Identification of novel variants for *KAP 1.1*, *KAP 8.1* and *KAP 13.3* in South African goats

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Highlights

- 108 South African goats were sequenced to evaluate variation within three KAP genes.
- Fourteen SNPs and 19 alleles newly identified for *KAP 1.1*, *KAP 8.1* and *KAP 13.3*.
- Unique alleles identified for Angora, Boer and AB crossbred populations.
- High heterozygosity levels may provide opportunity for improved selection strategy.

Abstract

The yield and quality of animal fibres such as mohair, cashmere and cashgora are primarily influenced by the expression of various keratin associated protein genes, such as *KAP* 1.1, *KAP* 8.1, and *KAP* 13.3. Recent developments in molecular genetics provide the opportunity to characterize *KAP* genes at a base-pair level, which can lead to improved selection and genetic progress in mohair fibre production. PCR and sequencing technology was used to identify and characterize *KAP* 1.1, *KAP* 8.1, and *KAP* 13.3 in 108 goats, representing the South African Angora, Boer and Angora x Boer

goat populations. The three genes showed varying degrees of polymorphism with between 4 and 18 alleles identified per locus. Some discrepancies in the current gene sequence of *KAP* 1.1 were discovered. Nineteen novel variants were identified in total, seven for *KAP* 1.1, one for *KAP* 8.1 and eleven for *KAP* 13.3. Observed heterozygosity values closely matched expected heterozygosity values, with Boer goats consistently having the lowest levels of expected and observed heterozygosity (approximately 0.5) for all three of the genes. The greatest variation for each gene existed between the Angora and Boer goat breeds, with F_{ST} values of 0.28, 0.13, and 0.24 for *KAP* 1.1, 8.1, and 13.3 respectively. Predominant alleles differed between the various populations, indicating the need for further research into possible allelic and fibre quality associations.

Keywords: heterozygosity, keratin associated protein, mohair, nomenclature, polymorphism

1. Introduction

Natural animal fibre production contributes approximately 40% to the global fibre market (Hochman, 2014). Luxury fibres produced by Angora and Cashmere goats primarily serve a niche market worldwide. The main countries producing mohair include South Africa, the USA and Turkey; while countries such as Argentina, Lesotho, New Zealand, France and Great Britain have relatively smaller industries (DAFF, 2012). The South African cashmere industry is very small, but Boer goats have been proven to produce limited quantities of good quality cashmere (Van Niekerk et al., 2004). Most of the world's cashmere is produced in traditional areas in Asia, and is marketed through informal trading channels. Cashgora is the fibre produced by crosses of Angora goats with meat, dairy, or Cashmere goats. The resulting fibre is intermediate between mohair and cashmere, dull, and mostly white in colour (Lupton, 2010). Research on Angora goats in South Africa has included projects to crossbreed Angora goats with Boer goats, at different proportions of each breed in a cross, to produce a hardy animal that produces a good quality fibre (Snyman, 2004).

Research on improving fibre production traits quality, such as fibre diameter and fleece weight, have primarily been focused on quantitative traits selection, with molecular technology only recently

becoming available (Visser & Van Marle-Köster, 2014). Further progress can now be attained by investigating the genetic background of economically important traits. Advances in molecular technology allow for easy and cost-effective sequencing and investigation of the genome. During the last decade there has been interest in DNA sequencing of genes hypothesized to influence traits associated with fibre production (Jin *et al*, 2011; Zhang *et al*, 2011; Gong et al., 2012b). Genes governing the formation of keratin and its associated proteins are responsible for the main structural components of fibres. Keratin intermediate filaments (KIF) and keratin associated proteins (KAP) cross-link to form rigid fibre structures (Powell & Rogers, 1996). Various keratin associated protein genes have been identified and hypothesized to have specific roles on the qualities of the resulting fibres, such as *KAP* 1.4 (Shah et al., 2013), *KAP* 6.2 (Zhao et al., 2008), *KAP* 7.1 (Jin et al., 2011), *KAP* 8.1 (Zhao et al., 2009), and *KAP* 9.2 (Yu et al., 2008). The majority of research regarding these genes has been performed on Cashmere breeds, with no results yet reported for Angora goats.

