

## Polymorphism of some Genes Associated with Meat-Related Traits in Egyptian Sheep Breeds

### Research Article

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### ABSTRACT

The genetic polymorphism of some genes related to meat production in three Egyptian sheep breeds (Barki, Rahmani and Osseimi) was studied. The candidate genes were: Calpastatin, Myostatin, Diacylglycerol-acyltransferase1, Insulin-like growth factor binding protein-3 and Booroola fecundity gene. The technique applied was the restriction fragment length polymorphism for the polymerase chain reaction products. Polymorphism was found in the genes: Calpastatin, Myostatin and Diacylglycerol-acyltransferase 1, while no polymorphism was exhibited by the other two genes, Insulin-like growth factor binding protein-3 and the Booroola fecundity in the three breeds under study. Calpastatin locus digested with *MspI* had two genotypes MM and MN. The highest allelic frequency was for allele M. The same locus Calpastatin digested with *NcoI* also exhibited two genotypes MM and MN. The NN genotype was absent with both the *MspI* and the *NcoI* enzymes in all breeds. Myostatin digested with *DraI* had two genotypes AB and BB. The AA genotype cannot be detected. The highest allelic frequency was for allele B. Diacylglycerol-acyltransferase1 digested with *AluI* showed two genotypes CC and CT. The highest allelic frequency was for allele C. The detected CT genotype might explain the moderate intramuscular fat content and muscle marbling in the Egyptian sheep breeds. Each of the remaining two loci (Insulin-like growth factor binding protein-3 and the Booroola fecundity) had only one genotype, BB genotype for Insulin-like growth factor binding protein-3 digested with *HaeIII* and ++ genotype for the Booroola fecundity digested with *AvaII* enzyme, therefore they are not recommended in the selection program. The result of Chi-square analysis indicated that the three Egyptian sheep breeds were in Hardy-Weinberg equilibrium.

**KEY WORDS** genetic polymorphism, PCR-RFLP, sheep.

### INTRODUCTION

Sheep is one of the most important domestic animals raised in Egypt. Sheep population in Egypt increased by 66.7% from 1961 to 2005 (FAO, 2007). There are almost 5.5 million head of sheep (FAOSTAT, 2011). Sheep are raised mainly for meat production with carpet sheep wool as a secondary product (Galal *et al.* 2002). The three major Egyptian sheep breeds are: Barki, Rahmani and Osseimi.

They represent about 65% of the total sheep population in Egypt (Elshennawy, 1995). The Egyptian sheep breeds are characterized as small-to medium-sized body, fat tailed and fleece is coarse wool (Aboul-Ela *et al.* 1987). Meat is an integral part of a healthy diet and meat consumption continues to increase steadily, while the world's producers of livestock for red meat production are finding it difficult to cope with the increase in demand (FAO, 2007). Sheep contribute 6% of the total red meat produced and are con-

sidered the second source of red meat in Egypt (Galal *et al.* 2005). At the present time, the consumers demand for sheep meat is not focused on quantitative traits only, but also qualitative traits of meat. Animal breeders could accelerate the rate of genetic improvement attained in carcass composition and meat quality traits through the application of gene-assisted selection (GAS), which is simply based on incorporating favorable candidate genes in traditional breeding programs (Andersson, 2001). The allele variation within candidate genes between breeds is warranted to differentiate them on genetic basis. The result of this variation may assist in choosing the best strategy of improvement options for these genetic resources (Tohidi *et al.* 2013). There are many of published articles on different genes associated with meat-related traits in varied sheep breeds, among these genes are *CAST*, *MSTN*, *DGATI*, *IGFBP3* and *FecB*.

Calpastatin (*CAST*) gene is an endogenous inhibitor of non-lysosomal proteases-calpains, present in cytosol, mapped to ovine chromosome 5 (OAR 5). It is essential for the formation of skeletal muscles during the postnatal period and proteolysis after slaughter, having therefore an effect on traits of particular importance to consumers such as meat tenderness or water binding (Padma *et al.* 2004). It was also proved that there is a relationship between polymorphism in the calpastatin gene in sheep and slaughter traits such as lamb's body weight at birth and its growth rate until weaning (Byun *et al.* 2008). Associations between variation in *CAST* gene with carcass and meat quality traits in sheep have been reported by several authors (Palmer *et al.* 1998; Zhou *et al.* 2007; Sumantri *et al.* 2008; Gabor *et al.* 2009).

