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Full Length Research Paper

Genetic polymorphism of five genes associated with growth traits in goat

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Genetic polymorphism studies in domestic animals aim at evaluating genetic variations within and across breeds mainly for conservation purposes. In this study, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect polymorphisms of five candidate genes in four Egyptian and Saudi goat breeds (Barki, Zaribi, Ardi and Masri), to detect the genotype of GH, IGF1, POUIF1, MSTN and BMP15 genes in the goat breeds and their allele frequencies. Results of GH gene which encloses a HaeIII endonuclease restriction site show four unique PCR-RFLP banding patterns (genotypes AA, AB, CC and CD). The frequencies of the A allele in the samples from the goat breeds varied from 0.410 to 0.620. While IGF-1gene revealed three fragments after digestion with HaeIII with genotype AA, AB and BB and the frequencies of allele A varied from 0.432 to 0.731. Furthermore, PCR-RFLP of POUIF1 gene showed two fragments after digestion by Pst1 endonuclease with genotype TT and CC and the frequencies of allele T varied from 0.250 to 0.840. The MSTN gene revealed three fragments after digestion with DraI with genotype AA, BB and AB and the frequencies of allele A varied from 0.240 to 0.630. Meanwhile, the BMP15 gene revealed one fragments of 112 bp for AA after digestion with Hinf1 enzyme.

Key words: Goats, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), GH, IGF-1, POUIF1, MSTN, BMP-15.

INTRODUCTION

The world goat population is 550 million; 94% of this total is found in the Mediterranean, Asia and Africa. The Egyptian goats are reported to be domesticated after migration from Asia along the present Iran–Iraq borders and then to Africa (Mason, 1981). In Egypt, there are 3.13 million goats raised mainly in three regions: the Nile Delta, Upper Egypt and in the desert rangelands, particularly in the northwest coastal zone. Production systems and breeds in the three zones are different. There are about 1.7 million goats, mainly in mixed flocks, with some goats kept as household animals. In the desert rangelands, 1.4 million sheep and goats are kept in extensive systems (Galal et al., 2005). In Saudi Arabian where the climate is suitable for goat, the number of these animals is believed to exceed about 7.5 million mainly of the Masri and Ardi breeds. The Ardi goats are more adapted to the arid region than the Masri goats (Salah et al., 1989).

For the last decade, molecular genetics has lead to the discovery of individual genes or candidate genes with substantial effects on the traits of economic importance. Candidate gene strategy has been proposed by direct search for quantitative trait loci (QTL). Application of molecular genetics for genetic improvement relies on the ability to genotype individuals for specific genetic loci. The information utility from candidate genes in breeding programs has potential to substantially enhance the accuracy of selection and increasing selection differential. The current advances in molecular genetics are leading to the discovery of individual genes or candidate genes with substantial effects on the traits of economic importance. Studying the genetic make-up of individuals at the DNA level and molecular genetics has given

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scientists the tools for opportunities for genetic improvement. Currently, molecular genetic techniques have already resulted in the discovery of several genes that have a major effect on some interesting quantitative traits and of genetic markers that are linked to QTL (Tambasco et al., 2003). Marker assisted selection (MAS), employed in conjunction with traditional selection methods, could accelerate the rate of change in economically important traits (Womack, 2005).

Growth hormone (GH) gene

This gene is physically located on goat chromosome 19q22 (Schibler et al., 1998; Pinton et al., 2000). It is encoded by 1,800 base pairs (bp). It consists of 5 exons and it is separated by 4 interonic sequences (Kioka et al*.,* 1989). This gene produces the growth hormone from the anterior pituitary; it is interesting that both growth hormone gene in sheep and goats are duplicated, one of the copies codes for pituitary GH, but the other one is expressed in the placenta, thus having also implications in pre-natal growth (Yamano et al., 1988) and this hormone is necessary for postnatal growth and metabolism in vertebrates (Ge et al*.,* 2003; Supakorn, 2009). This hormone is associated with both animals growth (Hua et al., 2009) and milk yields traits (Malveiro et al., 2001; Marques et al., 2003) in livestock animals including goats.

Insulin like growth factor I (IGF-I)

The IGFs signaling system, which is composed of IGF-I, IGF-II, IGF-I receptor, IGF-II receptor and six binding proteins (IGFBP-1–IGFBP-6), play an important role in development, growth and reproduction as well as ageing (Miller and Gore, 2001; Li et al., 2009; Lan et al., 2007c). IGF-I gene is encoded by a single gene located on chromosome 5 (Schibler et al., 1998), consisting of three leader exons (1 w, 1 and 1a) and three exons (3, 4 and 6), in which exon 3 and exon 4 encode the mature IGF-I peptide (Mikawa et al., 1995). IGF-I plays a key role in mammalian growth, lactation and metabolism (Zhang et al., 2008), by stimulating anabolic processes such as cell proliferation, skeleton and hair growth and protein synthesis.

Pituitary transcription factor-1 gene (POU1F1)

This is located on goat chromosomes 1q21– 22, comprised six exons containing the POU domain and homeo-domain domain (Woollard et al., 2000); the binding site of this gene is more than 350 kb downstream from the presumed start of transcription at exon 1 (Howard et al., 2009).

