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Research Article
Genetic Characterization of Myostatin and Callipyge Genes in Egyptian Small Ruminant Breeds

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Abstract

Background and Objective: Growth and meat quality are economically important traits in domestic animals. Due to the positive effect of *MSTN* and *CLPG* variation on the growth performance and quality of meat in different livestock, the aim of the present study is the identification of the genetic polymorphism and SNPs in these two genes in Egyptian sheep and goat breeds. **Methodology:** Genomic DNA was extracted from blood of 171 animal belonging to Barki, Rahmani and Ossimi sheep breeds as well as Baladi, Barki and Zaraibi goat breeds. **Results:** The PCR amplified fragments (337 bp) from *MSTN* gene were digested using *Hae*III restriction enzyme. The results declared that sheep and goat animals tested in this study are genotyped as "mm". After the digestion, the amplified products gave three fragments at 125, 118 and 94 bp resulted from the presence of two restriction sites (GG^CC) at positions 125^126 and 219^220. The nucleotide sequences of allele "m" for Egyptian goat and sheep *MSTN* were submitted to GenBank database with the accession No. KP120861 and KP120862, respectively. The *CLPG* gene amplified PCR fragments of 214 bp and were then digested with *Ava*II endonuclease. Due to the presence of a restriction site (G^GACC) at position 77^78, all tested animals were genotyped as "NN" where all fragments were digested to 137 and 77 bp fragments. The nucleotide sequences of allele "N" for *CLPG* gene in Egyptian goat and sheep have the accession No. KM597158 and KM569669, respectively in GenBank database. **Conclusion:** Results concluded that there was no polymorphism or variation in amplified fragments of *MSTN* and *CLPG* genes.

Key words: Sheep, goat, MSTN, CLPG, PCR-RFLP, nucleotide sequencing

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Growth performance and meat quality are economically important traits with the highest impact on the production of small ruminant. Recently, the development of molecular genetics approaches help for improvement of these traits. Generally, the detection of genetic markers for quantitative traits is the principal step to establish best selection programs[1]. Many studies were done in this area to detect the potential genes associated with these economic traits in different livestock, like cattle, sheep and chicken[2]. Myostatin (*MSTN*) and callipyge (*CLPG*) are the most well known major genes which are related with growth and meat quality traits in domestic animals.

The mammalian growth transforming family has some members, one of them is myostatin which has an essential role in the development of embryo and regulation of tissue homeostasis[3]. Due to the impact role of myostatin gene in muscle growth, it is considered as one of the important candidate genes in meat quality and growth traits in domestic animals[4,5]. Sequence variations in the *MSTN* gene can alter its expression and produce a non-functional protein, which leads to double-muscling phenomenon in many species[6]. This effect of a single gene on processing yields can open a best way to improve the processing yields of animals using knockout technology[7].

The muscular hypertrophy phenomena, callipyge phenotype are proclaimed in the pelvic limb muscles[8,9]. In lambs expressing callipyge, the muscles are enlarged by different degrees in some muscles while others are not affected. The weights of callipyge expressed muscles range from 14% in thoracic muscles to 50% in torso muscles. This muscle hypertrophy develops after 21 days from birth[10]. The muscle hypertrophy phenomena occur in the proportion as well as the gycolytic myofibers which lead to the increase of muscle size[11,12].

Due to the positive effect of *MSTN* and *CLPG* variations on the growth performance and quality of meat in different livestock, the aim of this study was to identify the genetic polymorphism and SNPs for these two economically important genes in major Egyptian small ruminant breeds.

MATERIALS AND METHODS

Blood samples and genomic DNA extraction: The whole blood samples were collected from 171 animals belonging to six native major small ruminant breeds, in sheep animals: 47 Barki, 30 Rahmani and 30 Ossimi, while for goat animals: 24 Baladi, 17 Barki and 23 Zaraibi.

Genomic DNA was extracted from the whole blood according to the method described by Miller *et al.*[13] with minor modifications. Briefly, blood samples were mixed with cold 2x sucrose-triton and centrifuged at 5000 rpm for 15 min at 4°C. The nuclear pellet was suspended in lysis buffer, sodium dodecyl sulfate and proteinase K and incubated overnight in a shaking water bath at 37°C. Nucleic acids were extracted with saturated NaCl solution. The DNA was picked up and washed in 70% ethanol. The DNA was dissolved in 1x TE buffer. The DNA concentration was determined, using Nano Drop1000 Thermo Scientific spectrophotometer and then diluted to the working concentration of 50 ng μL^{-1}.

