Effect of the Polymorphisms of Keratin Associated Protein 8.2 Gene on Fibre Traits in Inner Mongolia Cashmere Goats*

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ABSTRACT : The aim of the experiment was to detect polymorphisms in the keratin-associated protein 8.2 (KAP8.2) gene to determine associations between the genotype and fibre traits in Chinese Inner Mongolia cashmere goats. The fibre traits data investigated were cashmere fibre diameter, combed cashmere weight, cashmere fibre length and guard hair length. Five hundred and forty-two animals were used to detect polymorphisms in the complete coding sequence of the hircine KAP8.2 gene by means of PCR-SSCP. The results identified six genotypes, AA, BB, DD, AB, AD and BD, coded for by three different alleles A, B and D. Two SNPs in the coding region were confirmed by sequencing, which were A214G and T218C respectively. The relationships between the genotypes and cashmere fibre diameter, combed cashmere weight, cashmere fibre length and guard hair length were analyzed. There were significant differences (p<0.01) between the associations of the different genotypes with cashmere fibre diameter, cashmere weight and hair length. Cashmere length was the only trait that was not associated with the genotypes. The genotype AA (0.73) was found to be predominant in Inner Mongolia cashmere goats and the animals with this genotype had the thinnest cashmere fibre diameter compared with the other genotypes. These results suggested that polymorphisms in the hircine KAP8.2 gene may be a potential molecular marker for cashmere fibre diameter in cashmere goats. (**Key Words :** Inner Mongolia Cashmere Goats, Keratin Associated Protein 8.2, Polymorphisms, Fibre Traits, PCR-SSCP)

INTRODUCTION

Cashmere fibre diameter and cashmere weight are quantitative traits of economic importance in the cashmere goat industry. Fibre diameter is the most important factor to define the value per unit weight of cashmere fleece. Increasing cashmere production and maintaining lower cashmere fibre diameter are typically associated with higher economic returns to cashmere producers. Thus, selection strategies are needed to maximize the quantity of the fibre without affecting the fibre quality.

The high glycine-tyrosine (HGT) proteins in fibre vary significantly both within and between species ranging from 1% to 12% in sheep wool, 18% in mouse hair to more than 30% in echidna quill (Frenkel et al., 1974; Gillespie, 1990).

The wide range in the content of these proteins in wool and hair raises intriguing questions concerning the regulation and function of these proteins in the matrix structure of the fibre. They are subjected to variation by a variety of genetic, nutritional and physiological factors (Gillespie, 1990). The keratin-associated protein 8.2 (KAP8.2) is one member of the HGT proteins that belong to one of the three classes of KAP proteins (Kuczek and Rogers, 1987; Fratini et al., 1992; Aoki et al., 1997). The KAP8.2 gene has a coding sequence of 192 bp, which lacks introns and mainly is expressed in the wool follicle cortex. The KAP8 gene has been mapped to ovine chromosome 1 (Wood et al., 1992). Genes encoding HGT proteins have been located on chromosome 21q22.1 (Rogers et al., 2002) in humans. It has been reported that allelic variation at the KAP8 locus and fibre diameter was significant associated in sheep (Parsons et al., 1994). This suggests that the KAP8 gene may play an important role in determining the phenotypes for different cashmere quality and production traits.

The cashmere fibre from goats (*Capra hircus*), is the finest and softest animal fibre, with an average diameter of 15 μm, and is used exclusively in luxurious textile products (McCerthy, 1998). World market demands still exceed the