Using molecular genetics approach to study meat quality in sheep, Palmer *et al.* (1998) have suggested *CAST* gene as a candidate gene for meat quality in ovine species. Post-slaughter tenderization depends on the balance between the level of activity and inactivation of proteolytic enzymes (Warner *et al.* 2010). A mutation in *CAST* gene can influence on calpastatin activity followed by a level of proteolytic enzymes at slaughter. Myostatin (*MSTN*) gene, (also known as growth and differentiation factor 8, GDF8) is the major regulator of myogenesis and it functions as a negative regulator of muscle growth in mammals. Mutations in *MSTN* are associated with increased skeletal muscle mass (double muscling) in sheep (Clop *et al.* 2006; Kijas *et al.* 2007). The regulation of muscularity, adiposity and tendon structure potentially has important implications for sheep meat production. Ovine *MSTN* is located on OAR 2. Myostatin is known to directly influence muscular hypertrophy and carcass conformation (Nakev *et al.* 2013). Diacylglycerol-acyltransferase1 (*DGATI*) is a gene that encodes one of the key enzymes controlling the rate of triglyceride syn-

thesis in adipocytes (Winter *et al.* 2002). It plays a central role in the metabolism of cellular glycerolipids (Cases *et al.* 1998) and also acts as a key modulator in fat deposition of livestock breeds (Cui *et al.* 2011; Patel *et al.* 2009). Ovine *DGATI* has been localized to OAR 14. Over expression of *DGATI* leads to increased cellular triacylglyceride (TG) storage in white adipose tissue, skeletal muscle, and liver in a tissue specific manner and thereby limits the amounts of intracellular diacylglycerol (DAG) and free fatty acids (Roorda *et al.* 2005; Liu *et al.* 2007; Liu *et al.* 2009; Kamisaka *et al.* 2010).

Insulin-like growth factor binding protein-3 (*IGFBP3*) is a specific carrier protein that binds non-covalently to IGFs in circulation (Bale and Conover, 1992). It acts as a marker for different body functions such as growth, metabolism, reproduction, in controlling body weight, immunity and energy balance (Sharma *et al.* 2012). *IGFBP3* has been localized to OAR21 (Hastie *et al.* 2004). Polymorphism in *IGFBP3* has been studied in different live stocks. There are a few reports in cattle (Shukla, 2001), buffalo (Padma *et al.* 2004) and sheep (Kumar *et al.* 2006), which suggest the polymorphic/non-polymorphic nature of *IGFBP3* gene.

The Booroola fecundity gene (*FecB*) is a single autosomal gene, which increases ovulation rate and litter size in sheep (Davis, 2004). The *FecB* locus is located in the region of ovine chromosome 6. In recent years, many aspects of the *FecB* gene are reported including reproductive endocrinology (Zhong *et al.* 2006), ovary development (Yao *et al.* 2006), litter size (Chu *et al.* 2007; Guan *et al.* 2006), organ development and body measurements (Guan *et al.* 2006; Yu *et al.* 2008). Litter size and lamb growth are important economic values in sheep breeding and reproduction.

The aim of the present study is to identify the genetic polymorphism for five genes correlated with meat gain and traits (*CAST*, *DGATI*, *MSTN*, *IGFBP3* and *FecB*) in three Egyptian sheep breeds (Barki, Rahmani and Osseimi). Application of Polymerase Chain Reaction-Restriction Fragment Length polymorphisms (PCR-RFLPs) was carried out.

## MATERIALS AND METHODS

### Animals and blood collection

A total of 60 animals representing the three Egyptian sheep breeds under study (Osseimi, Barki and Rahmani) were randomly sampled (20 samples per breed). All animals were born and reared in the Agriculture Research Station, belonging to the Faculty of Agriculture, Cairo University. Blood samples were collected in tubes containing EDTA as anticoagulant and transported to the laboratory under cooled conditions.

### DNA isolation

DNA was extracted and purified from blood samples using the whole blood salting out technique described by Miller *et al.* (1988). DNA concentration and purity were determined using an UV spectrophotometer at optical density of 260 and 280 nm.

