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Short Communication

SHORT COMMUNICATION

A highly polymorphic caprine keratin-associated protein gene identified and its effect on cashmere traits

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Abstract

Five keratin-associated protein 6 genes (*KRTAP6*) have been identified in sheep and variation in some *KRTAP6* has been associated with wool fiber diameter-related traits, but none of these homologues have been identified in goats. In this study, we reported the identification of the sheep *KRTAP6-5* homologue on goat chromosome 1 and polymerase chain reaction (PCR)-single strand conformation polymorphism analysis in 300 Longdong cashmere goats revealed the existence of 12 variant sequences. Both coding region and 3′UTR of the putative caprine *KRTAP6-5* displayed a biggest sequence similarity to ovine *KRTAP6-5* gene. This suggested that the gene represents caprine *KRTAP6-5* sequences, and these sequences composed 23 genotypes, which was the most polymorphism gene in *KRTAPs* that have been studied. Among these sequences, 15 nucleotide substitutions and a 24-bp insertion/detection were identified. Variation in goat *KRTAP6-5* was associated with variation in mean-fiber diameter, suggesting that *KRTAP6-5* is worthy of further study in the context of variation in cashmere traits.

Key words: goat, keratin-associated protein KAP6-5 gene, mean-fiber diameter, PCR-SSCP, variation

Introduction

Cashmere fiber is a main product of cashmere goats that is produced by secondary hair follicles. The fiber of cashmere is soft, warm, and strong and is probably the finest and lightest among all animal fibers. Like wool, the cashmere fiber is composed of hair-keratins and keratin-associated proteins (KAPs) (Rogers, 2006). KAPs serve as a matrix that cross-links hair-keratins (Rogers et al., 2002) and are hence believed to play an important role in defining the physical and mechanical properties of the fiber (Powell and Rogers, 1997; Gong et al., 2016).

KAPs are a diverse group of proteins encoded by small intron less genes called *KRTAPs* (Gong et al., 2012). To date, over 100 *KRTAPs* have been identified across a range of mammalian species (Gong et al., 2010 and 2016; Khan et al., 2014), and human has reported 88 functional *KRTAPs* (Rogers and Schweizer, 2005; Rogers et al., 2007 and 2008). In sheep, while many of the human *KRTAP* orthologues await discovery, 30 *KRTAPs* have been identified. In goats, only 17 *KRTAPs* have been characterized to date, and these are *KRTAP1-1* (Andrews et al., 2017), *KRTAP1-2* (Zhao et al., 2021), *KRTAP1-4* (Shah et al., 2013), *KRTAP3-1* (Parris and Swart, 1975), *KRTAP7-1* (Jin et al., 2011), *KRTAP8-1*(Zhao et al., 2009), *KRTAP8-2* (Jin et al., 2011), *KRTAP9-2* (Wang et al., 2012) , *KRTAP11-1* (Jin et al., 2017), *KRTAP13-1* (Fang et al., 2010), *KRTAP13-3* (Andrews et al., 2017), *KRTAP15-1* (Zhao et al., 2019), *KRTAP20-1* (Wang et al., 2018), *KRTAP20-2* (Wang et al., 2017), *KRTAP24-1* (Wang et al., 2019),

Abbreviations

HGT	high glycine–tyrosine
KAP	keratin-associated protein
KRTAP	keratin-associated protein gene
ORF	open reading frame
PCR-SSCP	PCR-single strand conformation polymorphism

KRTAP27-1 (Zhao et al., 2020), and *KRTAP28-1* (Wang et al., 2020). Despite there being *KRTAP* sequences reported as caprine *KRTAP6-2* (AY316158 and EU145019; Yin et al., 2004; Zhao et al., 2008), these sequences are very different to *KRTAP6-n* sequences from sheep and human and are unlikely to represent caprine *KRTAP6-n*, suggesting that not any gene from KAP6 family has been actually identified in goats.

KAP6 is a multigene subfamily with five family members having been identified in sheep (Zhou et al., 2016). Variation in *KRTAP6-1 and KRTAP6-3* has been reported to affect fiber diameter-associated traits in sheep (Zhou et al., 2015; Li et al., 2017b; Li et al., 2019). Given the potential effect of *KRTAP6* on wool traits (Plowman et al., 2019), investigation is needed to identify *KRTAP6-n* in goats and to determine whether variation in *KRTAP6s* affects cashmere traits. In this study, we attempted to identify the caprine *KRTAP6-5*, to search for potential variation in the gene, and to investigate its effect on cashmere fleece traits.

Materials and Methods

The collection of blood from of goats was approved by the Animal Care Committee of Gansu Agricultural University and was conducted under the guidelines for the care and use of experimental animals established by the Ministry of Science and Technology of China (Approval number 2006-398).

