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Identification of novel genetic variants for KAP1.1, KAP1.3 and K33 genes in some of indigenous goat breeds of Turkey

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Abstract: The animal fibres such as mohair, cashmere and cashgora have a complex structure and affected by genetic variation of keratin associated protein genes as KAP 1.1 (Keratin Associated Protein 1.1, formerly known as B2A), KAP1.3 (Keratin Associated Protein 1.3, formerly known as B2C) and K33 (Keratin Intermediate Filaments Type I, formerly known as KRT1.2). Keratin-associated proteins play a significant role in identifying structural and mechanical properties of the hair and wool fibres. This study was conducted to detect genetic variation at the KAP1.1, KAP1.3 and K33 genes in indigenous Turkish goat populations using DNA sequencing method. The DNA of 100 individuals selected from 5 different native goat breeds (Hair, Honamlı, Kilis, Norduz, and Angora) that reared different regions of Turkey were used as materials. A total of 59 nucleotide variations and indels (insertion/deletion) of KAP1.1 gene, 15 nucleotide variations and indels of KAP1.3 gene, 16 nucleotide variations of K33 gene were determined in the studied samples. These nucleotide variations and indels have been causing changes in the number and sequence of amino acids. It is necessary to determine the relationships with mohair yield, quality and polymorphisms that are determined in KAP1.1, KAP1.3 and KRT1.2 genes.

Key words: Mohair, KAP genes, goat, Turkey

1. Introduction

The total number of goats in the world is estimated at approximately one billion heads of which ten million are raised in Turkey, which corresponds almost half of the entire Europe [1]. In Turkey, while goat breeding is mainly based on Hair goats (98%) reared for dual purpose (meat and milk yield), Angora goats estimated at 210.000 heads (2%) are raised for mohair production. In 2018, 6000 tons of hair and 370 tons of mohair were produced from Hair and Angora goats, respectively [2]. The countries that produce mohair are South Africa, the USA, Turkey, Argentina, Lesotho, New Zealand, France and Great Britain [1].

The mohair is produced from domestic Turkish goats such as Angora and hair goat, while hair is produced from Honamlı, Kilis and Norduz goat in Turkey [2,3]. Angora goat is reared for its angora in Central Anatolia in Turkey. Angora goats have small body size and usually white, but the angora colour can be brown, black, grey, gold and beige in Siirt, Mardin and Şırnak provinces of Turkey. Cashgora is the fibre produced by Angora goat or crosses of Angora goat with local goat breeds. The cashgora fibre is intermediate between mohair and angora and generally white in colour [4]. Kilis goats are crosses of Hair goat and Halep goat breeds from Syria and generally have black hair and mostly bred in the Mediterranean and the Southeastern Anatolia Regions of Turkey [5]. Kilis goat has advantages because of its adaption to arid pastures, lowlands and elevated temperatures in Southeastern Anatolia Region of Turkey [6]. Honamlı goats are raised by the people living as a nomad in the Mediterranean region (Antalya, Isparta, Burdur and Konya provinces) of Turkey, and their colour is mostly black. Norduz goats are bred in the Van province of Turkey and mostly black in hair colour [7].

The variation in hair and mohair traits are affected by both genetic and environmental factors; therefore, it is an important principle that to detect the genes that determine hair and mohair production characteristics and quality. Keratin proteins have two different types as keratin-associated proteins (KAP) and keratin intermediate filament proteins (KIF or KRT). Keratin-associated proteins play a significant role in identifying structural and mechanical properties of the hair and mohair fibres. Physical traits of hair such as fibre diameter, length and medullation are significant in the textile industry [8]. The animal fibres such as mohair, cashmere and cashgora have