POUIF1 (also named PIT-1 or GHF-1) is mainly expressed in the pituitary and plays an important role in the expression of growth hormone (GH), prolactin (PRL), and thyroid- stimulating hormone β (TSH-β) in mammals. Accordingly, mutations on this gene possibly result in deficiency of GH, PRL and TSH (Cohen et al., 1997; Li et al., 1990). The earlier published articles reported that genetic polymorphisms of POU1F1 gene were significantly associated with growth, development and lactation in mammalians including goats (Lan et al., 2007b, d; Li et al., 1990). Moreover, the genetic variation of this gene in exon and flanking regions is associated with economic traits and production performance such as growth, carcass, milk and wool in meat, dairy and cashmere industries, respectively (Lan et al., 2007b,d).

Caprine myostatin (MSTN) gene [also named as growth and differentiation factor-8 (GDF-8) gene]

This gene was physically mapped to goat chromosome 2q11-q12 (Schibler et al., 1998; Pinton et al., 2000). It has been considered as an important candidate gene for growth and development of domestic animals due to its key role in muscle growth and its potential applications in goat breeding (Supakorn, 2009; Zhang et al., 2012). The MSTN gene has been considered as an important candidate gene for growth and development of domestic animals because of its key role in muscle growth and its potential application in animal husbandry. This gene is studied in not only ruminant but also non-ruminant animals. The researchers reported that nine base variations affected amino acids in cattle (Miranda et al., 2002). These changes gave rise to double muscling. Moreover, point mutations of the MSTN gene in swine influenced improvement of average daily gain (Jiang et al., 2002). In chickens, Gu et al. (2002) found 5 SNPs in the 5’ and 3’ untranslated region. These polymorphic sites were strongly associated with production traits ($P < 0.05$). Research identified SNPs of this gene in goats (Li et al., 2006); the authors found 8 polymorphic sites and 2 haplotypes in a part of intron 2 and exon 3. However, the association between these SNPs and production traits was not reported.

Bone morphogenetic protein (BMP) genes

These are members of the transforming growth factor-beta (TGF-β) super-family which are multifunctional cytokines with a 2-fold function and are expressed in a variety of cells. BMPs were originally identified on the basis of their ability to produce ectopic bone formation when implanted within soft tissue *in vivo*. They also play roles in embryonic development, homeostasis, repairing of various tissue patterning, cell differentiation and apoptosis (Wozney et al., 1988; Supakorn, 2009). This gene is located on the X chromosome (Ghoreishi et al.,

Table 1. The identification of the primers and restriction enzymes.

Gene	The primer sequence	Annealing temperature	Restriction enzyme	Reference
GH	GH1: TCAGCAGAGTCTTCACCAAC CAACAACGCCATCCTCAC	56°C	HaeIII	Hua et al. (2009)
	GH2: CTCTGCCTGCCCTGGACT GGAGAAGCAGAAGGCAACC	55°C	HaeIII	
IGF-1	CACAGCGTATTATCCCAC GACACTATGAGCCAGAAG	56°C	HaeIII	Liu et al.(2010)
POU1F1	CCATCATCTCCCTTCTT AATGTACAATGTGCCTTCT	54.5°C	Pst1	Lan et al. (2009)
Myostatin	TGGCGTTACTCAAAAGCAAA AACAGCAGTCAGCAGAGTCG	58°C	Dra I	Li et al. (2008)
BMP-15 *(FecX^G)*	CACTGTCTTCTTGTTACTGTATTTCAATGAGAC GGATGCAATACTGCCTGCTTG	63°C	Hinf1	Hamid et al. (2009)

2011). The BMP-15 regulates granulose cell proliferation and differentiation by promoting granulose cell mitosis, suppressing follicle-stimulating hormone receptor expression and stimulating kit ligand expression. This protein plays a pivotal role in female fertility in mammals (Juengel et al., 2002; Moore et al., 2003).

In the present study, the restriction fragment length polymorphism for the polymerase Chain Reaction products (PCR–RFLP) technique was used to detect the genetic polymorphism within five genes associated with growth trait; GH, IGF-1, POUIF1, MSTN and BMP15 in Egyptian and Saudi goats.

MATERIALS AND METHODS

DNA samples and data collections

Individual blood samples were drawn from 20 non-relative goats of each breed. The samples were collected from Barki breed located in the research farm of the Department of Animal Production, Faculty of Agriculture, Cairo University, and from Zaraibi breed located in the Agriculture research station (El-Serow, Domiatta) of the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture while in Saudi, 15 samples of each breed (Ardi and Masri) were collected from different Farms in Faculty of Science, King-Abd El-Aziz University.

DNA isolation from blood samples

Blood samples were collected from the jugular vein of the goats; the sample volume was 10 ml and collected on 0.5 ml ethylenediaminetetraacetic acid (EDTA) (0.5 M) as an anticoagulant reagent in a sterile tube. The DNA was isolated from goat whole blood using salting out method described by Miller et al. (1988).

Polymerase chain reaction (PCR) runs with specific primer

A PCR cocktail consists of 1.0 µM forward and reverse primers and 0.2 mM dNTPs (Biotechnology, Cairo, Egypt), 10 mM Tris (pH 9), 50 mM KCl (Ran Baxy, New Delhi, India), 1.5 mM $MgCl_2$ (Sigma), 0.01% gelatin (Merk), 0.1% Triton X-100 (Merk) and 1.25 units of Taq polymerase (Bioron, Germany). The cocktail was aliquot into tubes with 100 ng DNA. The reaction ran in a Perkin Elmar apparatus. The reaction was cycled for 1 min at 94°C, 2 min at optimized annealing temperature for each primer (Table 1) and 2 min at 72°C for 30 cycles. The PCR reaction products were electrophoreses on 1.5% agarose gel stained with ethidium bromide to test the amplification success. The optimized amplification cycle was the following: initial denaturation at 94°C for 5 min to completely denature the DNA template, followed by 35 cycles of denaturing at 94°C for 45 s, annealing for min at temperatures specific for the marker and extension at 72°C for 1 min, with a final extension step at 72°C for 5 min followed by storage at 4°C forever.

