Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Genetic polymorphism of the growth hormone (GH5) gene in small ruminants

Mohamed A. Abdelhafez*, Noha M. Osman, Neama I. Ali and Karima F. Mahrous

Department of Cell Biology, National Research Centre, El Buhouth Street, 12311, Dokki, Egypt

Abstract

The present study was conducted to detect polymorphism of growth hormone (GH5) gene in different ovine and caprine breeds. A 468 bp fragment of growth hormone gene spanning over 5'UTR, exon1, intron1 and partial of exon 2 were amplified and digested with *Hae*III endonuclease restriction enzyme to identify polymorphism at this locus and the results were confirmed by DNA sequence analysis. Screening polymorphisms within three caprine (n=52), and four ovine (n=67) breeds for GH5 gene was performed. For goats, Damascus and Zaraibi carried both the G and H alleles, but Barki breed displayed only the G allele. Both Damascus and Zaraibi goats carried both the G and H alleles with allele frequency of 0.80, 0.72 and 0.20, 0.28, respectively, and the GG genotype was the utmost prevailing in the examined breeds (70% in Damascus and 56% in Zaraibi). Nevertheless, Barki disclosed only the G allele. In sheep, all studied breeds appeared to carry only the H allele. Moreover, eight single nucleotide polymorphisms (SNPs) and one Indel were observed in Egyptian goat breeds and Damascus breeds in comparison to goat sequence accession No. D00476.1. While four single nucleotide polymorphisms (SNPs) were detected in different Egyptian sheep breeds in comparison to sheep sequence accession No M37310.1. These results may be employed to survey the interrelation of the detected SNPs with desirable traits in small ruminants.

Keywords: growth hormone gene; sheep; goat; *Hae*III endonuclease enzyme; single nucleotide polymorphisms (SNPs)

Introduction

Regarding the economic value, sheep and goat breeds are marked by their output of meat, milk, wool and hide. These products are the prime supply in nutritional and skin industries. Moreover, Small ruminants consider an integral portion of the pastoral production system, because of their short gestation period, high prolificacy, rapid growth rate, high feed conversion efficiency, high diseases resistance capacity, as well as easy marketing [1].

In Egypt, both sheep and goat hold a prime share of Egyptian livestock industry. There are several Egyptian sheep and goat breeds. The most common sheep breeds include Baki, Rahmani and Ossimi while, goat breeds include Baladi, Baki and Zaraibi [2].

Currently, there is an increasing interesting in technologies based on DNA-markers which are widely used in breeding programs of some countries especially developing countries which have significant impact to improve production performance in livestock [3, 4].

Growth Hormone (GH), Growth Hormone receptor (GHR) and insulin-like growth factor1 (IGF1) are considered as potential regulators of growth traits in small ruminants [5]. The GH gene is a master candidate gene for traits with economic importance such as milk traits [6], meat quality [7] and wool production [8] as well as metabolism [9, 10]. Therefore, recently many studies investigate the structure and function of the GH gene [11].

Meanwhile, the Caprine GH gene has been mapped on the short arm of goat chromosome 19 (Capra hircus 19q22) [12]. It is encoded by 2.5 kbp. Caprine GH gene consists of five exons and four intervening introns [13]. This gene encodes GH protein which is anabolic hormone which belongs to somatolactogenic hormones and synthesized by the anterior lobe of the pituitary gland [14]

In vision of the crucial role of GH in animal growth and development, GH gene may be used as a candidate gene for studying its polymorphism and association in relation to growth traits [12]. Over the last decade, several studies have performed to assess

*Corresponding author email: banhawy09@yahoo.com (Mohamed Abdelfattah M. Abdelhafez)
Receive Date: 31 October 2021, Revise Date: 09 January 2022, Accept Date: 11 January 2022
DOI: 10.21608/ejchem.2022.103528.4802

the association of GH gene polymorphisms with production traits in different livestock species [15, 16].

On the basis of studies on a restriction fragment length polymorphism (RFLP), genetic polymorphisms at the exon 2 and exon 3 of GH gene have been reported in Sirohi and Barbari breeds of goat [17]. In addition, Dettori et al. [18] reported some polymorphisms in GH gene in sheep associated with milk traits such as ss748770547 CC associated with higher fat percentage and ss748770553 GG had higher milk yield and lactose percentage than AG and AA genotypes. Moreover, two polymorphic sites located in GH gene exon 2 A781G and A1575G were found to be associated with growth traits in Boer goat bucks breed [19].