### Polymerase chain reactions (PCR)

Reactions were performed using specific primers for each gene under study. Details of the primer sequences are listed in Table 1. Amplification reaction was carried out in a 25  $\mu$ L volume containing 100 ng genomic DNA, forward and reverse primer (both at concentration 10 pmol/ $\mu$ L), 1U *Taq* polymerase, 2.5  $\mu$ L *Taq* polymerase buffer, four dNTPs (each at final concentration of 2.5 mM/ $\mu$ L) and de-ionized double distilled H<sub>2</sub>O up to a total volume of 25  $\mu$ L. The general PCR program for the amplification of the genes included in the current study was: initial denaturation: 95 °C for 3 min., 95 °C for 15 sec. (denaturation), 58-63 °C for 30-60 sec. (annealing depending on the gene) and 72 °C for 30 sec. up to 35 cycles, then final extension at 72 °C for 5 min. and finally storage at 15 °C. For PCR optimization the temperature and the time of the annealing temperature were changed. The success of PCR was tested after running some of the products on 2% horizontal agarose gel electrophoresis, and staining with ethidium bromide.

### Restriction fragment length polymorphism (RFLP)

Ten  $\mu$ L from the PCR products were digested with 5 units of the fast restriction enzyme including specific buffer (Fermentas, Germany) specific for each gene (Table 1) in a final reaction volume of 15  $\mu$ L. The reaction mixture was incubated at 37 °C in water bath for 30 minutes. After restriction digestion, the restricted fragments were visualized after running in horizontal gel electrophoresis (2-3% agarose) and staining with ethidium bromide, except *IGFBP3* reaction mixtures were running in vertical polyacrylamide gel electrophoresis (since the cut fragment size was small). 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 (Bio-Rad, USA). The allele sizes were determined using free software named Lab. Image V2.7. It is dispersed free from Proland company (Germany), from the internet through the web page: <http://www.labimag-ing.com/servlet/engine/home/start.html>.

### Statistical analysis

The genotypic and allelic frequencies, the observed and expected heterozygosities and the  $\chi^2$  test for Hardy-Weinberg equilibrium (HWE) were calculated using Pop Gene 32.1 package (Yeh *et al.* 1999).

## RESULTS AND DISCUSSION

### Genetic polymorphism of *CAST* gene digested with *MspI*

Digestion of 622 bp PCR product of *CAST* gene with restriction endonuclease (*MspI*) differentiated alleles N and M. The *MspI* digestion of the PCR products produced fragments of 336 bp and 286 bp for allele M, but the allele N was not digested (Figure 1). The homozygous genotype NN (622 bp) was not observed in the three sheep breeds studied. The heterozygous genotype NM (622 bp, 336 bp and 286 bp) and the homozygous genotype MM (336 bp and 286 bp), both were detected in all breeds involved. Polymorphism of *CAST* gene has been reported in Kurdi sheep in Iran (Nassiry *et al.* 2006), Karakul sheep in Iran (Shahroudi *et al.* 2006), Merino, Corriedale, Romney, Poll Dorset, and crossbred NZ sheep in New Zealand (Zhou *et al.* 2007).

Our results are in agreement with the polymorphism detected in *CAST* gene that was previously observed by Palmer *et al.* (1998) in Dorset sheep, Chung *et al.* (2001) in mixed population sheep of Tsigai, Improved Valachian, East Friesian, Lacaune and Lacaune  $\times$  Tsigai breed and Gabor *et al.* (2009) in local sheep raised in Slovakia. They found only two genotypes MM and NM. Palmer *et al.* (1998) detected 3 genotypes MM, MN, NN of *CAST* gene in Corriedale rams; also Shahroudi *et al.* (2006) found the same three genotypes in Iran Karakul sheep, while Sumantri *et al.* (2008) found only NN and NM genotypes in Indonesian Jonggol sheep.

There was a tendency of the three Egyptian sheep breeds to have higher frequencies of M allele than the N allele. Other studies also showed similar high frequency of allele M and low frequency of allele N (Table 2). Shahroudi *et al.* (2006) reported that the frequencies for M and N alleles of *CAST* gene digested with *MspI* in Karakul sheep were 0.79 and 0.21 respectively. Gabor *et al.* (2009) also reported that the genotyping results of *CAST* gene locus *MspI* in Tsigai sheep, Improved Valachian, Lacaune, East Friesian, and Tsigai sheep  $\times$  Lacaune sheep had allele frequencies of M 0.94 and N 0.06. In addition, Suleman *et al.* (2012) found that the frequency of M allele was higher than that of N allele in Thalli, Lohi and Kajli Pakistan sheep. In contrast, the result of the current study differs from that reported by Sumantri *et al.* (2008) who reported that the allele N frequency was higher than allele M in Indonesian Jonggol sheep breed. The highest genotypic frequency NM was found in Barki breed and the highest genotypic frequency MM was found in Osseimi breed.