RFLP and agarose gel electrophoresis

20 µl of PCR products were digested with 10 units of the restriction enzyme (Fermentas, Germany) specific for each gene (Table 1) in a final reaction volume of 25 µl. The reaction mixture was incubated at 37°C in water bath for 5 h. After restriction digestion, the restricted fragments were analyzed by electrophoresis on 2.5% agarose/1X TBE gel stained with ethidium bromide. The 100-bp ladder was used as molecular size marker. The bands were visualized under UV light and the gels were photographed using digital gel documentation system. The size length and allele identification were identified for the obtained photos using specific software named Labimag; it is downloaded from the company web page: www.labimage.com.

RESULTS AND DISCUSSION

There is a considerable interest in the application of

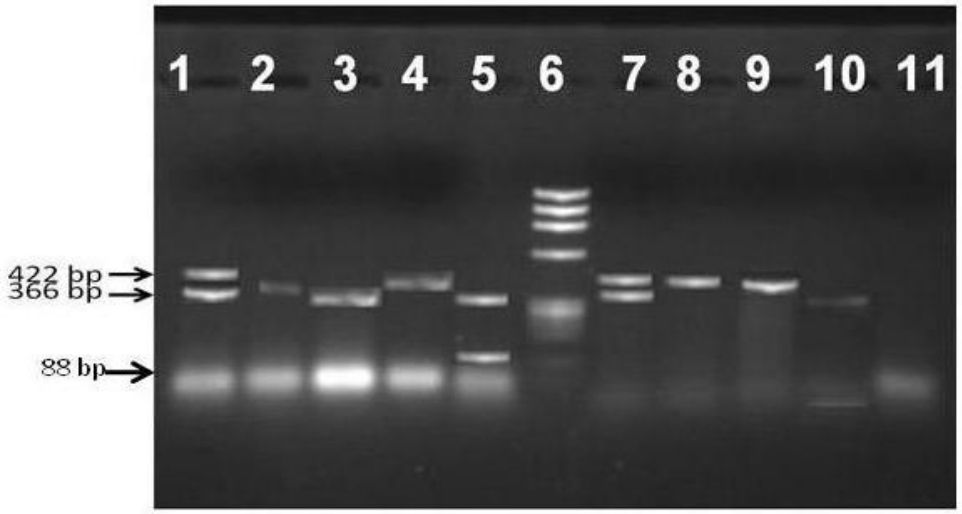

Figure 1. 422-bp PCR amplified GH products digested with HaeIII. Lane 6, DNA marker; lanes1 to 7, restriction digested PCR products from goat breeds DNA showed two fragments of 422 and 366 bp (AB genotype); lanes 2,4, 8,9, one fragment of 422 bp (AA genotype); lane 3, one fragment of 366 bp (BB genotype); lane 5, two fragments of 366 and 116 bp.

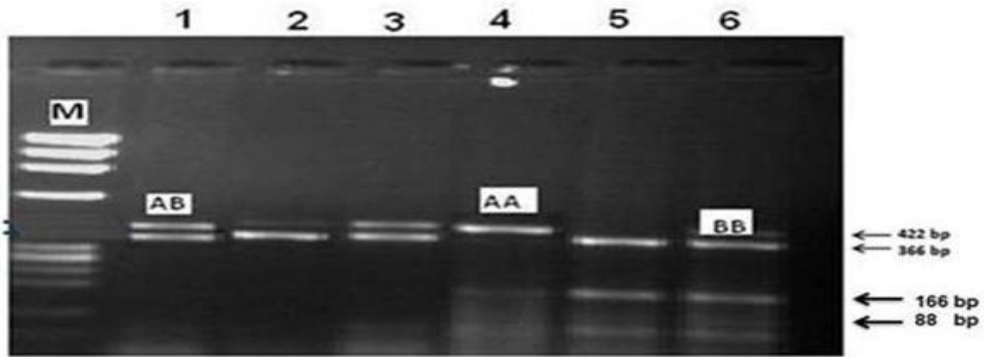

Figure 2. 422-bp PCR amplified GH products digested with HaeIII. Lane 1, DNA marker; lanes1,3, restriction digested PCR products from goat breeds DNA showed two fragments of 422 and 366 bp (AB genotype); lane 4, one fragment of 422 bp (AA genotype); lane 2, one fragment of 366 bp (BB genotype); lanes 5 ,6; three fragments of 366, 116 and 88 bp (CD genotype).

molecular genetics technologies in the form of specific DNA markers that are associated with various productivity traits to promote more efficient and relatively easy selection and breeding of farm animals with an advantage for inheritable traits of meat and milk productivity. Many candidate genes have been identified and selected for analysis based on a known relationship with productivity traits (Spelman and Bovenhius, 1998).

Growth hormone gene

RFLP analysis was conducted to detect polymorphism for two different mutations for this gene in Egyptian and Saudi goat breeds.

