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Correlation analysis of four KRTAP gene polymorphisms and cashmere fiber diameters in two cashmere goat breeds

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Abstract

Fiber diameter, a quantitative trait, is controlled by minor effect polygenes. Keratin-associated proteins (KRTAPs) are an important part of hair, and their rich polymorphisms facilitate the mining of cashmere trait molecular markers. In this study, Jiangnan and Tibetan cashmere goats were taken as the research object; multiplex PCR and exome sequencing technology were used to identify the exon regional polymorphisms of cashmere goats KRTAP15-1, KRTAP13.1, KRTAP27-1, and KRTAP24-1. The effects of mutation sites on the fiber diameter of cashmere were analyzed by least square method. The results showed that there were 28 mutation sites in the four KRTAP genes in Jiangnan cashmere goats and Tibetan cashmere goat populations. Among them, the KRTAP13.1, KRTAP27-1, and KRTAP24-1 gene polymorphisms were found to be significantly related to the fiber diameter of Jiangnan cashmere goats. The exploration of molecular markers in this study will help to improve the fiber diameter of the down, while the identification of gene polymorphisms will provide original data for the utilization and protection of germplasm resources of cashmere goats.

Key words: Jiangnan cashmere goat, Tibetan cashmere goat, cashmere fiber diameter, KRTAPs

Résumé

Le diamètre de la fibre, une caractéristique quantitative, est contrôlé par des polygènes à effet mineur. Les protéines associées à la kératine (KRTAPs — « keratin-associated proteins ») sont parties importantes des poils, et leurs polymorphismes riches facilitent le minage des marqueurs moléculaires des caractéristiques du cachemire. Dans cette étude, les chèvres à cachemire de Jiangnan et du Tibet ont été utilisées comme objets de recherche. La PCR multiplexe et la technologie de séquençage d'exons ont été utilisées afin de déterminer les polymorphismes régionaux des exons des chèvres à cachemire KRTAP15-1, KRTAP13.1, KRTAP27-1, et KRTAP24-1. Les effets des sites de mutation sur le diamètre de la fibre du cachemire ont été analysés par la méthode des moindres carrés. Les résultats ont montré qu'il y avait 28 sites de mutation dans les quatre gènes KRTAP dans les populations de chèvres à cachemire de Jiangnan et du Tibet. Parmi ceux-ci, les polymorphismes de gènes KRTAP13.1, KRTAP27-1, et KRTAP24-1 se sont avérés reliés de façon significative au diamètre de la fibre des chèvres à cachemire de Jiangnan. L'exploration des marqueurs moléculaires dans cette étude aidera à améliorer le diamètre de la fibre du duvet, tandis que l'identification des polymorphismes des gènes offrira des données originales pour l'utilisation et la protection des ressources du patrimoine génétique des chèvres à cachemire. [Traduit par la Rédaction]

Mots-clés : chèvre à cachemire de Jiangnan, chèvre à cachemire du Tibet, diamètre de la fibre de cachemire, KRTAPs

Introduction

Jiangnan cashmere goat is a white cashmere goat breed cultivated in China, and its main area of production is Aksu, Xinjiang. Xinjiang Cashmere Goat Core Breeding Base (Akesu Comprehensive Experimental Station) is located in arid desert and semidesert grassland at an altitude of 2000 m. The annual average temperature is 6.5 °C, the rainfall is 200 mm, 90% of the grassland is desert grassland, and the vegetation coverage rate is about 10%–30% (Qin et al. 2016). Tibetan cashmere goats are a local goat breed that has been retained after long-term natural and artificial selection and adapted to the special environment of high-altitude areas. They are also known as Tibetan Kashmir cashmere goats (Ci 2012; Fu 2021).

In recent years, with the increase in demand in the mutton market and the fall in grassroots breeding work, there has been a loss of original fine cashmere traits in both Jiangnan cashmere and Tibetan cashmere goats, seriously affecting the quality of cashmere and the sales price of cashmere (Tonin et al. 2002). Cashmere goats are the main source of income for local herdsmen. The breeding and popularization of high-quality cashmere goats has become an important means for these people to rise above poverty and become rich based on local resources. It is thus necessary for local people to breed ultrafine cashmere goats that are suitable for the extreme climates of Xinjiang and Tibet. Therefore, it is particularly important to find an effective and convenient breeding method which can accelerate the breeding process of cashmere goats in a short period of time. The classical breeding method mainly employs crossing, but this method has a slower genetic progress and a longer breeding cycle. With the rapid development of molecular biology theory and technology, molecular genetics and genetic engineering methods combined with traditional marker-assisted selection methods can provide better solutions to breeding problems (Niu and Hong-Bin 2008; Yang et al. 2017). The search for molecular markers related to cashmere traits is our primary focus (Hailemariam and Yadeta 2020).

In the hair cortex, hair keratins are embedded in an interfilamentous matrix consisting of keratin-associated proteins (KRTAPs), which are important for the formation of hair shaft as a result of disulphide bonds between cysteine residues. The genes encoding KRTAP are usually rich in polymorphism, which may be related to the cashmere traits of cashmere goats. At present, the polymorphisms of KRTAP15-1 (Wang et al. 2017*b*), KRTAP13.1 (Wu et al. 2018), KRTAP27-1 (Zhao et al. 2020), and KRTAP24-1 (Zhou et al. 2012) have been confirmed to affect fiber traits in some sheep and goat breeds, including Xinji fine-wool sheep, Merino × Southdown-cross sheep, Longdong cashmere goats, and Xinjiang local goat. However, it is unclear whether these genes can affect the fiber diameter of Jiangnan and Tibetan cashmere goats. Therefore, this study analyzed the effects of four gene polymorphism (KRTAP15-1, KRTAP13.1, KRTAP27-1, and KRTAP24-1) on cashmere fiber diameter of Jiangnan and Tibetan cashmere goats. The aim of this study was to obtain the molecular markers of cashmere fineness of cashmere goats, which would lay the foundation for the protection, development, and utilization of cashmere genetic resources, and provide theoretical basis for cashmere molecular breeding.

