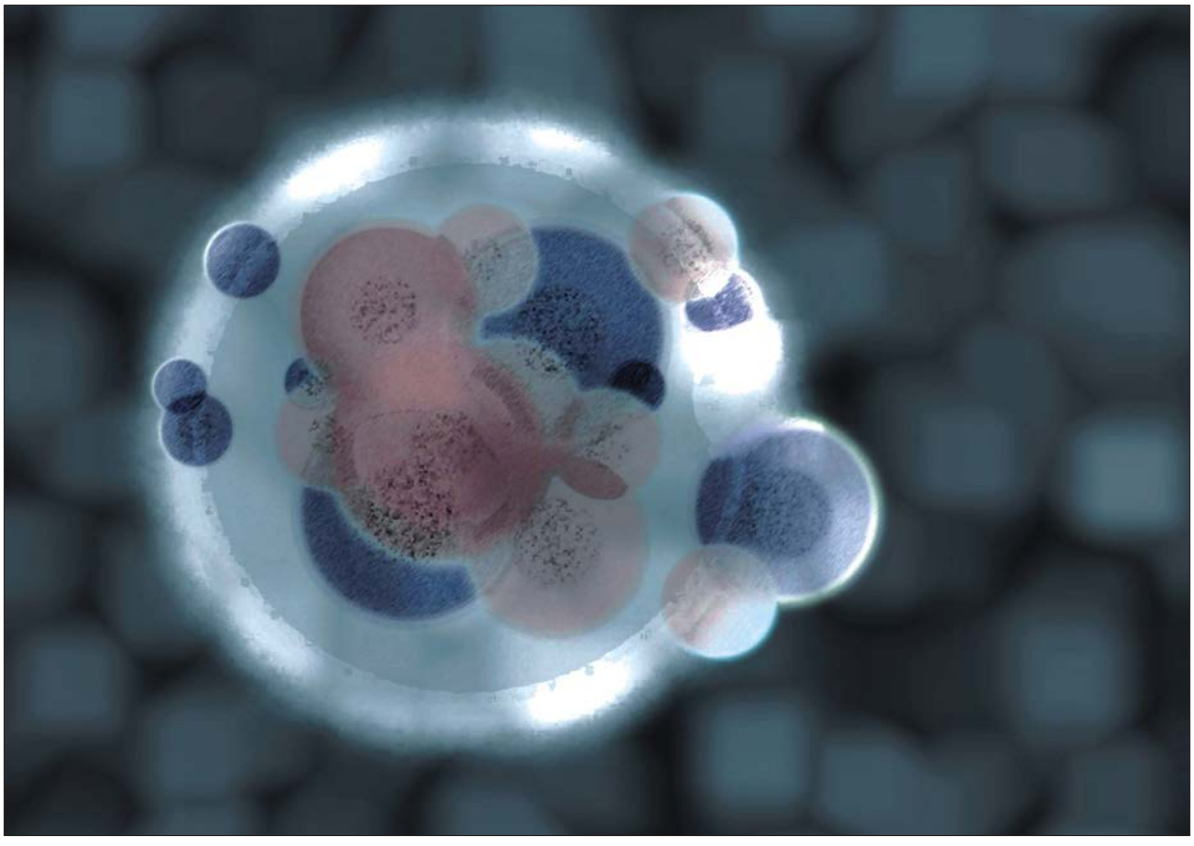

Journal of Biological Sciences

ISSN 1727-3048

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OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2018.158.164

Research Article
Effect of Genetic Polymorphisms of the KAP1.1 and KAP1.3 Genes on Wool Characteristics in Egyptian Sheep

[1]Ibrahim Mohamed Farag, [1]Hassan Ramadan Darwish, [1]Ahmed Mohamed Darwish, [2]Haidan Mostafa El-Shorbagy and [3]Ramadan Wael Ahmed

[1]Department of Cell Biology, National Research Centre, Dokki, 12622 Giza, Egypt
[2]Department of Zoology, Faculty of Science, Cairo University, Giza, Egypt
[3]Department of Wool Production and Technology, Animal and Poultry Division, Desert Research Centre, Cairo, Egypt