In the present study, two regions of the GH gene were amplified from goat genomic DNA; the first region were from the exon 2 while the second were from exon 4. The mutation or the single nucleotide polymorphisms (SNP) of each region were identified after digesting the PCR product with the HaeIII enzyme.

Exon 2 amplification produced 422 bp fragments; when these fragments were digested with the restriction enzymes, it produced three bands: 422 (non-cut), 366 and 56 bp representing the alleles A and B respectively. Exon 4 amplification produced 116 bp fragments; when these fragments were digested with the restriction enzymes, it produced three bands: 116 (non-cut), 88 and 28 bp representing the alleles C and D, respectively. Figures 1 and 2 show that the different alleles resulted from digestion of the PCR products with the HaeIII restriction enzyme after running on agarose gel

Table 2. Allelic frequencies at the GH locus in all goat breed.

Breed	Number of animals	Allelic frequency			
		GH1		GH2	
		A	B	C	D
Barki	20	0.410	0.590	0.000	0.000
Zaribi	20	0.620	0.380	0.580	0.420
Ardi	15	0.430	0.570	0.000	0.000
Masri	15	0.540	0.460	0.000	0.000

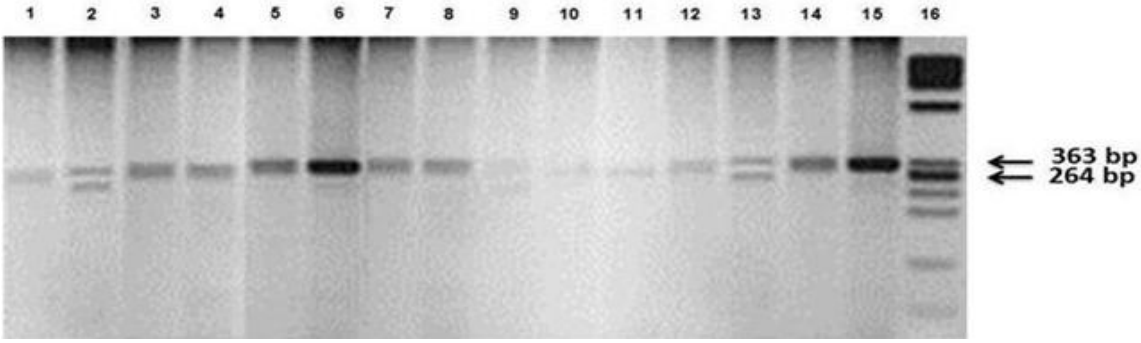

Figure 3. 363-bp PCR amplified IGF-1products digested with HaeIII. Lane 16, DNA marker; lanes 2,9, 13, restriction digested PCR products from goat breeds DNA showing two fragments of 363 and 264 bp (AB genotype); lanes 3, 4, 5, 6, 7, 8, 10, 12, 14, 15, one fragment of 363 bp (AA genotype); lanes 1, 11, one fragment of 264 bp (BB genotype).

electro-phoresis. Table 2 shows the different genotypes and allele frequencies in the different breeds studied.

Our results are similar to that reported earlier in different world goat breeds (Malveiro et al., 2001; Hua et al., 2009).

The allelic frequencies of allele A, B and C in GH1 (exon 2) and GH2 (exon 4) were 0.41, 0.59, and 0.00 for Barki breeds respectively. The allelic frequencies of allele of Zaribi breeds were 0.62, 0.38 and 0.58, respectively while the allelic frequencies of Saudi breeds (Ardi and Masri) were 0.43, 0.57 and 0.00 for Ardi breed and 0.49, 0.51 and 0.00 for Masri breed (Table 2).

Some publications have reported that polymorphism of this gene has been identified in the regulatory region, untranslated regions and exons. Indeed, a few of these polymorphic sites have been precisely characterized for nucleotide and amino acid changes (Ge et al., 2003; Yu et al., 2004). In addition, two polymorphic sites were located in each of exon 1 and exon 2; 4 sites in exon 3, 7 sites in exon 4 and 5 sites in exon 5 (Missohou et al., 2006).

Insulin –like growth factor-1(IGF-1) gene

It is known from past studies (Froesch et al., 1996) that insulin-like growth factor (IGF-1) is a peptide of the molecular weight 7.5 kDa built of 70 amino acids. IGF-1 plays an important stimulatory role in skeletal growth, cell differentiation and metabolism. In addition, there is further demonstration that the IGF-1 gene is important in the control of hair cycles (Philpott et al., 1995; Nixon et al., 1997) and believed to be involved in growth of wool fiber. So this dissertation mainly specializes in the association between polymorphisms in insulin-like growth factor-1 (IGF-1) and cashmere traits data with four local goat breeds in each of Egypt and Saudi.

In the past, some researchers investigated the association between polymorphisms of IGF gene and livestock production traits. For example, the study of Lan et al. (2007a) detected for the first time the polymorphisms of goat IGFBP-3 gene by PCR-SSCP and DNA sequencing methods. The associations of the HaeIII and XspI PCR-RFLPs of goat IGFBP-3 locus with milk traits were analyzed in dairy goat, but the significant statistical results were not found between them ($P > 0.05$). Other study (Kumar et al., 2006) was carried out to study nucleotide sequencing and DNA polymorphism by PCR-RFLP of IGFBP-3 gene in sheep and its comparison with cattle and buffalo. There was approximately 93% similarity in the amino acid sequence of sheep with cattle and buffalo In this study, PCR-RFLP was adopted to analyze polymorphism of IGF-1gene in four goat breeds in each of Egypt and Saudi; the amplified products were digested with HaeIII. The results reveal three fragments of 363, 264 and 99 bp after digestion with HeaIII (Figures 3, 4 and 5); hence the samples had three genotypes (named as genotype AA, AB and BB).

