International Journal of Animal Breeding and Genetics Vol. 5 (6), pp. 001-006, June, 2016. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Sequence variant in the KAP 1.1 gene associate with cashmere trait in two cashmere goat breeds

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Accepted 19 March, 2016

KAP1.1 gene played an important role in determining phenotypes for cashmere quality and productive traits. Here, polymorphisms analysis in the keratin-associated proteins 1.1 (KAP1.1) gene was applied by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and DNA sequencing methods in 540 individuals from Liaoning cashmere goat and Inner Mongolia white cashmere goat. One SNP was detected at the KAP1.1 gene and resulted in three different genotypes. DNA sequencing analysis showed, the sequence of CC genotype was the same to the sequence (X01610) in GeneBank, a novel SNP (g.688T>C) was found in the sequence of TT genotype, and caused synonymous mutation for the amino acid sequence. Statistical analysis demonstrated for cashmere yield, body weight and cashmere fineness of Liaoning cashmere and Inner Mongolia White cashmere goat , TT genotype was significantly higher than CC and CT genotype for cashmere yield (TT: 847.36 g; 716.90 g; CT: 583.66 g) and body weight (TT: 37.25 kg; CC: 31.27 kg; CT: 32.41 kg), there was no difference between three genotypes for cashmere fineness, these results showed that TT genotype could be a favorable marker for early breeding selection of the Liaoning and Inner Mongolia White cashmere goat.

Key words: Cashmere goat, KAP gene, PCR-SSCP, productive trait.

INTRODUCTION

The major structural proteins of mammalian hair are composed of the hair keratins and their associated proteins. The hair keratins belong to the large keratin multi-gene family. The keratins could be divided into two groups: the keratin intermediate filament (KIF) proteins and the keratin-associated proteins (KAP). KIF was formed by the hair keratin in the trichocytes, about 8 to 10 nm in diameter, is surrounded by the matrix, KAP are the major component of the matrix between the KIF, are thought to form the rigid hair shaft through a cross-linked network with the KIF(Powell and Rogers, 1996).The KAP could be divided into three major groups based on their protein sequencesthe high glycine-tyrosine group; the high sulphur group (16 to 30% cysteine content); the ultra-high sulphur group(>30% cysteine content).

To date, more than 100 KAP genes have been isolated from human and other mammalians species, in general, the KAP gene is composed of a single exon of less than 1000 bp. KAP protein controlling loci play an important role in determining phenotypes for wool quality and productive traits (Purvis et al., 2005). The observed variation pattern of high sulphur proteins was different among Merino, Romney and Corriedale sheep, suggesting that high sulphur proteins was related with wool trait (Flanagan et al., 2002). Jin et al. (2010) detected the KAP7.1 and KAP8.2 gene expression in the primary and secondary hair follicles, indicating that KAP7.1, KAP8.2 may have an important role in regulating the fiber diameter. Fang et al. (2010) revealed the polymorphism of KAP13.1 gene might be relevant to fiber diameter and other cashmere traits.

There are several proteins in KAP1 family (Powell et al., 1986). The KAPl proteins range in size from 151 to 181 amino acids, are neutral-basic and contain on average 22% cysteine residues. The amino acid sequences are highly conserved and differ mainly in the number of tandem copies of the 10 amino acid repeat,