The *KAP* 1 family contains various proteins that only differ in the number of tandem repeats of a 10 amino acid (30 basepair) sequence, SIQTSCCQPT, in the N-terminal half of the gene (Powell & Rogers, 1986). *KAP* 1.1, 1.2, 1.3, and 1.4 have four, three, two, and five repeats, respectively. Previously published primers for these different *KAP* 1 genes will thus lead to amplification of any of the four genes. This has resulted in some confusion with regards to the nomenclature of the reference gene sequences submitted to NCBI. A total of 16 alleles have been submitted for *KAP* 1.1 and 1.4, across 2 species (Genbank, NCBI). Scrutiny of these sequences has shown that sequences with 100% homology have been classified as different genes, while other *KAP* 1.1 and *KAP* 1.4 sequences have simply been classified incorrectly with regards to the number of repeats present in the sequence. Zhang et al. (2011) identified a beneficial genotype, TT, for *KAP* 1.1 in the Liaoning Cashmere and Mongolia White goat breeds which resulted in higher cashmere yields and higher body weights, without affecting the cashmere fineness.

KAP 8.1 has been identified in ovine and caprine species, and belongs to the high glycine-tyrosine KAP family. Together with *KAP* 6 and 7, *KAP* 8.1 is one of the first genes to be expressed during fibre formation (Zhao et al., 2009). A 357 basepair fragment was previously identified using SSCP analysis, with only two SNPs identified; at positions bp 105 (G/T) & bp 108 (Liu et al., 2011).

A favourable BB genotype has been identified in Liaoning Cashmere and Inner Mongolian goats, resulting in high cashmere yield and long, fine fibres (Zhao et al., 2009 & Liu et al., 2011). The previous research on *KAP* 8.1 has identified genotype BB (Liu et al., 2011) in Inner Mongolian goats as favourable, resulting in longer cashmere fibres, which in turn led to higher cashmere yields without any adverse effect on fibre diameter. This could indicate that this gene might allow an increase in fibre yield without the usual concurrent increase in fibre diameter.

KAP 13.3 has been identified to have eight allelic variants in the Liaoning Cashmere goat (Li et al., 2013). A 559 basepair sequence has been identified with the use of SSCP analysis. This DNA segment had only one exon, showing high homology (98-99%) to the sheep *KAP* 13.3 sequence identified by Gong et al. (2011). Only eight variants have been discovered thus far, but with nine SNPs, more combinations and thus more variants are possible (Li et al., 2013). The alleles discovered by Li et al., (2013) did not match any of the five alleles discovered by Gong et al. (2011) due to differences in the SNP positions. This may demonstrate that even though the sequences show high homology, sequences and alleles may be specific to each population, breed, or species.

Recent characterization of various keratin associated proteins have shown promising results with regards to their effects on wool and cashmere fibre formation, and association of certain alleles with certain phenotypes, with the possibility of seeing these effects in other fibre-producing animals as well (Liu et al., 2011). To remain competitive it is important to ensure that high quality fibres will be produced using selection programs based on the most recent technology. The primary aim of this study was to investigate three keratin associated protein genes, namely *KAP*1.1, *KAP*8.1 and *KAP*13.3, in South African Angora goats.

2. Materials and methods

Blood samples of 48 unrelated Angora goats were provided by Grootfontein Agricultural Development Institute (GADI) Bio-Bank (Lashmar, 2014; EC130618-060 & EC104-13). An additional 30 blood samples from a cross between fibre and non-fibre breeds, namely Angora X Boer goats, that experience alopecia during lactation, were provided by GADI. 30 Boer goat samples were

included as a meat producing outgroup. Blood samples for the 48 Angoras and 30 Angora X Boer goats were transported on ice from the GADI Biobank to the University of Pretoria's Animal Breeding and Genetics laboratory where they were stored at 4°C until DNA extraction was performed. Blood samples for the 30 Boer goats were extracted from the jugular vein into 10ml EDTA tubes (EC140403-024).