Materials and methods

Animal care

All animal experiments were strictly performed according to the guidelines established by the Animal Care and Use Committee of Xinjiang Academy of Animal Science (Approval number 2019009). Sample collection was carried out under license in accordance with the Guidelines for Care and Use of Laboratory Animals of China.

Experimental animals

A total of 353 two-year-old female Jiangnan cashmere goats were selected from the breeding center of Wenshu County, Aksu Prefecture, Xinjiang Province. They were from the Aerken group ($n = 144$), Samusak group ($n = 79$), Tuniazi group ($n = 54$), and Yiming group ($n = 79$). A total of 299 two-year-old female Tibetan cashmere goats were selected from the original breed of cashmere goats in Ngari Prefecture (Ritu County, $n = 132$; Gaize County, $n = 113$) and Nagqu Prefecture (Nima County, $n = 57$). Before the experiment, all cashmere goats are healthy and raised by grazing.

Sample collection

Cashmere samples were collected from Jiangnan and Tibetan cashmere goats at the 10 cm posterior margin of the scapula above the left midline of the body. Cashmere is naturally dried after washing according to its conventional washing process. A fiber diameter optical analyzer (OFDA2000) was used to determine the cashmere mean fiber diameter (MFD), fiber diameter standard deviation (FDSD), and coefficient of variation of fiber diameter (CVFD) under the conditions of a constant temperature of 20 ± 2 °C and a humidity of $65\% \pm 4\%$.

Corresponding to the cashmere sample, we collected 5 mL of experimental goat blood in an anticoagulation tube and stored it in a refrigerator at −20 °C. A blood genomic DNA extraction kit (TIANGENG, USA) was used to extract DNA from cashmere goats. The quality and concentration of DNA were determined using 1.0% agarose gel electrophoresis and the Qubit 2.0 (Thermo, USA).

Multiple PCR and exome sequencing

According to the sequence published in the NCBI goat chromosome 1 accession number (NC_030808.1), the exon regions of KRTAP15-1, KRTAP13.1, KRTAP27-1, and KRTAP24-1 were selected. A Primer pool containing four exon regions of target genes was designed using Primer 5.0 and synthesized by Shanghai Sangong. The list of primers is shown in Table S1. Then, the target SNP sequences of 353 Jiangnna cashmere goats and 299 Tibetan cashmere goats were amplified by two-step PCR and Illumina sequencing library was prepared. The two-step PCR system is shown in Tables S2 and S4, and the reaction procedure is shown in Tables S3 and S5. The final PCR product was purified and recovered using AMPure XP magnetic beads. Each PCR product was equally mixed and se-

quenced using a Hiseq XTEN sequencer (Illumina, San Diego, CA, US).

Sequencing data analysis and validation

Raw reads were filtered according to two steps: (*i*) removing any adaptor sequence the reads contained using cutadapt (v 1.2.1); (*ii*) removing low-quality bases from reads 3′ to 5′ ($Q < 20$) using PRINSEQ-lite (v 0.20.3). Additionally, the remaining clean data were mapped to the reference genome by BWA (version 0.7.13-r1126) with default parameters. Samtools (Version: 0.1.18) was used to calculate each genotype of the target site. Annovar was used to detect genetic variants.

The heterozygous individuals with four mutation sites selected for each gene were verified using first-generation sequencing technology. The first-generation sequencing results were assembled and corrected using the SeqMan program of the DNASTAR software, and the peak maps were compared with the BioEdit software.

Statistical analysis

The Popgene software was used to calculate the minor allele frequency (MAF) and Hardy–Weinberg equilibrium (HWE) of SNPs. The Linkage Format function of the Haploview software was used to analyze the linkage disequilibrium of SNPs. The GLM model in the SAS 9.2 software was used to analyze the influence of different SNPs genotypes on the cashmere fiber diameter. The results are expressed in the form of least squares mean (±standard error), and the linear model is

$$Y_{\mathrm{ick}} = \mu + G_{\mathrm{i}} + F_{\mathrm{c}} + e_{\mathrm{ick}}$$

In the formula, Y_{ick} denotes the individual phenotypic value of cashmere goats, μ the population mean, G_i the genotype SNP effect, F_c the group effect, and e_{ick} denotes the random error.

Results

Descriptive statistics of fiber diameter traits

A descriptive statistical analysis of the MFD, FDSD, and CVFD of Jiangnan cashmere goats and Tibet cashmere goats was performed. The basic statistics are shown in Table 1. The MFD, FDSD, and CVFD of Jiangnan cashmere goats were 15.69 μm, 3.26 μm, and 20.86%, respectively. The MFD, FDSD, and CVFD of Tibetan cashmere goats were 15.10 μm, 3.25 μm, and 21.53%, respectively. It is not difficult to see that, compared with Jiangnan cashmere goats, Tibetan cashmere goats have a finer MFD and a higher CVFD.