Goats investigated and data collection

Three hundred Longdong cashmere goats from 11 unrelated sires-lines farmed at Yusheng Cashmere Goat Breeding Company in Huan County of China were investigated. Cashmere fibers were collected at 1-yr-old goats by combing, and their greasy fleece weight (GFW) was measured. Cashmere samples were collected from the mid-side region of each goat and then the mean crimped fiber length (MCFL) and mean fiber diameter (MFD) were analyzed using an optical-based fiber length and diameter analyzer OFDA4000 (EPCO, Shanghai, CHN), at Inner Mongolia Agricultural University, China.

The blood samples from all Longdong cashmere goats were collected onto FTA cards (Whatman BioScience, Middlesex, UK), and genomic DNA from dried blood spots was purified using the two-step method described in Zhou et al. (2006).

Search for the Caprine KRTAP6-5

Using the ovine *KRTAP6-5* sequence (GenBank KT725841.1), a BLASTN search in GenBank of the Caprine Genome Assembly ASM170441 V1 (NC030808.1) was undertaken. Of all the sequences found by the BLASTN search, the sequence with the greatest similarity to the ovine *KRTAP6-5* sequence was assumed to be caprine *KRTAP6-5*.

Polymerase chain reaction (PCR) amplification of caprine KRTAP6-5

Using the goat sequence identified above, two PCR primers were designed to amplify the putative caprine *KRTAP6-5*. The sequences of the primers were 5′-AATGGCATGAAGGTGTGGTG-3′and 5′-TGCTGTGGGATAAGCATCAC-3′.

The PCR amplifications were performed in a 20-μL reaction including the purified genomic DNA from one 1.2-mm punch of dried blood spot, 0.25 μM of each primer, 1 × PCR buffer, 150 μM of each deoxyribonucleoside triphosphate (dNTP) (Bioline, London, UK), 2.5 mM Mg^{2+}, and 0.5 U of Taq DNA polymerase (Takara). Reaction was carried out in an Applied Biosystems 2720 thermal cycler (Bio-Rad, Hercules, CA, USA). The thermal profile consisted of 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C, with a final extension of 5 min at 72 °C.

Screening for sequence variation

The PCR amplicons were screened for DNA sequence variation using SSCP analysis. For each amplicon, a 0.7-μL aliquot was add to 7 μL of loading dye (0.025% bromophenol blue, 98% formamide, 10 mM EDTA, and 0.025% xylene-cyanol) and after denaturation at 95 °C for 5 min, the mixtures were then cooled on ice and loaded on 16 × 18 cm, 10% acrylamide: bisacrylamide (37.5:1) (Bio-Rad) gels. Electrophoresis was performed in 0.5× TBE using ProteanII xi cells (Bio-Rad) and the electrophoretic conditions were 290 V for 16 h under the temperature of 20 °C. Then the SSCP gels were silver-stained using a method described by Byun et al. (2009).

Sequencing of amplicons and sequence analysis

If the amplicons produced PCR-SSCP banding patterns that suggested the goat was homozygous in the amplified region, then a representative sample for each pattern was sequenced in both directions at Shanghai Shenggong biological Co., LTD (China). While for heterozygous, PCR amplicons were sequenced using an approach described by Gong et al. (2011). Briefly, a band corresponding to the allele was excised as a gel slice from the polyacrylamide gel, macerated, and then used as a template for re-amplification with the original primers. This second amplicon was then sequenced. Sequence assembly, alignment, and translation were carried out using DNAMAN version 8 (Lynnon BioSoft, Vaudreuil, Canada).

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics version 24.0. General linear mixed-effects models (GLMMs) were used to compare the various cashmere traits among *KRTAP6-5* genotypes and with a Bonferroni correction being applied to reduce the chances of obtaining false positive results during the multiple comparisons. Gender and sire were found to affect ($P < 0.05$) all the fiber traits and were included in the models as fixed and random factors, respectively. All P values were considered statistically significant when $P < 0.05$.

Results

Identification of KRTAP6-5 in Longdong cashmere goats

Using coding sequences of ovine *KRTAP6-5* homologous regions, a BLAST search of the caprine Genome Assembly ASM170441 V1 (NC030808.1) revealed a 252-bp open reading frame (ORF) with 99% sequence identity on caprine chromosome 1 (nt3527973_nt3528224). Twelve previously identified caprine KAP genes were also found near this ORF and these from centromere to telomere were *KRTAP11-1*, *KRTAP7-1*, *KRTAP8-1*, *KRTAP8-2*, *KRTAP20-2*,

KRTAP20-1, *KRTAP15-1*, *KRTAP13-1*, *KRTAP13-3*, *KRTAP27-1*, *KRTAP28-1*, and *KRTAP24-1*.