Although many studies have already reported about association between polymorphisms in GH gene and the growth traits in small ruminants, detection of markers responsible for the phenotypic variation in production traits remains a major challenge in genetic improvement programs [20].

In this regard, the present study was undertaken to identify GH5 gene polymorphism in diverse sheep and goat breeds understudy for a better understanding of the mechanisms underlying the phenotypic variations.

Material and Methods

Animals and Blood sampling

The current study was carried out on 119 animals including 67 Egyptian sheep (Barki, n=16; Ossimi, n=20; Rahmani, n=19 and Saidi n=12) and 52 goats (Barki, n=14; Damascus, n=20 and Zaraibi, n=18). These animals were reared at an Agricultural Experiment Station, Faculty of Agriculture, Cairo University. Blood samples were collected from jugular vein of animals into vacuum tubes containing 0.25% ethylene-diamine-tetra-acetic acid (EDTA), as an anticoagulant then these samples was stored at −80 °C until DNA extraction.

DNA extraction

Genomic DNA was extracted from the whole blood using salting out procedure described by Miller *et al.* [21]. The DNA concentrations were determined using Nano-drop 1000 (Thermoscientific) and then were adjusted to 50 ng/µL for PCR.

Polymerase Chain Reaction (PCR)

Primers used were designed by Amie-Marini *et al.* (2012). The sequences of primers were GH5-F: 5'-GCCAGTGGTCCTTGCATAAA-3', GH5-R: 5'-AGTCCAGGGCAGGCAGAG-3', for forward and reverse primer respectively. Thermal cycling conditions were as follows: 94°C for 10 min; 35 cycles of 95°C for 30 sec, 62°C for 30 sec, and 72°C for 30 sec, followed by a final step of 72°C for 5 min. Five microliters of each PCR product were subjected to 1.5% agarose gel electrophoresis run on 0.5X TBE buffer and stained with ethidium bromide. The gels were visualized under U.V. and photographed using gel documentation system (Bio-Rad, Laboratories Inc., USA).

Restriction Fragment Length Polymorphism (RFLP)

All the amplified fragments were subjected to digest with *Hae*III restriction enzyme (MBI Fermentas, Germany) according to the manufacturer's protocol. The resulting products were separated in 3% agarose gels containing ethidium bromide in 1X TBE buffer and visualized on U.V. trans-illuminator to determine the different genotypes.

Sequence analysis and SNPs identification

Purified PCR products were sequenced by Macrogen Incorporation (South Korea) using forward and reverse primers. The specificity of the nucleotide sequences was carried out using BLAST (Basic Local Alignment Search Tool) https://blast.ncbi.nlm.nih.gov/Blast.cgi [22]. Sequences were analysed by multiple alignments **using Clustal Omega** https://www.ebi.ac.uk/Tools/msa/clustalo/ **[23] to** determine polymorphic sites, which confirmed by visual examination of sequence's charts.

Results

GH5 genotyping and alleles frequencies

The primers amplified a 468 bp fragment which consists of 5'UTR, exon1, intron1 and partial of exon 2 of GH gene in sheep and goat (Fig. 1).

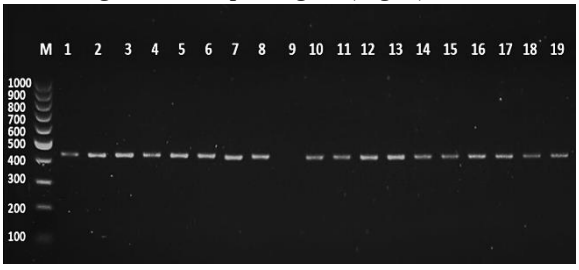


Fig. 1. Agarose gel electrophoresis of *GH*5-PCR fragment (468bp). Lane M, 100 bp DNA ladder. Lanes (1→8 goats), Barki, (10→19 sheep).

These PCR amplified fragments (468 bp) were digested with *Hae*III endonuclease, depending on the presence or absence of the restriction site (GG^CC). Three genotypes GH (228, 150, 78, 53 bp), GG (228, 78, 53 bp) and HH (150, 78, 53 bp) were yielded. In sheep, there was no polymorphism among the studied breeds (Barki, Ossimi, Rahmani and Saidi), all of them were found to carry only the H allele (100%), and only one HH homozygous genotype (Fig. 2).