Polymerase Chain Reaction (PCR): A PCR cocktail consisted of 1.0 mM upper and lower primers specific for each tested gene (Table 1), 0.2 mM dNTPs and 1.25 units of *Taq* polymerase. The cocktail was aliquoted into PCR tubes with 100 ng of sheep or goat DNA. The reaction was cycled for 1 min at 94°C, 1 min at an optimized annealing temperature that was determined for each primer (Table 1) and 1-2 min at 72°C for 35 cycles. The PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide to test the amplification success.

Restriction Fragment Length Polymorphism (RFLP): The PCR products for the two tested genes were digested with specific restriction enzyme for each gene (Table 1). Ten microliters of PCR product were digested with 1 µL of FastDigest restriction enzymes specific for each tested gene for 15 min at the optimum temperature for maximum activity of each restriction enzyme. For *CLPG* gene, the restriction fragments were subjected to electrophoresis in 2% agarose/ethidium bromide gel in 1x TBE buffer (0.09 M tris-boric acid and 0.002 M EDTA). Gels were visualized under UV light and

Table 1: Sequences and information of primers used in this study

Gene	Primer sequence 5'--------3'	PCR conditions (30 cycles)	PCR product size (bp)	Restriction enzyme used	References
MSTN	CCG GAG AGA CTT TGG GCT TGA	94°C 1 min	337	*Hae*III	Dehnavi *et al.*[2]
	TCA TGA GCA CCC ACA GCG GTC	60°C 1 min			
		72°C 2 min			
CPLG	GGA ATC ATC GTG TCC TGG TC	94°C 1 min	214	*Ava*II	Qanbari *et al.*[15]
	CCA GCA GGA TAC TCC GTG TC	58°C 1 min			
		72°C 1 min			

documented in FX Molecular Imager apparatus (BIO-RAD). Whereas the digestion products of *MSTN* gene were separated by electrophoresis on 12% non-denaturing polyacrylamide gels then stained by silver nitrate staining method[14].

Sequence analysis: The PCR products of each tested gene were purified and sequenced by Macrogen Incorporation (Seoul, Korea). Sequence analysis and alignment were carried out using ClustalW2. Results of endouclease restriction were carried out using FastPCR. The nucleotide sequences of the two tested genes in Egyptian sheep and goat were submitted to GenBank (NCBI, BankIt).

RESULTS AND DISCUSSION

Despite of the fast progress in breeding programs of farm animals in recent years, little attentions was focused on the meat quality performance[15,16]. Growth performance and meat quality are economically important traits in domestic animals and there are some factors; like breed and genotype that affect on them[17]. Recently many studies were carried out to test some genes, markers and chromosome regions which are related with these economic traits in many farm animals.

Myostatin (*MSTN*) and callipyge (*CLPG*) are the most well known major genes which are related to growth performance as well as meat quality traits. Due to the positive effect of these two genes on farm animal breeding, this study aimed to genetically characterize *MSTN* and *CLPG* genes in 6 main Egyptian sheep and goat breeds as a step towards the improvement of their productivity traits.

Myostatin (*MSTN*) gene: Myostatin (also known as *GDF*8) is a member of the mammalian growth transforming family (TGF-beta superfamily). This super-family includes differentiation and growth factors which have major roles in embryonic development regulating and maintainability of tissue homeostasis in adult animals. The major regulation of myogenesis is controlled by myostatin gene which negatively regulates muscle growth in mammals[18].

Three exons and two introns are the major components of myostatin gene[19] which maps to sheep chromosome 2[18]. Muscular hypertrophy allele (mh allele) in the double muscle breeds was attributed to mutations within myostatin gene[20]. Knockout technology was used to improve the processing yields of animals after notifying such a major effect of a single gene[7]. Therefore, myostatin gene characterization in farm animals is essential for future direction of selection programs, especially marker-assisted selection for economic traits.

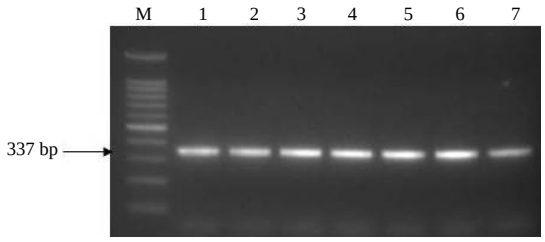

Fig. 1: Ethidium bromide-stained gel of PCR products representing amplification of *MSTN* gene in Egyptian sheep and goat animals, Lane M: 100 bp ladder marker, Lane1-7: 337 bp PCR product amplified from sheep and goat DNA

Table 1 presents the primers investigated in this study, which were found to flank a 337 bp segment of sheep and goat *MSTN* gene exon 3. The segments amplified in all tested sheep and goat DNA gave the expected fragment of 337 bp (Fig. 1).