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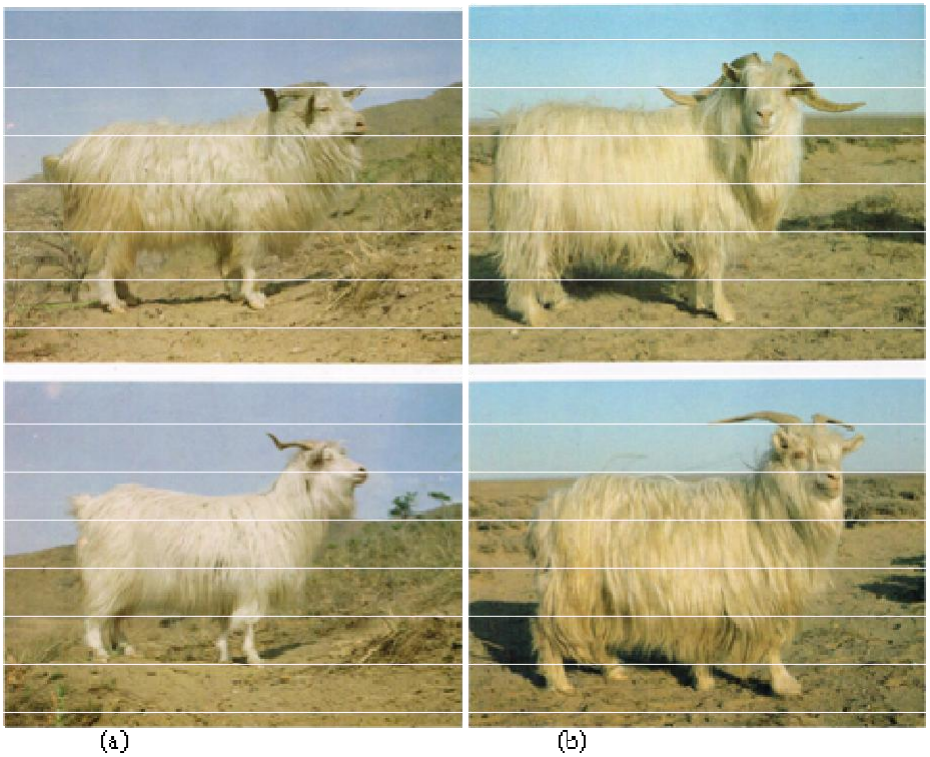


Figure 1. The picture of two cashmere goat. (a) Liaoning cashmere goat ♂/♀ (b) Inner Mongolia white cashmere goat ♂/♀.

SIQTSCCQPT, that is located at the N-terminal half of the protein **,** there is 4, 3, 2 and 5 repeat for KAP1.1,1.2,1.3 and 1.4, respectively. Many studies have revealed the polymorphism of KAP 1.1 gene (Shimomura et al., 2002a; Shimomura et al., 2002b). Liaoning cashmere goat (Figure 1a) and Inner Mongolia White cashmere goat (Figure 1b) distribute in north of China. Both of them has the merits of high production of cashmere, widely adaptability and stable hereditability, and has a significant value for improving the productive trait of other cashmere goat breeds. Genetic selection within those breeds aimed at the improvement of production traits might be the best strategy to assure its preservation. Moreover, its preservation would contribute to the biodiversity conservation in the world.Based on blood protein in cashmere goats, the association between structural gene and productive traits were studied, and many QTLs linked to productive traits of cashmere goat were found (Geng et al., 2000; Allain et al., 1998).

By using micro-satellite markers, the association between the cashmere goat's diversity and their productive traits were revealed (Li et al., 2002; Liu et al., 2005; Jin et al., 2006), however, there were no reports about KAP gene used as a candidate gene to find the association between KAP gene and the cashmere goat productive traits (Rogers et al., 1994; Xiao et al., 2005; Pruett et al., 2004). In this study, the KAP gene was firstly used as a candidate gene to study the association between the cashmere goat's polymorphism and their productive traits. The polymorphism of KAP 1.1 gene in two cashmere goat breeds were detected by PCR-SSCP in order to establish an association between the genotypes with cashmere yield, body weight and cashmere fineness trait.

MATERIALS AND METHODS

Goats and DNA sources

The Liaoning cashmere goat (*N* = 254) and Inner Mongolia White cashmere goat (*N* = 286) samples were obtained from Liaoning province and the centers of cashmere goat reproduction in Inner Mongolia Autonomous Region, respectively. Genomic DNA utilized in PCR amplification was extracted from blood according to Sambrook and Russell (2001).

Data

The cashmere samples were collected, and body weight of each goat was measured as well. The cashmere fineness was measured by scanning electron microscope.