Neucleotide sequence homology analysis revealed that the ORF sequences were 96.8%, 98.6%, 89.4%, 97.7%, and 99.1% identity with ovine *KRTAP6-1*, *KRTAP6-2*, *KRTAP6-3*, *KRTAP6-4*, and *KRTAP6-5*, respectively (Figure 1a). The comparison of the 3′ flanking sequences of ovine *KRTAP6-s* with that of putative caprine *KRTAP6-5* showed that the sequence of goat was exactly the same as that of ovine *KRTAP6-5* (Figure 1b). This suggests that this ORF represents the caprine *KRTAP6-5*.

Amino acid composition of caprine KAP6-5

The caprine KAP6-5 sequences would encode a polypeptide of 83 amino acid residues. This polypeptide would have a high content of glycine (38.5 mol%) and tyrosine (21.7 mol%), and moderate levels of cysteine (12.1 mol%) and serine (10.8 mol%). The putative caprine KAP6-5 protein would therefore appear to be a basic protein, with a predicted isoeletric point (*pI*) value of approximately 8.2.

Sequence variation in caprine KRTAP6-5

PCR-SSCP analysis of the 300 goats revealed 12 unique SSCP patterns, with either one or a combination of two banding patterns observed for each goat. DNA sequencing revealed that these 12 PCR-SSCP patterns represented 12 nucleotide acid sequences (named *A-L*) and were deposited into GenBank with accession numbers MW762477–MW762488. Twenty-three SSCP bands represent 23 genotypes (*AA*, *BB*, *CC*, *DD*, *GG*, *AB*, *AC*, *AD*, *AE*, *AF*, *AG*, *AI*, *AJ*, *AK*, *BD*, *BG*, *CG*, *CE*, *CK*, *CH*, *CI*, *CL*, and *DG*; Supplementary Figure 1).

Fifteen nucleotide substitutions (c.-36A>C, c.-25A>G, c.-3A>C, c.27T>C, c.45A>G, c.51C>T, c.59G>C, c.83C>G, c.84C>T, c.85T>G, c.103C>T, c.117C>T, c.135T>C, c.238G>A, and C.*77C>A) and a 24-bp insertion/deletion were detected when comparing the 12 sequences (Supplementary Figure 2). Of these SNPs, five of them were non-synonymous (c.59G>C, c.83C>T, c.85T>G, c.103C>T, and c.238G>A) and would nominally result in amino acid changes (p.Gly20Aly, p.Ser28Cys, p.Cys29Gly, p.Arg35Cys, and p.Gly80Ser). The 24-bp insertion/deletion was located in a region encoding a repeated amino acid sequence "SRPLYGCG."

Variant frequencies and genetic polymorphism of KRTAP6-5 in Longdong cashmere goat

The variant frequencies of caprine *KRTAP6-5* in Longdong cashmere goat were *A*: 61.83%; *B*: 4.67%; *C*: 19.16%; *D*: 6.33%, *E*: 0.50%; *F*: 0.17%; *G*: 5.33%; H: 0.17%; I: 0.67%; *J*: 0.17%; K: 0.83%; and L: 0.17%. Of the 23 genotypes, with *AA* being the most common genotype (at a frequency of 38.00%), and to ensure adequate sample size (>10), only *AA*, *AB*, *AC*, *AD*, *AG*, and *CC* were used in the genotype association analyses.

Associations between KRTAP6-5 variation and cashmere traits

Goats with genotype *AA*, *AB*, *AC*, and *AD* produced cashmere fibers with higher MFD than *CC* ($P < 0.05$); meanwhile, MFD of goats with genotype *AA* was also higher than that of *AG* ($P < 0.05$; Table 1).

Discussion

This study reports the identification of a new caprine high glycine-tyrosine KAP (HGT-KAP). This gene was located between *KRTAP 20-2* and *KRTAP 20-1*, and this is consistent with the location of ovine *KRTAP6-s*. Both coding region and 3′UTR of the putative caprine *KRTAP6-5* displayed a biggest sequence similarity to ovine *KRTAP6-5* gene. This suggests that the gene represents caprine *KRTAP6-5* sequence. The identification of caprine *KRTAP6-5* brings the total number of HGT-KAPs identified in goats, from five to six.

If transcribed and translated, the amino acid sequence that would be produced from the caprine KAP6-5 sequence would contain 83 amino acids and more than half of these amino acids would be glycine and tyrosine and contain moderate levels of cysteine and serine. This is consistent with the defining characteristic of the HGT-KAPs.