The alignment of nucleotide sequences of the amplified fragments from Egyptian goat and sheep *MSTN* showed 100% identity in both species (Fig. 2) and were given the accession No. KP120861 and KP120862, respectively through the submission to nucleotide sequences database NCBI/ Bankit/GenBank.

Restriction endonuclease *Hae*III was used to digest the amplified PCR fragments (337 bp). The differentiation between the different genotypes could be performed depending on the presence or absence of the restriction site (GG^CC), at positions 125^126 and 219^220, where 3 different genotypes: "MM" with one undigested fragment at 337 bp, "mm" with three digested fragments at 125, 118 and 94 bp and "Mm" with four fragments at 337, 125, 118 and 94 bp. The results showed that all 171 tested sheep and goat animals, investigated for this gene, were genotyped as "mm" (Fig. 3 and 4) due to the presence of the restriction site (GG^CC) at positions 125^126 and 219^220 (Fig. 5).

Four goat populations were used to investigate *MSTN* polymorphism using DNA sequence analysis and PCR-SSCP[21] which revealed two SNPs (368A>C) and (4911C>T). Female Boer goats with AA genotype showed increased body weight and greater withers height compared to those with AC genotype. The biochemical and physiological functions of *MSTN* along with the obtained results suggested that the gene might play crucial role in affecting goat growth traits.

