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Polymorphism of growth hormone gene in three goat breeds in Egypt

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Abstract

Background: In Egypt, like other developing countries, goats are prime resources of meat. So, selection of goats for superior growth rate is advantageous. Growth hormone (GH) is the main regulator of animal growth, and encoded by *GH* gene that exhibits active gene variants improving growth. The objective of this study was to identify *GH* gene variants in three goat breeds (Barki, Damascus, and Zaraibi), via polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and gene sequencing analyses.

Results: Three loci on GH gene named *GH1*, *GH2*, and *GH6* polymorphisms were analyzed. *GH1*-*Hae*III/RFLP showed only two genotypes (AB and BB) in all breeds, with absent AA genotype. Both Barki and Zaraibi exhibited the highest GC genotype frequency (0.95). *GH2*-*Hae*III/RFLP produced only two homozygous genotypes AA in Damascus and BB in both Barki and Zaraibi, with the absence of AB genotype in the three breeds. However, digestion of *GH6* by *Hae*III was monomorphic; it exhibited different single-nucleotide polymorphisms (SNPs), detected by DNA sequencing, among the studied goat breeds.

Conclusions: The revealed SNPs could be employed as useful markers, helping goat breeders in selection of goats for high growth performance. Further analyses with larger sample size are needed for investigating the relationship between the different genotypes and growth traits.

Keywords: Goat, Polymorphism, PCR-RFLP, SNP, Growth hormone gene

Background

Goat is one of the main meat-producing animals in Egypt, and has a considerable contribution to the resource poor section of society for their livelihood (Abdelaziz et al. 1995). There are more than three million heads of goats in Egypt, raised primarily in three regions: the Upper Egypt, Nile Delta, and in the desert rangelands (FAOSTAT 2011). There are about 1.7 million goats, mostly in mixed flocks with sheep, buffalo, and cattle (Galal et al. 2005). In Egypt, there are five indigenous goat breeds: Baladi (the common breed in Nile Delta), Barki or Sahrawi (the predominant breed in desert), Sinaoy (also called Bedouin goat, found mainly in Sinai peninsula), Saidi (distributed widely in Upper Egypt), and Zaraibi (also known as Egyptian Nubian, the common breed in Nile Valley and Delta) (Galal et al. 2005). Apart of the five goat breeds recognized in Egypt, Anglonubian breed, which is produced through crossing Egyptian Nubian breed with some British breeds (Latif et al. 1987). Genetic improvement schemes of goats in Egypt have also involved different crossbreeding trials with foreign breeds like Damascus goat, which is originated from Syria (Galal 2005).

Growth is an intricate biological process that comprises the regulated coordination of a wide diversity of neuro-endocrine pathways, including a coordinated action of several hormones (like growth, thyroxine, insulin, and prolactin hormones) secreted by the key endocrine glands and controlled by the action of their corresponding genes (Ge et al. 2003; Rasouli et al. 2016; An et al. 2015; Othman et al. 2014; Silveira et al. 2008; Zhang et al. 2013). It is well established that animals with high levels of these hormones exhibit enhanced growth performance (Jia et al. 2014; Sodhi et al. 2007; Sharma et al. 2013; Amie Marini et al. 2010; Ayuk and Sheppard 2006). So, selection of breeding animals based on their

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performance helps to explore superior individuals for growth traits. This superiority could be a result of receiving active gene variant(s) from their ancestors. The study of the structure and function of genes at the molecular level in a breeding population can help to determine the similarity of the genetic material carried by populations and the genetic variation they possess. Several techniques have been evolved to estimate the genetic variance or polymorphism within and among populations (Okumus and Mercan 2007). One of the most commonly used techniques is polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). It is a powerful method for detecting nucleotide sequence variance in the amplified deoxyribonucleic acid (DNA).

