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Polymorphism of growth hormone gene and its association with wool traits in Egyptian sheep breeds

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Growth hormone (GH) gene has been described as a candidate gene for marker-assisted selection in different farm animals. The present study was designed to identify the polymorphism in GH gene and its association with variation of wool traits in Egyptian sheep breeds. Wool and blood samples were collected from 42 animals including two breeds (Barki and Rahmani) and one crossbred (Rahmani x Awase). Measurements of wool traits were analyzed and involved staple strength (Str), staple length (STL), fiber diameter (FD) and clean fleece yield (CFW). DNA was extracted from blood samples and a 365-bp fragment from exon V was amplified by polymerase chain reaction (PCR). Single strand conformation polymorphisim (SSCP) analysis showed two conformational patterns. The pattern I was recorded to be more frequent (83.3, 92.86 and 90%) than pattern II (16.7, 7.14 and 10%) in Barki, Rahmani and crossbred, respectively. The sequence analysis showed one single nucleotide polymorphism (C/T). The pattern I (allele T) has been found to affect CFW and FD than pattern II (allele C). Whereas, C allele was more pronounced for Str and STL. These traits are the most important parameters determining commercial values of wool that are preferred for clothing or carpets industry. The nucleotide sequences of C and T alleles were submitted to GenBank and have the accession numbers: KT250511 and KT250512, respectively. In conclusion, the present results provide evidence that there is a single nucleotide polymorphism within GH gene in Egyptian sheep breeds. This mutation was found to have some effects on wool traits. Therefore our data show interesting prospects in future selection programs for improving wool industry.

Key words: Sheep, wool, growth hormone (GH) gene, polymorphism, single strand conformation polymorphism (SSCP).

INTRODUCTION

Wool is widely used throughout the world by clothing and carpets industry. The efficiency of wool processing is dependent on the quality of wool fibers, but considerable diversity exists both within and between fleeces and this

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is observed even in inbred lives of sheep (Itenge-Mweza et al., 2007; Itenge et al., 2010). This variation might be better controlled by using the genetic markers that contribute to the wool fiber (Itenge et al., 2010).

The use of genetic markers in the wool industry would allow earlier assessment of an animal's potential and increase the accuracy and efficiency of selection (Itenge-Mweza et al., 2007). Several studies suggested that the growth hormone (GH) gene may influence wool quality and quantity (Sami et al., 1999; Beh et al., 2001; Yousefi and Azari, 2012). GH gene plays an important role in growth regulation and development in sheep (Boyd and Bauman, 1989; Bathaei and Leory, 1998). The ovine GH is about 1.8 kb long and contains five exons and four introns (Hajihosseinlo et al., 2013). Ayuk and Sheppard (2006) reported that GH is an anabolic hormone synthesized and secreted by the somatotroph cells of the anterior lobe of the pituitary gland in a circadian and pulsatile manner. Boyd and Bauman (1989) found that the effects of GH on growth were in several tissues including bone, muscle and adipose tissue. These authors observed that these effects result from both direct action of GH on the partition of nutrients and cellular multiplication as well as IGF-1 mediated action stimulating cell proliferation and metabolic processes and associated with protein deposition.

Generally, in farm animals, the detection of the patterns (polymorphisms) of GH gene might play an important role in growth (Breier, 1999), development (Akers, 2006), lactation (Baldi, 1999), reproduction (Scaramuzzi et al., 1999) and metabolism (Bauman, 1999). Pereira et al. (2005) reported that the polymorphism of GH gene in the Canchim beef cattle, significantly had effects on yearling weight (YW) ($P < 0.05$); these effects were positive and associated with Leucine/Valine (L/V). Also, several studies in bovine, found that there are correlation between polymorphism in GH gene and milk traits (Peel and Bauman, 1987; Lucy et al., 1993; Hoj et al., 1993; Ng-Kwai-Hang, 1997). These researchers showed that the variations of GH gene are considered to be molecular markers for molecular assisted selection to increase milk production and improve milk composition. In sheep, Allain et al. (1998) reported that GH gene is located on chromosome 3, and found segregation for coefficient of variation of wool traits (fiber diameter and staple length) on chromosomes 3 and 4 in a composite sheep line (INRA401). Also, Yousefi and Azari (2012) reported three different conformational patterns in exon V of the GH gene in Zel sheep breed at frequencies of 19% for pattern 1 (G1), 51% for pattern 2 (G2) and 30% for pattern 3 (G3). These results showed that there was a significant ($P < 0.05$) effect of pattern 1 (G1) on staple strength of wool traits.