Fifteen nucleotide substitutions and a 24-bp insertion/deletion were detected in caprine *KRTAP6-5* gene and produced 12 unique variant sequences. Till now, caprine *KRTAP6-5* is the most polymorphism gene among *KRTAPs* that have been studied, and six alleles were ovine *KRTAP6-5* in 96 Merino sheep and Romney sheep. Deletions/insertions that maintain the reading frame have been reported for other *KRTAPs*, such as ovine *KRTAP1-1* (Rogers et al., 1994), *KRTAP5-4* (Gong et al., 2010), *KRTAP 6-n* (Zhou et al., 2016; Li et al., 2017b), and human *KRTAP1-n* (Shimomura et al., 2002) and *KRTAP4-n* (Kariya et al., 2005). The identification of this new deletion in *KRTAP6-5* supports the notion that length variation is a structural hallmark of the *KRTAPs* (Zhou et al., 2015).

The mutation c.59G>C (p. Gly20Ala), c.85T>G (p.Cys29Gly), c.238G>A (p.Gly80Ser), and insertion/deletion mutation would result in the change of glycine. The presence of glycine may affect the secondary structure of proteins, which mainly destroy the helical structure in the polypeptide chain. Glycine is also known as the "helix-breaker," which leads to a more flexible structure of HGT-KAPs and form a compact protein structure (Monné et al., 1999). The SNPs c.84C>T (p.Ser28Cys), c.85T>G (p.Cys29Gly), c.103C>T (p.Arg35Cys), and insertion/deletion mutation would result in the gain or loss of cysteine. Cysteine is considered to be a rate-limiting amino acid for sheep wool growth (Wickham, 1970), and it is a significant form of disulphide bonds between wool and cashmere fiber growth. Eventually, cysteine is very important in fiber growth and the ability of cashmere fiber protein to cross-link. On behalf of these, it can be predicted that the mutations of *KRTAP6-5* may have influence on fiber traits in Longdong cashmere goat, and this is supported by subsequent analysis.

In the six genotypes analyzed, the *CC* goats showed a lower MFD than four genotype (*AA*, *AB*, *AC*, and *AD*) goats. Among *AA*, *AC*, and *CC* goats, as there was no difference in the marginal means for these traits between *AA* and *AC* goats, this suggests that *C* has a recessive effect, while *A* has a dominant effect on these traits. The presence of *A* was significantly associated with an increase in MFD. The fiber of lower MFD would be more desirable in the market, which means that reducing the frequency of *A* on goat group would fit the market demand. But as the number of *CC* goats was a little small, more goats samples will be needed to conform whether the association identified could have utility as a gene-marker for cashmere traits. The effect of other variants (*E*, *F*, *H*, *I*, *J*, *K*, and *L*) on cashmere traits still needs to explore in the future, especially the *J* and *L*, which were produced by length variation.

Given that the only difference between variants *A* and *C* is a single synonymous SNP, the difference in wool traits between genotypes *AA* and *CC* may be due to this synonymous SNP. Although synonymous mutations lead to no change in amino acid sequence, they may affect the gene function in other ways.