Abstract

Background and Objective: Some wool traits were found to be affected by KAPs genes. So, this investigation was carried out to detect polymorphisms in KAP1.1 and KAP1.3 genes and to determine their effects on wool characteristics in sheep breeds. **Materials and Methods:** Blood and wool samples were obtained from 112 animals. Some economically wool characteristics were measured such as staple strength (STr), staple length (STL), clean fleece weight (CFW) and fiber diameter (FD). PCR-agarose gel electrophoresis, PCR-SSCP and nucleotide sequences were analyzed. Data were analyzed using two-way analysis of variance followed by Tukey test for comparisons among subclass means. **Results:** The A, B and C alleles were detected in KAP1.1 gene at 341, 311 and 281 bp, respectively with five genotypes. AB genotype in ewes had longer STL. BC genotype in ewes had the lowest value of MFD and greater mean STr in rams. AC genotype in rams had superior values of CFW. Also, AA genotype in ewes had high yield (CFW) over other genotypes. SSCP analysis of KAP1.3 gene recorded 10 patterns. Sequence analysis on such patterns confirmed seven alleles A, B, C, D, F, G and J with ten genotypes. The highest STL, CFW and STr as well as the lowest MFD were associated with DJ, FG, AB and CC genotypes, respectively. **Conclusion:** The present study showed an association between genetic polymorphisms in KAP1.1 and KAP1.3 genes and improving wool characteristics.

Key words: KAP1.1 gene, KAP1.3 gene, genetic polymorphism, wool characteristics, Egyptian sheep

Received: November 09, 2017 Accepted: February 28, 2018 Published: April 15, 2018

Citation: Ibrahim Mohamed Farag, Hassan Ramadan Darwish, Ahmed Mohamed Darwish, Haidan Mostafa El-Shorbagy and Ramadan Wael Ahmed, 2018. Effect of genetic polymorphisms of the KAP1.1 and KAP1.3 genes on wool characteristics in Egyptian sheep. J. Biol. Sci., 18: 158-164.

Corresponding Author: Ahmed Mohamed Darwish, Department of Cell Biology, National Research Centre, Dokki, 12622 Giza, Egypt Tel: +201100098225

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The major genes that were known to affect structure of wool fiber are keratin genes[1]. The main structure of wool fiber was found to be made up of the proteins that were referred to as KAPs that means keratin intermediate-filament-associated proteins and KRTs that means keratin intermediate-filament proteins[2]. KAPs are considered to be a major structure component of wool fiber[3]. Based on the amino acid composition, the KAPs can be divided into three groups: High sulphers (HS; <30 mol% cysteine), ultra-high sulphur (UHS; >30 mol% cysteine) and high glycine-tyrosine (HGT)[4]. Moreover, according to nucleotide and amino acid sequence homology, 27 families of KAPs across different mammal species have been identified. Of these families, KAP1-3, KAP10-16 and KAP23-27 were found to be high sulfur[5]. Since the wool fiber is mainly made up of the protein keratin, it was anticipated that the presence of variation at the gene codes for KAPs could affect wool quality and quantity. In Romney sheep, staple strength of wool fiber was associated with the polymorphism in KAP1.1 and KAP1.3 genes[6]. Also, fiber diameter was affected by KAPs polymorphisms in Merino sheep[7,8]. Moreover, some wool characteristics such as staple length, staple strength, fiber diameter, yield of greasy fleece weight (GFW) and wool color in Merino sheep had been improved by inducing the polymorphisms in KAP1.1 and KAP1.3 genes[9]. Egyptian sheep breeds were found to be among the farm animals that have been firstly domesticated. These animals were revealed to have important role in wool industry. Therefore, the improving wool characteristics in such animals are an important issue for Egyptian economy. However, information on genes affecting wool traits (especially KAPs genes) of Egyptian sheep breeds is limited. So, this study aimed to detect the genetic polymorphisms in the KAP1.1 and KAP1.3 genes and to identify the favorable alleles or genotypes associated with improving wool characteristics in sheep.