The polymorphisms of ovine GH have been reported by utilizing different techniques including restriction fragment length polymorphism (RFLP) using restriction endo-nucleases TaqI and PvuII (Gootwine et al., 1996; Ofir and Yossefi, 1996) and EcoRI (Gootwine et al., 1998) as well as PCR- single strand conformation polymorphisim (SSCP) technique (Santos et al., 2004). However, several studies proved that SSCP analysis is an effective technique for the detection of polymorphisms, (Sheffield et al., 1993; Neibergs et al., 1993; Barroso et al., 1999). Comparing with other methods, for instance, denaturing gradient gel electrophoresis, temperature gradient gel electrophoresis and chemical and ribonuclease cleavage, SSCP has several advantages; it does not require specific equipment, it is technically simpler and faster, it can be used in most laboratories and is not very expensive. Thus, SSCP analysis is the technique of choice when screening for point mutations and minor deletions within a given fragment is concerned (Neibergs et al., 1993). Therefore, the present study was designed to identify potential variation in GH gene using PCR-SSCP method and its association with wool traits in Egyptian sheep breeds.

MATERIALS AND METHODS

Sheep

This study used two breeds and one cross-bred of Egyptian domestic sheep. These two breeds are Barki (7 males and 11 females) and Rahmani (7 males and 7 females), whereas, the crossbred was Baladi x Awase (10 females). Barki breed were maintained at Nubaria Farm, National Research Centre, Egypt. Rahmani sheep were sourced from two Animal Production Farms. These farms belong to Faculty of Agriculture, Ain Shams University and Faculty of Agriculture, Al-Azhar University, Egypt. Crossbred (Baladi x Awase) samples were collected from South Sinai (Special Farm, Egypt).

Blood sample collection and DNA extraction

Blood samples were collected from all selected animals by vacuum glass tubes containing EDTA-Na_2 as an anticoagulant reagent. The whole blood samples were stored at -20°C until the time of DNA extraction. DNA was extracted from 100 µl of blood as described by Boom et al. (1990). After estimating the DNA concentration and its purity by spectrophotometer, DNA was diluted in sterile water to be a final concentration of 50 ng/ul before PCR amplification. DNA was also examined by loading samples on 0.7% agarose gel and visualizing the band under gel documentation system.

PCR amplification of the GH locus

The 365-bp fragment of the ovine GH gene was amplified. Based on the ovine GH gene sequence, one pair of oligonucleotide primers were designed to amplify this fragment using the primer 5.0 software (www.primerbiosoft.com). These sequences of the primers used in PCR were F: 5'-GAAACCTCCTTCCTCGCCC-3' and R: 5'-CCAGGGTCTAGGAAGCCACA-3'. These sequences had also previously been reported by Barracosa (1996). To obtain 365 bp fragment of GH exon V, the following PCR mix was used. Each reaction mixture consisted of 12.5 µl of the master mix, 1 µl of the DNA solution (50 ng/µl), 1 µl of each primer (5 pmol) and some de-ionized water making up a final volume of 25 µl. The protocol of PCR amplification was used according to Yousefi and Azari (2012) as follows: An initial denaturation step at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 62°C for

50s and extension at 72°C for 90s and a final extension of 72°C for 10 min.

Single-strand conformation polymorphism analysis

This analysis was done according to Yousefi and Azari (2012) as follows: 5 µl of each amplified product was added to 15 µl of denaturizing solution (95% formamide, 10 mM NaOH, 0.05% xylene cyanol and 0.05 bromophenol blue, 20 mM EDTA). The samples were heat-denatured at 95°C for 5 min, chilled on ice and loaded onto 8% polyacrylamide gel (39:1). The gels were run at 249-280 V for 6-8 h at 4°C. The electrophoresis was carried out in a vertical unit in 1 x TBE buffer. Gels were silver-stained according to the method of Sanguinetti et al. (1994).

Sequence analysis

PCR products representative of different SSCP patterns at GH gene exon 5 were purified and sequenced by Sigma Corporation. Sequence analysis and alignment were carried out using ClustalW2. The nucleotide sequences of detected patterns of GH gene in Egyptian sheep were submitted to GenBank (NCBI, BanKit).

Measurements of wool traits

Wool samples collection

Wool samples were obtained from the left mid-side position of each animal. Ten staples were taken from each greasy sample and used for measuring the percentages of staple length, fiber diameter, staple strength, clean wool yield (El-Gabbas, 1998) and amino acid analysis. These measurements are as follows:

Staple length: Staple length was the average of 10 staples; measurements were made from the base to the dense part of the tip of the staple to the nearest 0.5 cm (Chapman, 1960).

Fiber diameter: Short sections of at least 300 fibers were prepared and mounted in paraffin oil on glass slides and covered with glass cover using the method adopted by ASTM (1974). 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. The average fiber diameter (FD) and standard error (SE) of each sample were calculated.