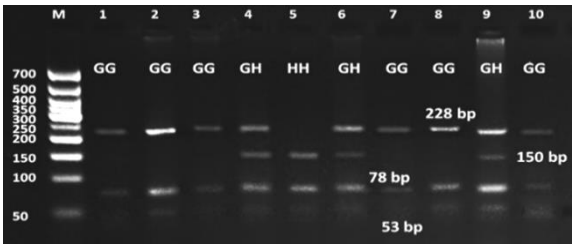


Fig. 2. Agarose gel electrophoresis of *GH*5-*Hae*III/PCR-RFLP fragments. Lane M; 50 bp DNA ladder. Lanes (1, 2, 3, 7, 8, 10) genotype GG (228, 78 & 53 bp). Lanes (4, 6, 9) genotype GH (228, 150, 78 & 53bp). Lane (5) genotype HH (150, 78 & 53 bp).

In goats, the Barki breed was found to carry only the G allele and one genotype was revealed (GG homozygous), While, Damascus and Zaraibi breeds carried both the G and H alleles with an allelic frequency of 0.80, 0.72 and 0.20, 0.28 ,respectively, and three RFLP patterns were present, the homozygote (G), heterozygote (GH) and the homozygote (H) alleles in frequencies of 0.70, 0.56 ; 0.20, 0.33 and0.10, 0.11, respectively (Table 1).

GG genotype was the most common genotype in the studied Egyptian goat breeds.

GH5 sequencing

Once sequenced reads were obtained, sequences were analyzed and compared with the GenBank Reference sequences NC_030826.1 (47736669-47737117) and NC_040262.1 (14849004-14849451) for goats and sheep, respectively.

Nucleotide sequencing of the amplified fragment of GH5 gene of Barki, Damascus and Zaraibi goat breeds and Barki, Ossimi and Rahmani sheep breeds were submitted to the GenBank "NCBI" under accession numbers: MW829141, MW829142, MW829143, MW829144, MW829145, MW829146, MW829147, MW829148, MW829149, MW829150 and MW829151.Submission ID were BankIt2444638, BankIt2444644, BankIt2444647 and BankIt2444648.

Sequence data resulted from sequencing of 5'UTR, exon1, intron1 and exon2 fragments was aligned against goat sequence database (D00476.1). The aligned results revealed eight nucleotide substitutions, and one Indel in Damascus breeds as shown in table (2).The one nucleotide substitution was detected in 5' UTR R (A > G) at 154 bp and eight nucleotide substitutions were detected in intron I in GH5 gene of Damascus breed with one indel GG between 221-222bp while no variants were detected in these fragments in GH gene of Barki and Zaraibi goat breeds as shown in Figure 3.

In addition to, sequence data resulted from sequencing of amplified GH5 fragments from different sheep breeds were aligned against sheep sequence database (M37310.1). The aligned results revealed four nucleotide substitutions in these fragments among Ossimi, Barki, Rahmani and Saidi sheep breeds as shown in table (3).The first nucleotide substitution (C>T) was detected at 125 bp in 5' UTR in the four sheep breeds. The second nucleotide substitution (G>A) was detected at 124 bp in 5' UTR in Ossimi sheep breed.The third nucleotide substitution (C>T) was detected at 218 bp in intron1 in Barki sheep breed while the last nucleotide substitution (G>A) was detected at 317bp in intron 1 in Barki and Rahmani sheep breeds as illustrated in Figure 4.

Table 1. Alleles and genotypes frequencies of *GH*5 gene for the studied goat and sheep breed

	Breed	No.	*GH*5				
			Genotype no. and frequency			Allele frequency	
			GG	GH	HH	G	H
Goat (n=52)	Barki	14	14	0	0	1.00	0.00
			1.00	0.00	0.00		
	Damascus	20	14	4	2	0.80	0.20
			0.70	0.20	0.10		
	Zaraibi	18	10	6	2	0.72	0.28
			0.56	0.33	0.11		
Sheep (n=67)	Barki	16	0	0	16	0.00	1.00
			0.00	0.00	1.00		
	Ossimi	20	0	0	20	0.00	1.00
			0.00	0.00	1.00		
	Rahmani	19	0	0	19	0.00	1.00
			0.00	0.00	1.00		
	Saidi	12	0	0	12	0.00	1.00
			0.00	0.00	1.00		

Table 2. Nucleotide variants in *GH*5 gene in wild goat sequence (D00476.1) and studied goat breeds