Quality control of sequencing data

The genomic DNA of cashmere goats was detected using 1% agarose gel electrophoresis, and the DNA bands were bright, as shown in Fig. S1. A nucleic acid protein detector was used to detect DNA, and the OD ratio at 260–280 nm was between 1.8 and 2.1, indicating that the quality and purity of DNA meet the requirements for subsequent library construction. Quality control was performed on the data after the HiSeq XTen sequencer was sequenced. The average coverage ratio (Coverage) of each fragment and the target area sequence comparison was 96.09%; the average coverage depth (Mean_depth) was 4838.75; and the sequencing error percentage (Error_ratio) was 4.52%. It can be seen that the quality of the sequencing data is relatively high, which can satisfy the follow-up experiment. In addition, our sequencing results were submitted to the NCBI public database (PRJNA738549).

Classification results annotation and verification

Combined with multiplex PCR technology and exome sequencing, a total of 28 mutation sites were obtained in the four genes of Jiangnan cashmere goats and Tibet cashmere goats, including 19 missense mutations and 9 synonymous mutations (Table 2). KRTAP15-1, KRTAP13.1, KRTAP27, and KRTAP24 genes had 5, 10, 7, and 6 mutation sites, respectively. It is worth noting that the SNP12 and SNP18 mutations were missing in the Jiangnan cashmere goat population. There was no SNP17 mutation in the Tibetan cashmere goat population. Heterozygous individuals with mutation sites were randomly selected for first-generation sequencing; the results are shown in Fig. 1. It can be seen from the figure that the high-throughput typing results are consistent with the first-generation sequencing results, indicating that the exome sequencing results are reliable.

Statistical analysis of SNP

The results of the genotyping and MAF and HWE analyses of 28 mutation sites in two cashmere goat populations are shown in Table 3. The MAFs of SNP11, SNP12, SNP16, SNP17, SNP18, SNP24, and SNP26 in the Jiangnan cashmere goat population were all less than 0.03. The MAFs of SNP11, SNP17, SNP18, SNP24, and SNP26 in the Tibetan cashmere goat population were all less than 0.03. SNP12 and SNP18 were not classified in Jiangnan cashmere goats, while SNP17 was not classified in Tibetan cashmere goats. Excluding SNP7, the remaining 27 SNPs complied with the Hardy–Weinberg equilibrium ($P > 0.05$).

Linkage disequilibrium analysis

We used the Haploview software to analyze the linkage disequilibrium of 28 SNP loci. When the MAF > 0.03 and HWE > 0.05, the linkage disequilibrium analysis of 21 SNPs was as shown in Fig. 2. The results indicate that we detected the existence of five blocks. SNP1, SNP2, SNP3, and SNP4 constitute block 1; SNP5, SNP6, SNP8, and SNP10 constitute block 2; SNP13 and SNP14 constitute block 3; SNP19, SNP20, SNP21, and SNP22 constitute block 4; and SNP23, SNP25, SNP27, and SNP28 constitute block 5.

The Tag SNP can be predicted using the linkage disequilibrium relationship between SNPs. The predicted Tag SNP can cover all SNP sites. When the R2 value >0.8, the 12 Tag SNPs are as listed in Table 4. The two Tag SNPs predicted by the KRTAP15-1 gene were SNP2 and SNP5. The KRTAP13.1 gene has four Tag SNPs, which are SNP6, SNP9, SNP10, and SNP14. The KRTAP27-1 gene has five Tag SNPs—namely, SNP16,

Table 1. Descriptive statistics on fiber diameter traits of two Chinese cashmere goat breeds.

	Mean		SD		Range	
Traits	JN	TT	JN	TT	JN	TT
MFD (μm)	15.69	15.10	1.19	1.02	11.70–19.40	12.50–18.50
FDSD (μm)	3.26	3.25	0.25	0.28	2.40–4.00	2.50–4.10
CVFD (%)	20.86	21.53	1.44	1.45	16.90–25.20	16.80–25.60

Note: JN, Jiangnan cashmere goat; TT, Tibetan cashmere goat.

Table 2. Nucleotide variant information of candidate genes.

Genes	SNPs	Location (bp)	Nucleotide variant	Amino acids variant
KRTAP15-1	SNP1	3853276	G → A	S
	SNP2	3853292	T → C	N-S
	SNP3	3853370	G → A	S-F
	SNP4	3853386	A → G	S-P
	SNP5	3853482	T → C	T-A
KRTAP13.1	SNP6	3908845	C → G	C-S
	SNP7	3908888	C → T	G-R
	SNP8	3908898	G → C	R
	SNP9	3909003	T → C	T
	SNP10	3909033	C → T	R
	SNP11	3909103	G → C	T-S
	SNP12	3909151	C → T	C-Y
	SNP13	3909160	G → A	T-I
	SNP14	3909162	G → C	S-R
	SNP15	3909169	A → G	V-A
KRTAP27-1	SNP16	3968769	C → T	R-W
	SNP17	3968770	G→A	R-Q
	SNP18	3968790	G → A	V-I
	SNP19	3968799	G → A	D-N
	SNP20	3968858	C → T	V
	SNP21	3969019	C → T	A-V
	SNP22	3969101	C → T	S
KRTAP24-1	SNP23	4034036	C → T	N
	SNP24	4034039	G → T	S
	SNP25	4034085	T → C	C-R
	SNP26	4034100	G → A	V-I
	SNP27	4034103	G → A	G-R
	SNP28	4034201	C → T	L

SNP19, SNP20, SNP21, and SNP22. The KRTAP24-1 gene had only one Tag SNP, which was SNP28.