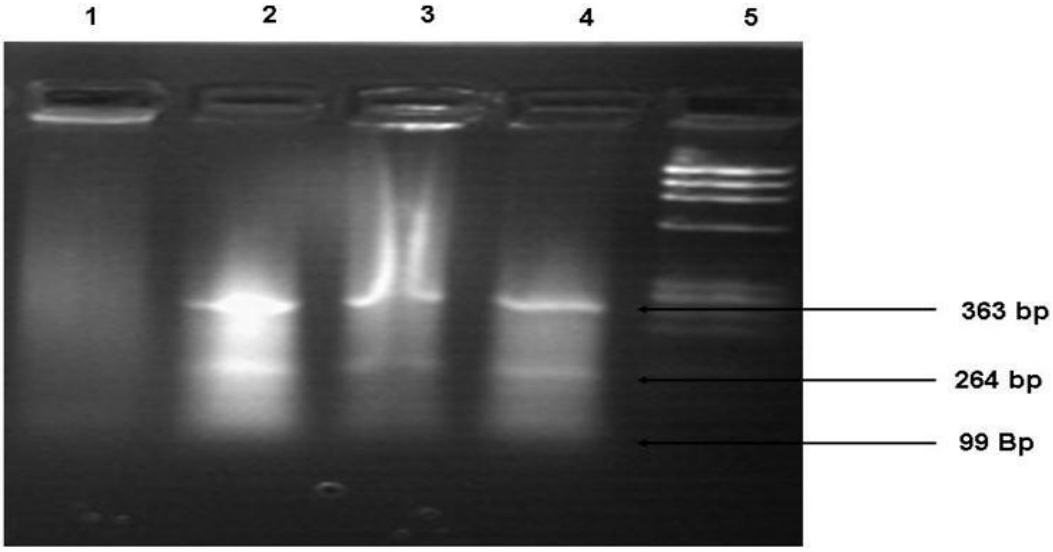

Figure 4. 363-bp PCR amplified IGF-1 products digested with HaeIII. Lane 5, DNA marker; lanes 2, 3, 4, restriction digested PCR products from goat breeds DNA showing three fragments of 363, 264 and 99 bp (BC genotype).

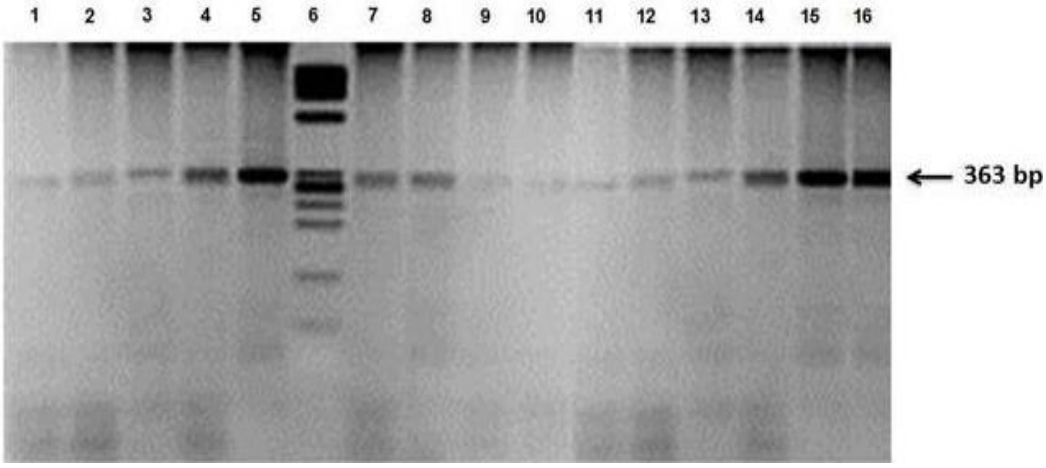

Figure 5. 363 bp PCR amplified IGF-1 products digested with HaeIII. Lane 6, DNA marker; lanes 2, 3, 4, 5, 7, 8, 9, 12, 13, 14 and 15, restriction digested PCR products from goat breeds DNA showing one fragments of 363 bp (AA genotype); lanes 1, 10, 11, 16 showing one fragment of 264 bp (BB genotype).

However, among the three genotypes, the Zaribi goat populations only had genotype AA and BB but the genotype AB disappeared. There may be two reasons; first, because the Zaribi goat populations should only have genotype AA and BB and the other is because the samples of genotype AB could not be collected. On the other hand, the genotypes AA of two Saudi breeds disappeared also. The same genotyping pattern was reported; the cashmere fineness of AA genotype individual was significantly lower than that of AB genotype in Xinjiang goat breeds in China (Qiong et al., 2011).

The results from the community genetics angle, and the genotypic frequencies of different genotype in four goat breeds were calculated (Table 3). Table 3 indicates that allelic frequencies of IGF-1: A, B were 0.731 and 0.269 in Barki breed, 0.432 and 0.568 in Zaribi goats, 0.615 and 0.385 in Ardi breed and 0.473 and 0.527 in Masri goat breed (Table 3).