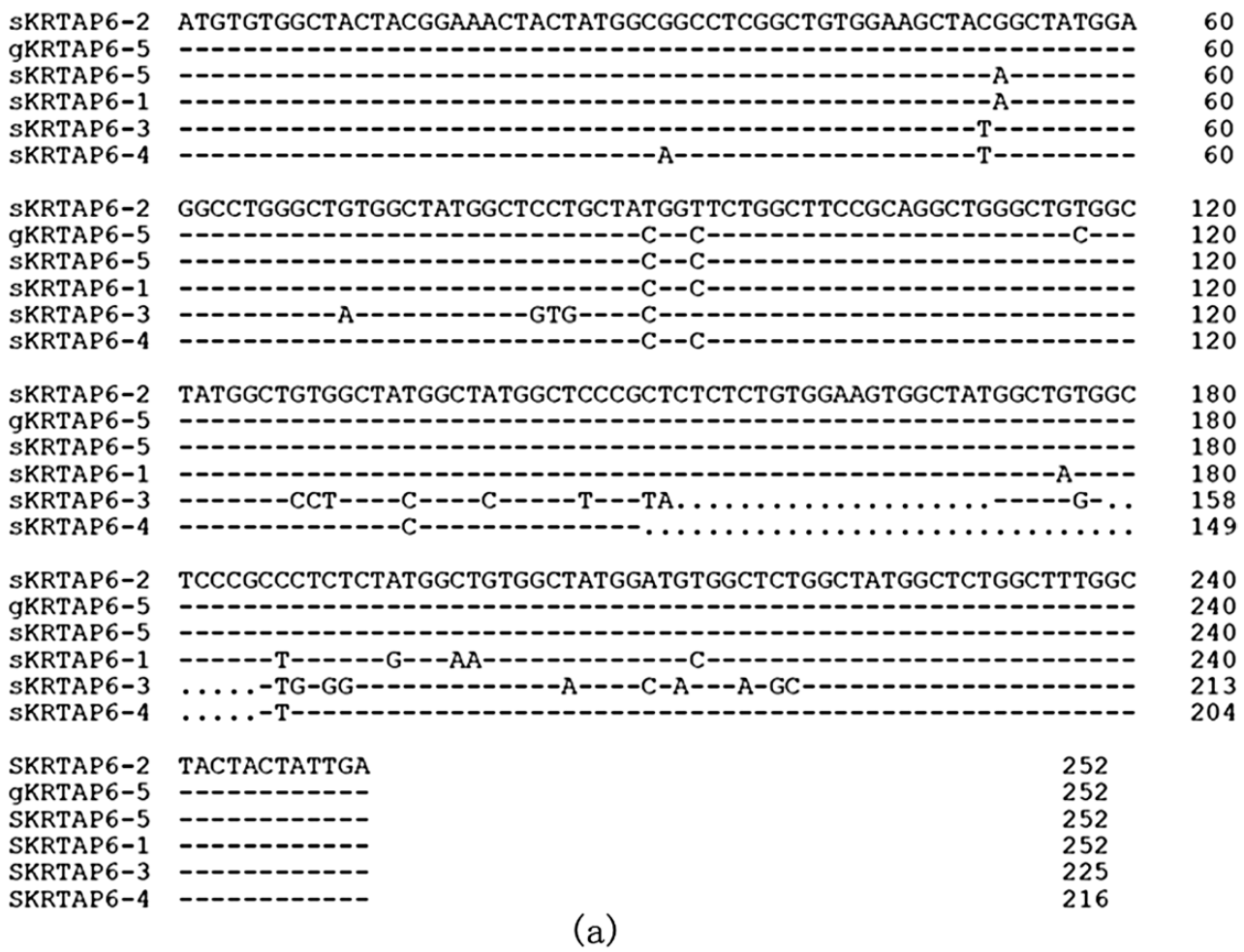

Figure 1. Comparison of the ORF (a) and flanking sequences (b) of the new identified gene with other ovine *KRTAP6-s*. Dashes mean sequences identical to the first sequence and dots are used as spacers to improve the alignments. The GenBank accession numbers for ovine *KRTAP6-s* are NM_001193399 (*KRTAP6-1*), KT725832 (*KRTAP6-2*), KT725837 (*KRTAP6-3*), KT725840 (*KRTAP6-4*), and KT725845 (*KRTAP6-5*).

Table 1. The association of *KRTAP6-5* genotype and various cashmere traits

Traits[1]	Mean ± SE[2]						P-value
	AA (*n* = 116)	*AB* (*n* = 17)	*AC* (*n* = 80)	*AD* (*n* = 22)	*AG* (*n* = 12)	*CC* (*n* =13)	
GFW (g)	418.13 ± 4.35	417.47 ± 10.38	415.25 ± 5.23	411.30 ± 8.86	413.33 ± 12.31	395.94 ± 11.79	0.590
MFD (μm)	13.62 ± 0.04^{a}	13.61 ± 0.09^{ab}	13.63 ± 0.05^{ab}	13.59 ± 0.08^{ab}	13.57 ± 0.11^{bc}	13.25 ± 0.11^{c}	0.030
MCFL (cm)	4.23 ± 0.05	4.26 ± 0.12	4.23 ± 0.06	4.22 ± 0.10	4.13 ± 0.14	4.28 ± 0.14	0.979

[1]GFW, greasy fleece weight; MFD, mean fiber diameter; MCFL, mean crimped fiber length.
[2]Estimated marginal means and standard errors (SE) of those means derived from general linear mixed-effects models. "Gender" and "sire" were included in the models as a fixed factor and a random factor, respectively.

There is some evidence showing that synonymous mutation may have a direct impact on gene function because they affect mRNA stability, folding, transport or translation; protein folding; and miRNA-based regulation of expression (Goymer, 2007; Gotea et al., 2015). Research on sheep *KRTAP22-1* and *KRTAP6-3* also found that the synonymous SNP had effect on wool traits which confirmed this viewpoint again (Li et al., 2017a; 2017b).