Table 1. PCR primers used for characterization of the hircine KAP8.2 gene

Primers	Sequence	Starting nucleotide position (GenBank no. AY510123)	Fragment length (bp)	Annealing temperature (°C)
1	5'-CCAGGAAACGGGGTCTTTG-3' 5'-AAGCCACCATATCCGTATCC-3'	3	193	60
2	5'-AGCACCTATTCCCCAGTGG-3' 5'-GGCTTCAGTTACAGTTCAGTTC-3'	146	271	65
3	5'-TTGAACTGTGCAAAGTAGA-3' 5'-GGGTTAAGGTATATTTATTAGAAT-3'	359	194	51

supply, so prices are consistently stable and higher than wool and mohair. China is the largest world producer of cashmere. About 30% of the total production comes from the Inner Mongolian population of cashmere goats that vary in production traits among individuals and flocks (Zhou et al., 2003). It is one of the major cashmere goat breeds in China (Chen et al., 2001; Bai et al., 2006), which has significant characteristics of fine cashmere performance quality, including well brightness, white color, big elasticity and average diameter under 15 μm. A number of genes having major effects on traits of the skin and fleece were identified in sheep (McLaren et al., 1997; Henry et al., 1998; Parsons et al., 1999; Bray et al., 2002; Arora and Bhatia, 2006). However, few candidate genes for cashmere production traits have been reported in cashmere goats. The objective of this study was to identify polymorphisms in the KAP8.2 gene and to evaluate the association of these polymorphisms with cashmere fibre characteristics.

MATERIALS AND METHODS

Animals and DNA samples

The blood samples of 542 female goats were collected from the breeding cashmere goat farm in the southwest of the Inner Mongolia region of China. The animals were kept extensively on a desert pasture all year round with similar rearing and feeding conditions. Genomic DNA was extracted from blood samples using the phenol-chloroform method.

Collection of cashmere phenotypic data

The fibre data including combed cashmere weight, cashmere fibre length and guard hair length, were collected from farm records during the period 1998 to 2005.

Moulted cashmere was harvested once a year by combing in late April, and weighed using an electronic scale. Cashmere and guard hair lengths were measured prior to combing on the same side of the shoulder without stretching the fibre. Patch samples of 10 cm² moulted cashmere on the side of the shoulder were obtained before combing in 2005. Hairs in the samples of moulted cashmere were separated and the cashmere samples were washed in ether solution to remove contaminants such as soil and grease. The cashmere fibre diameters were measured using an Auda 2000 optical

wool fiber gauging instrument (Shenaoda Technology Company, Shijiazhuang China) in the Wool Analysis Laboratory of the Liaoning Cashmere Breeding Center.

PCR amplification and SSCP analysis

PCR was performed to amplify sequences fragments of KAP8.2. Three pairs of PCR primers (Table 1) were designed using the software Oligo 6.0, according to the mRNA sequence of KAP8.2 (GenBank accession no. AY510123). The three overlapping PCR fragments covered the complete coding region and the 3' un-translated region of the hircine KAP8.2 gene. The 25-μl PCR volume included 50 ng DNA template, 0.2 mM dNTP, 1.5 mM MgCl$_2$ and 0.5U TaqDNA polymerase (Dingguo Biotechnology Company, Beijing, China). The PCR protocol was 94°C for 5 min followed by 35 cycles of 94°C for 30 s, annealing (Table 1) for 30 s, 72°C for 30 s and a final extension at 72°C for 10 min. The PCR products were then directly genotyped by SSCP. PCR product (1 μl) of each individual was mixed with 5 μl denaturing buffer (98% formamide, 0.05% xylene, cyanole FF and 0.05% bromophenol blue) and denatured at 98°C for 5 min, followed by a rapid chill on ice for 10 min. The denatured PCR products were electrophoresed for 16 h at 8 V/cm on 12% acrylamide gels (Jia et al., 2005). DNA bands on the gel were visualized by the silver staining technique.

Cloning and sequencing of PCR products to confirm mutation

The PCR products of individuals that showed different conformation patterns were purified using the DNA Fragment Quick Purification/Recover Kit (Dinggou Biotechnology Company, Beijing, China), and cloned into pGEM-T Easy Vector Kit (Promega, Madison, WI, USA), then sequenced using an 3730 sequencer (Applied Biosystems 3730xl DNA Analyzer).