Growth hormone (GH) plays a vital role in different biological processes, such as growth, metabolism, lactation, and reproduction of farm animal species (Malveiro et al. 2001; Ola et al. 2008; Boutinaud et al. 2003; Seevagan et al. 2015; Katoh et al. 2008; Reinecke et al. 1993). *GH* is released from the anterior lobe of pituitary gland, where its main effects are associated with the stimulation of growth of bones and skeletal muscles, through the action of insulin-like growth factor (IGF-1) (An et al. 2011), as well as its great role in milk production (Akers 2006). Consequently, it has been observed that high-yielding animals reveal greater *GH* levels in comparison to low-yielding ones (Akers 2006). The Caprine *GH* gene has been mapped on the short arm of goat chromosome 19 (*Capra hircus 19q22*) (Supakorn 2009). It is encoded by 2.5 kbp. Caprine *GH* gene consists of five exons and four intervening introns (Accession: D00476) (Missohou et al. 2006). The aim of the present study was to screen the genetic polymorphism of goat *GH* gene in three common goat breeds in Egypt (Barki, Damascus, and Zaraibi) employing two genetic tools; PCR-RFLP and DNA sequencing.

Methods

Sample collection and DNA extraction

Blood samples were taken from the jugular vein of 20 healthy animals that represent each 1 of the 3 goat breeds under study; Barki, Damascus, and Zaraibi. All the 60 animals, including males and females, involved in the present study were born and reared in the Agricultural Experiment Station, Faculty of Agriculture, Cairo University. Blood samples were collected in 10 ml tubes containing 2.7% EDTA as an anticoagulant and kept at 4 °C till use. Genomic DNA was extracted and purified from whole blood collected samples by salting out procedure illustrated by Miller et al. (1988). The concentration of genomic DNA isolated was measured using Ultraviolet (U.V.) spectrophotometer at wavelength of 260 nm.

Polymerase chain reaction

Three pairs of primers were used for amplifying *GH1*, *GH2*, and *GH6* loci of goat *GH* gene using primers suggested by Amie Marini et al. (2012) (Table 1).

Amplification reaction was carried out in an 25 μl volume containing 100 ng genomic DNA, forward and reverse primer (both at concentration 10 pmol/μl), 1 U *Taq* polymerase, 2.5 μl Taq polymerase buffer, four dNTPs (each at final concentration of 2.5 mM/μl), and de-ionized double-distilled H_2O up to a total volume of 25 μl. The PCR program included 94 °C for 10 min, 35 cycles of 95 °C for 30 s, 62 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 7 min. The amplicons were analyzed by 1.5% agarose gel electrophoresis. The gels were stained with ethidium bromide and visualized by U.V. transilluminator.

Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) was carried out in 15 μl of reaction mixture of each sample containing 5 μl of PCR product, 9.5 μl of 10 X buffer, and 0.5 μl of fast restriction enzyme (MBI Fermentas, Germany) specific for each gene (Table 1). The reaction mixture was incubated at 37 °C for 10 min. The digested products were initially separated by electrophoresis in 2.5% agarose gel, followed by 12% polyacrylamide gel. Thereafter, the gels were stained with ethidium bromide. The bands were visualized by U.V. transilluminator. The gels were photographed using digital gel documentation system (ChemiDoc™ XRS+ System with Image Lab™ Software from Bio-Rad, USA).

Gene sequence analysis

PCR products of the tested *GH6* locus were purified and sequenced by Macrogen Incorporation (Seoul, Korea). Sequence analysis and alignment were performed using NCBI/BLAST/blastn suite to identify the single-nucleotide polymorphisms (SNPs) among different patterns and alleles.

Statistical analysis

Genotype and allele frequencies for each locus were calculated using *POPGENE Software* for Population Genetic Analysis (PopGene version 3.2) (Yeh et al. 1999).