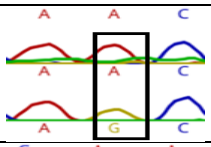
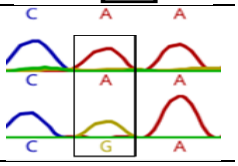
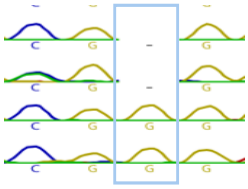
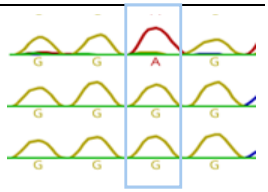
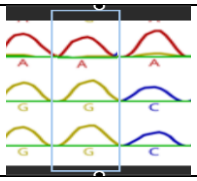
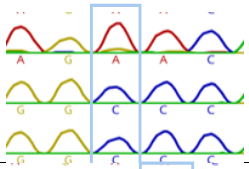
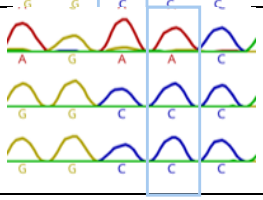
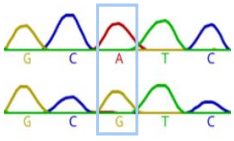
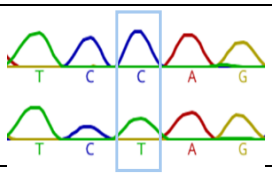
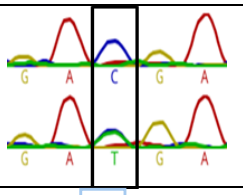
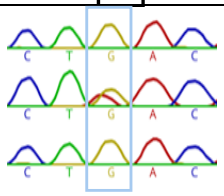
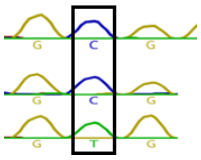
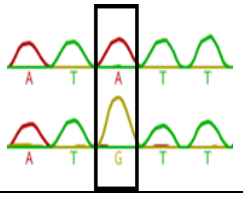
Genomic region	Wild sequence	Barki	Zaribi	Damascus	SNP position in goat breeds (bp)	SNP position in genomic sequence of *GH* gene (bp) (acc.No. D00476.1)	Chromatogram
5'UTR	**A**	**A**	**A**	**R(A > G)**	**154**	**429**	
Intron 1	**A**	**A**	**A**	**R (A > G)**	**180**	**455**	
Intron 1	**GG**	**-**	**-**	**GG**	**insertion between 221-222**	**-**	
Intron1	A	A	A	R(A > G)	222	497	
Intron1	**A**	**A**	**A**	**R (A > G)**	**223**	**498**	
Intron1	**A**	**A**	**A**	**W(A > C)**	**224**	**499**	
Intron1	A	A	A	W (A > C)	225	500	
Intron1	**A**	**A**	**A**	**R(A > G)**	**368**	**643**	
Intron1	**C**	**C**	**C**	**Y(C/T)**	**371**	**646**	

Table 3. Nucleotide variants in *GH*5 gene in wild sheep sequence (M37310.1) and studied sheep breeds

Genomic region	Wild sequence	Ossimi	Barki	Rahmani	Saidi	SNP position in the three sheep breeds (bp)	SNP position in genomic sequence of *GH* gene (bp) (acc.No.M37310.1)	Chromatogram
5'UTR	C	Y(C/T)	Y(C/T)	Y(C/T)	Y(C/T)	125	988	
5' UTR	**G**	**R(G/A)**	**G**	**G**	**G**	**142**	**1005**	
Intron 1	C	C	Y(C/T)	T	T	218	1081	
Intron 1	G	G	R(G/A)	R(G/A)	G	317	1280	