Dehnavi *et al.*[2] used PCR-RFLP and PCR-SSCP methods to investigate the association between Zel sheep yearling weight records and Myostatin gene polymorphism. Three fragments: 337, 222 and 311 bp were amplified using polymerase chain reaction, comprising a part of exon 3, intron 1 and intron 2

```
Goat   1   CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGTCGTTA  60
Sheep  1   CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGTCGTTA  60
           ************************************************************

Goat  61   CCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGATA  120
Sheep 61   CCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGATA  120
           ************************************************************

Goat  121  TAAGGCCAATTACTGCTCCGGAGAATGTGAATTTTTATTTTTGCAAAAGTATCCTCATAC  180
Sheep 121  TAAGGCCAATTACTGCTCCGGAGAATGTGAATTTTTATTTTTGCAAAAGTATCCTCATAC  180
           ************************************************************

Goat  181  CCATCTTGTGCACCAAGCAAACCCCAAAGGTTCAGCCGGCCCTTGCTGTACTCCTACAAA  240
Sheep 181  CCATCTTGTGCACCAAGCAAACCCCAAAGGTTCAGCCGGCCCTTGCTGTACTCCTACAAA  240
           ************************************************************

Goat  241  GATGTCTCCAATTAATATGCTATATTTTAATGGCAAAGAACAAATAATATATGGGAAGAT  300
Sheep 241  GATGTCTCCAATTAATATGCTATATTTTAATGGCAAAGAACAAATAATATATGGGAAGAT  300
           ************************************************************

Goat  301  TCCAGGCATGGTAGTAGACCGCTGTGGGTGCTCATGA                        337
Sheep 301  TCCAGGCATGGTAGTAGACCGCTGTGGGTGCTCATGA                        337
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Fig. 2: Nucleotide sequences and alignment between Egyptian sheep and goat *MSTN* gene

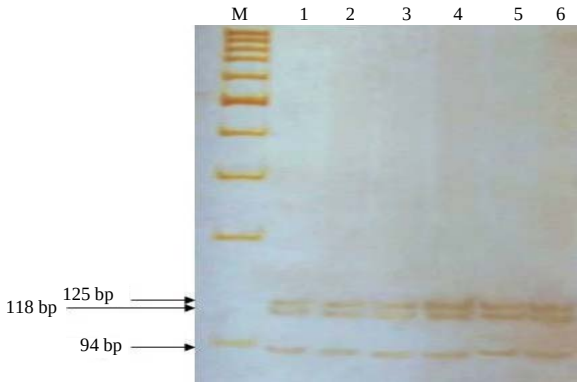

Fig. 3: Electrophoretic pattern (polyacrylamide gel) obtained after digestion of PCR amplified fragment of *MSTN* gene from sheep and goat DNA with *Hae*III restriction enzyme. Lane M: 100 bp ladder marker, Lanes 1-6: "mm" homozygous genotype with three digested fragment at 125, 118 and 94 bp

CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGTCGTT
ACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGA
TATAAGG^CCAATTACTGCTCCGGAGAATGTGAATTTTTATTTTTGCAAAAGTATCCTCAT
ACCCATCTTGTGCACCAAGCAAACCCCAAAGGTTCAGCCGG^CCCTTGCTGTACTCCTAC
AAAGATGTCTCCAATTAATATGCTATATTTTAATGGCAAAGAACAAATAATATATGGGAA
GATTCCAGGCATGGTAGTAGACCGCTGTGGGTGCTCATGA

Fig. 4: Endonuclease restriction of amplified fragment from sheep and goat *MSTN* using FastPCR GG^CC shows the restriction site in red

of myostatin gene, respectively. The RFLP method was conducted to digest exon 3 by *Hae*III enzyme, while SSCP

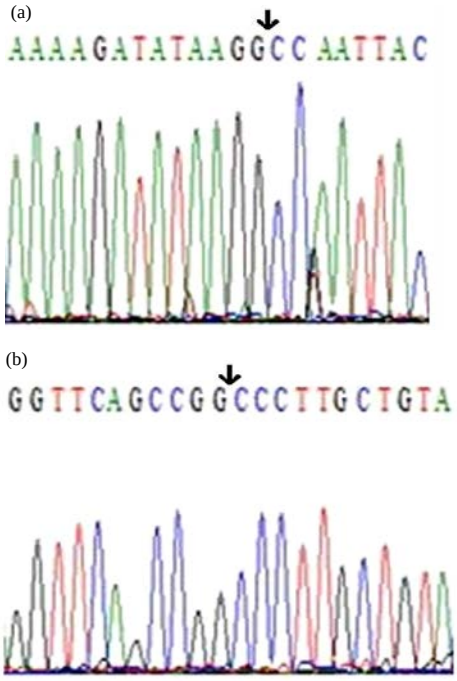

Fig. 5(a-b): Restriction site GG^CC at position (a) 125^126 and (b) 219^220

was used to study introns 1 and 2. All samples displayed "mm" genotype with RFLP method, while intron 1 was found to be monomorphic using SSCP method and intron 2 was polymorphic (AA, AB and BB). The A and B showed allelic frequencies of 75.5 and 24.5%, respectively. Yearling weights were found not to be significantly affected by myostatin gene.

Farhadian *et al.*[22] identified four SSCP patterns, representing four different genotypes in intron I of *MSTN* gene in Iranian Makoei sheep. The genotypes showed frequencies of 0.413, 0.293, 0.130 and 0.163 for AD, AC, AE and BC, respectively. These genotypes were investigated to test their effect on some traits and birth weight was found to be associated with the AD genotype. Other genotypes showed no phenotypic associations.

Two novel single nucleotide polymorphisms: 197G>A and 345A>T were detected in MSTN gene of two goat breeds by Zhang *et al.*[23], where the 5'-untranslated region showed three potential genotypes (AA, AB and BB) with the substitution 197G>A. Exon I segregated the polymorphism (CC and CD) of substitution 345A>T. Boer goat and Anhui white goat showed significant associations between the genotypes and body length, weight and height ($p<0.05$).

Han *et al.*[24] identified 28 nucleotide substitutions through *MSTN* gene in New Zealand sheep breeds, where the promoter region showed 3 substitutions, while the 5'UTR showed 3; 11 in intron 1, 5 in intron 2 and 5 substitutions were detected in the 3'UTR. One substitution in exon 1 (101G>A) potentially resulted in an amino acid substitution of glutamic acid (Glu) with glycine (Gly) at codon 34. This study revealed genetic variation that suggests that MSTN gene is more variable and can be considered as a foundation for future investigation of the effect of this variation on muscle and growth traits.

The results of the study showed that all tested Egyptian sheep and goat animals are genotyping as "mm" genotype for *MSTN* gene. This result agrees with the findings obtained by Dehnavi *et al.*[2] where there is no RFL polymorphism in exon 3 of *MSTN* gene in Iranian Zel sheep breed. Also, there is no report about the presence of nucleotide variation in exon 3 of *MSTN* gene in small ruminant breeds with the exception of the findings of An *et al.*[21] who reported one SNP (4911C>T) in Chinese goat breeds with frequency of allele C ranged from 0.76-0.82 against allele T with frequencies ranged from 0.18-0.24.

Callipyge (*CLPG*) gene: Selecting animals with superior reproduction capacities is considered to be the main focus of breeding schemes for increased meat production. Reproductive traits have been primarily the main concern of many studies directed at the genetic improvement of sheep and goat. Another trait that could be important in sheep and goat breeding is the improvement of growth efficiency and meat quality[25]. Callipyge gene is the most well known major gene concerned with these traits. The locus of *CLPG* gene was mapped to the telomeric region of ovine chromosome 18[26].

Several desirable production qualifications and meat quality traits are exhibited by callipyge lambs. Documentations for callipyge carcasses showed superior lean composition, larger longissimus (loin eye) areas, higher dressing percentages and leg scores[8,9]. These superior carcass traits reflect improved yields of wholesale leg, loin, rack and shoulder from callipyge animals by 11.8, 4.7, 2.5 and 2.3%, respectively, over normally muscled lambs[27]. It was also documented that callipyge lambs show superior feed efficiencies and lower daily feed intakes[10], which leads to lower production expenses.

The primers tested in this study (Table 1) covered a fragment of 214 bp of sheep and goat *CLPG* gene. The amplified fragments resulting from all tested sheep and goat DNA gave the expected fragment at 214 bp (Fig. 6).

Three nucleotide substitutions were detected between Egyptian sheep and goat *CLPG* at positions 60 (C/A), 175 (T/C) and 193 (A/G), after sequencing the amplified fragments (Fig. 7). Accession No. KM597158 and KM569669, respectively were given to the nucleotide sequences of Egyptian goat and sheep submitted to NCBI/Bankit/GenBank.

Restriction endonuclease *Ava*II was used to digest the PCR amplified fragments (214 bp). The presence or absence of the restriction site (G^GWCC) (W = A or T) at position 77^78 allowed the differentiation between 3 different genotypes: "MM" with one 214 bp undigested fragment, "NN" with two 137 and 77 bp fragments and "MN" with three 214, 137 and 77 bp digested fragments.

The obtained results indicated that all 171 sheep and goat animals tested for this gene showed the presence of the restriction site G^GACC at position 77^78 and therefore were genotyped as "NN" (Fig. 8-10).

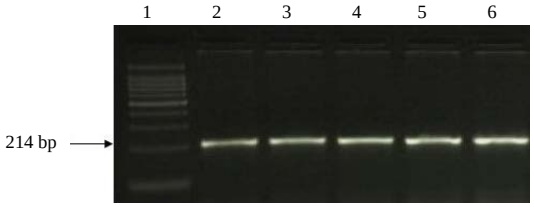

Fig. 6: Ethidium bromide-stained gel of PCR products representing amplification of *CLPG* gene in Egyptian sheep and goat animals. Lane 1: 100 bp ladder marker, Lanes 2-6: 214 bp PCR products amplified from sheep and goat DNA