DNA was extracted from 108 blood samples with a QIAGEN DNeasy® Blood & Tissue kit (Qiagen – Whitehead Scientific [Pty] Ltd, Cape Town, South Africa, www.qiagen.com) following the manufacturer's protocol. DNA quantification was performed using the Nanodrop spectrophotometer, all samples exceeded a concentration of 50ng/µl.

PCR using three sets of previously published primers was performed. The reactions contained a solution of 3µl Taq buffer containing dNTPs, 6.1µl molecular water, 0.3µl each forward and reverse primers (10pmol/µl), 0.3µl MyTaq polymerase enzyme, and 5µl DNA. The thermocycler program was optimized as follows: denaturation at 94°C for 10 minutes, followed by 33 cycles of amplification consisting of three steps - 94°C for 45s, annealing temperature for 80s, and 72°C for 60s and a final extension step at 72°C for 5 minutes.

An ethanol precipitation cleanup procedure was performed on successful samples to remove leftover primers, dNTPs, *Taq* polymerase, and other non-specific amplifications. DNA pellets were reconstituted with 15µl molecular water and electrophoresed on a 3% Agarose gel. A dye terminator reaction was performed to label the individual nucleotides fluorescently for sequencing. A solution of 0.5µl primer (10pmol/µl) (either forward or reverse), 1µl BigDye, 1µl sequencing buffer, 4µl molecular water, and 3.5µl DNA was made. The PCR program was as follows: denaturation at 96°C for 1 minute, 25 cycles of amplification consisting of three steps, namely 96°C for 10 seconds, annealing temperature for 5 seconds, and 60°C for 4 minutes. The samples were cleaned up with the NaOHAc and ethanol precipitation method and the clear DNA pellet was sequenced with the ABI PRISM 3130xl or 3500xl Genetic Analyzers designed by Applied Biosystems©.

Sequence data received were viewed in Seqscanner (Sequence Scanner, Version 1.0, Applied Biosystems, 2005). The sequences were edited using CLC Bio Main Workbench (Version 6.9, www.clcbio.com) and assembled to form contigs from which a consensus sequence was extracted.

Reference sequences created from the NCBI database were annotated with the positions of the various SNP's. New SNP positions or alleles were marked and named following the established nomenclature for that specific gene. BLAST searches were performed on the NCBI GenBank database to confirm that the identified sequences were the correct genes.

Allelic and genotypic frequencies were calculated by direct counting, while Arlequin software (Version 3.5.1.3, Excoffier et al., 2005) was used to calculate expected and observed heterozygosity, a pairwise F_{ST} test, and an Analysis of Molecular Variance (AMOVA) for each *KAP* gene. All tests were performed at a significance value of $p = 0.01$.

3. Results

A total of 7, 4, and 18 alleles were observed across all three populations for *KAP* 1.1, *KAP* 8.1, and *KAP* 13.3 respectively. *KAP* 13.3 was the most diverse of the three genes, with 18 alleles in total, while *KAP* 8.1 was the least diverse with only 4 alleles. All observed SNP's were found within the coding region of each gene.

For *KAP 1.1*, the sequences obtained matched both *KAP* 1.1 and *KAP* 1.4 reference sequences, which highlights the current nomenclature problem. Seven alleles were identified across the three populations in this study, a result of the combination of five SNPs at basepair positions 134, 455, 537, 651, and 668 (Table 1). None of these were a 100% match for any of the previously identified alleles of *KAP* 1.1, but could still be aligned to *KAP* 1.1 sequences in GenBank. Differences were observed in the position of SNPs, and the fragment size appears to be approximately 60 basepairs shorter than the fragments amplified by Zhang et al. (2011) as well as other *KAP* 1.1 sequences found in the NCBI Genbank (Genbank NM_001159760.1). Differences in the SNP positions were not related to the shorter length of the sequence. This would suggest that some of the previously identified alleles were from a different gene in the *KAP* 1 family. For the purposes of this study, and to avoid confusion with previous results and alleles, alleles for this study were identified and named as new alleles under *KAP* 1.1 (Alleles D-J), following the alphabet.