The effect of *KRTAP6-5* on cashmere traits detected in this study is consistent with the previous finding of a quantitative trait locus (QTL) on chromosome 1 for MFD in medium wool Merinos (Beh et al., 2001), and is similar to the findings reported for ovine *KRTAP6-1* and *KRTAP6-3* in which variation in these genes was also found to be associated with variation in MFD (Zhou et al., 2015; Li et al., 2019; 2017b). This is probably not surprising given that both genes are clustered next to each other on chromosome 1, and that they belong to the same subfamily gene with a very similar amino acid sequence. The possibility exists that the effects observed for caprine *KRTAP6-5* may be due to its linkage to other *KRTAPs* on the same chromosome. Together, this suggests that *KRTAP6-5* is worthy of further study in the context of variation in cashmere traits.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

Acknowledgments

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Literature Cited

Andrews, M., C. Visser, and E. Van Marle-Köster. 2017. Identification of novel variants for KAP 1.1, KAP 8.1 and KAP 13.3 in South African goats. *Small. Rumin. Res.* **149**:176–180. doi: 10.1016/j.smallrumres.2017.02.014

Beh, K. J., M. J. Callaghan, Z. Leish, D. J. Hulme, I. Lenane, and J. F. Maddox. 2001. A genome scan for QTL affecting fleece and wool traits in Merino sheep. *Wool Technol. Sheep Breeding*. **49**:88–97

Byun, S. O., Q. Fang, H. Zhou, and J. G. Hickford. 2009. An effective method for silver-staining DNA in large numbers of polyacrylamide gels. *Anal. Biochem.* **385**:174–175. doi:10.1016/j.ab.2008.10.024

Fang, Y., W. J. Liu, F. Q. Zhang, Y. G. Shao, and S. G. Yu. 2010. The polymorphism of a novel mutation of KAP13.1 gene and its associations with cashmere traits on Xinjiang local goat breed in China. *Asian. J. Anim. Vet. Adv.* **5**(1):34–42. doi:10.3923/ajava.2010.34.42

Gong, H., H. Zhou, J. M. Dyer, and J. G. Hickford. 2011. Identification of the ovine KAP11-1 gene (KRTAP11-1) and genetic variation in its coding sequence. *Mol. Biol. Rep.* **38**:5429–5433. doi:10.1007/s11033-011-0697-2

Gong, H., H. Zhou, G. W. McKenzie, J. G. Hickford, Z. Yu, S. Clerens, J. M. Dyer, and J. E. Plowman. 2010. Emerging issues with the current keratin-associated protein nomenclature. *Int. J. Trichology* **2**:104–105. doi:10.4103/0974-7753.77519

Gong, H., H. Zhou, G. W. McKenzie, Z. Yu, S. Clerens, J. M. Dyer, J. E. Plowman, M. W. Wright, R. Arora, C. S. Bawden, Y. Chen, J. Li, and J. G. Hickford. 2012. An updated nomenclature for keratin-associated proteins (KAPs). *Int J Biol Sci* **8**(2):258–264. doi: 10.7150/ijbs.3278

Gong, H., H. Zhou, R. H. J. Forrest, S. Li, J. Wang, J. M. Dyer, Y. Luo, and J. G. H. Hickford. 2016. Wool keratin-associated protein genes in sheep—A review. *Genes*. **7**(6):24. doi: 10.3390/genes7060024

Goymer, P. 2007. Synonymous mutations break their silence. *Nat Rev Genet*. **8**, 92. doi: 10.1038/nrg2056

Gotea, V., J. J. Gartner, N. Qutob, L. Elnitski, and Y. Samuels. 2015. The functional relevance of somatic synonymous mutations in melanoma and other cancers. *Pigment Cell Melanoma Res.* **28**:673–684. doi:10.1111/pcmr.12413

Jin, M., Q. Cao, R. Wang, J. Piao, F. Zhao, and J. Piao. 2017. Molecular characterization and expression pattern of a novel Keratin-associated protein 11.1 gene in the Liaoning cashmere goat (Capra hircus). *Asian-Australas. J. Anim. Sci.* **30**:328–337. doi:10.5713/ajas.16.0078

Jin, M., L. Wang, S. Li, M. X. Xing, and X. Zhang. 2011. Characterization and expression analysis of KAP7.1, KAP8.2 gene in Liaoning new-breeding cashmere goat hair follicle. *Mol. Biol. Rep.* **38**:3023–3028. doi:10.1007/s11033-010-9968-6

Kariya, N., Y. Shimomura, and M. Ito. 2005. Size polymorphisms in the human ultrahigh sulfur hair keratin-associated protein 4, KAP4, gene family. *J. Invest. Dermatol.* **124**:1111–1118. doi:10.1111/j.0022-202X.2005.23662.x

Khan, I., E. Maldonado, V. Vasconcelos, S. J. O'Brien, W. E. Johnson, and A. Antunes. 2014. Mammalian keratin associated proteins (KRTAPs) subgenomes: disentangling hair diversity and adaptation to terrestrial and aquatic environments. *BMC Genomics* **15**:779. doi:10.1186/1471-2164-15-779

