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Research Article
Genetic Polymorphism of KRT1.2 Gene and its Association with Improving of Some Wool Characteristics in Egyptian Sheep

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Abstract

Background and Objective: KRT genes were found to be the major factors that affect structure component of wool fiber. So, this study focused on the genetic polymorphism in KRT1.2 gene and identified the favorable genotypes associated with improving wool characteristics in some Egyptian sheep breeds. **Methodology:** One hundred and twelve animals were used to collect blood and wool samples. Measurements of important economically wool traits that involved staple length (STL), fiber diameter (FD), clean fleece weight (CFW) and staple strength (STR) were analyzed. DNA were extracted from whole blood samples. PCR-single strand conformational polymorphism (PCR-SSCP) was used to identify sequence variation. All allelic variation was confirmed by DNA sequencing. **Results:** SSCP analysis recorded 8 unique banding patterns. Some of these patterns, P5, P7, P2 and P3 were associated with strong effects on wool traits, STL, FD, CFW and STR, respectively. Nucleotide sequence analysis identified seven alleles A, B, C, D, E, F and G (F and G alleles are considered to be new ones) with eight genotypes, AB, DC, BE, DD, DE, DF and DG. The longer STL, lowest FD, high yield of CFW and improvement of STR were associated with DF (P5), DE (P7), DG (P2) and DD (P3) genotypes, respectively. **Conclusion:** The present study revealed that there is an association between genetic polymorphism of K33 gene and improving wool traits in Egyptian sheep.

Key words: Genetic polymorphism, KRT1.2 genes, wool traits, PCR-SSCP, genotypes

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

INTRODUCTION

In Egypt, the improvement of wool traits has become necessary for development of textile industry and enhancement of its competitiveness. The production of high quality of wool yield is accomplished through the genetic improvement of sheep. Identification of gene markers of the candidate genes approach that are thought to be responsible for the phenotypic variance of wool traits are very important in animal breeding program. The use of these genetic markers associated with wool quality and quantity lead to genetic improvement by allowing selection choices and eliminating the animals that do not meet breeding goals before they express their phenotypes. The major genes that were known to affect structure of wool fiber are keratin genes[1]. Wool characteristics like oily fleece weight, clean yield, fiber diameter also its coefficient of variation are very notable choice aims in sheep breeding methods, furthermore modern traits like as staple strength and staple length are of increasing importance in the wool industry[2]. Fiber diameter as well as staple strength is major wool quality characteristics that help to rate difference approaching to their act on fiber processing characteristics also the perfect level of the products. Staple strength is more costly to calculate, however due to the great association with the co-operative of difference of fiber diameter, it can be revealed at a part of the value[3]. Wool characteristics have commonly high (fleece weight and fiber diameter) or medium (staple strength) degrees of heritability, yet experiment into definite wool quality genes or targeting particular wool quality characteristics (staple strength, staple length, position of break) is critical for the processing of the product[4].

The opportunity exists to utilize knowledge of major genes that influence the economically important traits in wool sheep. Genes with Mendelian inheritance have been identified for many notable characteristics in wool sheep[5]. The genetic polymorphisms of K33 (KRT1.2) gene was associated with variations in some wool traits such staple length, staple strength, fiber diameter, yield of greasy fleece weight (GFW) and wool colour (brighter and whiter) in Merino sheep and crossbred lambs (Merino × Romney ram × Merino ewes)[6] and variation in fiber diameter of Perendale sheep[7]. So, the aim of the present study was to detect the genetic polymorphisms in the K33 gene and identify the favorable alleles or genotypes associated with improving wool traits in Egyptian domestic sheep breeds.

MATERIALS AND METHODS

Animals: The present study was carried out through year 2016 at National Research Centre. Sheep utilized in this study were four purebreds and two crossbreds. The purebreds included Barki, Rahmani, Osseimi and Awase. The crossbreds involved Ousseimi × Barki and Baladi × Awase. These sheep were sourced from Animal Production Farms in Egypt. These farms located at Faculties of Agriculture (belonging to Universities of Al-Azhar, Ain Shams and Cairo)and National Research Center (that involved Nubaria and South Sinai Stations).