Statistical analysis

All data were subjected to general linear models analysis using the SAS software package (version 8.2). The mixed linear model was as follows:

$$Y_{ijkl} = \mu + G_i + Y_j + A_k + S_l + e_{ijkl}$$

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Table 2. Genotype and allele frequencies of hircine KAP8.2 gene

Item	Genotypes						Alleles		
	AA	AB	AD	BB	BD	DD	A (A-T)	B (A-C)	D (G-T)
No. of animals	298	99	94	7	16	28			
Frequency	0.55	0.18	0.17	0.013	0.03	0.05	0.73	0.12	0.15
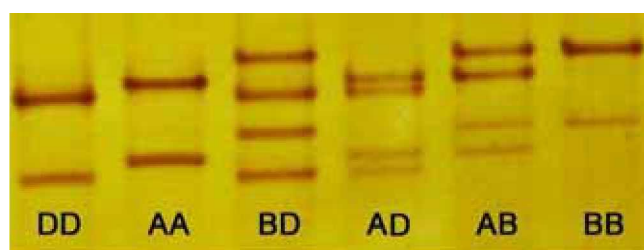
Figure 1. Genotypes of SSCP in the hircine KAP8.2 gene.

Where Y_{ijkl} is an observation of the dependent variable (cashmere fibre diameter, combed cashmere weight, cashmere fibre and guard hair length); μ is the population mean for the variable; G_i is the fixed effect of ith genotype (i = 1 (AA), 2 (AB), 3 (AD), 4 (BB), 5 (BD), 6 (DD)); Y_j is the fixed effect of jth year (j = 1, 2, ..., 8); A_k is the fixed effect of kth age (k = 1, 2, ..., 8); S_l is the random effect of l sire and e_{ijkl} is the random residual term. Treatments comparisons were made using least square means by p-values for differences options (SAS version 8.2).

The allele and genotype frequencies were calculated and Hardy-Weinberg equilibrium was tested by comparing expected and observed genotype frequencies using a Chi-square test.
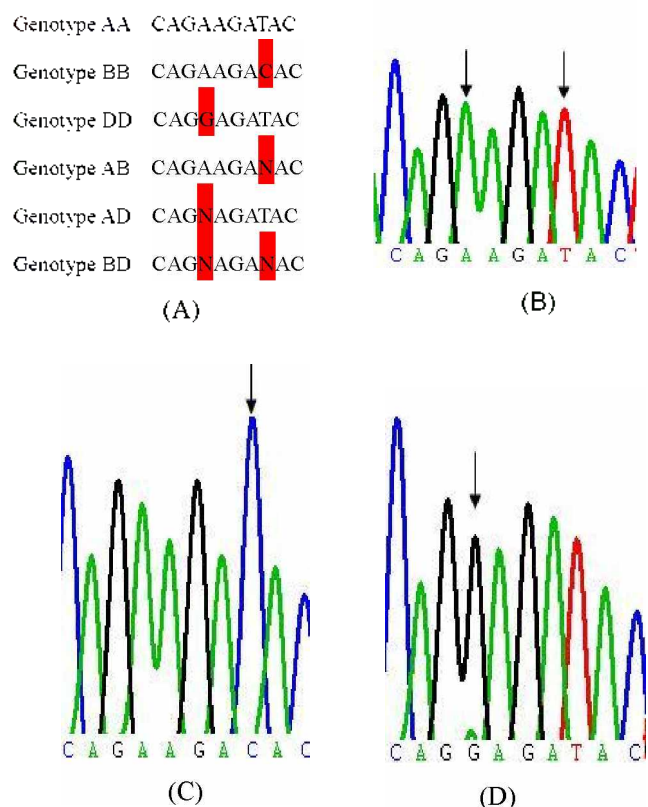
RESULTS

The analysis of polymorphism

Three fragments, amplified by PCR using the primers described in Table 1, showed the expected lengths. The polymorphisms of the PCR products in all individuals were analyzed by PCR-SSCP. Only the products of primer 2 exhibited polymorphisms. In the primer 2 fragment, six conformation patterns were identified, denominated AA, AB, AD, BB, BD and DD genotypes by analyzing the SSCP results (Figure 1).

Nucleotide sequence

The nucleotide sequence analyses of the six genotypes revealed that three different alleles, A (A-T), B (A-C) and D (G-T), which contained two unreported SNPs (Figure 2) were identified in the KAP8.2 mRNA sequence (GenBank accession no. AY510123). Using allele A as a reference, the allele B had a T→C transition at the 218th position of the nucleotide sequence. Allele D had a silent mutation (A→G) at the 214th position of the nucleotide sequence.