There are limited studies for association between polymorphism of *CAST* gene and growth and meat characteristics in sheep.

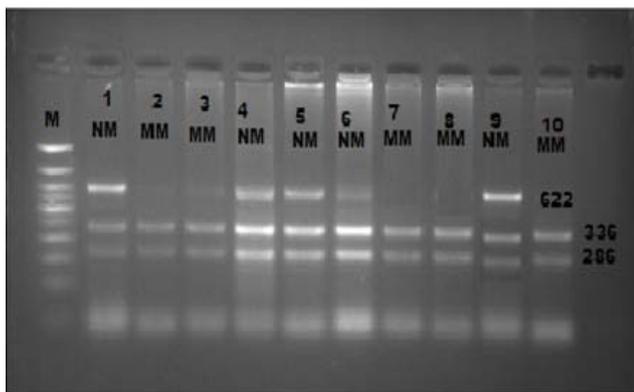
**Table 1** List of primer sequences and restriction enzymes of candidate genes

Gene symbol	Primer séquences	Restriction enzyme	Reference
CAST	TGGGGCCCAATGACGCCATCGATG	<i>MspI</i>	Shahroudi <i>et al.</i> (2006)
	GGTGGAGCAGCACTTCTGATCACC	<i>NcoI</i>	
MSTN	'TGGCGTACTCAA AAGCAAA AACAGCAGTCAGCAGAGTCG	<i>DraI</i>	Xianglong <i>et al.</i> (2008)
DGATI	GCATGTTCCGCCCTCTGG GTCCTAAATAGGTCCTCTCG	<i>AluI</i>	Xu <i>et al.</i> (2008)
IGFBP3	CCAAGCGTGAGACAGAATAC' AGGAGGGATAGGAGCAAGAT	<i>HaeIII</i>	Ali <i>et al.</i> (2009)
FecB	CCAGAGGACAATAGCAAAGCAAA CAAGATGTTTTTCATGCCTCATCAACACGG	<i>Avall</i>	Guan <i>et al.</i> (2006)

**Table 2** Genotypic frequency, allelic frequency, observed heterozygosity (Ho), expected heterozygosity (He) and  $\chi^2$  estimates of CAST gene digested with *MspI* and *NcoI*

Breed	Restriction Enzyme	Genotypic Frequency			Allelic Frequency		Ho	He	$\chi^2$
		MM	NM	NN	N	M			
Barki	<i>MPSI</i>	0.65	0.35	0	0.32	0.68	0.65	0.45	4.22
	<i>NcoI</i>	0.50	0.50	0	0.75	0.25	0.50	0.38	1.96
Rahmani	<i>MPSI</i>	0.40	0.60	0	0.20	0.80	0.40	0.32	1.07
	<i>NcoI</i>	0.55	0.45	0	0.77	0.23	0.45	0.35	1.47
Osseimi	<i>MPSI</i>	0.30	0.70	0	0.15	0.85	0.30	0.26	0.508
	<i>NcoI</i>	1.00	0.00	0	1	0.00	0.30	0.00	0.00

However, [Diyono \*et al.\* \(2007\)](#) demonstrated an association between MN genotype with live body weight. They reported that male sheep with MN genotype had higher body weight in comparison to NN genotype.



**Figure 1** DNA electrophoretic pattern of CAST amplicon after digestion with *MspI* endonuclease. Lane M, 100 bp DNA ladder, lanes genotype NM (1, 4, 5, 6, 9); (622, 336 and 286 bp) and lanes (2, 3, 7, 8, 10) genotype MM (336 and 286)

Regarding the heterozygosity values for this gene in the breeds under study, in general, allelic frequency of the observed heterozygosity was higher than the expected heterozygosity in all breeds. The highest (0.65) was in Barki, while the lowest value (0.30) was in Osseimi (Table 2). The CAST locus *MspI* had  $\chi^2$  value of 4.22, 1.07 and 0.508 for Barki, Rahmani and Osseimi breeds respectively (Table 2). These values shown are non-significant deviation from HWE ( $P > 0.05$ ).