Pituitary specific transcription factor-1 (POU1F1) gene

The PCR products of this gene (450 bp) were digested by

Table 3. Allelic frequencies at the IGF-1locus in all breeds.

Breed	Number of animals	Allelic frequency	
		A	B
Barki	20	0.731	0.568
Zaribi	20	0.432	0.385
Ardi	15	0.615	0.527
Masri	15	0.473	0.269

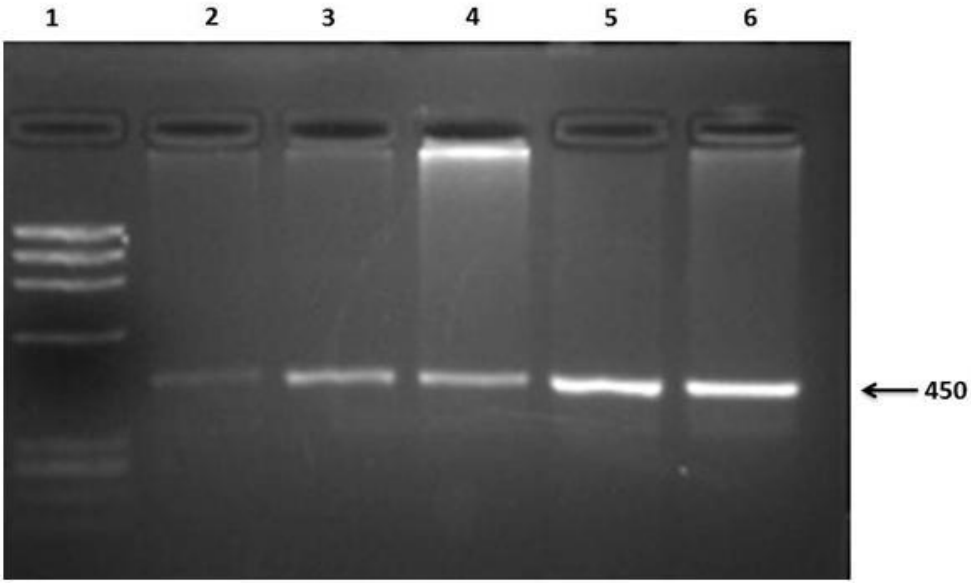

Figure 6. 450 bp PCR amplified POU1F1 products digested with *Pst1.* Lane 1, DNA marker; lanes 2, 3, 4, restriction digested PCR products from goat breeds DNA showing one fragments of 450 bp (TT genotype); lanes 4, 5, showing one fragment of 370 bp (CC genotype).

restriction enzyme *Pst1* and resolved using horizontal gel electrophoresis. The restriction fragment length polymorphism determined by Pst1 endonuclease revealed two fragments of 370 and 80 bp (Figures 6 and 7) hence all samples of goat were typed as T allele (450 bp) and allele C (370 and 80 bp). Our results or obtained alleles are similar to that reported earlier in Chinese goats by Lan et al. (2009).

In the present study, screening for polymorphisms in the analyzed region revealed the presence of a polymorphic site in POUIF-1 (Table 4). The mutation was detected at POUIF-1 locus (Figures 6 and 7). Frequencies of POUIF1-T alleles were 0.84, 0.25, 0.51 and 0.35 for Barki, Zaribi, Ardi and Masri breeds in Egypt and Saudi (Table 4)

The PstI mutation found might not be the causal mutation by itself, but might be in linkage disequilibrium with the causal mutation which could affect either the POU1F1 gene or other genes near to the POU1F1 locus. Thus, the present work presents preliminary results which open a new field of study and suggests that the TT genotype could be used as a molecular marker for superior cashmere yield. However, further analysis should be performed in order to validate both the association found and the physiological significance of the PstI mutation at position 110 in the POU1F1 (Lan et al., 2007a). Mutations on this gene possibly result in deficiency of GH, prolactin, and thyroid stimulating hormone (Li et al., 1990). The genetic variation of this gene in exon and flanking regions is associated with economic traits and production performance such as growth, carcass, milk and wool in meat, dairy and cashmere industries, respectively (Lan et al., 2007b, d).

Caprine myostatin (MSTN) gene

In our study, the genotyping of MSTN gene were done after digesting the PCR product (497 bp) by the restriction enzyme Dra 1 and running the digested fragment on horizontal agarose gel electrophoresis. The fragments obtained were: 497 for AA genotype, 427 and

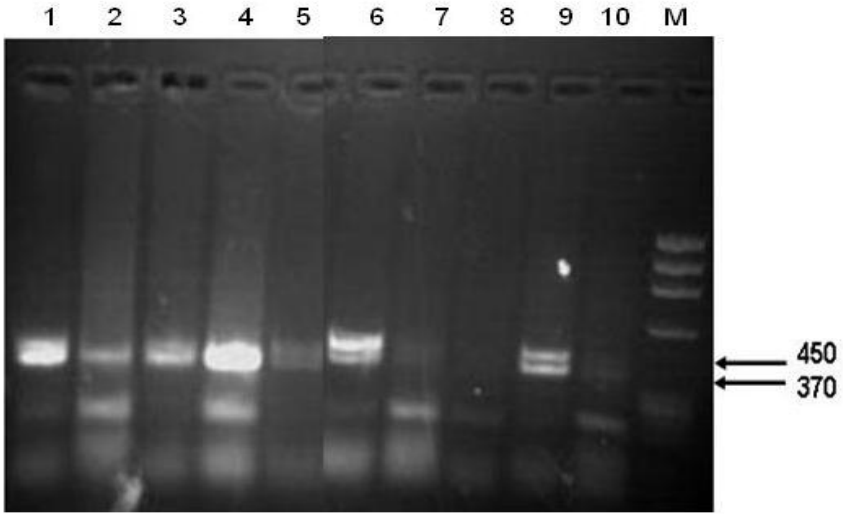

Figure 7. 450-bp PCR amplified POU1F1 products digested with *Pst1.* Lane 1, DNA marker; lanes 1, 3, 5, 6, 9, restriction digested PCR products from goat breeds DNA showing two fragments of 450 bp and 370 (TC genotype); lanes 2, 7 showing one fragment of 450 bp (TTgenotype).