PCR amplification

One pair of PCR primers forward (5′AGA TGC AGA AGG TGG AGC CAA AAC 3′) and reverse (5′GAA TGG TTC TTG AGA GAT

TC TC TT TC TT TT TT TC CC TT TC TC TT TT TC
TC CC TT TT

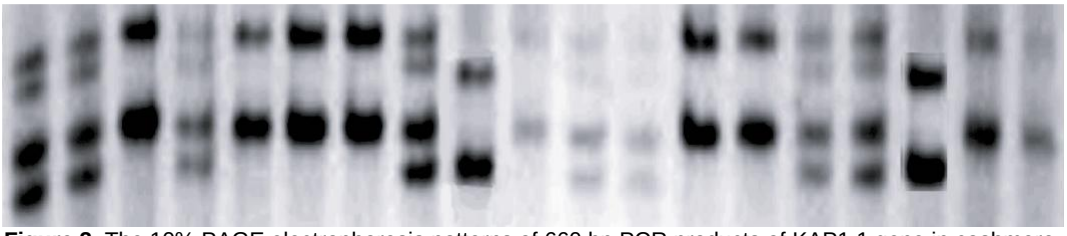


Figure 2. The 10% PAGE electrophoresis patterns of 660 bp PCR products of KAP1.1 gene in cashmere goat.

Table 1. Genotype distribution and allelic frequencies of the KAP1.1gene in two cashmere goat breeds.

Breed	Observed genotype			Total	Allele frequencies	
	CC	CT	TT		C	T
Liao Ning cashmere goat	56	84	114	254	0.3858	0.6142
Inner Mongolia White cashmere goat	78	95	113	286	0.4388	0.5612

CAG GGC 3′) was designed to amplify a 660 bp PCR product including the entire CDS region and its flanking region (GenBank Accession No.: X01610). PCR reactions were performed in PTC-200 PCR machine (M J Research Inc., MA, USA) according to the following program: initial denaturation for 5 min at 94°C, and then 34 cycles (94°C 30 s; 58°C 30 s; 72°C 45 s), final extension for 10 min at 72°C. The PCR reaction mix in a total volume of 12 µl contained: 2 µl (50 ng/µl) genomic DNA, 1.0 µl dNTPs (2.5 mmol/L), 1.0 µl primer (5 pmol/µl), 2.0 µl Taq DNA polymerize (0.5 U/µl), 1.5 µl 10×PCR buffer (Mg^{2+} plus), 4.5 µlH_2O. The products of each amplification was analyzed by electrophoresis on 1% agarose gel (5V/cm), 1×TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA) using ethidium bromide staining (1 µg/ml).

SSCP analysis

For SSCP analysis, 2 µl PCR products were mixed with 5 µl denaturation solution (95% formamide, 0.5 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C, and chilled on ice (Zhao et al., 2008). Denatured DNA was subjected to PAGE (200 × 125 × 1.00 mm) in 1 × TBE buffer and constant voltage (110 V) for 12 h. The gel was stained with 0.1% silver nitrate. The PCR products from different SSCP genotypes were sub-cloned to T-vector (Promega) and sequenced by Shanghai Sangon Biological Engineering Technolog and Service Co., Ltd. For the same genotype, three samples from different individuals were sequenced independently.

Statistical analysis

The calculation of Ho, He, Ne and PIC

Ho, He, Ne and PIC was calculated with the following equation:

$$PIC = 1 - \sum_{I=1}^{M} P_I^2 - \sum_{I=1}^{M-1} \sum_{J=I+1}^{M} 2P_I^2 P^2{}_J$$

$$HO = \sum_{I=1}^{M} P_I^2 \text{ , } HE = 1 - \sum_{I=1}^{M} P_I^2 \text{ , } NE = 1/\sum_{I=1}^{M} P_I^2$$

Where p_i and p_j was the gene frequency; m was the number of allelic; Ho was gene homozygosity; He was gene herterozygosity; Ne was effective allele number; PIC was polymorphism information content.