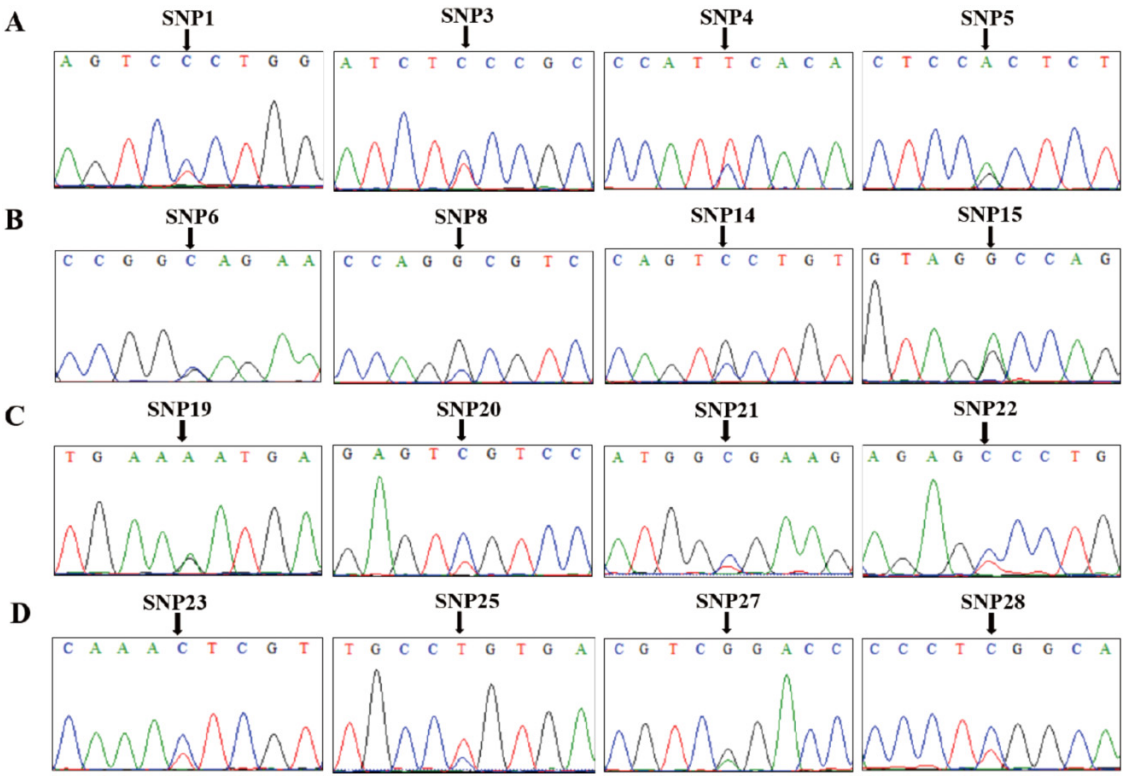
SNP effect analysis

The correlation analysis between Tag SNP and the diameter of cashmere fiber using the SAS 9.2 software is shown in Table 5. In Jiangnan cashmere goats, Tag SNP6, Tag SNP19, and Tag SNP22 significantly affected the MFD ($P < 0.01$); Tag SNP9, Tag SNP14, and Tag SNP21 significantly affected the MFD ($P < 0.05$); and Tag SNP21 significantly affected the FDSD ($P < 0.05$). Tag SNP2 and Tag SNP19 significantly affected the CVFD ($P < 0.05$). In Tibetan cashmere goats, none of the mutation sites had any significant effects on MFD, FDSD, and CVFD.

Discussion

The price of cashmere is influenced by the color and fineness of cashmere. Age, feeding management, and environment can affect the fiber diameter of a cashmere goat (Zhou et al. 2003). However, in addition to these nongenetic factors, genes are the fundamental causes of the fineness of cashmere. Recently, major cashmere goat breeds in China, such as the Liaoning cashmere goat (Yu et al. 2014; Zheng et al. 2019, 2020*b*), the Inner Mongolia cashmere goat (Zheng et al. 2020*a*), and the Tibetan cashmere goat (Fu et al. 2020), have been studied in terms of their fiber diameter at the transcriptomic level. In terms of genome, we prefer the use of genome-wide association analysis (Li et al. 2017; Zheng et al. 2020) and candidate gene polymorphism analysis (Zhao et al. 2008;

Fig. 1. Validation of high-throughput sequencing results. (A) SNP1, SNP3, SNP4, and SNP5 of the KRTAP15-1 gene. (B) SNP6, SNP8, SNP14, and SNP15 of the KRTAP13.1 gene. (C) SNP19, SNP20, SNP21, and SNP22 of the KRTAP27 gene. (D) SNP23, SNP205, SNP27, and SNP28 of the KRTAP24-1 gene.



Wang et al. 2017a, 2018; Zhang et al. 2019) to explore genes and molecular markers associated with cashmere fiber diameter.

In the hair cortex, hair keratin intermediate filaments are embedded in an interfilamentous matrix, consisting of hair KRTAP, which is essential for the formation of a rigid and resistant hair shaft through extensive disulfide bond cross-linking with the abundant cysteine residues of hair keratins (Strasser et al. 2015). KRTAP13, KRTAP15, KRTAP24, and KRTAP27 are all high-sulfur keratin-associated proteins (HS-KRTAP; <30 mol% cysteine) (Gong et al. 2012, 2016). Studies have shown that a change in amino acids may cause the loss of phosphorylation sites in the process of the post-translational modification of proteins, as well as leading to changes in the net charge of proteins (Gong et al. 2011).