MATERIALS AND METHODS

Animals and samples collection: The present study was carried out during year 2016 at National Research Centre in Egypt. Blood and wool samples were collected from 112 animals of domestic Egyptian sheep including four breads (Barki, Rahmani, Osseimi and Awase) and two crossbreds (Osseimi×Barki and Baladi×Awase). These animals were obtained from Animal Production Farms that are located at Colleges of Agriculture (Al-Azhar, Ain Shams and Cairo Universities) and National Research Center (Nubaria and South Sinai Stations).

Chemicals: DNA extraction kit, agarose, all salts and primers were purchased from Sigma Aldrich Company.

DNA extraction: DNA extraction was done according to manufacture protocols and then stored at -20°C until use.

Primers: The primer used to amplify a 311 bp fragment of KAP1.1 locus and 598 bp region of KAP1.3 gene were designed by Itenge-Mweza *et al.*[9]. Primers for KAP1.1 gene: F (5'-CAA CCC TCC TCT CAA CCC AAC TCC-3'), R (5'-CGC TGC TAC CCA CCT GGC CAT A-3'). Primers for KAP1.3 gene: F (5'-GGG TGG AAC AAG CAG ACC AAA CTC-3'), R (5'-TAG TTT GTT GGG ACT GTA CAC TGG C-3').

PCR amplification: In a total volume of 25 µL, PCR contained 50-100 ng μL^{-1} DNA, 2.5 µL 1×PCR reaction buffer with 0.25 µL *Taq* polymerase, 10 pmol primer, 175 µM dNTPs and 1.5 mM of $MgCl_2$ (Promega, USA). Amplification conditions were as follows: 5 min at 95°C followed by 35 cycles at 94°C for 40 sec; 65°C for 40 sec and 72°C for 45 sec and 72°C for 7 min.

Detection of KAP1.1 genotypes: A 5 µL of PCR product was mixed with 12.5 µL of loading dye [0.1% bromophenol blue, 0.1% xylene cyanol, 20% (w/v) sucrose]. This mixture was loaded on 2% agarose gel.

Detection of KAP1.3 genotypes by SSCP: Amount of PCR product (5-10 µL) was diluted in denaturing solutions that consisted of A and B types. Solution A was prepared of 95% formamide, 10 mM NaOH, 0.05% xylene-cyanol and 0.05 bromophenol blue. Solution B was as the same as solution A, plus 20 mM of EDTA (pH 8). Then, the samples were denatured at 95°C for 5 min, chilled on ice and loaded onto polyacrylamide gel (39:1). A 10% SSCP gel mixture (30 mL) was prepared through acrylamide-bisacrylamide (37.5:1), tetramethylethylenediamine (TEMED) (30 µL) and 10% ammonium persulfate (0.8 mL) in a 1×TBE (90 mM Tris-borate at pH 8.3, 4 mM EDTA) and a voltage of 300 V, running time (6-8 h) and running temperature at 4°C. The gels were stained with ethidium bromide.

Sequence analysis: Different genotypes of KAP1.1 and KAP1.3 genes were purified from PCR product and sequenced by

Marcogen Incorporation, Seoul, Korea. Sequence analysis and alignment were done by cluster wide analysis using Codon Code Aligner software, Codon Code Corporation, USA.

Measurements of wool traits: Wool samples were obtained from mid side from each sheep. Staple length and clean fleece weight were measured according to Chapman[10]. Microscope was used to estimate fiber diameter and image captured by image analysis software (Video Pro, Leading Edge Ltd., S. Aust.). Staple strength was measured according to Caffin[11].

Statistical analysis: The obtained data were analyzed by the least square Mean±SE technique using the general linear model procedure (two-way ANOVA) of SAS software to determine the total variance. Comparisons between subclass means had been made using Tukey test at 0.05 significant level[12].