Figure 2. Nucleotide sequence comparison of the PCR products amplified by primer 2. (A) BLAST results of the nt sequence of the six genotypes in this study with nt sequence in GenBank; (B) nt sequence of genotype AA (arrows' positions are consistent with nt sequence in GenBank), (C) and (D) nt sequence of genotype BB and DD (the arrow pointed to the mutation site). AA genotype contains two 214A218T single strands (allele A); BB genotype contains two 214A218C single strands (allele B), DD genotype contains two 214G218T single strands (allele D); AB genotype contains one 214A218T single strand and one 214A218C single strand; AD genotype contains one 214A218T single strand and one 214G218T single strand; BD genotype contains one 214A218C single strand and one 214G218T single strand.

Association between genotypes and fibre traits

Five hundred and forty-two cashmere goats were used to estimate the frequencies of the alleles and genotypes (Table 2). The frequency of genotype AA was the highest, followed by AB, AD and DD, while BD and BB were the lowest in the population. The allele A was the most frequent allele and the allele B was the least frequent in the population.

Statistical analyses showed that the different genotypes

Table 3. Associations between the genotypes of the KAP8.2 gene and cashmere fibre traits (LSM±SE)

Genotype	Fibre diameter (μm)	Cashmere weight (g)	Cashmere length (cm)	Hair length (cm)
AA	14.74±0.13^{aA}	575.69±19.26^{aA}	5.76±0.092	16.64±0.56^{ab}
AB	15.22±0.15^{bB}	576.54±20.45^{aA}	5.75±0.097	16.19±0.60^{b}
AD	15.19±0.14^{bB}	570.40±20.31^{aA}	5.69±0.097	16.98±0.59^{a}
BB	15.28±0.32^{abAB}	733.91±40.39^{bB}	6.02±0.202	14.21±1.42^{c}
BD	15.37±0.25^{bB}	588.55±29.25^{aA}	5.81±0.135	16.03±0.92^{b}
DD	15.19±0.20^{bB}	566.73±23.70^{aA}	5.69±0.117	17.43±0.69^{a}

Means within a column with no common superscript capital letters differed significantly (p<0.01) and means within a column with no common superscript lowercases differed significantly (p<0.05).

Table 4. Associations between the genotypes of the KAP8.2 gene and cashmere fibre traits (LSM±SE)

Genotype	Fibre diameter (μm)	Cashmere weight (g)	Cashmere length (cm)	Hair length (cm)
AA	14.74±0.13^{aA}	577.09±19.48	5.75±0.095	16.60±0.56
AC	15.20±0.14^{bB}	576.72±19.57	5.72±0.096	16.56±0.56
CC	15.25±0.17^{bB}	575.43±22.25	6.01±0.112	16.90±0.65

Means within a column with no common superscript capital letters differed significantly (p<0.01) and means within a column with no common superscript lowercases differed significantly (p<0.05).

were significantly associated with cashmere fibre diameter (p<0.01), cashmere weight (p<0.01) and hair length (p<0.01) except for cashmere length (p>0.05) (Table 3). Goats with genotype AA exhibited the thinnest fibre diameter (<15 μm) when compared with those from animals with other genotypes (p<0.01). The mean difference was about 0.5 microns in fibre diameter between genotype AA and other genotypes. Genotypes with allele D or B had significantly increased fibre diameter. The cashmere goats with genotype BB had the highest cashmere weight than the others genotypes (p<0.01). The goats with genotype BB had over 145 gram more than other genotypes in cashmere weight (p<0.0001). The goats with genotype AA exhibited the longer hair length (p<0.01), while those with genotype BB had the shortest hair length (p<0.01) when compared with the other genotypes.