We identified a total of 28 mutant sites in both Jiangnan and Tibetan cashmere goats. The SNP12 and SNP18 mutations were absent in the Jiangnan cashmere goat population. At present, the SNP18 mutation site has not been reported in other goat breeds. The MAF of SNP12 and SNP18 in the Tibetan cashmere goat population was low. Similarly, the SNP17 mutation was found to be missing in the Tibetan cashmere goat population, and the MAF was also low in Jiangnan cashmere goats. On the one hand, this suggests that we need to expand our sample size in future research; on the other hand, this also reflects the diversity of species.

There are five mutation sites (SNP1-SNP5) in the KRTAP15-1 gene of both Jiangnan and Tibetan cashmere goats, among which SNP1 is a synonymous mutation and SNP2-SNP5 is a missense mutation. These five mutations were also found in Tan sheep and Hu sheep (Wang et al. 2017*b*). Among them, SNP1–SNP4 are strongly linked ($D' = 1$). The analysis of Tag SNP2 shows that it is significantly correlated with the CVFD of the Jiangnan cashmere goat population ($P > 0.05$), and the haplotype "GTGA" is the dominant genotype. Among 396 Merino × Southdown-cross sheep, the KTAP15-1 polymorphism was found to be significantly correlated with wool yield and fiber diameter standard deviation ($P < 0.05$) (Li et al. 2018). This suggests that KRTAP15-1 may have an effect on the villus traits of sheep and goats.

Recently, KRTAP 13.1 in both sheep and goats has received a lot of research. Sun et al. (2014) found that three synonymous mutations in the KRTAP13.1 gene were significantly related to wool length and wool yield on Xinji fine wool sheep ($P < 0.05$). Yu et al. (2014) used Solexa technology to find the differential expression of the KRTAP13-1 gene in the skin tissues of the fine cashmere group and the coarse cashmere group in Liaoning cashmere goats. This suggests that the expression level of KRTAP13-1 may affect the fiber diameter of cashmere goats. Li et al. (2013) found five mutation sites in the KRTAP13.1 gene in Hexi mountain cashmere goats, Huanxian cashmere goats, Liaoning cashmere goats, and Inner Mongolia cashmere goats, including the SNP11 mutation site in our research results. This has no significant effect on the fiber diameter of Liaoning cashmere goats ($P > 0.05$) but a significant effect on the level of cashmere production and

Table 3. The basic characters of 28 SNPs located in four genes.

Genes	SNPs	Genotype	JN	MAF_JN	HWE_JN	TT	MAF_TT	HWE_TT
KRTAP15-1	SNP1	GG/GA/AA	94/168/91	0.50	0.37	116/146/37	0.37	0.39
	SNP2	TT/TC/CC	94/168/91	0.50	0.37	116/146/37	0.37	0.39
	SNP3	GG/GA/AA	93/169/91	0.50	0.42	99/147/53	0.42	0.90
	SNP4	AA/AG/GG	92/169/92	0.50	0.42	99/145/55	0.43	0.88
	SNP5	TT/TC/CC	171/151/31	0.30	0.78	259/38/2	0.07	0.64
KRTAP13.1	SNP6	CC/CG/GG	220/116/17	0.21	0.73	188/100/11	0.20	0.61
	SNP7	CC/CT/TT	325/0/28	0.08	0.00	163/0/136	0.45	0.00
	SNP8	CC/GC/GG	48/160/145	0.36	0.71	77/150/72	0.49	0.95
	SNP9	TT/TC/CC	321/32/0	0.05	0.37	270/28/1	0.05	0.76
	SNP10	CC/CT/TT	259/83/11	0.15	0.18	152/121/26	0.29	0.78
	SNP11	GG/GC/CC	347/6/0	0.01	0.87	288/11/0	0.02	0.75
	SNP12	CC/CT/TT	353/0/0	0.00	-	284/15/0	0.03	0.66
	SNP13	GG/GA/AA	257/83/13	0.15	0.06	132/138/29	0.33	0.41
	SNP14	CC/GC/GG	52/161/140	0.38	0.61	108/143/48	0.40	0.95
	SNP15	AA/AG/GG	139/162/52	0.38	0.67	35/154/110	0.37	0.09
KRTAP27-1	SNP16	CC/CT/TT	338/15/0	0.02	0.68	268/30/1	0.05	0.87
	SNP17	GG/GA/AA	341/12/0	0.02	0.75	299/0/0	0.00	-
	SNP18	GG/GA/AA	353/0/0	0.00	-	286/13/0	0.02	0.70
	SNP19	GG/GA/AA	124/174/55	0.40	0.64	179/110/10	0.22	0.16
	SNP20	CC/CT/TT	233/110/10	0.18	0.49	115/145/39	0.37	0.52
	SNP21	CC/CT/TT	326/25/2	0.04	0.06	252/44/3	0.08	0.49
	SNP22	CC/CT/TT	171/157/25	0.29	0.17	281/18/0	0.03	0.59
KRTAP24-1	SNP23	CC/CT/TT	98/168/87	0.48	0.37	65/160/74	0.52	0.22
	SNP24	GG/GT/TT	342/11/0	0.02	0.77	296/3/0	0.01	0.93
	SNP25	TT/TC/CC	110/170/73	0.45	0.62	65/160/74	0.52	0.22
	SNP26	GG/GA/AA	342/11/0	0.02	0.77	296/3/0	0.01	0.93
	SNP27	GG/GA/AA	97/169/87	0.49	0.43	65/160/74	0.52	0.22
	SNP28	CC/CT/TT	97/169/87	0.49	0.43	65/160/74	0.52	0.22

Note: HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency.

body weight after fleecing ($P < 0.05$). Fang et al. (2010) used PCR-RFLP technology to study the correlation between the KRTAP13.1 gene and the cashmere traits of Jiangnan goats, and the results showed that there was no significant difference in cashmere fineness between the different genotypes ($P > 0.05$). Similarly, Shanaz et al. (2020) used PCR-RFLP technology and found that the KRTAP13.1 gene polymorphism had no significant effect on the cashmere traits of Changthangi goats ($P > 0.05$). The above two loci are not present in our research results, which indicates the rich polymorphism of the KRTAP13.1 gene. Wu et al. (2018) used the PCR-SSCP method to find that the polymorphism of the KRTAP13.1 gene was significantly correlated with the cashmere yield and hair length of Tibetan cashmere goats.