Table 1 *KAP* 1.1 Allele SNP combinations

All/Pos	134	455	537	651	668
D	T	C	C	T	A
E	T	C	T	C	A
F	T	C	T	C	G
G	C	C	T	T	A
H	C	C	T	C	G
I	C	T	T	C	A
J	C	T	T	C	G

In Table 2 the most prevalent allele in each population is indicated. The most frequently observed allele identified in the Angora goats was allele F, with a frequency of 0.44. Two unique alleles were present in the Angoras, namely allele E and allele J, both with frequencies below 0.05. The allele most frequently observed for the Boer goats was allele H (0.67), and for the AB crossbred goats allele I (0.48). No unique alleles were observed for the Boer goats or the AB crossbred goats.

Table 2 *KAP* 1.1 allelic frequencies for the three populations

Allele	Angoras	Boer goats	AB crossbred goats
D	0.02 (2/96)	0.00 (0/60)	0.17 (10/60)
E	0.04 (4/96)	0.00 (0/60)	0.00 (0/60)
F	**0.44*** (42/96)	0.10 (6/60)	0.12 (7/60)
G	0.00 (0/96)	0.02 (1/60)	0.02 (1/60)
H	0.09 (9/96)	**0.67** (40/60)	0.21 (13/60)
I	0.39 (37/96)	0.21 (13/60)	**0.48** (29/60)
J	0.02 (2/96)	0.00 (0/60)	0.00 (0/60)

*Allele with highest frequency in bold

Only four alleles were observed for *KAP* 8.1 (Table 3), with the GenBank accession codes given in brackets for the three previously identified alleles. The most prevalent allele for the Angora goats was allele C (0.47), while allele A was mostly observed for both the Boer goats and AB crossbred goats (0.65 and 0.57, respectively). A new allele was identified within the Boer goats and

was classified as allele D. Both alleles B and D were present at low frequencies (0.03) in the Boer goat population.

Table 3 *KAP* 8.1 SNP combinations and allele frequencies for the three populations

Allele	**SNP bp 105**	**SNP bp 108**	**Angoras**	**Boer goats**	**AB crossbred goats**
A (EU595395.1)	G	G	0.30 (29/96)	**0.65** (39/60)	**0.57** (34/60)
B (mRNA)	T	G	0.23 (22/96)	0.03 (2/60)	0.23 (14/60)
C (EU595394.1)	G	C	**0.47*** (45/96)	0.28 (17/60)	0.20 (12/60)
D	A	G	0.00 (0/96)	0.03 (2/60)	0.00 (0/60)

*Allele with highest frequency in bold

KAP 13.3 was the most polymorphic of the genes investigated, with a total of 17 SNP positions and 18 alleles present across the three populations investigated. Of these, eight SNPs were newly identified in this study, found at base-pair positions 103, 127, 155, 203, 222, 242, 282, and 285. Eleven of the corresponding alleles (allele I-S) were newly identified in this study, and named in accordance with the established nomenclature of the *KAP* 13.3 gene (Table 4).

Table 4 *KAP* 13.3 Allele SNP combinations, newly identified alleles marked with *

Allele	**Position**																
	43	58	62	107	103	127	158	155	203	222	242	282	285	323	343	370	373
A	C	C	A	A	G	C	A	T	G	C	G	C	T	T	A	G	T
B	T	C	C	A	G	C	G	T	G	C	G	C	T	C	A	G	T
C	C	T	A	A	G	C	A	T	G	C	G	C	T	C	T	A	T
D	C	C	A	A	G	C	A	T	G	C	G	C	T	C	A	G	T
E	C	C	A	A	G	C	G	T	G	C	G	C	T	T	A	G	T
G	C	C	C	A	G	C	G	T	G	C	G	C	T	T	T	G	T
H	C	C	A	G	G	C	A	T	G	C	G	C	T	C	A	G	T
I*	C	C	A	A	G	C	A	T	G	C	G	T	G	T	T	G	G
J*	T	C	C	A	G	C	G	T	G	C	G	C	T	T	T	G	T
K*	C	C	C	A	A	C	A	T	G	T	G	C	T	C	A	G	T