Collection of blood samples: One hundred and twelve animals of the mentioned sheep were used. For each sheep, 5 mL of whole blood was collected from jugular vein using vacuum tubes containing EDTA.

DNA extraction: DNA was extracted from 100 µL of blood according to manufacture instructions and then stored at -20°C until use.

Primers: The primers that utilized in this study to amplify 480 bp region of KRT1.2 gene were previously designed by Rogers *et al.*[7] and Iteng-Mweza[1]. The sequence of the primers are as follows: F5'-CAC AAC TCT GGC TTG GTG AAC TTG-3', R5'-CTT AGC CAT ATC TGG GAT TCC CTC-3'.

PCR amplification: To make up a find volume of 25 µL, 5 µL of DNA (50-100 ng μL^{-1}), 2.5 µL × PCR reaction buffer with 0.25 µL *Taq* polymerase, 175 µM dNTPs, 2.5 mM of $MgCl_2$ and 350 nM of each primer and some deionized water were used. Amplification was carried out under the following conditions: 5 min at 95°C followed by 35 cycles at 94°C for 40 sec; 65°C for 40 sec, 72°C for 45 sec and 72°C for 7 min.

SSCP analysis: A quantity of PCR product (5-10 uL) had been diluted in denaturing solutions that consisted of A and B types. Solution A consisted of 95% of formamide, 10 mM NaOH, 0.05% Xylene-cyanol and 0.05 bromophenol blue; while solution B is the same as solution A, plus 20 mM of EDTA (pH 8.0). A 10% SSCP gel mixture (30 mL) was prepared through acrylamide-bisacrylamide (37.5:1), TEMED (30 uL) 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. Each PCR reaction had been diluted in denaturing solution, denatured at 95°C for 5 min, chilled on ice and

resolved on non-denaturing polyacrylamide gel. The gels were stained with ethidium bromide.

Sequence analysis: The PCR products representing patterns and alleles of tested genes in the percent study were purified and sequenced by special Company (Marcogen Incorporation, Seoul, Korea). Sequence analysis and alignment were performed by cluster wide analysis using CodonCode Aligner software, CodonCode Corporation, USA.

Collection of wool samples and measurements: For each sheep, the samples of 10 staples of wool were taken from left mid side.

Measurements of staple length were made from the base to the dense part of the tip of the staple to the nearest 0.5 cm[8]. Fiber diameter was estimated using a microscope and image captured by image analysis software (Video Pro, Leading Edge Ltd, S. Aust.) and device (LEICAQ 500 MC) with lens 4/0.12. Determination of clean wool weight for each sample was carried out using method of Chapman[8]. Staple strength was estimated by measuring the force required to break the staple in Newton and dividing this value by the thickness of the staple[9].

Statistical analysis: The observed data were analyzed by two-way ANOVA using the general linear model procedure of SAS software. Turkey test was used to determine comparisons among subclass means[10]. Data were expressed in the form of Mean±SE.

RESULTS

The present results showed eight unique banding patterns (Fig. 1, Table 1) had been detected in K33 (KRT1.2) gene using PCR-SSCP typing methods. Some of these patterns (Table 1) were associated with strong effects on wool traits, STL, FD, CFW and STR. P5 had the longer value of STL especially in rams. Also, P7 had the lowest value of FD especially in rams. P2 had the high yield of CFW especially in rams. On the other hand the P3 had the highest value of STR especially in rams as compared to other patterns. So, to identify the favorable alleles associated with improving wool traits nucleotide sequence analysis has been performed on such patterns. This analysis confirmed that SSCP patterns represented seven alleles, A, B, C, D, E, F and G with eight genotypes, AB, DC, BE, EE, DD, DE, DF and DG (Fig. 1-3). A, B, C, D and E alleles were designated as in GenBank accession