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a complex structure and are affected by genetic variation of keratin associated protein genes and keratin intermediate filament as KAP 1.1, KAP 1.3 and K33. Until today, variations of KAP1.1, KAP1.3 and K33 have been analyzed by different molecular techniques such as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP), and DNA sequencing in order to investigate yield and quality of hair or mohair in different sheep and goat breeds across the world [9,10,11,12,13]. The genetic variation identification studies were mostly conducted on sheep breeds with these genes. Rogers et al. [9] and Itenge-Mweza et al. [10] defined genetic variation in the K33 gene region of Merino sheep using PCR-RFLP and PCR-SSCP methods, respectively. Afterward, Andrews et al. [11] have revealed KAP1.1 gene polymorphism in South African South African Angora, Boer and Angora × Boer goat populations using sequencing method. Shah et al. [12] analyzed the genetic variation of the KAP1.3 gene in pashmina and non-pashmina local goats with the single-strand conformation polymorphism (SSCP) method. Zhang et al. [13] revealed polymorphisms in KAP1.1 gene and its relation with growth and cashmere traits in the Liaoning and Inner Mongolia White Cashmere goat breeds with SSCP method. Farag et al. [14] and Itenge et al. [15] found that KAP1.1, KAP1.3 and K33 loci were useful gene markers related with fibre characteristics such as fibre diameter, fibre colour and mean staple length in Merino and Merino-cross sheep. Many scientists carried out researches on sheep to identify the genetic variations of KAP 1.1, KAP 1.3 and K33 genes and their associations with production traits, but there are a few articles studying on these genes on goats.

This study aimed to identify genetic variation in the KAP1.1, KAP1.3 and K33 gene regions in 5 different local goat breeds (Hair, Honamlı, Kilis, Norduz, and Angora) of Turkey.

2. Materials and methods

2.1. Blood collection and DNA extraction

In the present study, a total of 100 blood samples, 20 of which were taken from each breeds namely Hair, Honamlı, Kilis, Norduz and Angora, are raised in different provinces of Turkey (Figure 1). Five mL blood samples were collected into vacuum tubes, including ethylenediamine tetraacetic acid (EDTA) as anticoagulant, and stored at −80 °C till the DNA extraction. Genomic DNA was isolated by using a commercial DNA isolation kit (GeneJET Whole Blood Genomic DNA Purification Mini Kit, Thermo Fisher Scientific) according to the manufacturer's instructions. The quality and quantity of DNA were determined with NanoDrop Spectrophotometers (2000/2000c, Thermo Fisher Scientific).

2.2. PCR amplification and sequencing

Primer sequences of KAP1.1 F: 5'-CAACCCTCCTCTCAACCCAACTCC-3', R: 5'-CGCTGCTACCCACCTGGCCATA-3' [10], KAP1.3 F: 5'- CAAGCAGACCAAACTCAGAAAC-3', R: 5'-TGTCCACAGTAGGATGGGCGGC -3' [16] and K33

Figure 1. Locations of samples collected during the experiment in Turkey (Hair, Honamlı, Kilis, Angora and Norduz goat). The map should be downloaded from the Republic of Turkey, General Directorate of Mapping and the related reference should be given here as a footnote.

F: 5'-CACAACTCTGGCTTGGTGAACTTG-3', R: 5'-CTTAGCCATATCTGGGATTCCCTC-3' [9] are used for PCR amplification and sequencing. For amplification reactions, the 35 µL PCR volume contained: 20 ng genomic DNA, 0.5 µM of each primer, 1× PCR buffer ($(NH4)_2SO4$), 200 µM dNTP, 1.5 mM $MgCl_2$ and 1 U of Taq DNA polymerase (Taq DNA Polymerase, Thermo Scientific, USA). The cycling protocol was 4 min at 94 °C for initial denaturation, 35 cycles of amplification; 94 °C for 30 s, 56.1 °C annealing for 30 s, 72 °C for 2 min and 15 min at 72 °C for final extension. Afterwards, the PCR products were checked on 1 % agarose gel using horizontal electrophoresis, and the gels were stained using SafeView Classic (Applied Biological Material Inc., Canada).

A total of 327 bp of KAP1.1, 652 bp of KAP1.3 and 480 bp of K33 genes were sequenced on an Applied Biosystems 3500XL Genetic Analyzer System (Applied Biosystems, USA) in order to determine those genes sequence. PCR products of KAP1.1, KAP1.3 and K33 gene regions were sequenced in both directions. The obtained data were aligned to identify nucleotide variations on fragments by the ClustalW multiple alignment modules and MEGA 7 software (Molecular Evolutionary Genetics Analysis, version 6.0) [17,18].