Table 1 Primer sequences (5′ → 3′), PCR products (size and region), and restriction enzyme of *GH1*, *GH2*, and *GH6* genes

Gene	Primer sequence (5′ → 3′)	PCR product (size and region)
GH1	CTC TGC CTG CCC TGG ACT GGA GAA GCA GAA GGC AAC C	422 bp Exons 2 and 3
GH2	TCA GCA GAG TCT TCA CCA AC CAA CAA CGC CAT CCT CAC	116 bp Exon 4
GH6	CCA TCC AGA ACA CCC AGG T CCA AGC TGT TGG TGA AGA CTC	405 bp Exon 3

F forward, *R* reverse

A chi-square test (χ^2 test) was performed on the basis of Hardy-Weinberg law to measure the genetic equilibrium at the population level. Sequence analysis and alignment of sequence products were carried out using NCBI/BLAST/blastn and BioEdit software in comparison with GenBank Accession numbers to identify single nucleotide substitutions between different detected genotypes.

Results

Three loci on GH gene named *GH1*, *GH2*, and *GH6* polymorphisms were analyzed. *GH1*-flanked a422bp (Fig. 1a). Digestion of this fragment with the restriction enzyme *Hae*III resulted in two different alleles, A (uncut 422 bp fragment) and B (366 bp and 56 bp fragments). Only two genotypes, the homozygous BB (366 bp and 56 bp) and the heterozygous AB (422 bp with 366 bp or 56 bp), were found, with absence of the homozygous genotype AA (Fig. 1b). As shown in Table 2, the genotype AB had the highest frequency in all breeds. Also, the estimates of observed heterozygosity were considerably higher than that of expected heterozygosity for all breeds. Chi-square (χ^2) value showed significant deviation from Hardy-Weinberg Equilibrium [HWE] ($P < 0.05$) in both Barki and Damascus goats (Table 2).

Concerning *GH2* gene, PCR produced a DNA fragment of 116 bp (Fig. 1c). Digestion of this fragment with *Hae*III gave two alleles, A (uncut 116 bp fragment) and B (88 and 28 bp fragments) as shown in Fig. 1d, with a higher frequency of B allele (Table 3). Only two homozygous genotypes, AA (116 bp) and BB (88 and 28 bp), were found, with absence of AB genotype (116, 88, and 28 bp). All Damascus individuals analyzed showed only the genotype AA. On the other hand, the animals belonging to both Barki and Zaraibi breeds had only the genotype BB (Table 3).

In regard to *GH6* gene, the PCR amplification of the gene presented a DNA fragment of 405 bp (Fig. 1e). *Hae*III digestion produced only one allele (B), with the absence of allele A. Only one genotype BB (108, 78, 70, 48, 44, 40, and 17 bp) was shown in all breeds studied (Fig. 1f). No polymorphism among the three breeds was detected, indicating the homozygosity of *GH6* gene in the goat breeds under study.

DNA nucleotide sequencing of the amplified PCR product of *GH6* gene was analyzed in the goat breeds under study (Figs. 2 and 3). As shown in Fig. 3, the sequences of our goats GH6 were aligned with reference sequences for *Capra hircus*: KU976149, GU355686, KX032517, GU355688, KU935713, KU288612. The sequence alignment of Barki *GH6* gene amplicon (ACCN: KU935713.1, *Capra hircus*) with published sequence showed 98% identities with two gaps between positions 366 and 369, and six SNPs; (A/G) transition at position 57, (C/G) transversion at position 66, (C/T) transition at position 128, (T/G) transversion at position 282, (A/C) transversion at position 372, and (T/C) transition at position 402. Also, the sequence alignment of