Clean wool yield (YLD %): Determination of clean wool weight for each sample was carried out by using the method suggested by Chapman (1960) as follows:

$$\text{Clean scoured yield} = \frac{\text{Weight of clean scoured and dry wool}}{\text{Weight of greasy wool}} \times 100$$

Staple strength: 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 in Kilotex. Two staples have been plucked at random from each greasy sample to be prepared for measuring their strength using the Agritest Staple Breaker (Caffin, 1980) and to be in harmonious with the procedure displayed by El-Gabbas (1999).

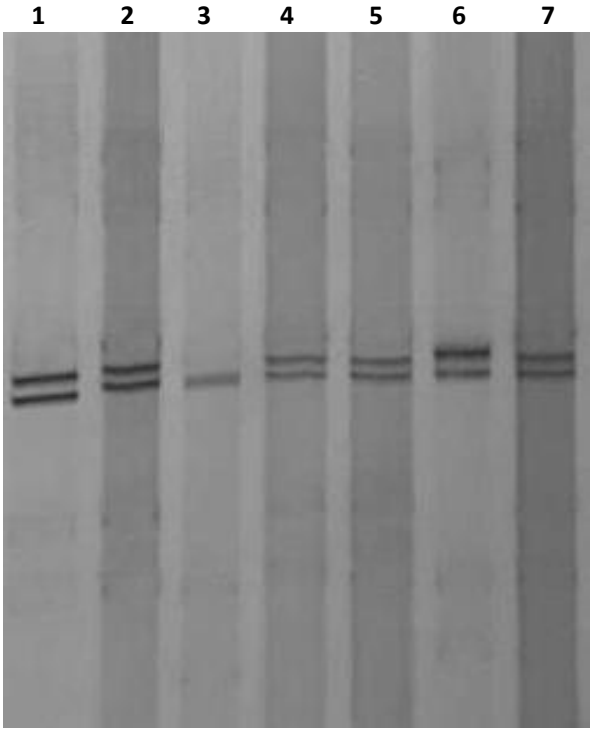

Figure 1. Representatives of PCR-SSCP analysis of 365 bp amplimer of GH gene showing two patterns identified in Barki males of sheep animals. Lanes 1, 2, 4, 5, 6 and 7: Pattern I. Lane 3: Pattern II.

Statistical analysis

Analysis of variance was carried out for the data to split the total variance into its components by the least square technique using the general linear model procedure of SAS (2004). Comparisons among subclass means were also carried out following Tukey test (SAS, 2004).

RESULTS

The PCR-SSCP analysis of 365 bp amplified fragments of GH gene revealed two patterns in forty-two tested Egyptian sheep animals (Figure 1). Pattern I was recorded with high frequency in all investigated sheep in this study as shown in Table 1. Its frequency in Barki males and females were 85.7 and 81.8%, respectively, whereas its frequency in Rahmani sheep was 85.7% in males and 100% in females. Cross-bred females (Baladi X Awase) recorded 90% of pattern I. Rahmani females possessed the highest frequency of pattern I (100%). On the other hand, the results showed the presence of pattern II with few frequencies in all tested sheep. Its frequency in Barki males and females were 14.3 and 18.2%, respectively. Also, the frequency of pattern II in Rahmani sheep was 14.3% in males and 0.0% in females. The females of crossbred (Baladi x Awase) possessed 10% of this pattern II.

Table 1. The pattern frequencies of GH gene in two breeds and one crossbred of tested sheep animals.

Breeds	No. of animals	Pattern frequencies			
		Pattern I		Pattern II	
		No	Frequency	No	Frequency
Barki males	7	6	85.7%	1	14.3%
Barki femals	11	9	81.8%	2	18.2%
Total Barki	18	15	83.3%	3	16.7%
Rahmani males	7	6	85.7%	1	14.3%
Rahmani females	7	7	100%	0	0.0%
Total Rahmani	14	13	92.86%	1	7.14%
Crossbred females (Baladi x Awase)	10	9	90%	1	10%