**Genetic polymorphism of CAST gene digested with *NcoI***

The digestion of 622 bp PCR product of CAST gene with restriction endonuclease *NcoI* differentiated alleles N and M. The *NcoI* digestion of the PCR products produced digestion fragments of 374 and 248 bp for allele N but the allele M was not digested. The size of fragment for allele M was 622 bp (Figure 2).



**Figure 2** DNA electrophoretic pattern of CAST amplicon after digestion with *NcoI* endonuclease. Lane M, 100 bp DNA ladder, lanes genotype MM (1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18, 19) (622 bp) and lanes (5, 14, 15, 16) genotype NM (622, 374 and 248)

The homozygous genotype MM (622 bp) was detected in all animal belonging to Osseimi breed and the heterozygous genotype MN (622 bp, 374 bp and 248 bp) was absent in Osseimi breed. The homozygous genotype NN (374 bp and 248 bp) was not observed in all sheep breeds, while MM was the common genotype (Table 2).

This result is similar to that obtained by Sutikno *et al.* (2011) where genotypes NN was absent in population of local sheep in JASTRU. On the contrary, Palmer *et al.* (1998) identified three genotypes MM, MN and NN in Dorset Down, Dorset Down × Coopworth, and Corriedale sheep.

The frequency of homozygous genotype MM had (0.50, 0.55 and 1) while heterozygous genotype MN had frequency (0.50, 0.45 and 0.00) this suggests a superiority of M allele (0.75, 0.77 and 1) than N allele (0.25, 0.23 and 0.00) in Barki, Rahmani and Osseimi, respectively (Table 2).

In general, low to moderate observed and expected heterozygosity estimates were obtained. The Barki had the highest expected heterozygosity (0.38) followed by Rahmani (0.35). No heterozygosity, either observed or expected could be noticed in Osseimi breed (Table 2). The value of the observed heterozygosity was higher than that of the expected heterozygosity in both Barki and Rahmani, this means that the inbreeding rate within each of these two breeds is low (Lewontin, 1974).

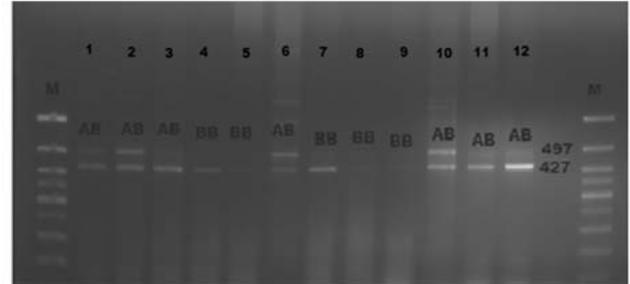
Genetic variation of breeds was evaluated by using  $\chi^2$ -test (Table 2). The *CAST* locus *NcoI* had  $\chi^2$  value of 1.96 and 1.47 for Barki and Rahmani.  $\chi^2$  showed non-significant deviation from HWE ( $P > 0.05$ ). This explains the high value of observed than expected heterozygosity in these breeds. Generally, HWE can be affected by inbreeding, assortative mating, natural selection and population subdivision (Nei and Kumar, 2000).

### Genetic polymorphism of *MSTN* gene digested with *DraI*

The ovine *MSTN* gene produced a DNA fragment of 497 bp. Digestion of this fragment by restriction endonuclease *DraI* generated only two different genotypes, AB and BB, no AA genotype was found. The uncut was related to the A allele, while the cut fragment (427 and 70 bp) was corresponding to the B allele (Figure 3). In general the goat A allele was characterized by the deletion of a small DNA fragment (TTTTA). This deletion unique for goats was found in 5'UTR of goat *MSTN* gene and causing a significant effect on body weight and size (Xianglong *et al.* 2008). The present study indicates that this deletion can be also seen in different sheep breeds and not limited to goats. It was observed that the BB genotype was the more common genotype in all breeds under study; this might be due to the low frequency noticed for the allele A (Table 3).

The values of the observed heterozygosity were higher than the expected ones in all breeds (Table 4). The frequency of the B allele was significantly higher than that of A allele, especially in Osseimi breed. This may explain the lowest heterozygosity in this breed.

Absence of AA genotype and presence of AB genotype in Egyptian sheep's may be due either to a mutation (deletion) in corresponding locus or allele A is naturally exists in the sheep genome. Further studies, especially sequencing of the heterozygous genotype (AB) is recommended.