RESULTS

KAP1.1 gene: The present results identified three bands (alleles) that varied in length (Fig. 1) for KAP1.1gene. These alleles were designated as A, B and C with five genotypes, AA, AB, BB, AC and BC. Nucleotide sequence analysis (Fig. 2) revealed that the length of each of these bands (alleles, A, B and C) were 341, 311 and 281 bp, respectively. As comparison to allele A (341 bp), the nucleotide missing in the B allele (30 nucleotides) is within coding region at position

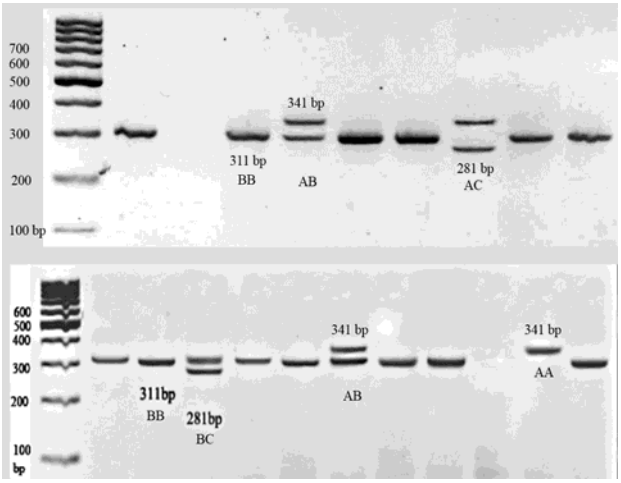

Fig. 1: Five genotypes AA, BB, AB, AC and BC of KAP1.1 gene separated in a 2% agarose gel electrophoresis at molecular weight 341, 311, 341, 281 and 281 bp, respectively

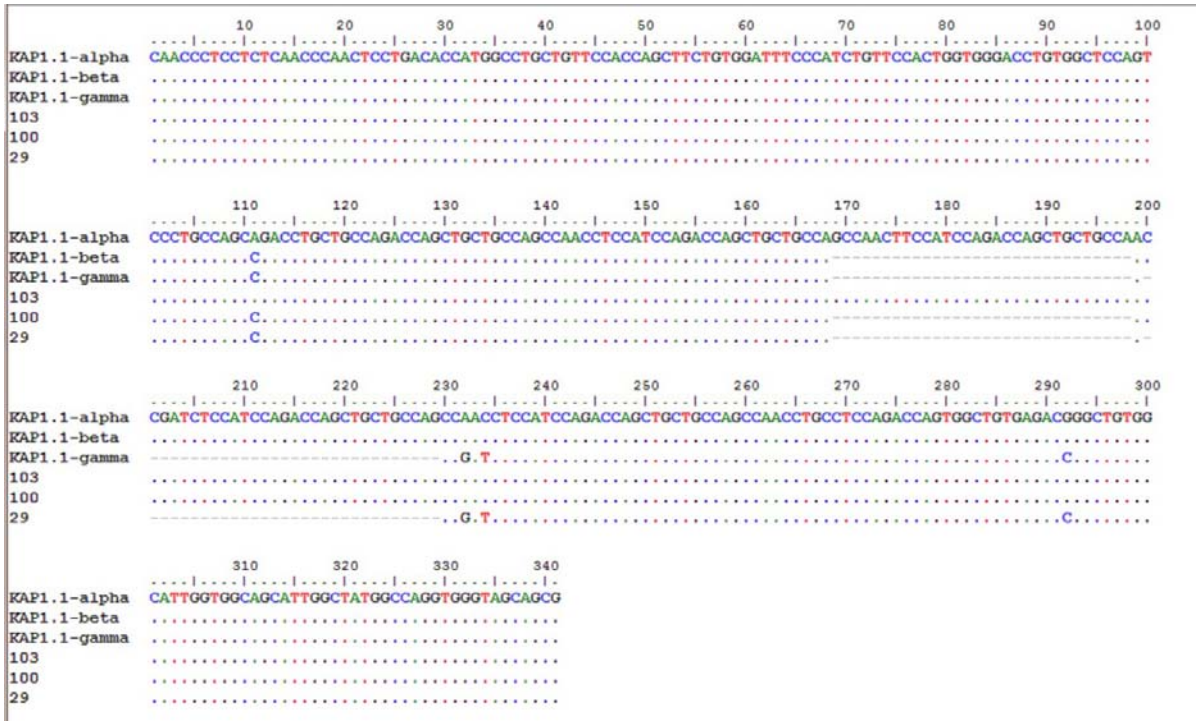

Fig. 2: Alignment of the KAP1.1 alleles sequences (A, B and C)