The least square statistics

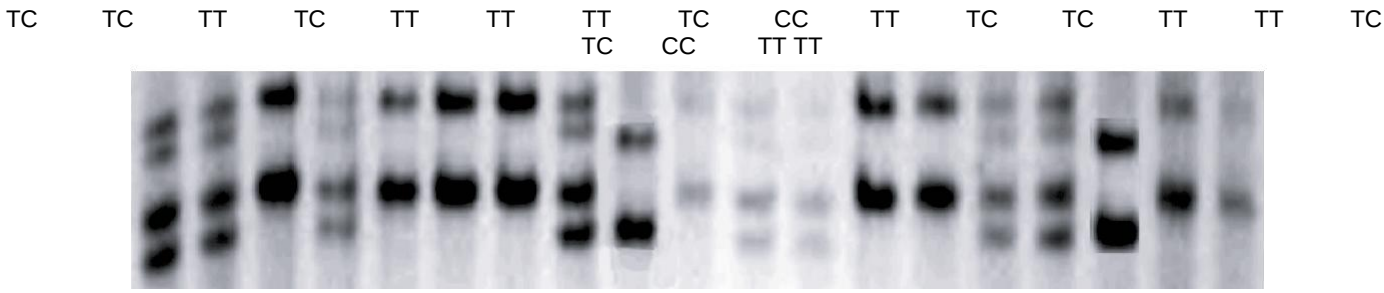
All data were analyzed by GLM procedure of the statistical software SPSS version 13.0. The association between KAP1.1 gene and productive traits of two cashmere goat breeds were analyzed by linear model, and calculated using the least square method. A fixed model was adopted according to the factors that affect phenotypic traits by using the following equation:

$Y_{ijkh} = \mu + Age_i + Sex_j + Marker_k + e_{ijkh}$

Where Y_{ijkh} was the phenotypic value of the individual detected, µ was the populations mean value, Age_i was the fixed effect of age, Sex_j was the fixed effect of sex, $Marker_j$ was the effect of marker genotypes from the population detected, and e_{ijkh} was the random errors.

RESULTS AND DISCUSSION

Although there was study related to polymorphism of KAP1.1 gene in Merion sheep, few studies focused on the goat polymorphism. However, the entire CDS region of KAP1.1 gene demonstrated polymorphism (named genotype CC, CT and TT) by PCR-SSCP method in both
Chinese cashmere goat breeds (Figure 2) .Frequencies of allele KAP1.1- C in Liaoning cashmere goat (*N*=254) and Inner Mongolia White cashmere goat (*N*=286) was
0.3858 and 0.4388 respectively; frequencies of allele KAP1.1-T was 0.6142 and 0.5612, respectively (Table 1).



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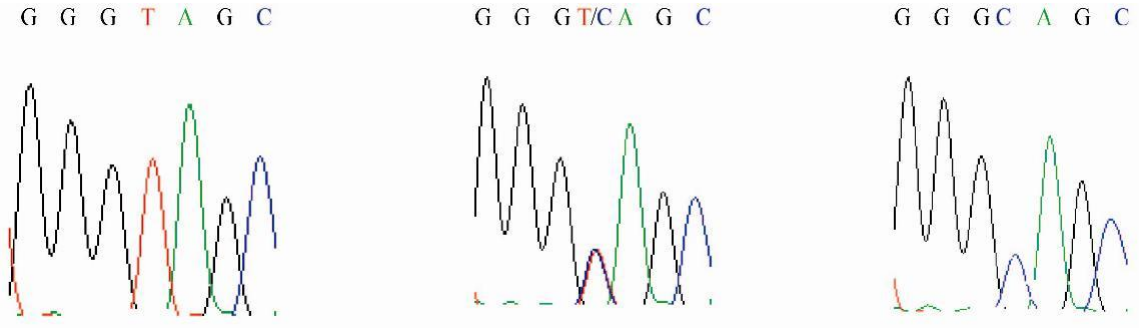

Figure 3. Sequence mapping of CC, CT and TT genotype in goat KAP1.1 gene.

DNA sequencing analysis showed that the sequence of CC genotype was the same to the sequence (X01610) in GeneBank, a novel SNP (g.688T>C) (Figure 3) was found in the sequence of TT genotype.

This result was not totally the same with previous study, Itenge-Mweza found three alleles (A, B and C) and more than three genotype for KAP1.1 gene in Merino sheep resulted from the insertion or deletion of 30 nt nucleotides (Itenge et al., 2007), which was in agreement with in Romney sheep (Rogers, 1994), however, we just found two alleles and three genotypes in both cashmere goat breeds, this difference could result from different species. According to Nei's methods (Nei, 1974), the population genetic index (Ho, He, Ne and PIC) were calculated (Table 2), both of the population showed the median polymorphism (low polymorphism if PIC<0.25; median polymorphism if 0.25<PIC<0.5; high poly-morphism if PIC>0.5). This reflected that both of the cashmere goat breeds were not a very high genetic diversity for the KAP1.1 gene, this might be caused by the cross-breeding, usually one excellent male cashmere goat was frequently used for cross-breeding in order to improve the cashmere quality and contributed to the population homogenous.