**Figure 3** DNA electrophoretic pattern of *MSTN* amplicon after digestion with *DraI* endonuclease. Lane M, 100 bp DNA ladder, lanes (1, 2, 3, 6, 10, 11, 12) genotype AB (497, 427 and 70 bp) and lanes (4, 5, 7, 8, 9) genotype BB (427 and 70). It was difficult to classify the 70 bp fragment on 2.5% agarose gel since it could not be seen

**Table 3** Genotypic and allelic frequencies of *MSTN* gene digested with *DraI*

Locus	Breed	Genotypic frequency			Allelic frequency	
		AA	AB	BB	A	B
<i>MSTN</i> ( <i>DraI</i> )	Barki	0	0.45	0.55	0.23	0.77
	Rahmani	0	0.35	0.65	0.17	0.83
	Osseimi	0	0.15	0.85	0.07	0.93

**Table 4** Observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ) and  $\chi^2$  estimates of *MSTN* gene digested with *DraI*

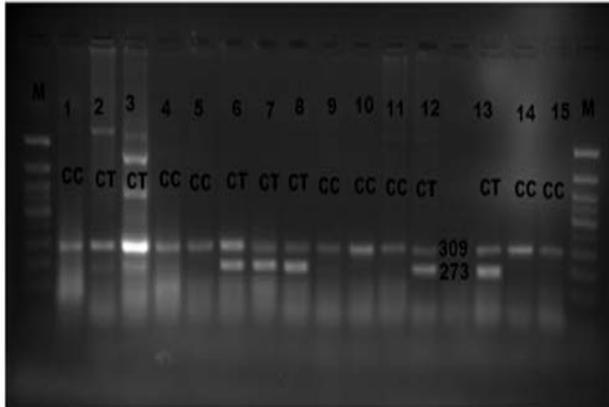
Locus	Breed	$H_o$	$H_e$	$\chi^2$
<i>MSTN</i> ( <i>DraI</i> )	Barki	0.45	0.36	1.47
	Rahmani	0.35	0.30	0.75
	Osseimi	0.15	0.14	0.08

The highest heterozygosity either observed (0.45) or expected (0.36) was in Barki sheep. The lowest values, 0.15 and 0.14 were in Osseimi but Rahmani has moderate value of 0.35 for ( $H_o$ ) and 0.30 for ( $H_e$ ) (Table 4). HWE of breeds was determined by using  $\chi^2$ -test. The *MSTN* locus *DraI* had  $\chi^2$  value of 1.47, 0.75 and 0.085 for Barki, Rahmani and Osseimi, respectively.  $\chi^2$  values indicate non-significant deviation from HWE ( $P > 0.05$ ) (Table 4).

### Genetic polymorphism of *DGTAI* gene digested with *AluI*

The size of PCR amplicon of *DGTAI* gene was 309 bp. The amplified segment includes part of exon 16, whole intron 16 and exon 17.

Xu *et al.* (2008) reported presence of a point mutation in exon 17 (C→T) which creates a cut site for endonuclease *AluI* (AGCT). The present locus has two alleles; C with 309 bp, and T with 272 and 37 bp. Two genotypes were generated after its digestion with *AluI*, which are CC (309 bp) and CT (309, 272 and 37 bp) (Figure 4).



**Figure 4** DNA electrophoretic pattern of *DGTA1* amplicon after digestion with *AluI* endonuclease. Lane M, 100 bp DNA ladder, lanes (1, 4, 5, 9, 10, 11, 14, 15) genotype CC (309 bp) and lanes (2, 3, 6, 7, 8, 12, 13) genotype CT (309, 273, 37). It was difficult to classify the 37 bp fragment on 2.5% agarose gel since it could not be seen

This SNP is a silent mutation {GCC(Ala)→ GCT(Ala)}, which creates a no substitution change for amino acid sequence of *DGTA1* protein (Yang *et al.* 2011). The genotypic and allelic frequencies in exon 17 of *DGTA1* gene of studied breeds are shown in Table 5.