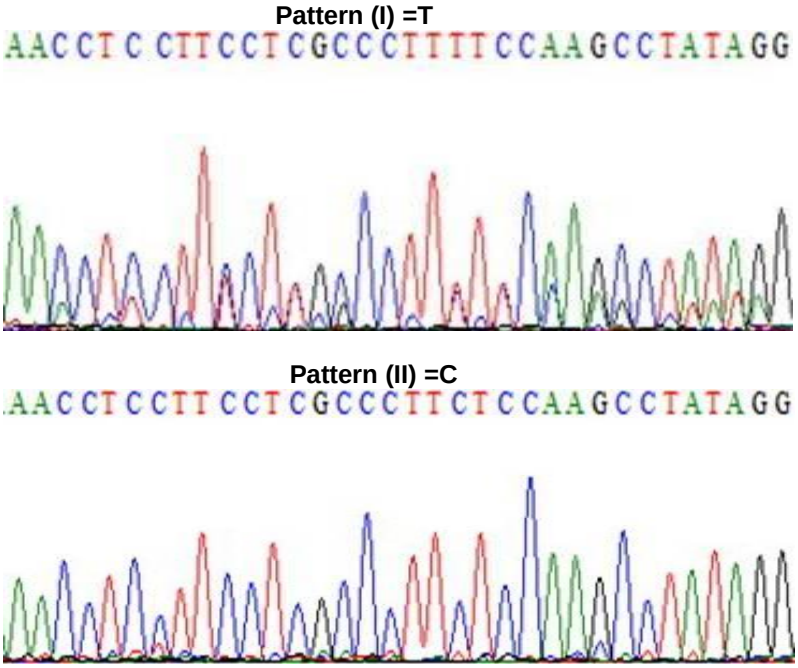

Figure 2. The sequences of the two different patterns.

The sequence analysis showed that there is one single nucleotide polymorphism (SNP) (C/T) in the amplified fragment (365-bp) of GH gene exon 5 (Figure 2). The sequence alignment between the two detected patterns showed the presence of this nucleotide substitution at position 22 of the amplified fragments (Figure 3). The nucleotide sequences of C and T alleles were submitted to GenBank and have the accession numbers: KT250511 and KT250512, respectively.

The association between wool traits and GH polymorphism

Statistical analysis of wool traits and their association with different detected alleles of GH gene is given in Table 2. Although there was only significant differences between pattern I (allele T) and pattern II (allele C) for staple length, however, the present results showed that the CFW and FD traits have been found to be more associated with pattern I (allele T) than pattern II (allele C). On the other hand, the STL and STR traits were more pronounced in pattern II (allele C) as compared to pattern I (allele T).

DISCUSSION

PCR-SSCP is used as a one of the preferred methods for screening samples to detect polymorphism on genetic marker (Itenge-Mweza et al., 2007; Itenge et al., 2009). In this technique, regions of the gene of interest are

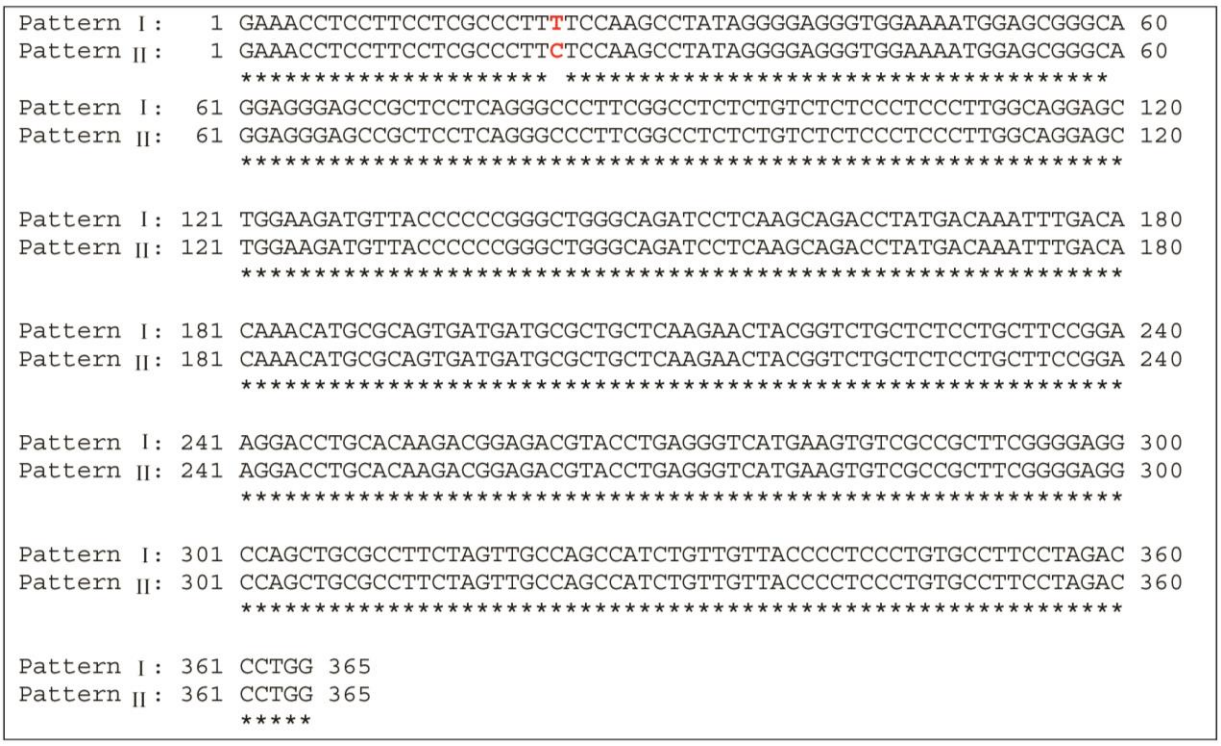

Figure 3. Sequence alignment between two detected patterns.