3. Results

The 327 bp of KAP1.1 gene region was amplified in all goat DNA samples except two samples, which were 389 bp. The nucleotide changes observed in the KAP1.1 gene compared with the reference sequences, and the animal numbers are given in Table 1. Insertions/deletions of length in 47 bp and 15 bp were observed in two individuals of Kilis and Norduz breeds. It is seen in the Figure 2 that the nucleotide changes from adenine to guanine at the 231st position in Kilis and Honamlı goat breeds. Similarly, 12 bp long insertions/deletions were observed in Hair goat. The NCBI RefSeq reference goat sequence (XM_018065086.1) of KAP1.1 consists of 102 amino acids, while the amino acid sequence of the studied samples consists of 127 amino acids due to insertions/deletions. It is seen in the Figure 3 that the 51 amino acids in the reference goat amino acid sequence are different from the ones in the samples studied, while 15 amino acids are added.

The 611 bp of KAP1.3 gene region was amplified in the studied samples with the primers that Itenge et al. [16] used for amplification of 598 bp sheep KAP1.3 gene. The 15 mutations and nucleotide changes observed in the KAP1.3 gene are compared with the reference sequences, and the animal numbers are given in Table 2. The sequences that found nucleotide variations in the studied samples were submitted to NCBI GenBank with the MT186779-92 accession numbers. The nucleotide changes at the 169th, 184th and 193th positions of the KAP1.3 gene region in the studied samples are shown in Figure 4. The nucleotide variations did not cause any amino acid changes in KAP1.3 gene region in the studied samples.

480 bp of K33 gene region was investigated with DNA sequencing method in some of indigenous goat breeds of Turkey. K33 gene region is located between 41204022 and 41204501 bp of NCBI reference genome of goat breed San Clemente chromosome 19 (NC_030826.1). The 16 mutations and nucleotide changes observed in the K33 gene compared with the reference sequences of goat and sheep are shown in Table 3. The studied gene region was included in the first exon of K33 gene, which coded 116 amino acids. K33 gene showed five synonymous mutations at the sites 310, 355, 358, 364 and 427 that didn't cause amino acid change (C>Y at cysteine, G>S at alanine, T>Y at proline, T>Y at leucine and C>Y at cysteine amino acid, respectively).

4. Discussion

The 327 bp of KAP1.1 gene region was amplified in all of goat DNA samples, besides Itenge et al. [15,16] amplified 311 bp gene regions in sheep DNA samples with the same primers. Itenge et al. [16] identified three alleles from A to C at the KAP1.1 locus. Similar to Itenge et al. [16], Rogers et al. [19] found that the A allele encodes one more decapeptide repeat than the B allele and two repeats more than the C allele at the KAP1.1 locus. Itenge et al. [16] found that the KAP1.1-A allele was associated with a higher wool yield than the B allele at 24 months of age. However, the KAP1.1 B allele was associated with an increased staple length. Firstly, Zhang et al. [13] amplified KAP1.1 gene region in Liaoning Cashmere goat and Inner Mongolia goat including same region used in the study of Itenge-Mweza et al [10]. Zhang et al. [13] revealed a novel SNP (g.688T > C) that located on KAP1.1 α allele identified before by Rogers et al. [19] and Itenge-Mweza et al [10]. Turkish indigenous goat breeds have not the same SNP with the one detected by Zhang et al. [13]. It is compared with sheep KAP1.1 gene region submitted by Itenge et al. [15] (AY835603) and goat reference KAP1.1 gene region (XM_018065086). In this study, 63 point nucleotide changes were found based on the results of the compression with sheep and goat KAP1.1 gene regions. All of these nucleotide changes exactly match the previously determined SNPs. It is found that Honamlı and Hair goat breeds have the highest genetic variations in the KAP1.1 gene region. Kilis breed has nucleotide variations at 10 points of KAP1.1 gene, which were not revealed before by Itenge et al. [15] (AY835603). Andrews et al. [11] identified seven new SNPs in Angora, Boer and AngoraXBoer crossbred goat in the KAP1.1 gene region. Additionally, they found 60 bp indels in the KAP1.1 gene region. According to the results of our study, similar to Andrews

Table 1. The locations of nucleotide changes in the KAP1.1 gene and the number of animals with these nucleotide changes.