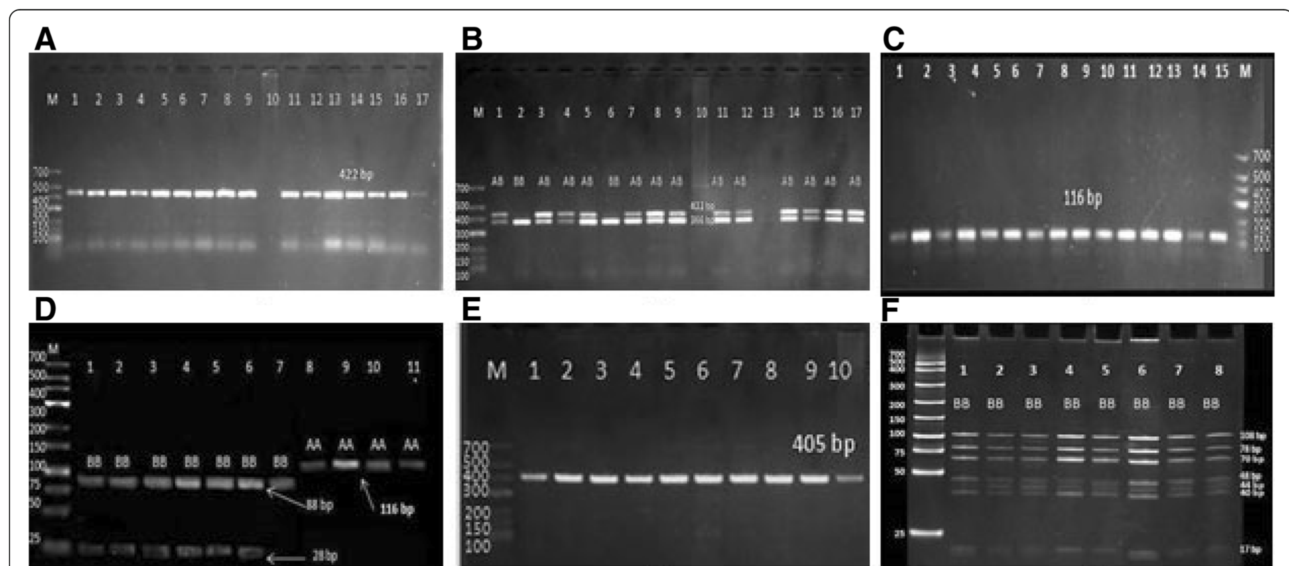

Fig. 1 Electrophoretic pattern of PCR-amplified fragment from three loci on GH gene named GH1, GH2, GH6 and their digestions. **a** Agarose gel electrophoresis of *GH1*-PCR fragment (422 bp). Lanes M (25 bp DNA ladder); 1, 2 3, 4, 5, 6 (Barki); 7, 8, 9, 11 (Damascus); and 12, 13, 14, 15, 16, 17(Zaraibi). **b** Agarose gel electrophoresis of GH1-HaeIII/RFLP fragments. Lanes M, 25 bp DNA ladder; lanes (1, 3, 4,5, 7, 8, 9, 11, 12, 14, 15, 16, 17) genotype AB (422, 366, and 56 bp); and lanes (2, 6) genotype BB (366 and 56 bp). **c** Agarose gel electrophoresis of *GH2*-PCR fragment (116 bp). Lane M, 25 bp DNA ladder. Lanes (1, 2, 3, 4, 5) Barki; (6, 7, 8. 9, 10) Damascus; and lanes (11, 12, 13, 14, 15) Zaraibi breeds. **d** Agarose gel electrophoresis *Hae*III/RFLP fragments. Lane M, 25 bp DNA ladder; lanes (1, 2, 3, 4, 5, 6, 7) genotype BB (88 and 28 bp); and lanes (8, 9, 10, 11) genotype AA (116 bp). **e** Agarose gel electrophoresis of *GH6*-PCR fragment (405 bp). Lane M, 25 bp DNA ladder. Lanes (1, 2, 3) Barki; (4, 5, 6) Damascus; and lanes (7, 8, 9, 10) Zaraibi breeds. **f** Polyacrylamide gel electrophoresis for *GH6-HaeIII*/RFLP. Lane M (25 bp DNA ladder) and 1 → 8 (genotype BB with 108, 78, 70, 48, 44, 40, and 17 bp)

Table 2 Genotype and allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), and χ^2 values of *GH1* gene digested with *Hae*III