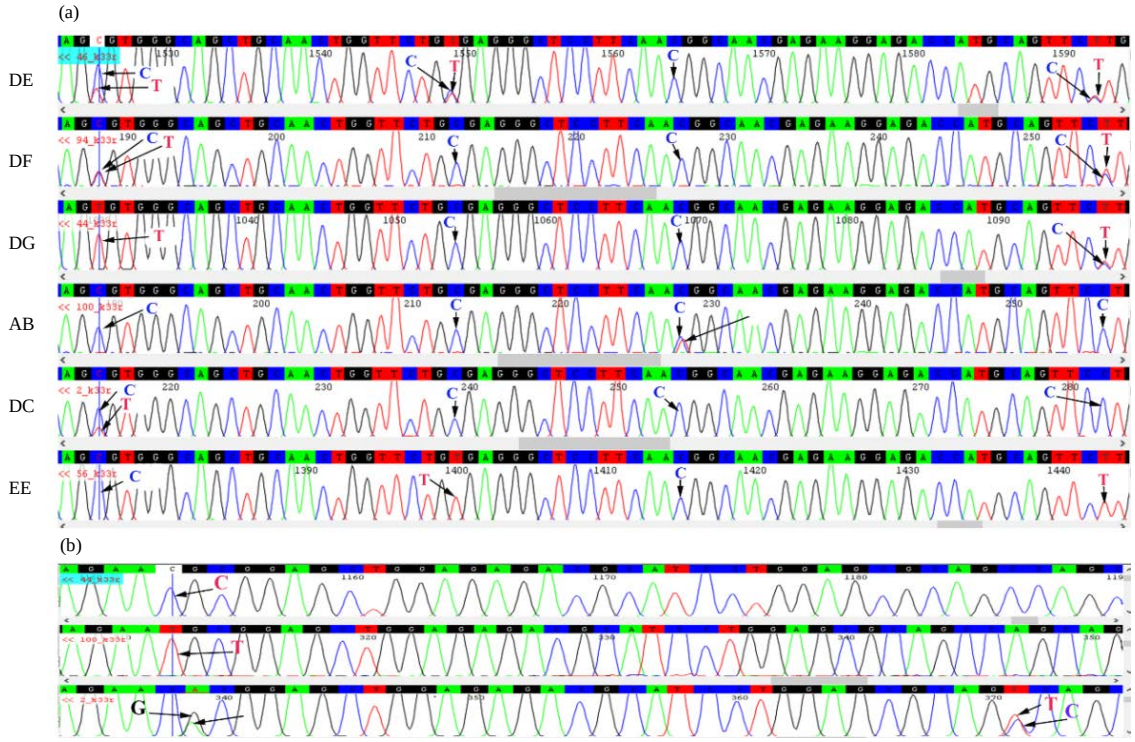

Fig. 1(a-b): Partial chromatograph alignment of different KRT1.2 genotypes. The arrows indicate to various nucleotide polymorphisms

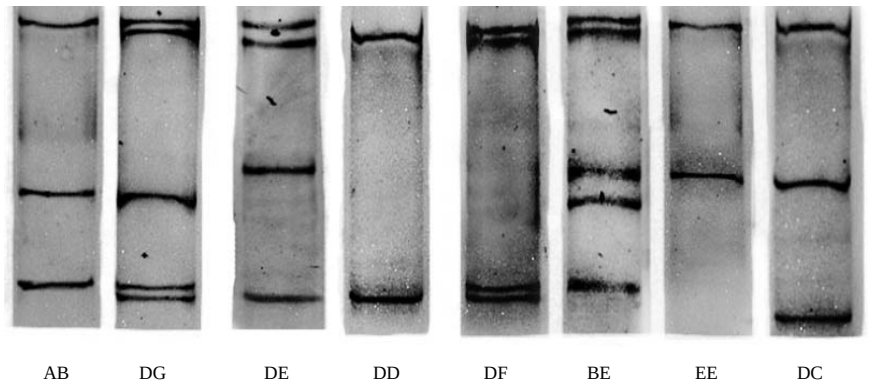

Fig. 2: Eight unique banding patterns were detected in PCR-SSCP analysis of the 480 bp amplimer of the K33 gene