Position	Sheep reference sequence*	Goat reference sequence*	Mutations	Number of animals	NCBI Accession Number#
16	-	A	-	-	All sequences
130	-	-	C	11 (Angora, Norduz, Kilis)	MW656238
149	-	-	A	1 (Kilis)	MW656237
154	-	-	A	1 (Kilis)	MW656235
155	-	-	G/A	11 (Angora, Norduz, Kilis)	MW656235
156	-	-	A	2 (Kilis, Norduz)	MW656235
163	-	-	C/T	11 (Angora, Norduz, Kilis)	MW656240
164	-	-	G	10 (Angora, Norduz, Kilis)	MW656242
179	-	-	G/C	11 (Angora, Norduz, Kilis)	MW656242
181	-	-	T/A/-	7 (Norduz, Kilis)	MW656236
182	-	-	T/G	11 (Angora, Norduz, Kilis)	MW656241
183	-	-	G/C/T	11 (Angora, Norduz, Kilis)	MW656245
213	T	T	C	10 (Angora, Norduz)	MW656243
231	G	G	A	2 (Kilis, Norduz)	MW656242
261	G	G	A	8 (Norduz)	MW656239
264	A	A	G	1 (Norduz)	MW656246
290	-	-	C	1 (Honamlı)	MW656244
292	A	A	G	2 (Honamlı, Hair)	MW656243
295	G	G	A	3 (Honamlı, Hair, Angora)	MW656243
297	T	T	C	3 (Honamlı, Hair, Norduz)	MW656236
300	C	C	G	2 (Honamlı, Hair)	MW656239
302	A	A	C	2 (Honamlı, Hair)	MW656238
313	C	C	T	2 (Honamlı, Hair)	MW656238
314	T	T	G	2 (Honamlı, Hair)	MW656238
319	C	C	T	2 (Honamlı, Hair)	MW656240
320	C	C	G	2 (Honamlı, Hair)	MW656240
323	C	C	A	2 (Honamlı, Hair)	MW656240
325	A	A	C	2 (Honamlı, Hair)	MW656239
326	A	A	G	2 (Honamlı, Hair)	MW656240
327	C	C	G	2 (Honamlı, Hair)	MW656239
331	C	C	T	2 (Honamlı, Hair)	MW656240
332	C	C	G	2 (Honamlı, Hair)	MW656240
333	T	T	G	2 (Honamlı, Hair)	MW656240
335	C	C	A	2 (Honamlı, Hair)	MW656238
336	A	A	T	2 (Honamlı, Hair)	MW656240
337	G	G	T	2 (Honamlı, Hair)	MW656238
338	A	A	G	2 (Honamlı, Hair)	MW656240
339	C	C	G	2 (Honamlı, Hair)	MW656238
340	C	C	T	2 (Honamlı, Hair)	MW656240
341	A	A	G	2 (Honamlı, Hair)	MW656239
343	T	T	C	2 (Honamlı, Hair)	MW656240

Table 1. (Continued).

344	G	G	A	2 (Honamlı, Hair)	MW656238
347	T	T	A	2 (Honamlı, Hair)	MW656240
348	G	G	T	2 (Honamlı, Hair)	MW656240
352	G	G	C	2 (Honamlı, Hair)	MW656238
354	C	C	A	2 (Honamlı, Hair)	MW656238
355	C	C	T	2 (Honamlı, Hair)	MW656238
359	T	T	C	2 (Honamlı, Hair)	MW656240
360	G	G	A	1 (Hair)	MW656239
361	T	T	G	1 (Hair)	MW656239
375	G	G	T	2 (Honamlı, Hair)	MW656238
376	C	C	G	2 (Honamlı, Hair)	MW656239
377	A	A	G	2 (Honamlı, Hair)	MW656240
378	T	T	G	2 (Honamlı, Hair)	MW656238
380	G	G	A	2 (Honamlı, Hair)	MW656239
382	T	T	C	2 (Honamlı, Hair)	MW656240
383	G	G	A	2 (Honamlı, Hair)	MW656238
386	A	A	G	2 (Honamlı, Hair)	MW656239
387	G	G	A	2 (Honamlı, Hair)	MW656240

*The NCBI (The National Center for Biotechnology) number of sheep reference sequence is AY835603, the NCBI number of goat reference sequence is XM_018065086.
#NCBI Accession Numbers that are submitted to NCBI GenBank database in this study.