Table 2. Least square mean of wool characteristics for different sheep breeds according to their gene pattern.

Genotype	STL	FD	CFW	STr
PI (37 animals)	4.12^{B}+0.26	29.92^{A}+0.78	70.79^{A}+1.39	27.89^{A}+2.17
PII (5 animals)	5.80^{A}+0.69	29.63^{A}+2.11	68.20^{A}+3.79	39.08^{A}+5.82

*Means with different capital superscript are differ significantly within the same column. PI = pattern I (allele T), PII = pattern II (allele C), STL: staple length, FD: fibers diameter, CFW: clean fleece weight, STr: staple strength.

amplified using PCR and the products denatured and then cooled rapidly to promote the formation of secondary structures due to internal base-pairing, which are in turn sequence dependent (Orita et al., 1989; Vignal et al., 2002). The folded single-strand DNA molecules are separated by polyacrylamide gel electrophoresis under non-denaturing conditions. Molecules that differ by even a single nucleotide may form different conformers under a given set of conditions and upon electrophoresis in a non-denaturing polyacrylamide gel, migrate differently (Orita et al., 1989; Vignal et al., 2002). So, those differences can be observed as a shift in the electrophoretic mobility (Hayashi, 1991). Also, Hayashi (1991) estimated PCR-SSCP analysis sensitivity (probability of detecting at least one strand shifted) as more than 99% for 100 to 300 bp fragments and 89% for 300 to 450 bp fragments. Thus, PCR-SSCP technique was used in this study to detect the polymorphism in exon 5 of GH sheep gene in two breeds and one crossbred of Egyptian domestic sheep.

The results showed two patterns of GH gene in tested animals with higher frequency of pattern I as compared to pattern II. Current results of GH polymorphism are similar to that reported in previous study by Bastos et al. (2001) on Portuguese indigenous sheep breed "Churra da Terra Quente". These authors identified two conformational patterns using SSCP analysis of exon 4 of the GH gene and they also observed five different conformational patterns in exon 5 of the same gene. Also, five ovine GH exons were analyzed by Marques et al. (2001) using PCR-SSCP in 200 Portuguese Serrada Estrela ewes. Their results revealed that all exons with the exception of exon 1 were polymorphic. Shiri et al. (2006) found three conformational patterns in exon 4 of GH gene in Kordian sheep. Tahmorespoor et al. (2011) and AhaniAzari et al. (2011) revealed three conformational patterns using the SSCP method in exon 5 of GH gene in Balouchi and Dalagh sheep breeds. Yousefi and Azari (2012) reported

three different conformational patterns (alleles) in exon 5 of the GH gene located on chromosome 3 in Zel sheep breed, 19% for pattern I (G_1), 51% for pattern 2 (G2) and 30% for pattern 3 (G3).

In the present study, the association between polymorphism in GH gene and variation in wool traits were also observed. The CFW and FD traits have been shown to be more associated with pattern I than pattern II. On the other hand, the STL and STr traits were pronounced in pattern II as compared to pattern I. The effect of GH polymorphism on wool traits was reported in some other studies. For example, Yousefi and Azari (2012) observed a significant effect ($P < 0.05$) between polymorphism at GH gene and wool staple strength in Zel sheep. Also, in previous study, Allain et al. (1998) found a segregation for coefficient of variation of wool traits (fiber diameter and staple length) on chromosomes 3 and 4 in INRA40/sheep, where GH gene is located on chromosome 3.

However, several studies in sheep and bovine showed the effect of GH polymorphism on other animal production traits. The association between polymorphism at GH gene and milk yield was observed in Serra da Estrela sheep by Marques et al. (2001). In Makooei sheep, a significant association between polymorphism at GH gene and growth traits (weaning weight, six month weight and nine month weight) was revealed by Moradian et al. (2013). Moreover, Koch et al. (2010) reported that ewes treated with GH had higher of fetal growth and development in lambs. Furthermore, in bovine, Lagziel et al. (1996, 1999) found an association between the percentage of milk protein and polymorphism at GH gene.

Falaki et al. (1996, 1997) revealed in Simmental and Holstein-Friesian cattle, the association between the polymorphism GH-Taq1 and milk traits. Also, the association of a polymorphism in exon 5 of GH with selection for milk yield was reported in Holstein-Friesian cattle by Lee et al. (1996).

In contrast, Folch et al. (2001) reported that the GH treatment did not significantly have effect on the percentage of ewes in estrus and the ovulation rate. Also, Yousefi et al. (2013) determined three (A, B and C) different conformational patterns in exon 5 at GH gene of Zel sheep. The frequency of pattern B (0.39) was higher than the pattern A (0.32) or C (0.29). However, their results revealed no significant association between lambing rate and conformational patterns of GH gene.