Table 4. Allelic frequencies at the *POU1F1* locus in goat breeds.

Breed	Number of animals	Allelic frequency	
		T	C
Barki	20	0.840	0.160
Zaribi	20	0.351	0.649
Ardi	15	0.512	0.488
Masri	15	0.250	0.650

70 for BB genotype and 497, 427 and 70 for AB genotype after digestion with Dra I (Figures 8 and 9). Frequencies of Myostatin allele A were 0.24, 0.63, 0.56 and 0.31 in Barki, Zaribi, Ardi and Masri breeds in Egypt and Saudi, respectively, while 0.76, 0.37, 0.44 and 0.69 for allele B in Barki, Zaribi, Ardi and Masri breeds in Egypt and Saudi, respectively (Table 5). The obtained results in our study agree with the previously obtained alleles (Li et al., 2006; Zhang et al., 2012).

Bone morphogenetic protein (BMP) gene

BMPs are members of the transforming growth factor-beta (TGF-β) superfamily which are multifunctional cytokines with a 2-fold function and are expressed in a variety of cells. BMPs were originally identified on the basis of their ability to produce ectopic bone formation when implanted within soft tissue *in vivo*. They also play roles in embryonic development, homeostasis, repairing of various tissue patterning, cell differentiation and apoptosis (Wozney et al., 1988).

The BMP-15 regulates granulose cell proliferation and differentiation by promoting granulose cell mitosis, suppressing follicle-stimulating hormone receptor expression and stimulating kit ligand expression. This protein plays a pivotal role in female fertility in mammals (Juengel et al., 2002; Moore et al., 2003).

In the present study, PCR-RFLP with HinfI digestion was used to investigate the genotyping of $FecX^{G}$ loci in exon 2 BMP-15 genes; the study reveal that the genotyping of BMP-15 gene by agarose gel electrophoresis revealed one fragments of 112 bp after digestion with Hinf1 enzyme (Figure 10). The basic finding of the current study was the absence of polymorphism at the loci of $FecX^{G}$ *in exon 2* BMP-15 gene in Egyptian goats and Saudi goats. All goats were monomorphic for exon 2 BMP-15 genes. The findings of the present study are in line with those of Hua et al. (2007) in Chinese goats who reported that none of the polymorphism of the BMP-15 gene was tested in goats from six breeds (or flocks). The same result was observed by Jamshidi et al. (2009) in Sangsari Sheep Breed of Iran. Also, none of the polymorphisms were found in the coding region of BMP- 15 mature peptide of Markhoz goats of Iran by using PCR-SSCP with three

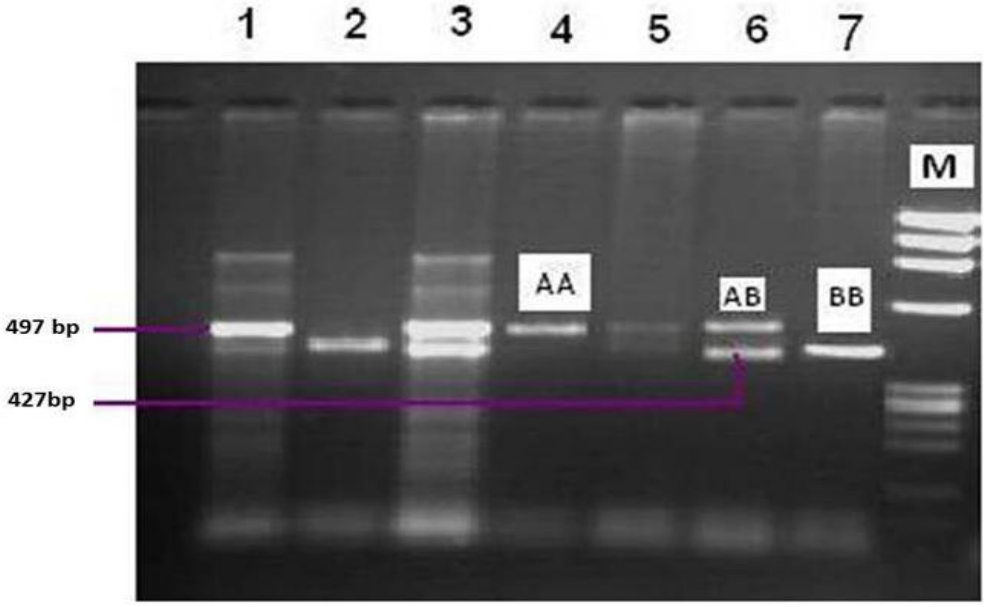

Figure 8. 497 bp PCR amplified Myostatin products digested with *Dra I*. Lane M, DNA marker; lanes 3,5, 6, restriction digested PCR products from goat breeds DNA showing two fragments of 497 and 427 bp (AB genotype); lanes 1, 4, showing one fragment of 497 bp (AA genotype); lanes 2, 7, showing one fragment of 427 bp (BB genotype).