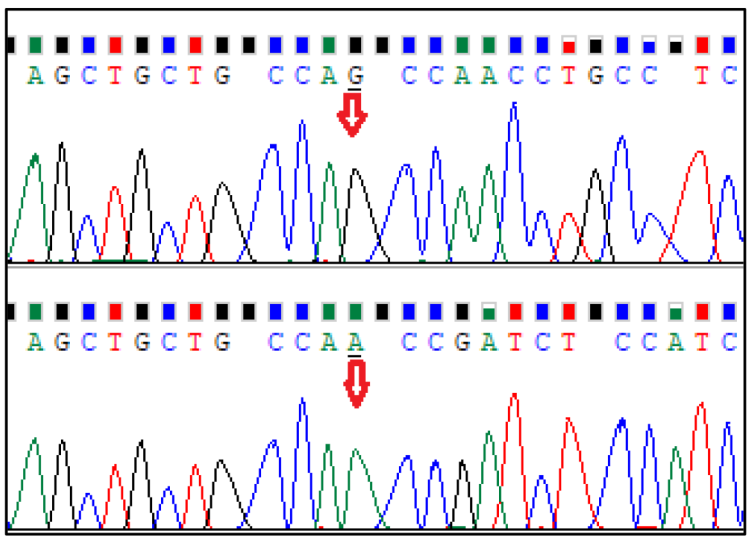

Figure 2. The nucleotide changes from G to A at the 231st position of KAP1.1 gene region in Kilis and Honamlı goat breeds. XM_018065086.1- Capra hircus keratin, high-sulfur matrix protein, mRNA
MACCSTSFCGFPICSTGGTCGSSPCQPTCCQTSCCQPTSIQTSCCQPTSIQ
TSCCQPTSIQTSCCQPISIQTSCCQPTCLQTSGCETGCGIGGSIGYGQVGS
Amino acid residues of the studied samples_MACCSTSFCGFPICSTGGTCG
SSPCQPTCCQTSCCQPTSMADQSAASQPAARPAAASQPPFRPAAASQPP
SRPAAASQPPSRPAAAQPISIQTSCCQPTCLQTSGCETGCETGCGIGGSIG
YGQVGS

XM_018065086.1- Capra hircus keratin, high-sulfur matrix protein, mRNA

MACCSTSFCGFPICSTGGTCGSSPCQPTCCQTSCCQPTSIQTSCCQPTSIQTSCCQP
TSIQTSCCQPISIQTSCCQPTCLQTSGCETGCGIGGSIGYGQVGS

Amino acid residues of the studied samples

MACCSTSFCGFPICSTGGTCGSSPCQPTCCQTSCCQPTSMADQSAASQPAARPA
AASQPPFRPAAASQPPSRPAAASQPPSRPAAAQPISIQTSCCQPTCLQTSGCETGC
ETGCGIGGSIGYGQVGS

Figure 3. The differences of amino acid residues of the KAP1.1 gene among the reference sequence and studied samples.

Table 2. The locations of nucleotide changes in the KAP1.3 gene and the number of animals with these nucleotide changes.