**Table 5** Genotypic and allelic frequencies of *DGTA1* gene digested with *AluI*

Locus	Breed	Genotypic frequency			Allelic frequency	
		CC	CT	TT	C	T
<i>DGTA1</i> ( <i>AluI</i> )	Barki	0.75	0.25	0	0.88	0.12
	Rahmani	0.60	0.40	0	0.80	0.20
	Osseimi	0.50	0.50	0	0.75	0.25

In the two breeds, Barki and Rahmani, the CC genotype was higher than that of CT, while in Osseimi breed, the two genotypes CC and CT were equal. The TT genotype was not exhibited by any of the three breeds. With regard to allelic frequencies, the C allele was the predominant one in all breeds. Its frequency was 0.88, 0.8 and 0.75 in Barki, Rahmani and Osseimi, respectively. On the other hand, the highest frequency for T allele was found in Osseimi (0.25) while the lowest one was seen in Barki (0.12) (Table 5). These results are in agreement with that obtained by Yang *et al.* (2011) whom reported that CC genotype was higher than that of CT and TT in Tan sheep, Oula sheep, Ganjia

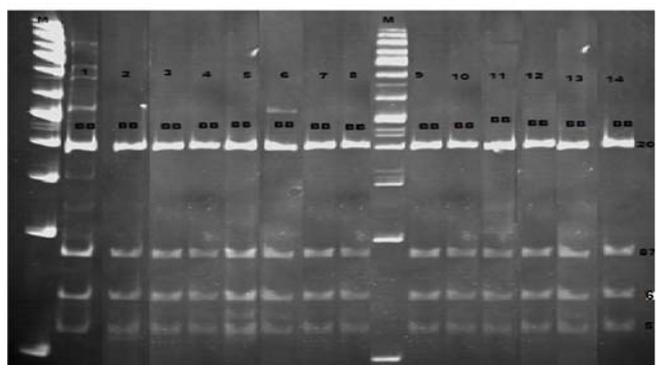
sheep and Qiaoke sheep. In addition, the SNP (C→T) in exon 17 of Chinese indigenous sheep breeds had a significant association with intramuscular fat content (IMF), muscle marbling and meat tenderness. Xu *et al.* (2008) reported that the mutated T allele of *DGTA1* gene had a positive effect on meat quality traits of sheep and the CT genotype had a moderate IMF content and marbling score. Detection of CT genotype and absence of TT genotype in the studied breeds may explain the moderated IMF content and muscle marbling in the Egyptian sheep breeds. The highest observed heterozygosity (0.5) was found in Osseimi breed and the lowest was (0.25) detected in Barki breed (Table 6). Regarding genetic variation, the values of Chi-square test were within HWE ( $P > 0.05$ ). This is similar to the result of Yang *et al.* (2011). The allele distribution in Tan sheep and Oula sheep were within Hardy-Weinberg equilibrium (Table 6).

**Table 6** Observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ) and  $\chi^2$  estimates of *DGTA1* gene digested with *AluI*

Locus	Breed	$H_o$	$H_e$	$\chi^2$
<i>DGTA1</i> ( <i>AluI</i> )	Barki	0.25	0.25	1.96
	Rahmani	0.40	0.33	1.47
	Osseimi	0.50	0.40	0.00

**Genetic polymorphism of *IGFBP3* gene digested with *HaeIII***

The length of the amplified product of ovine *IGFBP3* gene was 654 bp in sheep. The amplified 654 bp fragment comprises the last part of exon 2, complete intron 2, exon 3 and a part of intron 3. The exon-intron regions were assigned on the basis of the published reports of this gene in cattle (Maciulla *et al.* 1997). It is clear from *HaeIII* RFLP pattern represented in Figure 5 that, there was no polymorphism among the three Egyptian sheep breeds in respect to *IGFBP3* gene.



**Figure 5** Polyacrylamide gel electrophoresis for DNA of *IGFBP3* amplicon after digestion with *HaeIII* endonuclease. Lane M, 50 bp DNA ladder, all lanes (1-14) genotype BB (201, 201, 87, 76 and 57 bp). It was difficult to classify the (19, 16 and 8 bp) fragments on polyacrylamide gel

Digestion of the PCR product of *IGFBP3* gene with *HaeIII* revealed only one type of restriction pattern including fragments of sizes 201, 201, 87, 67, 57, 19, 16 and 7 bp (the first five fragments were visible) which represent the allele B only. This result indicates the homozygosity of this gene in the three sheep breeds studied. The present findings are similar to those reported by Kumar *et al.* (2006) who found no polymorphism in *IGFBP3* gene in five Indian sheep breeds. However, they obtained *HaeIII* restriction pattern of eight fragments of sizes 201, 201, 87, 67, 57, 19, 16 and 7 bp in all animals studied revealing absence of polymorphism in these Indian sheep breeds.