Furthermore, DNA sequencing analysis of GH gene in this study indicated that the sequences of PCR products are in correspondence with the sequences in the GenBank. SSCP analysis and sequencing allowed the detection of a single nucleotide change for GH gene exon 5. Sequence analysis revealed a point mutation (C to T) at position 22 in the amplified fragment with accession number KT250511 for C allele and KT250512 for T allele. To the best of the author knowledge, this is considered to be the first report on detection of the single nucleotide polymorphism C/T at GH gene exon 5 of the sheep. In another study, a nucleotide change C/T was detected in Baluchi sheep breed at nt 271 of IGF – 1 gene. The same mutation (in IGF – 1 gene) has been detected in 8 different sheep breeds selected throughout Europe (Pariset et al., 2006) and in three Italian dairy sheep (Scata et al., 2010). These researchers found that allele T exerted a positive effect on maintaining a constant milk yield level during lactation. In the present study, allele T in GH gene exon 5 has been found to be affected by CFW and FD traits than C allele. Whereas, C allele has been shown to be major agent for increasing the STr and STL traits. These traits are the most important parameters determining the commercial values of wool that are preferred for clothing or carpets industry (Itenge-Mweza, 2007). Also, the association between polymorphism in GH gene area (that are located on chromosome 3) and variation in fibre diameter and staple length has been reported in a composite sheep line (INRA 401) (Allain et al., 1998). Moreover, Yousefi and Azari (2012) detected three different conformational patterns (G1, G2 and G3) in exon V of GH gene in Zel sheep breed. Though researchers did not do the sequence analysis of DNA in the mentioned patterns, however, their results showed that these was a significant effect ($p < 0.05$) between pattern G1 and staple strength of wool traits.

Conclusion

In conclusion, the present results confirmed polymorphism within GH gene of Egyptian sheep breeds. This polymorphism may be considered to be important genetic marker to be used in selection programs for improving the wool industry.

Conflict of Interests

The authors have not declared any conflict of interests.

Abbreviations

PCR, Polymerase chain reaction; **SSCP,** single strand conformation polymorphisim; **RFLP,** restriction fragment length polymorphism; **GH,** growth hormone; **Str,** staple strength; **STL,** staple length; **FD,** fiber diameter; **CFW,** clean fleece yield; **EDTA,** ethylene diammine tetraacetic acid; **TBE,** tris-borate EDTA.

REFERENCES

AhaniAzari M, Yousefi S, Dehanvi E (2011). Evaluation of K-casein and Growth Hormone Gene Polymorphism in Native Dalagh Sheep.