Gene (restriction enzyme)	Breed	Genotype frequency			Allele frequency		Observed Het. (Ho)	Expected Het. (He)	χ^2
		AA	AB	BB	A	B			
GH1 (*Hae*III)	Barki	0.00	0.95	0.05	0.47	0.53	0.95	0.51	15.5*
	Damascus	0.00	0.90	0.10	0.45	0.55	0.90	0.51	12.6*
	Zaraibi	0.00	0.95	0.05	0.47	0.53	0.95	0.51	12.6

*$P \leq 0.05$

Damascus *GH6* gene amplicon(ACCN: KU288612.1) with published sequence (Fig. 3) showed 98% identities with one SNP; (T/C) transition at position 402, and eight gaps; three gaps between positions 363 and 367, two gaps between positions 369 and 372, and three ones between positions 382 and 386. Finally, the sequence alignment of Zaraibi *GH6* gene amplicon (ACCN: KU935713.1) with published sequence (Fig. 3) showed 99% identities with one SNP; (T/C) transition at position 402, and two gaps between positions 382 and 385.

Discussion

The findings of *GH1* gene are in consonance with those reported by Singh et al. (2015) who showed that both of Sirohi and Barbari goat breeds were polymorphic at the *GH1* locus treated with *Hae*III. Likewise, Othman et al. (2015) detected a SNP (G → A) at position 55 of *GH1* locus (422 bp) in the Egyptian Baladi, Barki, and Zaraibi goat breeds using *Hae*III/PCR-RFLP and gene sequencing. Moreover, these results are in agreement with those obtained in Savanna and Kalahari goats by Amie Marini et al. (2012). Furthermore, Hua et al. (2009) studied the polymorphism of *GH1* locus in Boer goat bucks, via gene sequencing and PCR-RFLP procedures, and its correlation with growth traits. They stated that the AA genotype (366 and 56 bp) resulted in a significant decrease in birth chest girth ($P = 0.03$) and weaning weight ($P = 0.014$), compared to AB genotype (422, 366 and 56 bp). Also, no homozygous BB (uncut 422 bp) individuals were identified. The same genetic polymorphism of *GH1* was recorded later in Matou and Boer Chinese goat breeds with a significant ($P < 0.05$) larger litter size for AB genotype (422, 366, and 56 bp) than for AA genotype (366 and 56 bp) (Zhang et al. 2011).

Table 3 Genotype and allele frequencies of *GH2-Hae*III/RFLP

Gene (restriction enzyme)	Breed	Genotype frequency			Allele frequency	
		AA	AB	BB	A	B
GH2 (*HaeIII*)	Barki	0.00	0.00	1.00	0.00	1.00
	Damascus	1.00	0.00	0.00	1.00	0.00
	Zaraibi	0.00	0.00	1.00	0.00	1.00

Conversely to the results of *GH2* locus, Amie Marini et al. (2012) studied the genetic polymorphism of *GH2* locus in Savanna and Kalahari goats via PCR-RFLP method. The authors found that both breeds were homozygous for CC genotype (88 and 28 bp), and that *GH2* gene was monomorphic. Also, Zhang et al. (2011) studied*GH2* gene polymorphism in Matou and Boer Chinese goat breeds by using the same method. They reported that the frequency of CC genotype (88 and 28 bp) was significantly ($P < 0.05$) higher than that of CD genotype (116, 88, and 28 bp). Also, litter size for CC genotype was significantly ($P < 0.05$) larger than that for CD genotype. Furthermore, Hua et al. (2009) analyzed the *GH2* locus polymorphism in Boer goat bucks, by gene sequencing and PCR-RFLP, and its correlation with some growth traits. They obtained two genotypes CC (88 and 28 bp) and CD (116, 88, and 28 bp). The CC genotype was associated with heavier weaning weight than CD genotype.