```
Goat   1   GGAATCATCGTGTCCTGGTCTATTTTCGGGCCTCTGCTGAGAGCGCAGGAATCCAGGCGC  60
Sheep  1   GGAATCATCGTGTCCTGGTCTATTTTCGGGCCTCTGCTGAGAGCGCAGGAATCCAGGCGA  60
           ************************************************************

Goat   61  AGGGGCCCGAGGGCTGGGACCACCTGTCAGATCCTTTCCCCAGCTGAAGGCAGGGTGTGG  120
sheep  61  AGGGGCCCGAGGGCTGGGACCACCTGTCAGATCCTTTCCCCAGCTGAAGGCAGGGTGTGG  120
           ************************************************************

Goat   121 GTGATCCAGGGCCGGAAAAAGTCAAGGCCACCTCCAAGCCTTCCAATTTTAGAGTTGCAC  180
Sheep  121 GTGATCCAGGGCCGGAAAAAGTCAAGGCCACCTCCAAGCCTTCCAATTTTAGAGCTGCAC  180
           ************************************************** *****

Goat   181 GTCTCCAGCTCCAGGACACGGAGTATCCTGCTGG                        214
Sheep  181 GTCTCCAGCTCCGGGACACGGAGTATCCTGCTGG                        214
           ************ **********************
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Fig. 7: Nucleotide sequences and alignment between Egyptian goat and sheep *CLPG* gene nucleotide substitutions are shown in red

