casual mutation, it showed missense mutation in *KAP13.1* protein leucine to arginine, which is essential for optimal growth in infancy and childhood and for nitrogen equilibrium in adults (Jmaes John, http://searchwarp.com/swa51880.htm). The genome is highly redundant in terms of tRNA species for each amino acids but enigmatically under-represents a number of specific codons (Shah *et al*., 2008), that may be linked to cashmere goat in the coding or regulatory regions of the gene which is not be a causal mutation for the cashmere goat growth traits. The polymorphic level of Xinjiang goat population and Bogeda cashmere goat population were lower than that of Nanjiang cashmere goat population.

The χ^2-test results obtained in our study may be caused by nonrandom mating and among the three Xinjiang local goat breed selected some of the excellent individuals could violate the Hardy-Weinberg equilibrium.

Despite all the available information concerning *KAP13.1* gene in human and mouse, there is no information known for cashmere goat or other livestock. Present results showed that the mutations of *KAP13.1* were associations with fiber traits and it was firstly detected in Xinjiang local goat breeds of China. Therefore, *KAP13.1* gene seems to be promising as it plays an important role in fiber diameter traits. Considering the economic importance of the cashmere production traits to the livestock industry, it appears clearly that essential further researches on *KAP13.1* gene in the livestock should be done.

CONCLUSION

In conclusion, the present study revealed a novel SNP in CDS region of *KAP13.1* gene. The SNP is significantly associated with fiber diameter and down cashmere thickness trait. The G allele was a recessive in the three goat breeds and it should be rejected from the breeding schemes of goat. Therefore, this study considered as a preliminary important research for improving the Xinjiang local goat breeds and the breeding of genuine cashmere in China.

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