L*	C	C	A	A	G	C	A	C	G	C	G	C	T	C	A	G	T
M*	C	C	A	A	G	T	A	T	G	C	G	C	T	T	A	G	T
N*	C	C	A	A	G	C	G	T	G	C	G	C	T	C	A	G	T
O*	C	C	A	G	G	C	G	T	G	C	G	C	T	T	A	G	T
P*	C	C	A	A	G	C	G	T	T	C	G	C	T	T	A	G	T
Q*	C	C	A	A	G	C	A	T	G	C	A	C	T	C	A	G	T
R*	C	C	A	A	G	C	A	T	G	C	G	C	T	T	T	G	G
S*	C	C	C	A	G	C	G	T	G	C	G	C	T	T	A	G	T

Alleles that were most or least prevalent differed among the three populations, with unique alleles present in both the Angora and Boer goat breeds. Only the five most prevalent alleles across populations are shown in Table 5. The most observed allele in the Angora group was allele H (0.23). Three unique alleles were present in the Angoras, namely alleles B, C, and O, which were all classified as rare alleles with minor allele frequencies (MAF) values of 0.01. The Boer goats had a high allele frequency of 0.63 for allele D, and a MAF of 0.02 for the two unique alleles, alleles M and S. The AB crossbred goat group had no unique alleles, and an allele frequency of 0.22 for allele J, which was the highest observed frequency for this group.

Table 5 Predominant *KAP* 13.3 allele frequencies for the three populations

Allele	**Angora**	**Boer goat**	**AB crossbred goats**
D	0.02 (2/96)	**0.63** (38/60)	0.15 (9/60)
H	**0.23*** (22/96)	0.00 (0/60)	0.05 (3/60)
J	0.10 (10/96)	0.02 (1/60)	**0.22** (13/60)
K	0.02 (2/96)	0.07 (4/60)	0.15 (9/60)
P	0.20 (19/96)	0.02 (1/60)	0.13 (8/60)

*Allele with highest frequency in bold

The Boer goats had the lowest level of expected heterozygosity for all three genes, with values between 0.5 (*KAP* 1.1 and *KAP* 8.1) and 0.58 (*KAP* 13.3). The Angora and AB crossbred group had higher values of 0.65 and 0.68 for *KAP* 1.1, and 0.64 and 0.59 for *KAP* 8.1, respectively. These two

populations showed very high heterozygosity for KAP 13.3, with values of 0.87 and 0.88, respectively. *KAP* 8.1 had the lowest F_{ST} values for all three population comparisons, while the greatest variation existed between the Angora and Boer goat group for *KAP* 1.1 (0.28), and the least variation between the Boer goats and Angora X Boer goats for *KAP* 8.1 (0.03).

4. Discussion

The primary objective of this study was to determine the presence and extent of variation of the *KAP* 1.1, *KAP* 8.1, and *KAP* 13.3 alleles in three South African goat populations. Previous research has identified KAP's and KIF's as integral to the formation and quality of fibres (Rogers, 2004). Identification of the gene variants that influence the formation and differentiation of these proteins, would provide the means to directly influence fibre production through improved selection in small stock. The focus of previous studies was primarily set on verifying the presence of the different *KAP* genes, as well as determining their allelic variation. Limited results have been obtained with regards to associations between different variants and production levels. *KAP* 1.1, *KAP* 8.1, and *KAP* 13.3 have been identified as being polymorphic in studies performed in other goat breeds (Zhao et al., 2009; Zhang et al., 2011; Li et al., 2013).

Of the three *KAP* genes investigated, *KAP* 1.1 is perhaps the gene most poorly understood at present, due to the varying results of previous research (Itenge-Mweza et al., 2007; Zhang et al., 2011). Previous *KAP* 1.1 research on sheep indicated that this gene was located on chromosome 11 and part of the high sulphur group (Gong et al., 2012a), with a basepair length of 660bp, depending on the number of tandem repeats of a 10 amino acid/30bp fragment within the gene. Results from the current study, using primers for *KAP* 1.1 published by NCBI, amplified a fragment of 600bp, with 30bp absent in the tandem repeat region, and another 30bp absent at the 3'-end of the sequence. The sequence obtained aligned with the *KAP* 1.1 reference sequence on NCBI. The absence of a tandem repeat could indicate that this sequence may in fact be classified as *KAP* 1.2 under nomenclature established by Powell & Rogers (1986), but for the purpose of the study, we will continue with it as *KAP* 1.1. This is in contrast to other gene sequences submitted on NCBI for *KAP* 1.1 in the ovine