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GGAATCATCGTGTCCTGGTCTATTTTCGGGCCTCTGCTGAGAGCGCAGGAATCCAGGCG
CAGGGGCCCGAGGGCTGG^GACCACCTGTCAGATCCTTTCCCCAGCTGAAGGCAGGGTG
TGGGTGATCCAGGGCCGGAAAAAGTCAAGGCCACCTCCAAGCCTTCCAATTTTAGAGTT
GCACGTCTCCAGCTCCAGGACACGGAGTATCCTGCTGG
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Fig. 8: Endonuclease restriction of amplified fragment from sheep and goat *CLPG* using FastPCR. G^GACC restriction site is shown in red

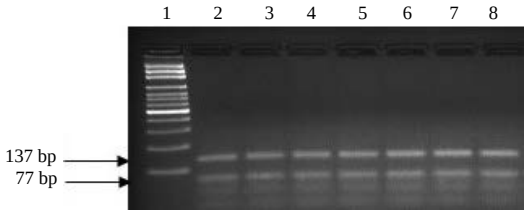

Fig. 9: Electrophoretic pattern obtained after digestion of PCR amplified fragment of *CLPG* gene from sheep and goat DNA with *Ava*II restriction enzyme. Lane 1: 100 bp ladder marker, Lanes 2-8: "NN" homozygous genotype with two digested fragments at 137 and 77 bp

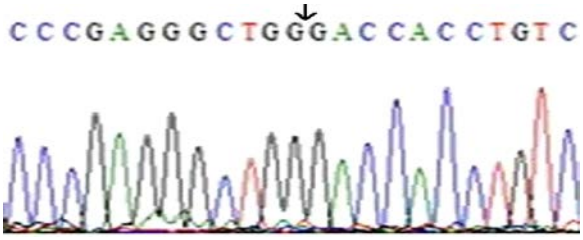

Fig. 10: Restriction site G^GACC at position 77^78

Sheep expressing *CLPG* mutation exhibited marked enlargement or hypertrophy of certain muscles, notably those of the hind legs and loin. The *CLPG* lambs showed greater percentages of total weight of excised muscles from the pelvic, torso and thoracic limbs by 42, 50 and 14%, respectively than in normally muscled lambs[9]. Interestingly there is no increased risk of dystocia for *CLPG* lambs since this muscle hypertrophy develops after about a few weeks of age[10]. Therefore, there would be increased profitability of the sheep industry and lowering of lamb costs for consumers through widespread production of *CLPG* lambs.

The polymorphism of *CLPG* gene was analyzed in Dorset, Suffolk and Xinjiang sheep breeds by Liu *et al.*[28]. The results showed that there was no PCR-RFLP polymorphism which suggested that hindquarters over-development was not controlled by the *CLPG* in Xinjiang meat sheep group. On the other hand, SNPs were detected by PCR-SSCP which resulted in the presence of three genotypes AA, AB and AC with AA being the major one. Results indicated that the AC genotype had a significant impact on the hindquarters hypertrophy, while the other two were not related to the muscling trait.

Qanbari *et al.*[15] surveyed the presence of responsible mutations in Afshari sheep breeding flock. Direct test for *CLPG* alleles were conducted on 58 DNA samples by PCR-RFLP assay. The banding patterns resulted from *Ava*II digestion of *CLPG* amplicons approved the absence of the mutations in this flock. On contrary, Li *et al.*[29] recognized one SNP (184C→T) of goat *CLPG* gene using *Fok*I. Boer goat was found to have the characteristics of double muscle, having higher T allele frequency (0.2465) and lower C allele frequency (0.7535) compared to other breeds. Therefore, it could be inferred that the double muscle characteristics of the Boer goat might be related to the 184 C→T mutation.

On the other hand, Cao *et al.*[30] identified the polymorphism in goat *CLPG* gene and its association with production traits. A partial DNA fragment of 250 bp was obtained from the goat callipyge gene which shared 96.04% with the corresponding regions of ovine. The results of sequencing showed no A→C mutation corresponding to the ovine *CLPG* gene, although one A→C transversion was located 147 bp downstream from the *CLPG* site. Allele A was found to be dominant in four of the goat populations, with the exception of Mongolia Alashan White cashmere goats. In last population where the allele C is dominant, least-square means of birth weight, production of cashmere and body weight gain from birth to weaning did not differ significantly between the AA and AC phenotypes.