Position	Sheep reference sequence*	Goat reference sequence*	Mutations	Number of animals	NCBI Accession Number#
24	C	C	A	1 (Honamlı)	MT186785
76	C	C	G	1 (Honamlı)	MT186785
91	C	T	T	All samples	MT186779
138	G	G	R	4 (Angora, Honamlı, Kilis)	MT186784
169	C	T	Y	10 (Angora, Honamlı, Norduz, Kilis, Hair)	MT186786,8
185	-	T	C/Y	26 (Angora, Honamlı, Norduz, Kilis, Hair)	MT186787,1
193	-	A	G/R	22 (Angora, Honamlı, Norduz, Kilis, Hair)	MT186790, 84
313	T	T	C/Y	34 (Angora, Honamlı, Norduz, Kilis, Hair)	MT186789,8
354	C	C	G	1 (Honamlı)	MT186785
363	G	G	R	3 (Honamlı, Hair, Kilis)	MT186782
404	T	T	K	2 (Honamlı)	MT186783
410	T	C	T/Y	4 (Angora, Norduz, Hair)	MT186791,80
418	C	C	Y	1 (Honamlı)	MT186781
548	G	G	A/R	21 (Angora, Honamlı, Norduz, Kilis, Hair)	MT186789,82
639	G	G	C	All samples	MT186792

* The NCBI (The National Center for Biotechnology) number of sheep reference sequence is AY835589, the NCBI number of goat reference sequence is JQ772533.
# NCBI Accession Numbers that are submitted to NCBI GenBank database in this study.

et al. [11], 47 bp and 15 bp length two insertions/deletions were observed in Kilis and Norduz breeds but not in Angora breed. Also, these insertions/deletions caused the addition of 15 amino acids. Farag et al. [14] have defined A, B and C alleles of KAP1.1 gene at 341, 311 and 281 bp, respectively with five genotypes in in Egyptian sheep. Different from Farag et al. [14], four alleles of KAP1.1 gene in 401, 371, 351 and 339 bp were detected in this study, and these sequences of alleles were submitted to NCBI GenBank with the accession numbers MW656235-45.

Itenge-Mweza et al. [10], similar to Powell et al. [20], determined 8 genotypes in Merino sheep (except B) from A to I in KAP1.3 gene region. Roger et al. [19] identified 6 alleles of KAP1.3 gene in Romney sheep. Itenge et al. [16] identified nine alleles (from A to I) at the KAP1.3 locus in Merino sheep. In this study, we amplified 611 bp of the KAP1.3 gene region using primers designed by Itenge et al. [16]. However, we have seen none of nine alleles from A to I in the KAP1.3 gene region of studied Turkish native breeds. We identified 14 novel SNPs in the KAP1.3 gene region of studied Turkish native breeds. Honamlı breed has high genetic diversity on the KAP1.3 gene that has most of the SNPs. Angora breed has 10 point nucleotide change on the KAP1.3 gene region. Shah et al. [12] have amplified

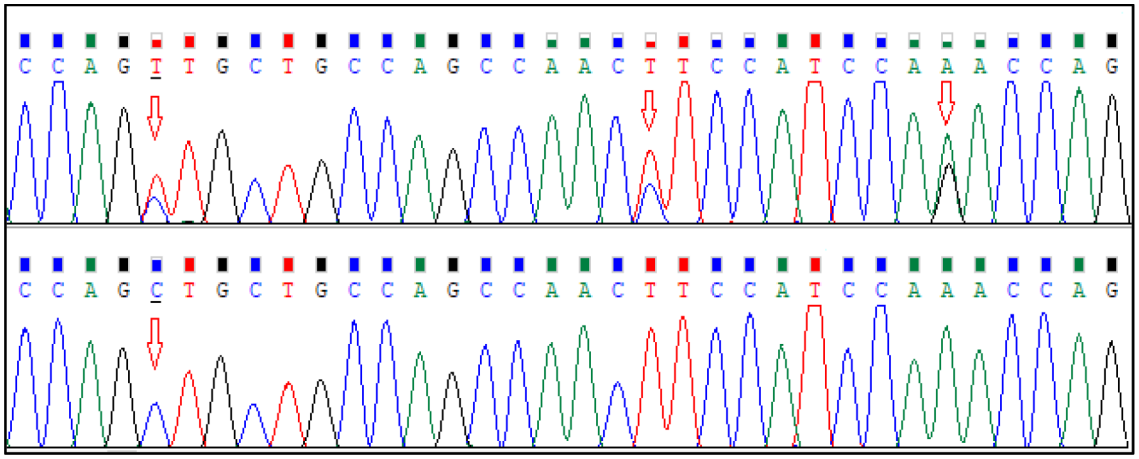

Figure 4. The nucleotide changes at the 169th (from T to C), 184th (from T to C) and 193th (from A to G) positions of KAP1.3 gene region in the studied samples.