Reversely to the outputs of *GH6* locus, Amie Marini et al. (2012) illustrated the genetic polymorphism of *GH6* locus in Savanna and Kalahari goats via PCR-RFLP. They found that both breeds were homozygous for genotype (JJ) (110, 80, 70, 50, 40, and 23 bp), and *GH6* gene was characterized as monomorphic. The inconsistency in band sizes between genotype (JJ) and genotype (BB) (108, 78, 70, 48, 44, 40, and 17 bp) in the current study may be due to breed difference (i.e., the fact that the different breeds/populations maintained under the different sets of environmental conditions are subject to different evolutionary forces to varying degree), and may also be the consequence of sampling of breeds understudy. Moreover, the authors detected a substitution of TCC to TCT at position 1148 located in exon three at *GH6*. This substitution did not change amino acid coding. This detection was done by gene sequencing only in Savanna goats, but not in Kalahari goats due to a limited sample size.

Conclusion

In this study, PCR-RFLP technique proved to be a convenient tool for screening gene polymorphism. *GH1* and *GH2* loci were found to be polymorphic, when digested with *Hae*III restriction enzyme, among the studied goat breeds; Barki, Damascus, and Zaraibi. These polymorphisms can

CCATCCAGAACACCCAGGTTGCCTTCTGCTTCTCTGAAACCATCCCAGCCCCCACGAGCAA
GAACCAGGCCCAGCAGAAATCAGTGAGTGGCCACCTAGGACCGAGGAGCAGGGGACCTC
CTTCATCCTAAGTAGGCTGCCCCAGCTCTCTGCACCGGGCCTGGGGTGGCCTTCTCCCTGA
GGTGGCAGAGGGTGTTGGATGGCAGTGGAGGATGATGGTTGGTGGTGGTGGCAGGAGGT
CCTCGGGCAGAGGCCGACCTTGCAGGGCTGCCCCGAGCCCGTGGCACCCACCAACCACCC
ATCTGCCAGCAGGACTTGGAGCTGCTTCGCATCTCACTGCTCCTTATCCAGTCGTGGCTTG
GGCCCGACTGAAGTTCCTCAGCAGAGTCTTCACCAACAGCTTGG

Fig. 2 The sequence analysis of goat*GH6* amplified fragment. Forward and reverse primers are shown in red and blue color, respectively