Slovak. J. Anim. Sci. 44(4):129-133.
Akers RM (2006). Major advances associated with hormone and growth factor regulation of mammary growth and lactation in dairy cows. J. Dairy Sci. 89(4):1222-1234.
Allain D, lantier I, Elsen JM, Francois D, Brunel JC, Weisbecker JL, Schibler L, Vaiman D, Cribiu E, Gautier A, Berthon P, Lantier F (1998). A design aiming at detecting QTL controlling wool traits and other traits in the INR401 sheep line. In Proc. 6th World Cong. Genet. Appl. Livest. Prod. Armidale. pp. 11-16.
ASTM. Standard, annual book (1974). Diameter of wool and other animal fibres by micro-projection, 32:418.
Ayuk J, Sheppard MC (2006). Growth hormone and its disorders. Postgrad. Med. J. 82(963):24-30.
Baldi A (1999). Manipulation of milk production and quality by use somatotropin in dairy ruminants other than cow. Domest. Anim. Endocrinol. 17:131-137.
Barracosa H (1996). Study of the genetic polymorphisms of k-casein and GH genes and their association with milk production capabilities in Algarve and Serra da Estrela sheep. Dissertation, University of Algarve, Faro, Portugal.
Barroso A, Dunner S, Canton J (1999). Technical note: use of PCR-single strand conformation polymorphism analysis for detection of bovine β-casein variants A^1, A^2 $A^3$and B. J. Anim. Sci. 77:2629-2632.
Bastos E, Cravador A, Azevedo J, Guedes-Pinto H (2001). Single strand conformation polymorphism (SSCP) detection in six genes in Portuguese indigenous sheep breed "Churrada Terra Quente". Biotechnol. Agron. Soc. Environ. 5:7-15.
Bathaei SS, Leroy PT (1998). Genetic and phenotypic aspects of the growthcurve characteristics in Mehraban Iranian fat-tailed sheep. Small Rumin. Res. 29:261-269.
Bauman DF (1999). Somatotropin mechanism in lactating cows: from basic science to commercial application. Domest. Anim. Endocrinol. 17:101-116.
Beh KJ, Callaghan MJ, Hulme DJ, Lenane I, Maddox JF (2001). A genome scan for QTL affecting fleece and wool traits in Merino sheep. Wool Technol. Sheep Breed. 48:88-89.
Boom R, Sol CJA, Salimans M (1990). Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. 28:495-503.
Boyd RD, Bauman DE (1989). Mechanisms of action for somatotropin in growth. In: Champion DR, Hausman GJ, Martin RI (eds). Animal Growth Regulation. Plenum Publishing Corporation, NewYork, NY, USA, pp. 257-293.
Breier BH (1999). Regulation of protein and energy metabolism by the somatotropic axis. Domest. Anim. Endocrinol.17:209-218.
Caffin RN (1980). The CSIRO staple strength/length system. I. Design and performance. J. Text. Inst. 71(2):65-70.
Chapman RE (1960). In the Biology of the fleece, edited by A. S. Fraser, and B. F. Short, Animal Research Laboratories Technical Paper No. 3. CSIRO, Australia.
El-Gabbas HM (1998). Wool fibre diameter and its distribution in Barki sheep with regard to the sampling positions and season. Egypt. J. Anim. Prod. 35(2):123-141.
El-Gabbas HM (1999). Compressional properties of the coarse wool Barki fleeces. Alex. J. Agric. Res. 44(2):45-55.
Falaki M, Prandi A, Corradini C, Sneyers M, Gengler N, Massarr S, Fazzini U, Burny A, Portetelle D, Renville R (1997). Relationship of growth hormone gene and milk production traits in Simmental cattle. J. Dairy Res. 64:47-56.
Falaki M, Sneyers M, Prandi A, Massart S, Corrandini C, Formigoni A, Burry A, Portetelle D, Renaville R (1996).TaqI growth hormone genepolymorphism and milk production traits in Holstein-Friesian cattle. Anim. Sci. 63:175-181.
Folch J, Ramn JP, Cocero MJ, Alabart JL, Beckers JF (2001). Exogenous growth hormone improves the number of transferable embryos in superovulated ewes. Theriogenology 55(9):1777-1785.
Gootwine E, Ofir R, Yossefi S (1996). Characterization of Pvull polymorphisms between the ovine growth hormone GH2-N and GH2-Z gene copies. Anim. Biotechnol. 7:135-143.
Gootwine E, Suttie JM, Mcewan JC, Veenvliet BA, Littlejohn RP, Fennessy PF, Montgomery GW (1998). The physiological effects of natural variation in growth hormone gene copy number in ram lambs. Dom. Anim. Endocrinol. 14:381-390.
Hajihosseinlo A, Semsarnejad A, Abollow E, Hasbrafi F, Negahdary M (2013). Effect of GH gene polymorphisms on biometric traits in Makooeisheep. Ann. Biol. Res. 4(6):351-355.
Hayashi K (1991). PCR-SSCP: A simple and sensitive method for detection of mutations in the genomic DNA. PCR Methods Appl. 1:34-38.
Hoj S, Fredhelm M, Larsen N, Nielsen V (1993). Growth hormone gene polymorphism associated with selection for milk fat production in lines of cattle. Anim. Genet. 24:91-96.
Itenge TO, Hickford JGH, Forrest RHJ, Mckenzie GW, Frampton C (2010). Association of variation in the Ovine KAP1.1, KAP1.3 and K33 genes with wool traits. Int. J. Sheep Wool Sci. 58(1):1-15.
Itenge TO, Hickford JGH, Forrest RHJ, Mckenzie GW, Frampton CM (2009). Improving the quality of wool through the use of gene markers. S. Afr. J. Anim. Sci. 39(1):219-223.
Itenge-Mweza TO, Forrest RHJ, Mckenzie GW, Hogan A, Abbtt J, Amoafo O, Hickford JGH (2007). Polymorphism of the KAP1.1, KAP1.3 and K33 genes in Merino sheep. Mol. Cell. Probes 21:338-342.
Koch JM, Wilinoth TA, Wilson ME (2010). Periconceptional growth hormone treatment alters fetal growth and development in lambs. J Anim. Sci. 88:1619-1055.
Lagziel A, Lipkin E, Ezra E, Soller M, Weller JI (1999). An M5pI polymorphism at the bovine growth hormone (bGH) gene is linked to a Locus affecting milk protein percentage. Anim. Genet. 30(4):296-299.
Lagziel A, Lipkin E, Soller M (1996). Association between SSCP haplotypes at the bovine growthhormone gene and milk protein percentage. Genetics 142:945-951.
Lee BK, Lin CF, Crooker BA, Murtaugh MP, Hansen LB, Cherster-Jones H (1996). Association of somatotropin (BST) gene polymorphism at the 5^{th} exon with selection for milk yield in Holstein cows. Domest. Anim. Endocrinol. 13:373-381.
Lucy M, Hauser S, Eppard P, Krivi G, Clark J, Bauman D, Collier R (1993). Variants of somatotropin in cattle: gene frequencies in major dairy breeds and associated milk production. Domest. Anim. Endocrinol. 10:325-333.
Moradian C, Mohamadi N, Razavi-Sheshdeh SA, Hajihosseinlo A, Asharfi F (2013). Effect of genetic polymorphism at the growth hormone gene on growth traits in Makooei sheep. Eur. J. Exp. Biol. 3(3):101-105.
Marques MR, Santos IC, Belo CC, Cravador A (2001). Associations between SSCPs in the GH gene and milk traits in "Serra da Estrela" ewes. I: Procedings of the IV. International Conference on Farm Animal Endocrinology. BASE. Gembloux, Belgium 5:57.
Neibergs H, Dietz A, Womack J (1993). Single-Strand Conformation Polymorphisms (SSCPs) detected in five bovine genes. Anim. Genet. 24:81-84.
Ng-Kwai-Hang K (1997). A review of the relationship between milk protein polymorphism and milk composition/milk production. In: Proceedings of the IDF Seminar. Palmerston North, New Zealand: IDF. Special issue 9702. pp. 22-37.
Ofir R, Yossefi S (1996). Characterization of Pvull polymorphisms between the ovine growth hormone GH2-N and GH2-Z gene copies. Anim. Bioechnol. 7:135-143.
Orita M, Iwahana H, Kanagawa H, Hayashi K, Sekiya T (1989). Detection of polymorphism of human DNA by gel electrophoresis as single strand conformation polymorphism. Proc. Natl. Acad. Sci. USA. 86:2766-2770.
Pariset L, Capuccio I, Ajmone-Marasan P, Bruford M, Dunner S, Cortes O, Erhardat G, Prinzenberg EM, Gutscher K, Joost S, Pinto-Juma G, Nijman IJ, Lenstra JA, Perez T, Valentini A, Consortium E (2006). Characterization of 37 Breed – Specific Single - Nucleotide Polymorphisms in Sheep. J. Hered. 97:531-534.
Peel C, Bauman O (1987). Somatotropin and lactation. J. Dairy Sci. 70: 474.
Pereira AP, de Alencar MM, de Oliveira HN, de Almeida Regitano LC (2005). Association of GH and IGF-1 polymorphisms with growth trails in a synthetic beef cattle breed. Genet. Mol. Biol. 28(2):230-236a.
Sami AJ, Wallis OC, Wallis M (1999). Production and characterization of deletion mutants of ovine growth hormone. J. Mol. Endocrinol. 23:97-106.