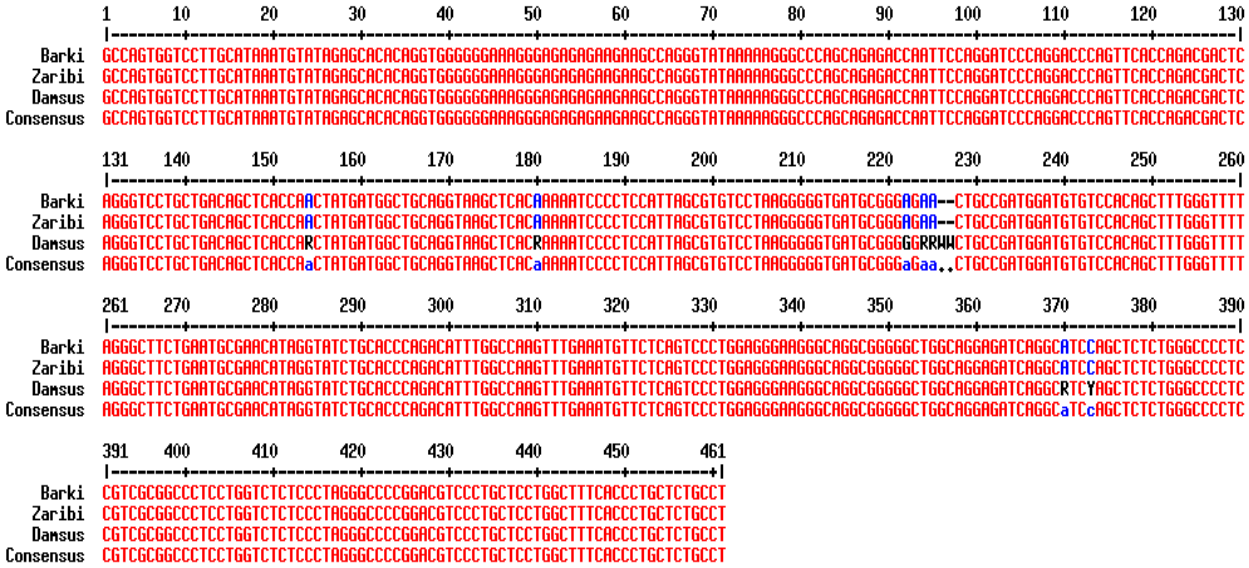


Fig. 3. Nucleotide sequences with single nucleotide polymorphisms of *GH*5 gene in the studied goat breeds. Different genotypes are illustrated in the blue color.

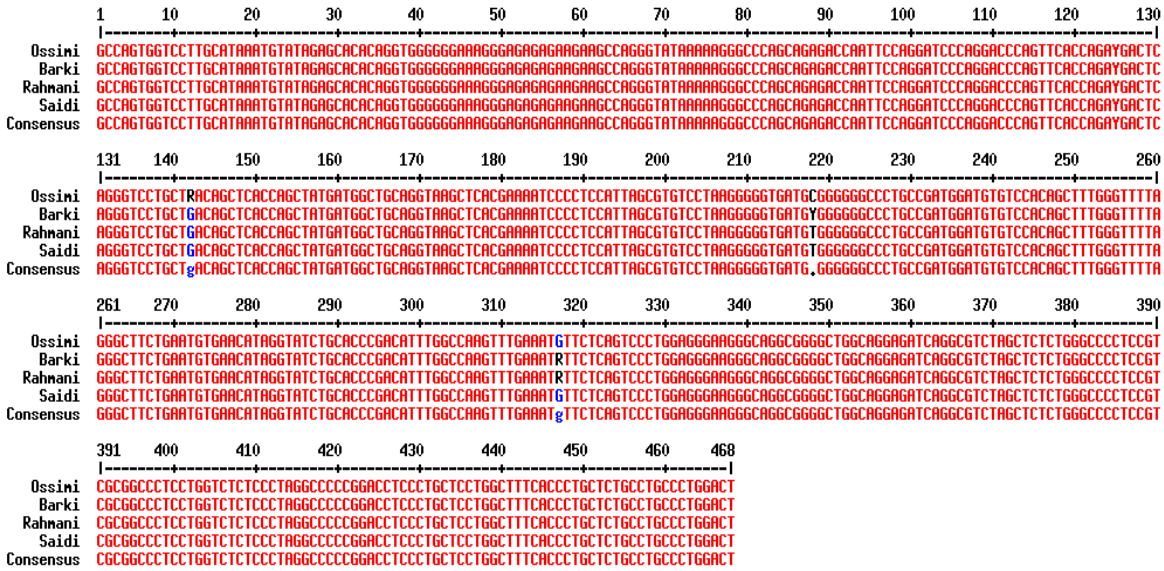


Fig. 4. Nucleotide sequences with single nucleotide polymorphisms of *GH*5 gene in the studied sheep breeds. Different genotypes are illustrated in the blue color.

Discussion

The current study aims to screen growth hormone (GH) gene variants and identifies the GH single nucleotide polymorphisms (SNPs) in different sheep and goat breeds. Recently, molecular genetics has an influential role in identifying gene structure and function, opening onto discovery of molecular markers, vital in assisting selection programs and improving livestock productivity.