Table 1: Least square Mean±SE of wool characteristics of different sheep gender according to their genotypes of KAP1.1gene

Genotypes	Sex	STL	FD	CFW	STr
AA	Female	3.0±2.84^{b}	30.3±4.59^{ab}	74.4±7.37^{a}	15.9±14.83^{b}
AB	Male	6.0±1.42^{a}	28.0±5.54^{ab}	53.3±8.88^{b}	15.0±17.88^{b}
	Female	7.7±1.11^{a}	30.7±1.80^{ab}	64.8±2.89^{ab}	32.9±5.81^{a}
BB	Male	6.4±0.60^{a}	33.1±0.98^{a}	67.8±1.57^{ab}	33.8±3.15^{a}
	Female	6.1±0.46	29.9±0.74^{b}	67.5±1.19^{ab}	35.6±2.40^{a}
AC	Male	3.9±2.38^{b}	30.1±3.85^{ab}	81.4±6.18^{a}	26.6±12.43^{ab}
	Female	6.2±1.04^{a}	30.3±1.68^{ab}	68.5±2.70^{ab}	32.6±5.42^{a}
BC	Male	5.1±1.33^{a}	30.3±2.15^{ab}	59.8±3.45^{b}	42.9±6.94^{a}
	Female	6.7±2.04^{a}	24.4±3.31^{b}	54.0±5.31^{b}	35.1±10.68^{a}

Means with small superscript are differ significantly between animal sexes within the same column at p = 0.05, STL: Staple length, FD: Fiber diameter, CFW: Clean fleece weight, Str: Staple strength

Table 2: Least square Mean±SE of wool characteristics of different sheep gender according to their genotypes of KAP1.3 gene

Patterns	Genotypes	Sex	STL	FD	CFW	STr
P1	DJ	Male	7.8±0.92^{a}	32.9±1.56^{ab}	60.4±2.92^{c}	31.6±5.15^{c}
		Female	8.9±0.92^{a}	29.8±1.56^{bc}	58.3±2.91^{c}	34.6±5.15^{bc}
P2	BD	Female	5.3±1.13^{b}	26.6±1.91^{c}	65.4±3.57^{abc}	25.9±6.30^{c}
P3	BG	Male	5.8±1.95^{abc}	37.3±3.32^{a}	69.1±6.19^{abc}	13.4±10.92^{d}
		Female	8.8±1.59^{abc}	32.8±2.71^{ab}	68.9±5.05^{abc}	37.5±8.91^{bc}
P4	FJ	Male	7.4±1.04^{abc}	29.7±1.77^{bc}	64.5±3.31^{bc}	38.9±5.83^{bc}
		Female	7.9±0.87^{abc}	27.5±1.48^{b}	63.4±2.77^{bc}	37.0±4.88^{bc}
P5	CJ	Male	7.1±0.98^{abc}	30.2±1.66^{abc}	69.0±3.09^{ab}	46.9±5.46^{a}
		Female	7.1±1.38^{abc}	29. 5±2.34^{abc}	68.7±4.38^{ab}	38.2±7.72^{bc}
P6	CC	Male	5.3±2.76^{abcd}	25.7±4.69^{abc}	70.1±8.75^{a}	44.8±15.44^{a}
		Female	6.0±1.95^{abcd}	33.3±3.32^{abc}	68.6±6.19^{ab}	62.7±10.92^{a}
P7	AB	Male	6.8±1.95^{abcd}	29.0±3.32^{abc}	65.4±6.19^{abc}	68.1±10.92^{a}
		Female	6.9±1.38^{abcd}	27.6±2.34^{bc}	65.8±4.38^{abc}	55.0±7.72^{a}
P8	DG	Male	5.9±1.95^{abcd}	30.8±3.32^{abc}	64.1±6.19^{bc}	27.8±10.92^{c}
		Female	6.7±1.59^{abcd}	33.8±2.71^{a}	73.1±5.05^{a}	53.4±8.91^{a}
P9	BC	Male	4.5±1.95^{bcd}	30.0±3.32^{abc}	63.8±6.19^{bc}	39.5±10.92^{bc}
		Female	5.9±1.23^{abcd}	29.7±2.10^{abc}	62.9±3.91^{bc}	36.8±6.90^{bc}
P10	FG	Male	3.3±1.23^{d}	30.4±2.10^{abc}	74.9±3.91^{a}	25.9±6.90^{c}
		Female	4.9±1.13^{cd}	31.5±1.91^{abc}	70.1±3.57^{a}	36.3±6.30^{bc}