Meanwhile, the association of the KAP1.1 gene polymorphism with the productive trait (cashmere yield, body weight and cashmere fineness) was revealed, for both cashmere goat breeds, TT genotype' cashmere yield (Liaoning cashmere goat: 847.36 g; Inner Mongolia White cashmere goat: 859.26 g) was significantly higher than CC and CT genotype (Table 3); TT genotype's body weight (Liaoning cashmere goat: 37.25kg; Inner Mongolia White cashmere goat: 36.48 kg) was significantly higher than CC and CT genotype (Table 3), this showed that TT genotype could be a favorable marker for early breeding selection of the Liaoning and Inner Mongolia White cashmere goat, in addition, that demonstrated allele T played

Table 2. Genetic diversity at KAP1.1 l gene in two cashmere goat breeds.

Breeds	Ho	He	Ne	PIC
Liao Ning cashmere goat	0.5260	0.4740	1.9009	0.3617
Inner Mongolia White cashmere goat	0.5074	0.4858	1.9708	0.3714

Table 3. Association of genotype at the KAP1.1 gene with cashmere yield, body weight and cashmere fineness trait in two cashmere goat breeds.

Breeds	Genotype	Cashmere yield (g) (Mean±SE)	Body Weight (kg) (Mean±SE)	Cashmere fineness(μm) (Mean±SE)
Liao Ning cashmere goat	CC	583.66±10.75^{a}	32.41±0.74^{a}	15.26±0.85
	CT	716.90±12.39^{b}	31.27±0.64^{a}	15.16±0.79
	TT	847.36±15.04^{c}	37.25±0.70^{b}	15.52±0.11
Inner Mongolia White cashmere goat	CC	577.01±7.52^{a}	32.27±0.75^{a}	15.51±0.11
	CT	741.50±11.90^{b}	32.25±0.68^{a}	15.32±0.87
	TT	859.26±13.68^{c}	36.48±0.77^{b}	15.16±0.79

a, b and c indicates significant difference.

an important role in association with cashmere yield and body weight. No statistically significant difference was observed among CC genotype (Liaoning cashmere goat: 15.26 μm; Inner Mongolia White cashmere goat: 15.51 μm) and CT or TT genotype for cashmere fineness (Table 3). Liaoning cashmere goat and Inner Mongolia White cashmere goat are special genetic resources in China. They have brought more productive benefits for local farmers due to its high cashmere yield. For male and female Liaoning Cashmere goat, they can produce 1,650.00 and 830.00 g per head in a year, respectively; Inner Mongolia White cashmere goat can produce 1,134.00 and 821.3 g per head in a year, respectively.

Cashmere yield, body weight and cashmere fineness were important productive traits in cashmere goats, which directly affected productive benefits of cashmere goats. Although traditional population genetics and quantitative genetics have been used for cashmere goat breeding, but little study has been made on the genetic mechanism from gene level. Then, the relationship between KAP gene and productive traits of two cashmere goat breeds was firstly studied, and one favorable genotypes (TT genotype) related to productive traits were found. Although further studies with more samples from the cashmere goat were needed, the results shown in this paper were useful for the breeding of two cashmere goat breeds.

In conclusion, our results provide evidence that the KAP1.1 gene has a potential effect on cashmere yield and body weight. Therefore, further work will be necessary to use this SNPs for marker-assisted selection (MAS) in a large population and to investigate whether KAP1.1 gene play an important role in cashmere productive traits.

ACKNOWLEDGMENTS

This work was supported by the Chinese National Program for Scientists focusing on Cashmere goat or Wool sheep industrial technology system (nycytx-40-11); we would like to thank Z.H. Wei for sample collection. Professor Z.F. Yuan and M.C. Guo gave valuable comments on data statistics.

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