Sanguinetti CJE, Neto D, Simpson AJ (1994). Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques 17:914-921.

Santos IC, Marques MR, Belo CC, Cravador A (2004). Polymorphism anlaysis at the growth hormone gene in Merino da Beira Baixa ewes. Biotechnol. Agron. Soc. Environ. 8:40-41.

SAS (2004). Statistical Analysis System. SAS Users Guide: Statistics. SAS Institute Inc. Editors, Cary, NC.

Scaramuzzi RJ, Murray JF, Downing JA, Campbell BK (1999). The effects of exogenous growth hormone on follicular steroid secretion and ovulation rate in sheep. Domest. Anim. Endocrinol. 17:269-277.

Scata MC, Catillo G, Annicchiarico G, De Matteis G, Napolitano F, Signorelli F, Moioli B (2010). Investigation on Lactation Persistency and IGF – I gene Polymorphisms in Dairy Sheep. Small Rumin. Res. 89:7 -11.

Sheffield V, Beck I, Kwitck A, Sandstron D, Stone E (1993). The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. Genomics 16:325-332.

Shiri SAK, Saghi DA, Nasiri MR, Emrani H, Montazer Torbati F, Mohammadzade M (2006). Survey of genetic diversity growth hormone and growth hormone receptor genes in Iranian indigenous sheep breed (koridan sheep using non-radioactive SSCP. 57th Annual Meeting of the European Association for Animal production, Antalya, Turkey.

Tahmorespoor M, Taheri A, Gholami H, Ansary M (2011). PCR-SSCP variation of GH and STAT5A Genes and Their Association with Estimated Breeding Values of Growth Traits in Baluchi Sheep. Anim. Biotechmol. 22(1):37-43.

Vignal A, Milan D, San-Cristobal M, Eggen A (2002). A review on SNP and other types of molecular markers and their use in animal genetics. Genet. Sel. Evol. 34:275-305.

Yousefi S, Azari MA (2012). Genetic effect of growth hormone gene on yearling weight and wool traits in Zel Sheep. Arch. Tierz. 55(3):303-306.

Yousefi S, Shahmohammadi L, Azari MA, Zerehdaran S, Dehnavi E (2013). Survey of Fex^{L} locus of BMP15 gene and growth hormone (GH) gene and their effects on lambing rate in Zel sheep. Iran. J. Appl. Anim. Sci. 3(2):351-355.