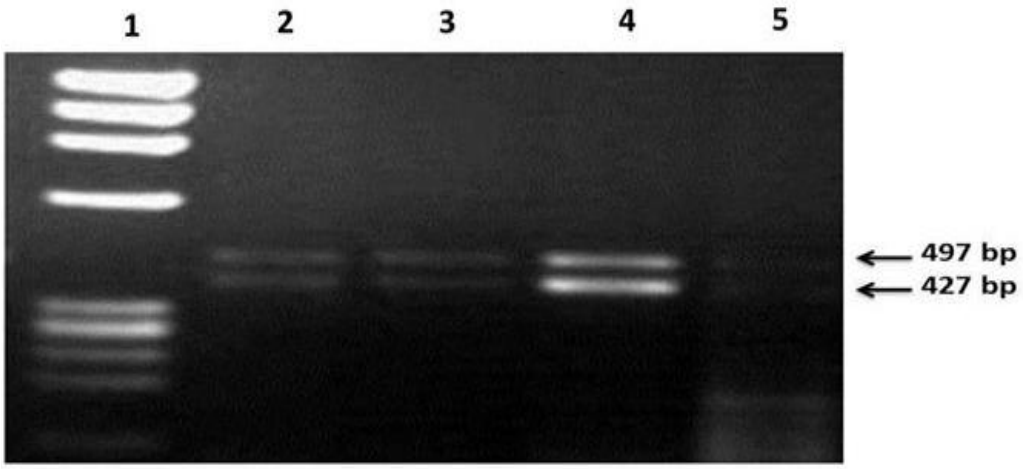

Figure 9. 497-bp PCR amplified Myostatin products digested with *Dra* I I. Lane 1, DNA marker; lanes 2, 3, 4, 5, restriction digested PCR products from goat breeds DNA showing two fragments of 497 and 427 bp (AB genotype).

primer pairs, PCR sequencing and PCR- RFLP with DdeI digestion (Arefnezhad, 2007). The absent of mutation in BMP-15 of six breeds of Chinese goats was reported by He et al. (2006). Zare et al. (2007) also detected no mutations in two points of BMP-15 gene from 240 bloodsamples of Shal ewes by using of PCR- RFLP. Hamid et al. (2009) showed that there was no genetic polymorphism of loci in BMP15 gene in Iranian native goats. Further investigation should be directed at other loci of BMP-15 gene or other genes, using larger sample sizes.

Conclusion

The AA, AB and CC, CD and TT, TC and AA, AB and AA, AB genotypes could be used as a molecular marker genes for growth traits (GH1 and CH2, IGF-1, POUIF1

Table 5. Allelic frequencies at the MSTN locus in all breeds

Breed	Number of animals	Allelic frequency	
		A	B
Barki	20	0.240	0.760
Zaribi	20	0.630	0.370
Ardi	15	0.560	0.440
Masri	15	0.310	0.690

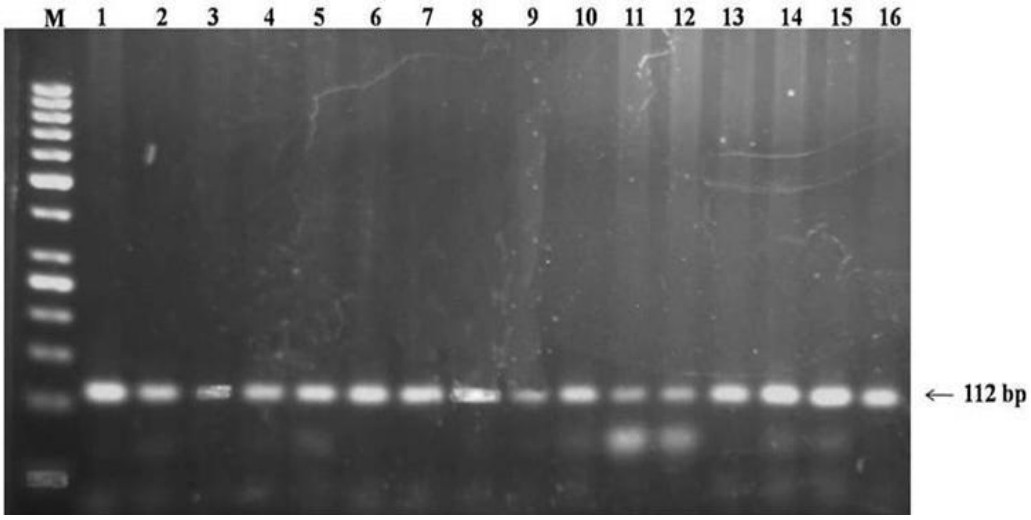

Figure 10. 112-bp PCR amplified *BMP-15* products digested with Hinf1. Lane M, 100 bp ladder marker; lanes 1 – 16, restriction digested PCR products from goat breeds showing one undigested fragment at 112 bp (AA genotype).

and MSTN) genes. However, further analysis should be performed in order to validate both the association found and the physiological significance of the Haelll, Haelll, PstI, DraI and Hinf1 mutations in the GH1 and CH2, IGF-1, POUIF1 and MSTN genes.

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