Means with small superscript differ significantly between animal sexes within the same column at p = 0.05, STL: Staple length, FD: Fiber diameter, CFW: Clean fleece weight, Str: Staple strength

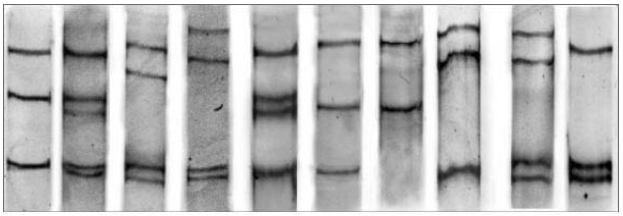

Fig. 3: Ten unique banding patterns were identified in PCR-SSCP analysis of the 598 bp amplimer of the KAP1.3 gene

169-198 bp. While the nucleotide missing in the C allele (60 nucleotides) is within coding region at position 169-198 and from position 200-229. The nucleotide sequence of A, B and C alleles shared 100% homology with GenBank accession numbers AY835603-AY835605, respectively and part of the published KAP1.1 gene sequence under accession number X01610. The present results (Table 1) revealed that AB genotype in ewes followed by BB genotype in rams had the longer value of STL than other genotypes. While, genotype BC had the lowest values of FD and highest values of STr, in female and male sexes, respectively. The results also showed that the genotype AC had the high yield of CFW especially in rams as compared to other genotypes. Also, AA genotype in ewes had high yield of CFW than other genotypes.

KAP1.3 gene: SSCP analysis of 598 bp amplified of KAP1.3 gene recorded 10 patterns in 112 of tested sheep (Fig. 3 and Table 2). Some of these patterns were found to have strong effect on improving wool traits. P1 had the longer value of STL especially in ewes. Whereas, P6 had the lowest value of FD especially in rams as compared to other genotypes. On the other hand, P10 had the high yield of CFW especially in rams or in the total of mean percentages of CFW for rams plus ewes. Also, P7 had the high value of STr especially in rams or in the