Table 3. The locations of nucleotide changes in the K33 gene and the number of animals with these nucleotide changes.

Position	Sheep reference sequence*	Goat reference sequence*	Mutations	Number of animals	NCBI Accession Number#
26	C	A	C	1 (Kilis)	MW656232
27	C	C	T	2 (Kilis, Hair)	MW656231
29	C	C	T	2 (Kilis, Hair)	MW656232
36	G	C	T/A	3 (Angora, Hair, Kilis)	MW656233
51	C	C	T	1 (Kilis)	MW656232
55	-	C	T	1 (Kilis)	MW656232
127	-	C	T/Y	81 (Angora, Honamlı, Norduz, Kilis, Hair)	MW656228
308	A	G	G	All samples	MW656225
310	T	G	S	17 (Angora, Honamlı, Norduz, Kilis, Hair)	MW656234
322	A	C	C	All samples	MW656229
340	T	C	C	All samples	MW656230
355	C	T	Y	1 (Honamlı)	MW656226
358	C	T	Y	1 (Honamlı)	MW656226
364	T	C	Y	1 (Honamlı)	MW656226
427	G	A	C/M	29 (Angora, Honamlı, Norduz, Kilis, Hair)	MW656227
441	C	A	C	15 (Honamlı)	MW656231

*The NCBI (The National Center for Biotechnology) number of sheep reference sequence is NC_040262.1, the NCBI number of goat reference sequence is NC_030826.1.
#NCBI Accession Numbers that submitted to NCBI GenBank database in this study.

622 bp of the KAP1.3 gene region using the same primers with Itenge-Mweza et al. [10] in Changthangi, Bakerwal and Kargil goat breeds. The authors determined six genotypes in KAP1.3 gene using the PCR-SSCP method. While C1C1 and C1C2 genotype frequencies were observed as 0.50 and 0.50 in Changthangi breed, C1 and C2 allele frequencies were determined as 0.75 and 0.25, respectively. We defined 15 different genotypes, none of which are the same as the ones defined by Shah et al [12], and these sequences of genotypes were submitted to NCBI GenBank with the accession numbers MT186779-92.

Itenge et al. [16] have determined five alleles as A, B, C, D and E at the K33 locus in Merino sheep. They have found that the B allele of K33 gene was associated with

staple strength at 12 months of age. Farag et al. [14] have defined seven alleles in K33 gene, which differ from Itenge et al. [16] F and G were new alleles with eight genotypes, AB, DC, BE, DD, DE, DF and DG by sequencing. They have found 8 different band patterns with SSCP analysis, which some of these patterns, P5, P7, P2 and P3, were related with staple length, fiber diameter, clean fleece weight and staple strength, respectively. Sumner et al. [11] reported five variants from A to E of K33 gene that are associated with the decreasing A allele and increasing B allele in the population, which caused increase in the fibre curvature and core bulk and decrease in the fleece weight, staple length and washing yield. Although the impact on fleece properties in sheep is important, there were not any published papers on the identification of the K33 gene on goats related hair traits. To the best of our knowledge, this study is the first research on the determination of genetic variation in somea certain part of the K33 gene in Turkish native goats. We identified 13 nucleotide changes on the studied K33 gene region, and these sequences were submitted to NCBI GenBank with the accession numbers MW656225-34. Kilis breed has a high genetic variation that has 9 of these SNPs of the K33 gene region.

In conclusion, the potential polymorphisms and indels of KAP1.1, KAP1.3 and K33 gene regions were detected in the five Turkish indigenous populations. Genetic identification and analysis showed that these gene regions have various polymorphisms and indels in the studied populations. Yet, it is necessary to prove the interactions with association studies. Therefore, it is speculated that these variations might be candidate genes for hair yield and traits.

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