Also, the present results showed only one genotype (BB) in extra the three Egyptian sheep breeds. Ali *et al.* (2009) reported the same result in the four Egyptian sheep breeds (Rahmani, Osseimi, Barki and Awassi) indicating the homozygosity of this gene in the four breeds studied. They also reported that all the sheep have intact *HaeIII* restriction site (GG↓CC) indicating the absence of polymorphism at this site.

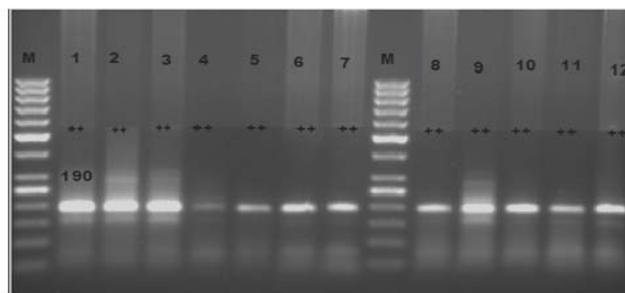
#### Genetic polymorphism of *FecB* gene digested with *Avall*

The PCR products of the *FecB* gene from the three Egyptian breeds produced a single band of 190 bp. The primer used in the present study was that used by Guan *et al.* (2006). It was specific for detection of the restriction site, G/GACC when the *FecB* gene products are digested with *Avall* enzyme.

Digestion of the amplified products of the *FecB* gene revealed only the same band (190 bp). No differences in the band patterns of digested products were detected. Only the wild type alleles (++) were recognized and all breeds for this locus were monomorphic (Figure 6). Similar results were reported by El-Hanafy and El-Saadani (2009). They found the 190 bp band in five Egyptian sheep breeds, among them were Rahmani, Barki and Osseimi.

Guan *et al.* (2006) studied polymorphism of *FecB* gene in nine sheep breeds. Seven out of the nine breeds had only the wild type (++) genotype, i.e. they have only the 190 bp band. One breed (the Hu sheep) was all homozygous for *FecB* gene (BB), the mutant form. In the remaining breed which is the Chinese Merino prolific meat strain, the three genotypes BB, B+ and ++ were found. In the present work, only the wild type (++) was detected in the three Egyptian breeds. The present findings and those reported by El-Hanafy and El-Saadani (2009) confirm the absence of the restriction site, G/GACC in the Egyptian breeds. Similar results were published by Abouheif *et al.* (2011) in Najdi and Naeimi sheep breeds of Saudi Arabia and by Al-Barzinji and Othman (2013) in five Iraqi sheep breeds (Hamdani, Karadi, Arabi, Naeimi and Awassi). The mean

litter size of ewes with genotype ++ was less than that of those having BB and B+ genotypes.



**Figure 6** DNA electrophoretic pattern of *FecB* amplicon after digestion with *HaeIII* endonuclease. Lane M, 100 bp DNA ladder, all lanes (1→12) genotype ++ (190 bp)

The body weights of ++ genotype lambs were lower than that of BB/B+ genotype lambs. In addition, the heart girth and chest width of ++ genotype lambs were shorter than those of the BB/B+ genotype lambs (Mulsant *et al.* 2001; Guan *et al.* 2006).

It was reported that the *FecB* gene has negative effects on fetal body weight, body size and development during pregnancy (Smith *et al.* 1996). The prevalence of the ++ genotype in the three Egyptian breeds under study may explain the low litter size of ewes, the low body weights and the short heart girth and chest width of lambs in these breeds. In this respect Galal *et al.* (1996) reported that Egyptian sheep breeds are of medium size and low growth rate.

## CONCLUSION

The three genes: *CAST* (*Mps1*, *Nco1*), *MSTN* (*Dra1*) and *DGTA1* (*Alu1*) were polymorphic in all breeds examined. Barki sheep breed showed the highest observed heterozygosity for both *CAST* and *MSTN* genes. The same breed also showed the highest genotypic frequency (AB) for *MSTN* gene, followed by Rahmani then Osseimi. Osseimi sheep showed the highest genotypic frequency (CT) and the highest observed heterozygosity for *DGTA1* gene, followed by Rahmani and Barki. The *IGFBP3* and *FecB* genes were monomorphic, therefore they are not recommended in the selection program. In order to have sharp molecular markers especially for economically important traits such as gain and meat traits in the Egyptian sheep breeds, studies must be carried out on the candidate genes on a large sample size from each breed.

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