The primers, used in this study, flanked a 468 bp fragment (GenBank: DQ461658.1) which represents 5' UTR (1 - 98), exon 1 (99 -169), Intron 1 (170-415) and part from exon 2 (416-468) in different Egyptian sheep and goat breeds. These PCR amplified fragments (468 bp) were digested with *Hae*III restriction enzyme. The results reported that GH5 gene showed three genotypes (GH, GG and HH) in Damascus and Zaraibi goat breeds. These current results are parallel to those found by Min *et al.* [24], recognizing three genotypes (AA, AB and BB) in the 5′ promoter region of the GH gene in Boer goats. Adding up, Singh *et al.* [17] reported that exon 2 and exon 3 of GH gene in Sirohi and Barbari goat breeds were polymorphic for restriction enzyme *Hae*III. Barki goat breed showed one genotype (GG) and the occurrence of genotype GG was higher than other genotypes in the goat breeds in the current study. These findings are in fluency with Othman *et al.* [25], who set forth that the existence of genotype GG frequency was 43.56%, in the Egyptian Baladi, Barki, and Zaraibi goat breeds, via *Hae*III/PCR-RFLP. As well, Gitanjli *et al.* [26] researched the GH gene polymorphism in Gaddi goat, across amplifying the four targeted regions (GH1, GH2, GH3 and GH4), employing *Hae*III/PCR-RFLP, and noticed that GH1, GH2 and GH3 loci were polymorphic, whereas GH4 was monomorphic. Besides, our results for goat breeds were similar to Amie Marini *et al.* [27] who revealed three GH5 genotypes (GH, GG and HH) in Savanna and Kalahari goats. The highest genotype frequencies in Savanna goats were 0.57 and 0.37 for GG and GH genotypes, respectively while the highest genotype frequencies in Kalahari goats were GH (0.5) and HH (0.36) genotypes. Mahrous *et al.* [28] documented a polymorphic pattern for exon 2 of GH gene in Egyptian Barki, Zaraibi and Damscus goat breeds.

PCR-RFLP monomorphic pattern for GH5 locus in sheep breeds was stated in our study, and exposed one genotype (HH) in Barki, Ossimi and Rahmani sheep breeds. These outputs harmonized with what reported earlier by Malewa [29], who found out monomorphism in GH5 locus, digested with *Hae*III in Palu sheep. At odds, Gorlov *et al.* [7] brought out three different genotypes (AA, AB and BB) of GH gene in Salsk sheep, utilizing a similar PCR-RFLP tool. Furthermore, genetic polymorphisms in 5' regulatory region, exon 4 and 3' untranslated region of GH, in Chinese sheep breeds, were issued by Jia *et al.* [14]. Lastly, Othman *et al.* [25] documented a polymorphic pattern for exon 2 and 3 of GH gene in Egyptian Barki, Rahmani and Ossimi sheep breeds.

In the current study, the analysis of the GH5 gene sequence of the three different RFLP genotype

patterns in the studied goat breeds, manifested two polymorphic sites in exon one, including nucleotide substitution at nt125 (c.125 C>T) and nt 142 (c.142 A>G). Additionally, two nucleotide substitutions at nt218 (c.218 C>T) and nt317 (c.317 A>G), appeared in intron 1. In opposing, there was no polymorphism, detected between Barki and Zaraibi goat Egyptian breeds in the sitting study, while eight polymorphic sites were uncovered in intron one of Damascus breed, with GG insertion between nts 222-225.

The finding of associations between polymorphism in GH gene of different animal breeds and growth traits is notable. Tahmoorespur *et al.* [30] displayed an association between GH gene variants and growth traits in Baluchi sheep breeds. Also, do Rosário Marques *et al.* [11] investigated polymorphism, in the 5' flanking region and five exons in growth hormone gene, in Serra da Estrela sheep breed, and they were highly polymorphic.

Conclusion

The current study provided a novel genetic information, regarding the genetic polymorphisms, in 5'UTR, exon1, intron1 and partial of exon 2 of GH gene in different sheep and goat Egyptian breeds. The results of this study can be used to study the association of discovered SNPs with desired traits in small ruminants.

Acknowledgements

The authors like to thank Cell Biology Department, the National Research Centre, Egypt, for providing necessary facilities for this study. Also, The authors sincere gratefulness to the Agricultural Experiment Station, for kind assistance in collecting the blood samples of the goat and sheep breeds understudy, needed for executing this research.

Authors' Contributions

All authors contributed equally. KFM conceived the idea and designed the experiment. MAA collected the blood samples. MAA, NMO and NIA performed the DNA sequence and variants analysis, and also the statistical analysis. MAA, NMO, KFM and NIA wrote the manuscript. All the authors revised, read, and approved the final manuscript.