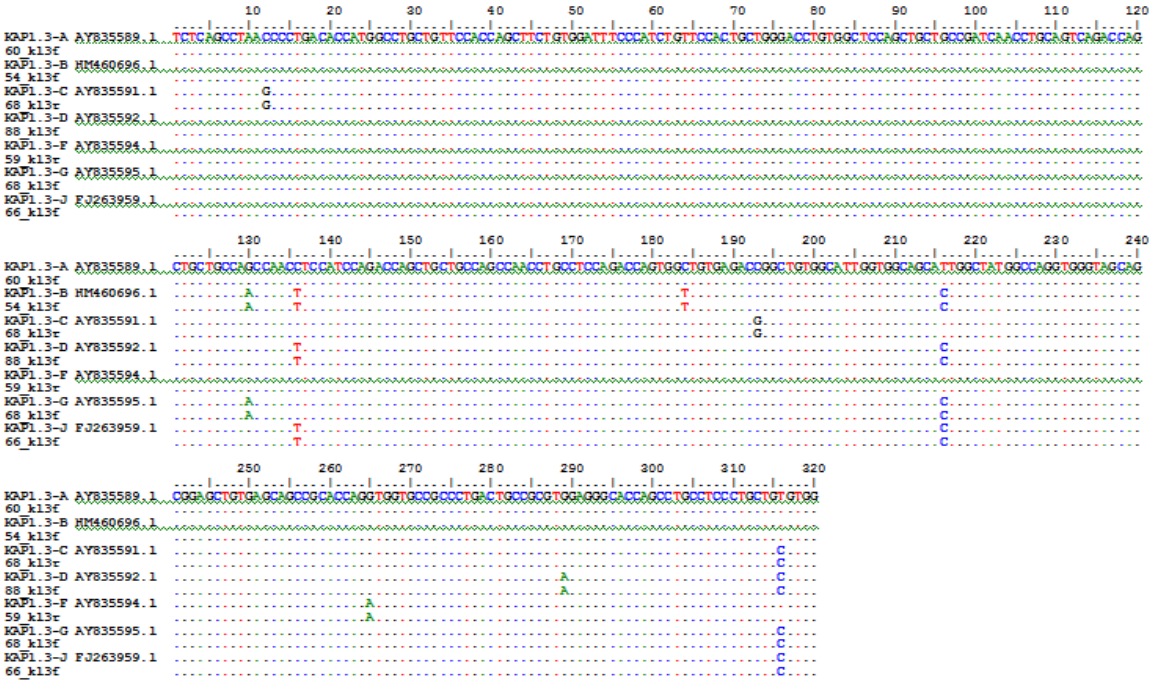

Fig. 4: Alignment of the KAP1.3 allele sequences (A, B, C, D, F, G and J)

total of mean percentages of STr for males plus females. Nucleotide sequence analysis (Fig. 4) on such patterns confirmed seven alleles, A, B, C, D, F, G and J. These alleles are designated as in GenBank accession numbers, HM460695-HM460697 for A, B and C alleles, respectively and AY835592, AY835594, AY835595 and FJ263959.1 for D, F, G and J alleles, respectively. These alleles were found to form ten genotypes, AB, DJ, CC, FJ, BC, DG, CJ, BD, BG and FG (Fig. 4). From the present results, according to nucleotide sequence analysis, it was found that the longer STL was associated with DJ genotype. Also, the improving STr was associated with AB genotype and the high yield of CFW was associated with FG genotype. The lowest fiber diameter (MFD) was associated with CC genotype. The major differences between alleles D and J in genotype DJ are the presence of four nucleotide substitutions within coding region at position 337 A→G. Also, the major differences between alleles A and B in genotype AB (Fig. 4) are the presence of four nucleotide substitutions within coding region at position 132 A→G, 138 T→C, 186 T→ C and 218 C→T. On the other hand, the major differences between alleles F and G in genotype FG are the presence of five nucleotide substitutions within coding region at position 132 A→G, 218 C→T, 267 G→A, 318 C→T and 334 C→T. The dots represent the same nucleotides as published in GenBank accession number HM460697.

DISCUSSION

In the present results, the nucleotide sequences of the KAP1.1 locus (alleles A, B and C) had also been published and can be found at GenBank accession numbers AY835603-AY835605, respectively and part of the published KAP1.1 gene sequence X01610[13]. The length polymorphism of the KAP1.1 gene that observed in this study has also been reported in Romney sheep[6], Merino×Romney×Merino backcross sheep[14], Merino sheep[9] and Merino and Merino-cross sheep[1]. The major difference between the KAP1.1 alleles is that the allele A encodes one more decapeptide repeat than the allele B and two repeats more than the allele C[9]. Rogers *et al.*[4] speculated that the length variation in the KAP1.1 gene was due to genetic events such as unequal crossover and gene conversion. Elleman[15] and Crewther[16] noted that the inserted 30 bp translate to the same consecutively repeated 10 amino acid unit of HS KAPs, this decapeptide repeat constitutes a structural repeat unit which is stabilized by covalent disulphide bonding of the double cysteine residues leading to protection of the wool fiber and inhibition proteolytic attacks. Decapeptide repeat unit might be involved in binding copper as a first step in the catalytic formation of disulphide bonds, where copper is important for the catalytic oxidation of cysteine sulfhydryl to sulphide in the Keratinization process during wool fiber formation[17,6]. Hemsley and Reis[18],