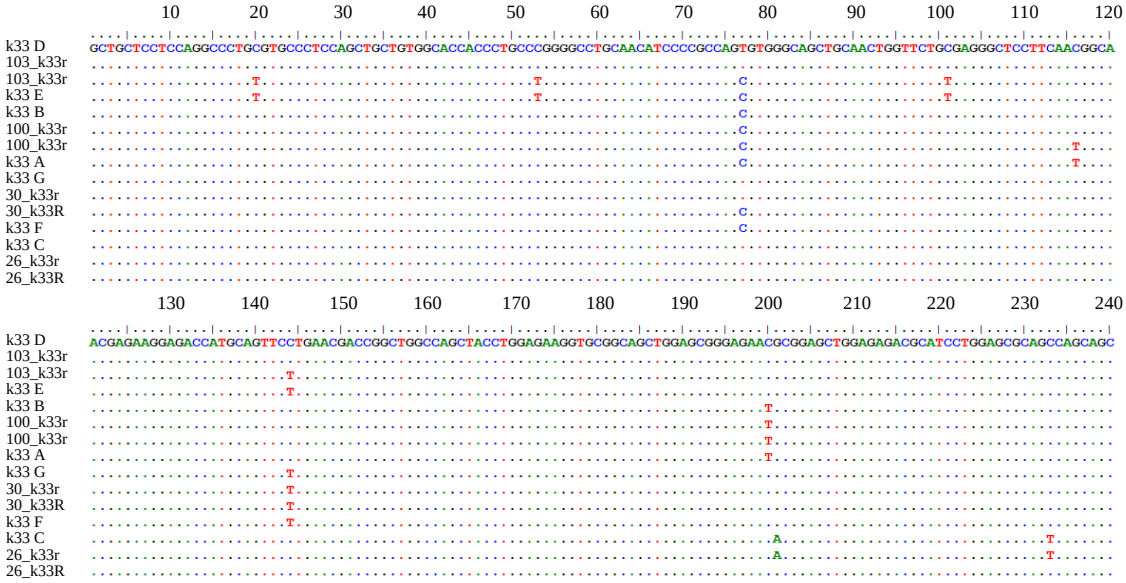

Fig. 3: Alignment of the KRT1.2 alleles (A, B, C, D, E, F and G). The dots represent the same nucleotides as published in GenBank under accession numbers AY835601 and M23912

Table 1: Least square mean±SE of wool characteristics of different sheep sexes according to their genotypes (K33)

Pattern	Genotype	Breed	STL	FD	CFW	STr
P1	AB	Male	5.1±0.77^{bc}	30.8±1.92^{b}	64.5±3.13^{ab}	31.7±5.89^{b}
		Female	5.9±0.65^{b}	30.1±1.64^{b}	68.3±2.68^{ab}	32.8±5.02^{b}
P2	DG	Male	2.9±1.37^{c}	30.7±3.43^{b}	76.3±5.60^{a}	18.3±10.5^{c}
		Female	6.0±1.14^{b}	30.4±2.86^{b}	75. 9±4.67^{a}	23.1±8.76^{c}
P3	DD	Male	5.5±1.54^{bc}	28.3±3.87^{b}	67.4±6.30^{ab}	50.7±11.8^{a}
		Female	9.9±0.72^{a}	34.1±1.81^{a}	58.9±2.96^{b}	30.5±5.56^{b}
P4	EE	Male	3.7±1.22^{c}	33.6±3.07^{a}	61.0±5.00^{b}	33.7±9.39^{b}
		Female	5.9±0.85^{b}	28.3±2.12^{b}	68.98±3.46^{a}	32.1±6.49^{b}
P5	DF	Male	11.1±0.67^{a}	36.4±1.69^{a}	64.5±2.76^{ab}	37.4±5.17^{b}
		Female	7.1±0.59^{b}	36.4±1.69^{a}	60.3±2.42^{b}	37.4±4.55^{b}
P6	DC	Female	5.6±1.13^{b}	26.6±1.49^{b}	63.3±4.63^{ab}	34.2±8.7^{b}
P7	DE	Male	5.0±1.13^{bc}	26.4±2.84^{b}	68.4±4.63^{a}	49.7±8.7^{a}
		Female	6.6±2.17^{b}	29.8±5.4^{b}	57.1±8.87^{b}	47.9±16.7^{a}
P8	BE	Male	2.9±0.93^{c}	28.2±2.3^{b}	66.8±3.78^{a}	22.5±7.1^{c}