We found 10 mutation sites (SNP6-SNP15) in the KRTAP13.1 gene, among which SNP8-SNP10 was synonymous and strongly linked ($D' = 1$), while SNP6, SNP7, and SNP11–SNP15 were missense mutations. These mutation sites were not annotated by the ensemble database (ENSCHIT00000017674.1), including SNP7, SNP8, and SNP11-15. It is worth noting that SNP7 has no heterozygous genotype in Jiangnan cashmere goat and Tibetan cashmere goat populations; the only two genotypes are CC and TT. And MAF of SNP11 in Jiangnan and Tibetan cashmere goats was less than 0.03; there was no correlation analysis between SNP11 and cashmere fiber diameter.

KRTAP15-1 and KRTAP24-1, like most KRTAP genes, do not contain introns. However, KRTAP13.1 and KRTAP27-1 have an intron. KRTAP27-1 is mainly expressed in skin tissues and is slightly or not expressed in longissimus dorsi muscle, heart, kidney, liver, lung, and spleen tissues (Zhao et al. 2020). This suggests that KRTAP27-1 may play a unique role in the skin. We found seven mutation sites on KRTAP27-1, of which SNP20 and SNP22 were synonymous mutations, while SNP16–SNP19 and SNP21 were missense mutations. Interestingly, we found a novel mutation site SNP18 in Tibetan cashmere goats. This suggests that the genetic resources of Tibetan cashmere goats have abundant genetic resources. Zhao et al. (2020) found SNP21 and SNP22 sites on Longdong cashmere goats, among which the SNP21 site significantly affected the fiber diameter of Longdong cashmere goats, which was consistent with the research results found for Jiangnan cashmere goats. This indicates that the KTAP27-1 polymorphism has a significant effect on the cashmere fiber diameter of cashmere goats.

We found six mutation sites (SNP23-SNP28) in the 200 bp fragment within the exon of KRTAP24-1 gene, of which SNP23, SNP24, and SNP28 are synonymous mutations and

Fig. 2. Blocks found in the linkage disequilibrium (LD) analysis of SNPs. The block with a black border is a completely linked haplotype block. The color of each block in the picture changes from blue to red, indicating that the degree of linkage is becoming higher and higher. Each square number indicates the D' value.

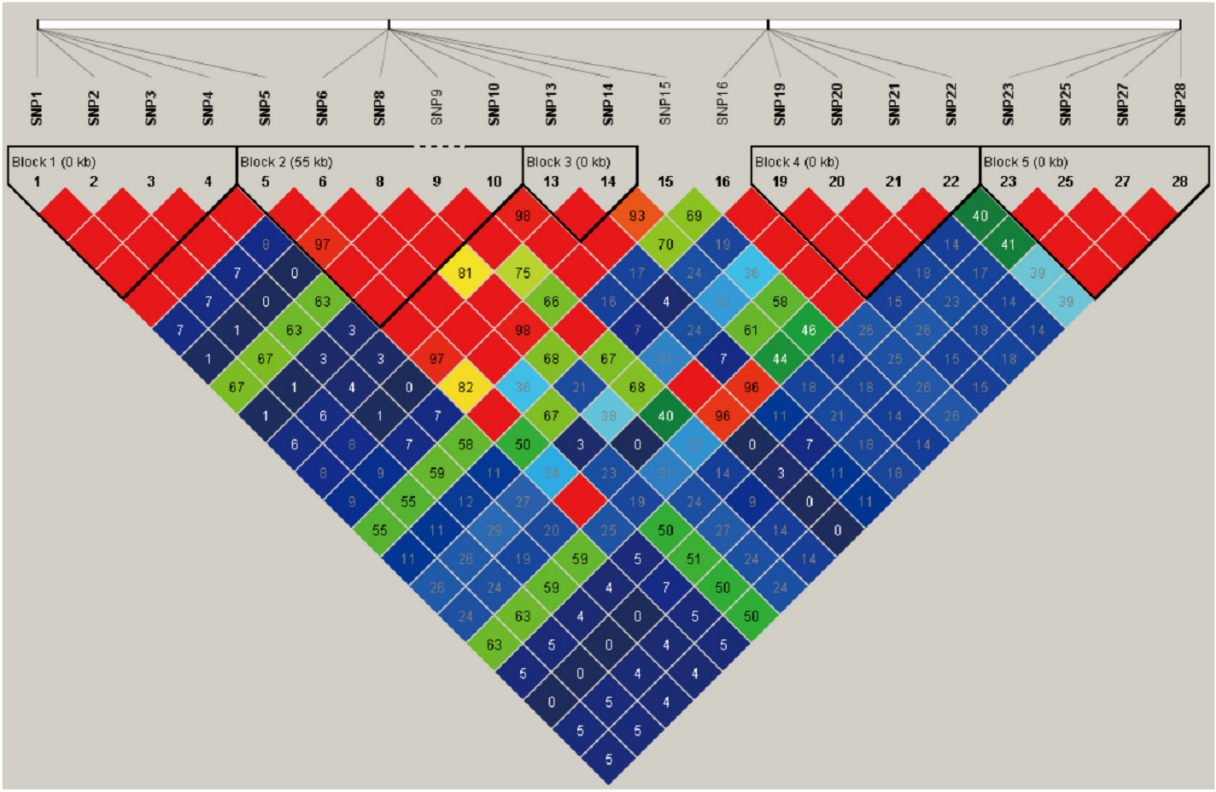


Table 4. Summary of predictive tag SNP information.