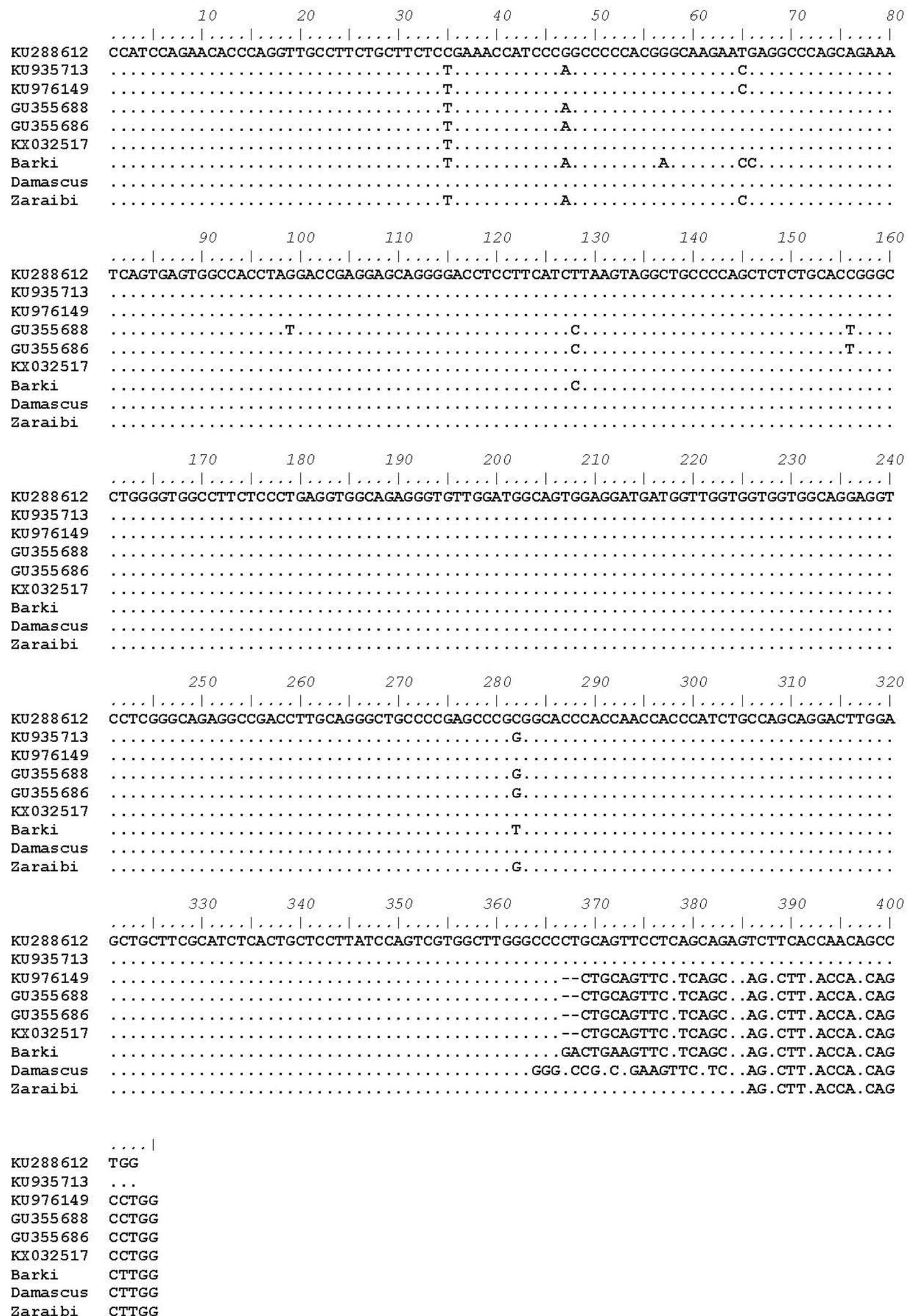

Fig. 3 Sequencing alignment for GH6 gene of three Egyptian goat breeds (Barki, Damascus, and Zaraibi) in comparison with Genbank entries to describing different new detected SNPs

be employed as effective markers for genetic discrimination between goat breeds. Although *GH6* locus was monomorphic among the studied breeds when digested with *Hae*III, it exhibited different SNPs using DNA sequencing. In the end, this study is considered to be a step advancing for further studies that may add to give additional information about the genetic polymorphism of meat and growth characters of Egyptian goat breeds and the improvement of these economically important traits.

Abbreviations

19q22: A position on the long arm of chromosome 19, region 2, band 2; ACCN: Accession number; AFLP: Amplified fragment length polymorphism; Bankit: A web-based submission tool for GenBank; BL: Length of body; BLAST: Basic Local Alignment Search Tool; bp: Base pair; DNA: Deoxyribonucleic acid; dNTP: A generic term referring to the four deoxyribonucleotides: dATP, dCTP, dGTP, and dTTP; GH: Growth hormone; *Hae*III: An endonuclease isolated from *Haemophilus aegyptius* bacteria; *IGF-I*: Insulin-like growth factor-I; Kbp: Kilobase pairs; M: Molar; NCBI: National Center for Biotechnology Information; OD: Optical density; P: Probability; PCR : Polymerase chain reaction; pH: Hydrogen ion concentration of a solution; RFLP: Restriction fragment length polymorphism; rpm: Round per minute; SNP: Single nucleotide polymorphism; U: Unit; U.V.: Ultraviolet; w/v: Weight:volume ratio; µg: Microgram; µl: Microliter; χ^2: Chi-square test; a statistical hypothesis test

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Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Authors' contributions

KFM developed the concepts of study, design, and material preparation. KFM and MAM A-H collected the literature research. KFM, SH. A-A, MAM. A-H, MA-M, and HER analyzed and interpreted the data and manuscript preparation. KFM, SH. A-A, MAM. A-H, and MA-M written the manuscript. AIE and FIE revised the manuscript. All authors read and approved the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors participated in this manuscript and have read this manuscript and accept publishing it.

Competing interests

The authors declare that they have no competing interests.

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