The results of the study showed that all tested Egyptian sheep and goat animals are genotyping as "NN" genotype for *CLPG* gene. This finding agrees with the previous results obtained by Qanbari *et al.*[15] which showed no mutation or polymorphism in this site of *CLPG* gene and Gui-Ling *et al.*[30] which showed the presence of dominant nucleotide A (allele N) in highest frequency than that of nucleotide C (allele M). Also the present results declared that Egyptian sheep is similar to Iranian Afshari sheep breeds where the polymorphism in this site of *CLPG* gene is absent[15].

CONCLUSION

It can be concluded that although there were no polymorphism or variation detected through the amplified fragments of *MSTN* and *CLPG* genes, further analysis will be needed to find out other molecular loci associated with muscular performance and meat quality in Egyptian sheep and goat breed, in order to use these markers in breeding programs as a step for improvement of carcass trait and meat quality in these native breeds.

ACKNOWLEDGMENT

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SIGNIFICANCE STATEMENT

This study contributes as a preliminary step for meat and growth trait improvement in native small ruminant breeds. It is the first study to focus on the genetic characterization, polymorphism and nucleotide sequencing of two important genes associated with meat and growth traits in Egyptian sheep and goat breeds. This molecular information for such economically important trait genes allows their improvement through marker-assisted selection based on favorable genotypes correlated with superior reproduction traits.

REFERENCES

1. Allan, M.F., R.M. Thallman, R.A. Cushman, S.E. Echternkamp and S.N. White *et al.*, 2007. Association of a single nucleotide polymorphism in *SPP1* with growth traits and twinning in a cattle population selected for twinning rate. J. Anim. Sci., 85: 341-347.
2. Dehnavi, E., M.A. Azari, S. Hasani, M.R. Nassiry and M. Mohajer *et al.*, 2012. Polymorphism of *Myostatin* gene in intron 1 and 2 and exon 3 and their associations with yearling weight, using *PCR-RFLP* and *PCR-SSCP* techniques in Zel sheep. Biotechnol. Res. Int. 10.1155/2012/472307
3. Sonstegard, T.S., G.A. Rohrer and T.P.L. Smith, 1998. *Myostatin* maps to porcine chromosome 15 by linkage and physical analyses. Anim. Genet., 29: 19-22.
4. Miranda, M.E., Y. Amigues, M.Y. Boscher, F. Menissier, S. Corte and S. Dunner, 2002. Simultaneous genotyping to detect *Myostatin* gene polymorphism in beef cattle breeds. J. Anim. Breed. Genet., 119: 361-366.
5. Zhang, C., Y. Liu, D. Xu, Q. Wen, X. Li, W. Zhang and L. Yang, 2012. Polymorphisms of *Myostatin* gene (*MSTN*) in four goat breeds and their effects on Boer goat growth performance. Mol. Biol. Rep., 39: 3081-3087.
6. Grisolia, A.B., G.T. D'Angelo, L.R. Porto Neto, F. Siqueira and J.F. Garcia, 2009. *Myostatin* (GDF8) single nucleotide polymorphisms in Nellore cattle. Genet. Mol. Res., 8: 822-830.
7. Kocabas, A.M., H. Kucuktas, R.A. Dunham and Z. Liu, 2002. Molecular characterization and differential expression of the *Myostatin* gene in channel catfish (*Ictalurus punctatus*). Biochimica Biophysica Acta (BBA)-Gene Struct. Express., 1575: 99-107.
8. Koohmaraie, M., S.D. Shackelford, T.L. Wheeler, S.M. Lonergan and M.E. Doumit, 1995. A muscle hypertrophy condition in lamb (callipyge): Characterization of effects on muscle growth and meat quality traits. J. Anim. Sci., 73: 3596-3607.
9. Jackson, S.P., R.D. Green and M.F. Miller, 1997. Phenotypic characterization of Rambouillet sheep expressing the *Callipyge* gene: I. Inheritance of the condition and production characteristics. J. Anim. Sci., 75: 14-18.
10. Jackson, S.P., M.F. Miller and R.D. Green, 1997. Phenotypic characterization of Rambouillet sheep expressing the *Callipyge* gene: II. Carcass characteristics and retail yield. J. Anim. Sci., 75: 125-132.
11. Carpenter, C.E., O.D. Rice, N.E. Cockett and G.D. Snowder, 1996. Histology and composition of muscles from normal and callipyge lambs. J. Anim. Sci., 74: 388-393.