Li, W., H. Gong, H. Zhou, J. Wang, S. Li, X. Liu, Y. Luo, and J. G. H. Hickford. 2019. Variation in KRTAP6-1 affects wool fibre diameter in New Zealand Romney ewes. *Arch. Anim. Breed.* **62**:509–515. doi:10.5194/aab-62-509-2019

Li, S., H. Zhou, H. Gong, F. Zhao, J. Wang, X. Liu, Y. Luo, and J. G. H. Hickford. 2017a. Identification of the ovine

keratin-associated protein 22-1 (KAP22-1) gene and its effect on wool traits, *Genes*. **8**(1):27. doi: 10.3390/genes8010027

Li, S., H. Zhou, H. Gong, F. Zhao, J. Wang, Y. Luo, and J. G. H. Hickford. 2017b. Variation in the ovine KAP6-3 gene (KRTAP6-3) is associated with variation in mean fibre diameter-associated wool traits. *Genes (Basel)*. **8**(8):204. doi: 10.3390/genes8080204

Monné, M., I. Nilsson, A. Elofsson, and G. Von Heijne. 1999. Turns in transmembrane helices: determination of the minimal length of a "helical hairpin" and derivation of a fine-grained turn propensity scale. *J. Mol. Biol.* **293**:807–814. doi:10.1006/jmbi.1999.3183

Parris, D., and L. S. Swart. 1975. Studies on the high-sulphur proteins of reduced mohair. The isolation and amino acid sequence of protein scmkb-m1.2. *Biochem. J.* **145**:459–467. doi:10.1042/bj1450459

Plowman, J., A. Thomas, T. Perloiro, S. Clerens, and A. M. de Almeida. 2019. Characterisation of white and black merino wools: a proteomics study. *Animal* **13**:659–665. doi:10.1017/S1751731118001647

Powell, B. C., and G. E. Rogers. 1997. The role of keratin proteins and their genes in the growth, structure and properties of hair. *Exs* **78**:59–148. doi:10.1007/978-3-0348-9223-0_3

Rogers, G. E. 2006. Biology of the wool follicle: an excursion into a unique tissue interaction system waiting to be re-discovered. *Exp.Dermatol.* **15**:931–949. doi:10.1111/j.1600-0625.2006.00512.x

Rogers, M. A., and J. Schweizer. 2005. Human KAP genes, only the half of it? Extensive size polymorphisms in hair keratin-associated protein genes. *J. Invest. Dermatol.* **124**:vii–vix. doi:10.1111/j.0022-202X.2005.23728.x

Rogers, G. R., J. G. Hickford, and R. Bickerstaffe. 1994. Polymorphism in two genes for B2 high sulfur proteins of wool. *Anim. Genet.* **25**(6):407–415. doi:10.1111/j.1365-2052.1994.tb00531.x

Rogers, M. A., L. Langbein, H. Winter, C. Ehmann, S. Praetzel, and J. Schweizer. 2002. Characterization of a first domain of human high glycine-tyrosine and high sulfur keratin-associated protein (KAP) genes on chromosome 21q22.1. *J. Biol. Chem.* **277**:48993–49002. doi:10.1074/jbc.M206422200

Rogers, M. A., L. Langbein, S. Praetzel-Wunder, and K. Giehl. 2008. Characterization and expression analysis of the hair keratin associated protein KAP26.1. *Br. J. Dermatol.* **159**:725–729. doi:10.1111/j.1365-2133.2008.08743.x

Rogers, M. A., H. Winter, L. Langbein, A. Wollschläger, S. Praetzel-Wunder, L. F. Jave-Suarez, and J. Schweizer. 2007. Characterization of human KAP24.1, a cuticular hair keratin-associated protein with unusual amino-acid composition and repeat structure. *J. Invest. Dermatol.* **127**:1197–1204. doi:10.1038/sj.jid.5700702

Shah, R. M., T. A. Ganai, F. D. Sheikh, S. Shanaz, M. Shabir, and H. M. Khan. 2013. Characterization and polymorphism of keratin associated protein 1.4 gene in goats. *Gene* **518**:431–442. doi:10.1016/j.gene.2012.12.021

Shimomura, Y., N. Aoki, J. Schweizer, L. Langbein, M. A. Rogers, H. Winter, and M. Ito. 2002. Polymorphisms in the human high sulfur hair keratin-associated protein 1, KAP1, gene family. *J. Biol. Chem.* **277**:45493–45501. doi:10.1074/jbc.M206398200

Wang, J., L. Che, J. G. H. Hickford, H. Zhou, Z. Hao, Y. Luo, J. Hu, X. Liu, and S. Li. 2017. Identification of the caprine keratin-associated protein 20-2 (KAP20-2) gene and its effect on cashmere traits. *Genes (Basel)*. **8**(11):328. doi: 10.3390/genes8110328