Funding

No fund was received for this work.

Data availability

All data generated and analyzed during this study are included in this paper.

Code availability

Not applicable.

Declarations

Ethics approval

This study does not require ethical approval. However, the blood samples were harvested as per standard sample collection procedure with no harm to animals. The authors obtained consent from the staff of the Agricultural Experiment Station, for sample collection.

Competing interests

The authors declare no conflict of interest.

Consent for publication

All authors read and approved the final manuscript.

Consent to participate

Not Applicable

References

[1] Sejian V, Bagath M, Krishnan G, Rashamol VP, Pragna P, Devaraj C, Bhatta R (2019) Genes for resilience to heat stress in small ruminants: A review. Small Rum Res 173: 42-53. http://dx.doi.org/10.1016/j.smallrumres.2019.02.009

[2] Galal SF, Abdel-Rasoul MR, Anous, Shaat I (2005) On-Station Characterization of Small Ruminant Breeds in Egypt. In: Characterization of Small Ruminant Breeds in West Asia and North Africa. Iniguez, L.C. (Ed.). Vol. 2, ICARDA, Aleppo, Syria, 141-193.

[3] Cauvery D, Sivaselvam SN, Karthickeyan SMK, Tirumurugaan KG, Kumanan K, Venkataramanan R (2016) Single nucleotide polymorphisms in GH (growth hormone) gene associated with growth traits in Nilagiri sheep of Tamil Nadu. IJEST 5: 4097-4103.

[4] Bahrami Y, Bahrami S, Rezvannejad E, Mousavizadeh SA (2018) The Polymorphism of FecXG Region Exon 2 of BMP15 Gene in Hisari Sheep. J Genet Resour *4*(1), 7-13. https://dx.doi.org/10.22080/jgr.2018.13627.1094

[5] El-Halawany N, El-Werdany A, El-Sayed YA, Abd-El-Monsif AS, Al-Tohamy AF, Abdel-Shafy H, Abd-El-Razek FM (2019) Characterization of growth hormone gene (GH) in three Egyptian goat breeds. Meta Gene 20: 100556. https://doi.org/10.1016/j.mgene.2019.100556

[6] Shakweer, El-Sayed WM, Abd EL-Rahman HH (2020) Cloning, nucleotide sequencing, and bioinformatics analyses of growth hormone mRNA of Assaf sheep and Boer goats reared in Egypt. J Genet Eng Biotechnol 18(1): 1-23. https://doi.org/10.1186/s43141-020-00046-6

[7] Gorlov IF, Kolosov YA, Shirokova NV, Getmantseva LV, Slozhenkina MI, Mosolova NI, Bakoev NF, Leonova MA, Kolosov AY, Zlobina EY (2017) Association of the growth hormone gene polymorphism with growth traits in

Salsk sheep breed. Small Rum Res 150: 11-14. https://doi.org/10.1016/J.SMALLRUMRES.2017.02.019

[8] Farag IM, Darwish AM, Darwish HR, AbdelAziz KB, Ramadan WA, Mohamed MI, Othman OE (2016) Polymorphism of growth hormone gene and its association with wool traits in Egyptian sheep breeds. Afr J Biotechol 15(14): 549-556. https://doi.org/10.5897/AJB2015.14928

[9] Vijayakumar A, Yakar S, LeRoith D (2011) The intricate role of growth hormone in metabolism. Front Endocrinol 2: 32. https://doi.org/10.3389/fendo.2011.00032

[10] Sedykh TA, Gizatullin RS, Dolmatova IY, Gusev IV, Kalashnikova LA (2020) Growth Hormone Gene Polymorphism in Relation to Beef Cattle Carcass Quality. Russian Agr Sci 46 (3): 289-294. http://dx.doi.org/10.3103/S1068367420030167

[11] do Rosário Marques M, Santos IC, Carolino N, Belo CC, Renaville R, Cravador A (2006) Effects of genetic polymorphisms at the growth hormone gene on milk yield in Serra da Estrela sheep. J Dairy Res 73(4): 394-405. https://doi.org/10.1017/s0022029906001932

[12] Supakorn C (2009) The Important Candidate Genes in Goats - A Review. Walailak J Sci Tech 6(1): 17-36. http://dx.doi.org/10.2004/wjst.v6i1.70

[13] Missohou A, Talaki E, Laminou IM (2006) Diversity and genetic relationships among seven West African goat breeds. Asian-Aust J Anim Sci 19(9): 1245-1251. https://doi.org/10.5713/ajas.2006.1245