Means with capital superscript are differ significantly between animal sexes within the same column

numbers AY835598, AY835599, AY835600, AY835601 and AY835602, respectively. Two new alleles named F and G were found in the present study under accession numbers, KY421398-421399, KY421400-421402, respectively. These alleles were found to form eight genotypes, AB, DC, BE, EE, DD, DE, DF and DG. From the present results, according to nucleotide sequence analysis, it was found that the longer STL was associated with DF genotype. Also, the improving STR was associated with DD genotype and the high yield of CFW was associated with DG genotype. The lowest fiber diameter (FD) was associated with DE genotype.

DISCUSSION

The present study confirmed genetic polymorphisms of K33 gene. These genetic polymorphisms represented in seven alleles, A, B, C, D, E, F and G (F and G alleles are considered to be new ones) with eight genotypes, AB, DC, BE, DD, DE, DF and DG. The longer STL, Lowest FD, high yield of CFW and improvement of STR were associated with DF (P5), DE (P7), DG (P2) and DD (P3) genotypes, respectively. Present study findings were similar with that reported in the previous studies. Itenge *et al.*[11] found five alleles (A-E) were detected at K33 gene in pure Merino lambs and Crossbred lambs (Merino × Romney ram × Merino ewes). All variations in these alleles resulted in from nucleotide substitutions that were found in coding region[11]. Moreover, diallelic polymorphism was found at 480 bp of the KRT1.2 locus using MspI PCR-RFLP in Romney sheep[7,12], the two alleles were identified as a result of the presence/absence of an MspI recognition site at nucleotide 259 bp (allele M/N respectively). Furthermore, Itenge-Maweza[1] searched the MspI restriction site within alleles A-E and revealed that E allele lacked the informative MspI site, while alleles A, B, C and D possessed this site[13].

The genetic polymorphism (nucleotide substitutions) of K33 gene that observed in this study was found to have a strong impact on improving wool traits in Egyptian domestic sheep. The present findings were supported by Kimchi-Sarfaty *et al.*[14] and Rogers *et al.*[15], who reported that the nucleotide substitutions (silent or non- silent) can affect gene expression and protein structure and consequently impact on the wool fiber traits. Also, Rogers *et al.*[15] showed evidence of a QTL for wool strength on chromosome II in the region of KAP1.1, KAP1.3 and K33 genes. In Merino sheep, the allele D in K33 gene was associated with longer staple length, while allele E was associated with brighter wool[13]. Moreover, the inheritance of K33 D was associated with longer staple length and tended to be associated with a decrease in wool brightness compared to inheritance of K33 E in pure Merino lambs and Crossbred lambs (Merino × Romney ram × Merino ewes)[11]. Five alleles of the KRT1.2 gene (A-E) were detected in perendale sheep[6] and presence of A allele was directly opposed to those arising from selection for increased core bulk, while the presence of B and C alleles had been associated with a slightly higher mean of fiber diameter. However, the other remaining alleles were unrelated to the phenotypic expression of any of the measured wool characteristics.

CONCLUSION

It is important to make such genetic polymorphisms as candidate gene markers for improving wool traits and could be identified before using successful breeding program. This program can contribute in development of Egyptian wool textile industry.

SIGNIFICANCE STATEMENT

This study discovers the genetic polymorphism effect of KRT1.2 gene that can be beneficial for wool characteristics. This study will help the researcher 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 SNPs may be arrived at.

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