Gene	Tag SNP	Alleles captured	Genotype
KRTAP15-1	SNP2	SNP1, SNP2, SNP3, SNP4	TT/TC/CC
	SNP5	SNP5	TT/TC/CC
KRTAP13.1	SNP6	SNP6	CC/CG/GG
	SNP9	SNP9	TT/TC/CC
	SNP10	SNP10, SNP13	CC/CT/TT
	SNP14	SNP8, SNP14, SNP15	CC/GC/GG
KRTAP27-1	SNP16	SNP16	CC/CT/TT
	SNP19	SNP19	GG/GA/AA
	SNP20	SNP20	CC/CT/TT
	SNP21	SNP21	CC/CT/TT
	SNP22	SNP22	CC/CT/TT
KRTAP24-1	SNP28	SNP23, SNP25, SNP27, SNP28	CC/CT/TT

SNP25–SNP27 are missense mutations. Zhou et al. (2012) found four missense mutations in New Zealand Romney-cross sheep. Sun et al. (2016) found that two missense mutations in the KRTAP24-1 gene had a significant effect on the wool yield and hair length of Xinji fine wool sheep, but no significant effect on the fiber diameter. Comparing with our results, we found that these mutations in sheep do not exist in Jiangnan and Tibetan cashmere goats. Wang et al. (2019) found eight SNPs in the coding region of the KTAP24-1 gene of Longdong cashmere goats, which contained six mutation sites (SNP23-SNP28) that we found in Jiangnan and Tibetan cashmere goats. Similar to our results, SNP24 and SNP26 had a lower MAF in Longdong cashmere goats. Meanwhile, the combination of SNP23, SNP25, SNP27, and SNP28 in Longdong cashmere goats forms two unique band types using PCR-SSCP technology. This is consistent with our results of a strong linkage between SNP23, SNP25, SNP27, and SNP28.

According to linkage disequilibrium analysis, we selected a small number of SNPs from the set of SNP loci to represent the overall SNP. The purpose of this was to minimize the number of genotypes, thereby greatly reducing the number of SNPs used for further association studies, which will be helpful in accelerating the mining of functional genes (Wang et al. 2017*c*). Our results show that Tag SNP9, Tag SNP14, Tag

Table 5. Correlation analysis between mutation sites and cashmere fiber diameter.