12. Carpenter, C.E. and N.E. Cockett, 2000. Histology of longissimus muscle from 2-week-old and 8-week-old normal and callipyge lambs. Can. J. Anim. Sci., 80: 511-514.
13. Miller, S.A., D.D. Dykes and H.F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res., 16: 1215-1215.
14. Benbouza, H., J.M. Jacquemin, J.P. Baudoin and G. Mergeai, 2006. Optimization of a reliable, fast, cheap and sensitive silver staining method to detect SSR markers in polyacrylamide gels. Biotechnol. Agron. Soc. Environ., 10: 77-81.
15. Qanbari, S., R. Osfoori and M.P.E. Nasab, 2007. A preliminary study of marker data applicability in gene introgression program for afshari sheep breed. Biotechnology, 6: 513-519.
16. Gao, Y., R. Zhang, X. Hu and N. Li, 2007. Application of genomic technologies to the improvement of meat quality of farm animals. Meat. Sci., 77: 36-45.
17. Rosenvold, K. and H.J. Andersen, 2003. Factors of significance for pork quality-a review. Meat. Sci., 64: 219-237.
18. Hickford, J.G.H., R.H. Forrest, H. Zhou, Q. Fang, J. Han, C.M. Frampton and A.L. Horrell, 2010. Polymorphisms in the ovine *Myostatin* gene (*MSTN*) and their association with growth and carcass traits in New Zealand Romney sheep. Anim. Genet., 41: 64-72.
19. Bellinge, R.H.S., D.A. Liberles, S.P.A. Iaschi, P.A. O'Brien and G.K. Tay, 2005. *Myostatin* and its implications on animal breeding: A review. Anim. Genet., 36: 1-6.
20. Kambadur, R., M. Sharma, T.P.L. Smith and J.J. Bass, 1997. Mutations in *Myostatin* (*GDF8*) in double-muscled belgian blue and piedmontese cattle. Genome Res., 7: 910-915.
21. An, X.P., J.G. Wang, J.X. Hou, H.B. Zhao and L. Bai *et al*., 2011. Polymorphism identification in the goat *MSTN* gene and association analysis with growth traits. Czech J. Anim. Sci., 56: 529-535.
22. Farhadian, M., A. Hashemi, K. Mardani, R Darvishzadeh and S. Jafari, 2012. Polymorphisms in the ovine *Myostatin* gene are associated with birth weight but not with weight gain in Iranian Makoei sheep. Genet. Mol. Res., 11: 3568-3575.
23. Zhang, Z.J., Y.H. Ling, L.J. Wang, Y.F. Hang and X.F. Guo *et al*., 2013. Polymorphisms of the *Myostatin* gene (*MSTN*) and its relationship with growth traits in goat breeds. Genet. Mol. Res., 12: 965-971.
24. Han, J., R.H. Forrest and J.G.H. Hickford, 2013. Genetic variations in the *Myostatin* gene (*MSTN*) in New Zealand sheep breeds. Mol. Biol. Rep., 40: 6379-6384.
25. Montgomery, G.W., S.M. Galloway, G.H. Davis and K.P. McNatty, 2001. Genes controlling ovulation rate in sheep. Reproduction, 121: 843-852.
26. Cockett, N.E., S.P. Jackson, T.L. Shay, D. Nielsen and S.S. Moore *et al*., 1994. Chromosomal localization of the *Callipyge* gene in sheep (*Ovis aries*) using bovine DNA markers. Proc. Natl. Acad. Sci. USA., 91: 3019-3023.
27. Busboom, J.R., T.L. Wahl and G.D. Snowder, 1999. Economics of callipyge lamb production. J. Anim. Sci., 77: 243-248.
28. Liu, G.Q., R. Dai, H.X. Ren, X.H. Wang, S.R. Liu, Y.L. Sun and L.G. Yang, 2006. [Polymorphism analysis of genes associated with hindquarters muscular development on chromosome 18 in Xinjiang meat sheep]. Yi Chuan, 28: 815-820.
29. Li, X., H. Wang, R. Zhou, G. Zheng, L. Li and Z. Shen, 2009. Variation of 184C→T of goat *Callipyge* gene in different populations and its effect on body weight. Front. Agric. China, 3: 319-324.
30. Cao, G.L., B. Li, H. Tang, P.R. Tang, J.M. Wang and Y.L. Jiang, 2009. Identification of polymorphism in the goat *Callipyge* gene (CLPG) and its associations with production traits. Chin. J. Agric. Biotechnol., 6: 235-240.