DISCUSSION

The KAP genes that code for the structural proteins of the wool fibre may be candidate genes with major effects on wool production variations. Wood et al. (1992) reported CA repeat variation at the KAP8 locus in sheep. It was also indicated that the allelic variation at the KAP8 locus and fibre diameter was significant associated (Parsons et al., 1994). In addition, Rogers et al. (1994) found DNA polymorphisms in the B2 high-sulfur proteins. These results suggest that the KAPs genes may be candidates for wool QTLs.

In the current study, six genotypes and three alleles were identified by SSCP in the KAP8.2 gene (Figure 1). The present results revealed that the frequency of genotype AA was the predominant in Inner Mongolia cashmere goats, up to 55%, whereas genotype BB was the least, only about 1.3%. The allele frequencies was A>D>B. The distribution of allele and genotype frequencies in our sample

significantly deviated from the Hardy-Weinberg equilibrium (x^2=26.80, p<0.01) indicating that the locus is under selection pressure. The differences between genotypes and alleles are possibly due to long-term artificial fertilization and selection towards thin fibre diameter.

The current results suggested that the polymorphisms of KAP8.2 were associated with cashmere fibre diameter, cashmere weight and hair length (Table 3). The goats with genotypes AA had over 3.1% thinner fibre diameter (<15 μm) than those of other genotypes. The animals with genotype BB had the thicker fibre diameter in the population, higher by 0.54 μm than genotype AA. However, this difference did not reach significance level (p=0.07) which might be caused by the population containing less homozygous mutation BB. The goats with genotype BB had distinctly largest cashmere weight and significantly shortest hair length in the population. This implies that the hircine KAP8.2 gene could be a potential candidate gene affecting fibre quality and performance. Further studies in larger populations and other breeds are needed to confirm this result.

The animals with genotype AA had the thinnest fibre diameter and lower cashmere weight; whereas, the animals with genotype BB had the highest cashmere weight and the thicker fibre diameter. Therefore, being of genotype AA might have an important effect on cashmere quality while genotype BB mainly affected production. Fibre diameter and fibre quantity are very highly and positively correlated, and genetic correlations between fibre diameter and fibre weight range from 0.62 to 0.83 (Pattie and Restall, 1989; Bigham et al., 1993; Bishop and Russel, 1996). In Inner Mongolia, cashmere phenotype, once the individual was high in cashmere production, was usually thick in fibre diameter and short in hair length. There existed a few individuals which had high cashmere production, thick cashmere fibre diameter and short hair length in the Inner

Mongolia cashmere population. In the present study, the goats with different genotypes followed this rule. These genetic correlations may indicate that multiple genes, and complex and subtle interactions between the genes, are responsible for fleece phenotype (Adelson et al., 2002).

Since the small number of observations on the genotype BB, BD and DD might not yield convincing statistical inference, we attempted to combine the allele B and D and analysed the association between genotypes and cashmere traits. The statistical results are given in Table 4 and suggested that the genotype AA was significantly associated with cashmere fibre diameter, which is consistent with the results in Table 3. Therefore, the genotype AA may be the most advantageous genotype for cashmere fibre diameter. Inner Mongolia cashmere goat is famous for its fine cashmere fibre diameter; in long-term artificial fertilization and selection the excellent trait is always primarily preserved.

The sequencing results showed that two unreported mutations, A214G and T218C were identified in the KAP8.2 gene. A214G did not change the amino acid, while T218C led to an amino acid change from the neutral amino acid tyrosine to the basic amino acid histidine at residue 57. These two mutations at different positions of the nucleotide sequence of a gene significantly associated with cashmere fibre diameter, indicated that they could be important functional sites for KAP8.2 and further studies are needed to investigate them. Investigations are required on the gene function at the cellular level and its interactions with other candidate genes.

CONCLUSIONS

Three alleles and six genotypes of the KAP8.2 gene were identified in Inner Mongolia cashmere goats. The study showed that the KAP8.2 gene was strongly associated with cashmere fibre diameter, cashmere weight and hair length. The genotype AA was found to be predominant in the population and the animals with this genotype had the thinnest cashmere fibre diameter, indicating that the polymorphisms in the hircine KAP8.2 gene may be a potential molecular marker for cashmere fibre diameter in cashmere goats.

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