Tag SNPs	Genotype	MFD_JN (μm)	FDSD _JN (μm)	CVFD_JN (%)	MFD_TT (μm)	FDSD _TT (μm)	CVFD _TT (%)
Tag SNP2	TT	15.72 ± 0.14	3.29 ± 0.03	20.98 ± 0.15^{ab}	14.98 ± 0.09	3.21 ± 0.03	21.50 ± 0.0.14
	TC	15.80 ± 0.10	3.25 ± 0.02	20.62 ± 0.11^{a}	15.09 ± 0.08	3.23 ± 0.02	21.46 ± 0.12
	CC	15.54 ± 0.13	3.26 ± 0.03	21.01 ± 0.15^{b}	15.14 ± 0.15	3.28 ± 0.04	21.63 ± 0.24
Tag SNP5	TT	15.58 ± 0.09	3.25 ± 0.02	20.92 ± 0.11	15.07 ± 0.06	3.23 ± 0.02	21.46 ± 0.10
	TC	15.84 ± 0.10	3.27 ± 0.02	20.71 ± 0.12	14.98 ± 0.15	3.23 ± 0.04	21.68 ± 0.24
	CC	15.84 ± 0.22	3.28 ± 0.05	20.80 ± 0.26	14.56 ± 0.66	3.19 ± 0.19	22.07 ± 1.04
Tag SNP6	CC	15.85 ± 0.08 A	3.27 ± 0.02	20.73 ± 0.10	15.07 ± 0.07	3.25 ± 0.02	21.58 ± 0.11
	CG	15.46 ± 0.11^{B}	3.23 ± 0.02	20.95 ± 0.13	15.01 ± 0.09	3.21 ± 0.03	21.42 ± 0.15
	GG	15.77 ± 0.29^{AB}	3.33 ± 0.06	21.15 ± 0.35	15.12 ± 0.28	3.16 ± 0.08	20.96 ± 0.44
Tag SNP9	TT	15.76 ± 0.07^{a}	3.27 ± 0.01	20.78 ± 0.08	15.07 ± 0.06	3.23 ± 0.02	21.48 ± 0.09
	TC	15.23 ± 0.21^{b}	3.22 ± 0.04	21.20 ± 0.26	14.87 ± 0.18	3.20 ± 0.05	21.58 ± 0.28
	CC	–	–	–	15.43 ± 0.93	3.68 ± 0.27	24.00 ± 1.45
Tag SNP10	CC	15.70 ± 0.08	3.26 ± 0.02	20.85 ± 0.09	15.01 ± 0.08	3.22 ± 0.02	21.53 ± 0.12
	CT	15.67 ± 0.13	3.25 ± 0.03	20.78 ± 0.16	15.07 ± 0.09	3.23 ± 0.03	21.42 ± 0.14
	TT	16.16 ± 0.36	3.30 ± 0.08	20.57 ± 0.43	15.32 ± 0.19	3.30 ± 0.05	21.65 ± 0.29
Tag SNP14	CC	15.71 ± 0.17^{ab}	3.27 ± 0.04	20.88 ± 0.20	15.16 ± 0.09	3.23 ± 0.03	21.34 ± 0.15
	GC	15.57 ± 0.10^{a}	3.25 ± 0.02	20.92 ± 0.12	14.94 ± 0.08	3.23 ± 0.02	21.60 ± 0.13
	GG	15.89 ± 0.10^{b}	3.28 ± 0.02	20.69 ± 0.13	15.16 ± 0.13	3.25 ± 0.04	21.53 ± 0.21
Tag SNP16	CC	–	–	–	15.04 ± 0.06	3.23 ± 0.02	21.52 ± 0.09
	CT	–	–	–	15.21 ± 0.17	3.22 ± 0.05	21.23 ± 0.27
	TT	–	–	–	15.24 ± 0.93	3.48 ± 0.27	22.58 ± 1.45
Tag SNP19	GG	15.66 ± 0.11^{AB}	3.27 ± 0.02	20.91 ± 0.13^{a}	15.07 ± 0.07	3.23 ± 0.02	21.45 ± 0.11
	GA	15.61 ± 0.09^{B}	3.25 ± 0.02	20.87 ± 0.11^{ab}	14.99 ± 0.09	3.23 ± 0.03	21.58 ± 0.14
	AA	16.16 ± 0.16 A	3.28 ± 0.03	20.44 ± 0.20^{b}	15.43 ± 0.29	3.29 ± 0.09	21.43 ± 0.46
Tag SNP20	CC	15.71 ± 0.08	3.25 ± 0.12	20.77 ± 0.10	15.08 ± 0.09	3.25 ± 0.03	21.59 ± 0.14
	CT	15.65 ± 0.12	3.27 ± 0.02	20.95 ± 0.14	15.03 ± 0.08	3.23 ± 0.02	21.49 ± 0.12
	TT	16.22 ± 0.38	3.33 ± 0.08	20.68 ± 0.46	15.08 ± 0.15	3.19 ± 0.04	21.25 ± 0.24
Tag SNP21	CC	15.74 ± 0.07^{a}	3.27 ± 0.01^{a}	20.82 ± 0.08	15.06 ± 0.06	3.23 ± 0.02	21.48 ± 0.10
	CT	15.20 ± 0.25^{b}	3.15 ± 0.05^{b}	20.83 ± 0.30	15.04 ± 0.14	3.24 ± 0.04	21.49 ± 0.22
	TT	16.17 ± 0.84^{ab}	3.60 ± 0.18^{a}	22.24 ± 1.02	14.47 ± 0.54	3.38 ± 0.16	23.42 ± 0.84
Tag SNP22	CC	15.89 ± 0.09 A	3.28 ± 0.02	20.70 ± 0.11	15.05 ± 0.06	3.23 ± 0.02	21.49 ± 0.09
	CT	15.52 ± 0.10^{B}	3.24 ± 0.02	20.92 ± 0.12	15.07 ± 0.22	3.27 ± 0.07	21.65 ± 0.35
	TT	15.67 ± 0.24^{AB}	3.29 ± 0.05	21.00 ± 0.29	–	–	–
Tag SNP28	CC	15.94 ± 0.12^{a}	3.28 ± 0.02	20.64 ± 0.15	15.21 ± 0.12	3.25 ± 0.03	21.38 ± 0.19
	CT	15.58 ± 0.09^{b}	3.25 ± 0.02	20.93 ± 0.11	14.96 ± 0.08	3.22 ± 0.02	21.59 ± 0.12
	TT	15.71 ± 0.13^{ab}	3.26 ± 0.03	20.81 ± 0.16	15.12 ± 0.11	3.24 ± 0.03	21.40 ± 0.17

Note: Data in the same column with different capital letters on the shoulder indicate very significantly different ($P < 0.01$), while different lowercase letters indicate significant differences ($P < 0.05$). The same letters on the shoulder indicate no significant difference ($P > 0.05$).

SNP19, Tag SNP22, and Tag SNP28 significantly or extremely significantly affect the average fiber diameter of cashmere in the Jiangnan cashmere goat population. However, we did not find any mutation sites significantly related to cashmere fiber diameter in Tibetan cashmere goats. However, it is not difficult to see that the trend of cashmere fiber diameter of Tibetan cashmere goats and Jiangnan cashmere goats in different genotypes of significant mutation sites is roughly the same. This indicates that these Tag SNPs have a certain influence on the fiber diameter. On the other hand, this also explains the genetic differences between the two breeds.

Conclusions

The genetic polymorphisms related to fiber diameter in Jiangnan and Tibetan cashmere goats were tentatively explored in this study. These molecular markers can provide a theoretical scientific basis for the improvement of cashmere goat breeds. Our sequencing results were submitted to the NCBI public database (PRJNA738549). The accumulation of original genome data is helpful for research on the germplasm characteristics of local cashmere goats and the protection and utilization of resources.

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Data availability

Data generated or analyzed during this study are available in the Sequence Read Archive (SRA) repository (PRJNA738549, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA738549.)

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Competing interests

The authors declare there are no competing interests.

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjas-2021-0112

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