Itenge-Mweza *et al.*[9] and Itenge *et al.*[1] suggested that the A allele (encoding extra decapeptide repeat of KAP1.1 gene) could favorably affect traits related to wool growth, such as greasy fleece weight (GFW) and staple length compared to alleles B or C. This suggestion might be due to that the decapeptide repeat unit contains two cysteine residues and consequently the increased availability of cysteine can increase wool growth[18]. In Merino sheep, the KAP1.1 A allele had a higher yield (79.13%) of greasy fleece weight (GFW) than KAP1.1 B allele (76.68%), while KAP1.1 B allele was associated with increased staple length as compared C allele, however KAP1.1 C allele was associated with brighter wool colour[9]. The inheritance of KAP1.1 A allele in pure New Zealand Merino sheep was associated ($p = 0.036$) with higher yield of wool as compared to KAP1.1 B allele, however the inheritance of the sheep (Merino × Romney ram × Merino ewes) KAP1.1 B allele was associated with increased ($P=0.006$) mean staple length compared to the KAP1.1 C allele and tended to be associated with a decrease ($p = 0.074$) in wool brightness compared to the inheritance of the KAP1.1 C allele[1]. Also, in the present study, the polymorphisms of KAP1.1 gene were found and represented in five genotypes, AA, AB, BB, AC and BC. The longer STL was associated with AB genotype. The high yield of CFW was associated with AC genotype. Whereas, FD and STr was affected by BC genotype. This observation can be explained by Johansson and Rendel[19], who reported that a single gene can be influencing two traits at the same time and such genetic correlation might be caused by the pleiotropy effect of the gene. For instance, in some sheep breeds, it was found a genetic correlation between staple length and fleece weight, where the increase in staple length was associated with increasing in fleece weight[20]. Also, it was found a positive genetic correlation ($r = 0.5$-0.8) between fine diameter and reduction of fleece weight in Romney and Perendale sheep breeds[21-23].

The present study revealed genetic polymorphisms of KAP1.3 gene. These genetic polymorphisms represented in seven alleles (A, B, C, D, F, G and J). Present study results were supported by Powell *et al.*[13], who published the nucleotide and amino acid sequences of KAP1.3 gene (allele B) at GenBank accession number XO2925. Also, six KAP1.3 alleles (α, β, g, V, S, t) of the 598 bp amplimer reported in Romney sheep using SSCP, these polymorphisms were mostly single nucleotide changes in the nucleotide sequences[24,25,6]. Moreover, Itenge-Mweza *et al.*[9] identified in Merino sheep ten alleles (A-J) of KAP1.3 gene. The sequence of nine alleles (A-I) have been published in the NCBI, GenBank with accession numbers: AY835589-AY835597, respectively. A new (tenth) allele named J had a very similar SSCP pattern to that of allele A but differ of the second set of primers. These authors also observed that the polymorphisms were predominantly single nucleotide substitutions. The genotypes DJ, AB, FG and CC that were shown in the present study had been associated with improving wool traits, STL, STr, CFW and MFD, respectively. The effects of genetic polymorphisms of KAP1.3 gene on wool traits were also observed in progeny of Merino sheep that inherited the KAP1.3 B or D alleles had an average yield of 76.58 or 79.12% GFW (greasy fleece weight), respectively, the results of such breed also found that KAP1.3 C allele was associated with lowest staple length, however, this allele was associated with brighter wool colour[9]. Moreover, in the other study, KAP1.3 D allele was tended to be associated with higher yield of wool (GFW) in pure New Zealand Merino Lambs when compared to B allele, however, KAP1.3 B allele was associated with whiter wool in respect to KAP1.3 D allele[1].

CONCLUSION

The present study proved strong association between genetic polymorphisms of KAP1.1 and KAP1.3 genes and amelioration of favorable wool characteristics in Egyptian sheep. These findings suggested making such genetic polymorphisms as candidate gene markers for amelioration of wool characteristics and could be identified before using successful breeding program.

SIGNIFICANCE STATEMENT

This study discovers the genetic polymorphism effect of KAP1.1 and KAP1.3 genes that can be beneficial for wool characteristics. This study will help the researchers to uncover the critical area of the variations between animals in wool characteristics that many researchers were not able to explore. Thus, a new theory on these Single Nucleotide Polymorphisms (SNPs) may be arrived at.

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