[14] Jia JL, Zhang LP, Wu JP, Ha ZJ, Li WW (2014) Study of the correlation between GH gene polymorphism and growth traits in sheep. Genet Mol Res 13(3): 7190-7200. https://doi.org/10.4238/2014.september.5.5

[15] Amiri, Sihem, Bayrem J, Mohamed AF, Hajer J, Rekik B, Abderrahmane BG (2018) Assessment of growth hormone gene polymorphism effects on reproductive traits in Holstein dairy cattle in Tunisia. Arch Anim Breed 61(4): 481-489. https://doi.org/10.5194/aab-61-481-2018

[16] El-Komy SM, Saleh AA, Abd El-Aziz RM, El-Magd MA (2021) Association of GH polymorphisms with growth traits in buffaloes. Domest Anim Endocrinol 74: 106541. https://doi.org/10.1016/j.domaniend.2020.106541

[17] Singh PP, Tomar SS, Thakur MS, Kumar A (2015) Polymorphism and association of growth hormone gene with growth traits in Sirohi and Barbari breeds of goat. Vet world 8(3): 382–387. https://dx.doi.org/10.14202%2Fvetworld.2015.382-387

[18] Dettori ML, Pazzola M, Pira E, Paschino P, Vacca GM (2015). The sheep growth hormone gene polymorphism and its effects on milk traits. J Dairy Res 82(2): 169-176. https://doi.org/10.1017/s0022029915000047

[19] Hua GH, Chen SL, Yu JN, Cai KL, CJ Wu et al (2009) Polymorphism of the growth hormone gene and its association with growth traits in Boer goat bucks. Meat Sci 81: 391-395. https://doi.org/10.1016/j.meatsci.2008.08.015

[20] Anwar S (2017) Association of growth hormone gene polymorphism with quantitative characteristics of thin-tailed sheep using PCR-RFLP in Jambi province. Afr J Biotechol 16(20): 1159-1167. https://doi.org/10.5897/AJB2016.15783

[21] Miller SA, Dykes DD, Polesky HF (1988): A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16(3): 1215. https://dx.doi.org/10.1093%2Fnar%2F16.3.1215

[22] Altschul SF, Warren G, Webb M, Eugene WM, David JL (1990) Basic local alignment search tool. J Mol Biol 215 (3): 403-410.

[23] Thompson, JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22 (22): 4673-4680. https://doi.org/10.1093/nar/22.22.4673

[24] Min LJ, Li MY, Sun GQ, Pan QJ, Chen H (2005) Relationship between polymorphism of growth hormone gene and production traits in goats. Yi Chuan Xue Bao 32(6):650-654.

[25] Othman, O.E., Alam, S.S., Abd El-Kader, H.A. and Abd-El-Moneim, O.M., 2015. Genotyping of growth hormone gene in Egyptian small ruminant breeds. Biotechnol 14: 136-141. https://dx.doi.org/10.3923/biotech.2015.136.141

[26] Gitanjli G, Sankhyan V, Thakur YP, Dogra PK (2020) Effect of growth hormone gene polymorphism on growth traits in migratory Gaddi goats of Western Himalayas, India. Trop Anim Health Prod 52(4):2091-2099. https://doi.org/10.1007/s11250-020-02227-4

[27] Amie Marini AB, Aslinda K, Mohd-Hifzan R, Muhd-Faisal AB, Musaddin K (2012) *Hae*III-RFLP Polymorphism of growth hormone gene in Savanna and Kalahari goats. Malays J Anim Sci 15: 13-19.

[28] Mahrous KF, Abdel-Aziem SH, Abdel-Hafez MA, Abdel-Mordy M, Rushdi HE (2018) Polymorphism of growth hormone gene in three goat breeds in Egypt. Bulletin NRC 42(1): 1-7. http://dx.doi.org/10.1186/s42269-018-0035-0

[29] Malewa AD (2019) Diversity of the Palu Sheep Growth Hormone Gene using the PCR-RFLP Method. AgriSains 20(3): 144-153. Available online at http://jurnal.untad.ac.id/jurnal/index.php/AGRISAINS

[30] Tahmoorespur M, Taheri A, Gholami H, Ansary M. PCR-SSCP (2011) Variation of GH and STAT5A genes and their association with estimated breeding values of growth traits in Baluchi sheep. Anim Biotechol 22(1):37-43. http://dx.doi.org/10.1080/10495398.2011.544205