Wang, J., Z. Hao, H. Zhou, Y. Luo, J. Hu, X. Liu, S. Li, and J. G. H. Hickford. 2018. A keratin-associated protein (KAP) gene that is associated with variation in cashmere goat fleece weight. *Small. Rumin. Res.* **167**:104–109. doi: 10.1016/j.smallrumres.2018.08.014

Wang, X., Z. D. Zhao, H. R. Xu, L. Qu, H. B. Zhao, T. Li, and Z. Y. Zhang. 2012. Variation and expression of KAP9.2 gene affecting cashmere trait in goats. *Mol. Biol. Rep.* **39**:10525–10529. doi:10.1007/s11033-012-1937-9

Wang, J., H. Zhou, Y. Luo, M. Zhao, H. Gong, Z. Hao, J. Hu, and J. G. H. Hickford. 2019. Variation in the Caprine KAP24-1 Gene Affects Cashmere Fibre Diameter. *Animals (Basel)*. **9**(1):15. doi: 10.3390/ani9010015

Wang, J., H. Zhou, J. G. H. Hickford, M. Zhao, H. Gong, Z. Hao, J. Shen, J. Hu, X. Liu, S. Li, and Y. Luo. 2020. Identification of caprine KRTAP28-1 and its effect on cashmere fiber diameter. *Genes (Basel)*. **11**(2):121. doi: 10.3390/genes11020121

Wickham, G. A. 1970. Wool growth in relation to sulphur-containing amino acid administration to sheep. *Proceedings of the New Zealand Society of Animal Production* **30**: 209–215. New Zealand Society of Animal Production

Yin, J., T. Hu, J. Li, C. Zhang, Z. Guo, H. Zhou. 2004. Construction of a skin cDNA library of cashmere goat and cloning of KAP6-2 full-length cDNA. *Zool. Res.* **25**(2):166–171

Zhao, M., H. Chen, X. Wang, H. Yu, M. Wang, J. Wang, X. Y. Lan, C. F. Zhang, L. Z. Zhang, Y. K. Guo, B. Zhang, and S. R. Hu. 2009. aPCR-SSCP and DNA sequencing detecting two silent SNPs at KAP8.1 gene in the cashmere goat. *Mol Biol Rep.* **36**(6):1387–1391. doi: 10.1007/s11033-008-9325-1

Zhao, M., H. Zhou, J. G. H. Hickford, H. Gong, J. Wang, J. Hu, X. Liu, S. Li, Z. Hao, and Y. Luo. 2019. Variation in the caprine keratin-associated protein 15-1 (KAP15-1) gene affects cashmere fibre diameter. *Arch. Anim. Breed.* **62**(1):125–133. doi:10.5194/aab-62-125-2019

Zhao, M., H. Zhou, Y. Luo, J. Wang, J. Hu, X. Liu, S. Li, Z. Hao, X. Jin, Y. Song, X. Wu, L. Hu, and J. G. H. Hickford. 2020. Variation in the caprine keratin-associated protein 27-1 gene is associated with cashmere fiber diameter. *Genes (Basel)*. **11**(8):934. doi: 10.3390/genes11080934

Zhao, M., H. Zhou, Y. Luo, J. Wang, J. Hu, X. Liu, S. Li, K. Zhang, H. Zhen, and J. G. H. Hickford. 2021. Variation in a newly identified caprine KRTAP gene is associated with raw cashmere fiber weight in Longdong cashmere goats. *Genes.* **12**(5):625. doi: 10.3390/genes12050625

Zhou, H., H. Gong, S. Li, Y. Luo, and J. G. Hickford. 2015. A 57-bp deletion in the ovine KAP6-1 gene affects wool fibre diameter. *J. Anim. Breed. Genet.* **132**:301–307. doi:10.1111/jbg.12138

Zhou, H., H. Gong, J. Wang, J. M. Dyer, Y. Luo, and J. G. Hickford. 2016. Identification of four new gene members of the KAP6 gene family in sheep. *Sci. Rep.* **6**:24074. doi:10.1038/srep24074

Zhou, H., J. G. Hickford, and Q. Fang. 2006. A two-step procedure for extracting genomic DNA from dried blood spots on filter paper for polymerase chain reaction amplification. *Anal. Biochem.* **354**:159–161. doi:10.1016/j.ab.2006.03.042

Zhao, M., X. Wang, H. Chen, X. Y. Lan, Y. K. Guo, J. Y. Li, T. B. Wei, Y. J. Jing, S. Q. Liu, M. H. Zhang, and Q. W. Gao. 2008. The PCR-SSCP and DNA sequencing methods detecting a large deletion mutation at KAP6.2 locus in the cashmere goat. *Small Rumin. Res.* **75**(2):243–246. doi: 10.1016/j.smallrumres.2007.10.007