species, and yet similar to *KAP* 1.4 sequences for the ovine species. This indicates that there may be confusion with the classification and naming of gene sequences previously submitted to NBCI. Seven new alleles were observed for *KAP* 1.1 due to the combination of five SNPs identified at new positions in the gene fragment, as well as the absence of one tandem repeat of 10 amino acids (30bp). The difference in different predominant alleles between/among populations may indicate that a specific allele could be associated with fibre yield and / or quality, and should be further investigated using an association study,

KAP 8.1 is a 357bp gene found on chromosome 1, and part of the high glycine-tyrosine group (Zhao et al., 2009). The alleles that were obtained for *KAP* 8.1, matched those previously identified in Cashmere goats by Zhao et al. (2009) and Liu et al. (2011), with the additional discovery of a novel SNP position and concurrent new allele in the Boer goat breed. The novel SNP found in the Boer goat population may be the result of a random mutation within the population, or possibly a rare allele. The predominant allele differed for each of the populations, as well as from the previous research of Zhao et al., (2009) and Liu et al., (2011), which stated the allelic prevalence as A>B>C for Cashmere goats, with allele frequencies of 0.6, 0.3, and 0.1 respectively. The predominant allele for the Angoras in this study was however the C allele (0.47), and for the Boer goats allele A (0.65). The B allele is present at moderate frequencies in the Angora and AB Cross goat populations, indicating a possible association with improved fibre quality, while the Boer goat population, a meat breed, has allele B present at a very low frequency.

The *KAP* 13.3 gene is a 559bp of the high sulphur group, and is located on chromosome 21. *KAP* 13.3 was the most polymorphic of the genes investigated, with a total of 18 different observed alleles, in contrast to the eight alleles discovered by Li et al., (2013). The discovery of new SNP positions may indicate that the gene sequence itself is highly variable and prone to mutations, or that the different goat breeds have different alleles that are being discovered with new research. The allelic variation was greatest in the Angora goats, with a total of 16 alleles, compared to 10 each for the Boer goats and AB Cross goats. Such a great variety in the small Angora sample group may indicate the absence of selection pressure based on this gene, which in turn would imply that the effect of the gene

is not clearly identifiable yet. The current low frequencies may limit the use of these alleles as markers, until a clear association has been identified between alleles and fibre quality.

No association studies have been performed on *KAP* 13.3, which has only recently been identified in Cashmere goats (Li et al., 2013). The resulting polypeptide sequence has a high prevalence of serine and threonine residues that are able to phosphorylate. Phosphorylation is known to influence keratin formation and structure (Li et al., 2013), which in turn will affect the resulting fibre structure. The potential effects of phosphorylation, as well as the ability to apply selection pressure to the highly polymorphic populations, may provide a method to influence the quality of hair fibres.

5. Conclusion

The South African Angora goat population is currently the leader in mohair production world-wide, but progress is hampered by unfavourable genetic correlations between different mohair traits. Advances in molecular genetics and genomics, such as the identification and characterization of candidate genes on a base-pair level, would provide the means to overcome some of the limitations of quantitative traits selection. A total of 19 unique variants at three genes of keratin associated proteins mainly responsible for structure and quality of hair fibres, namely *KAP* 1.1, *KAP* 8.1, and *KAP* 13.3, have been identified in three South African goat populations. Although no clear associations could be found between different alleles and populations, the high levels of observed heterozygosity are promising for selection based on favourable allelic associations, provided that further experiments with larger population samples will be designed. The South African Angora goat population could benefit from additional research focused on *KAP* genes used as candidate genes in selection programs for improved fibre production. Fibre production in the Boer goat breed could be exploited as an additional product from this breed, thus contributing to improved livelihood and economic security in rural communities.

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