CHARACTERISATION OF *MYOGENIN*(*MYOG*) GENE IN NATIVE GOAT BREEDS OF KERALA

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ABSTRACT

Myogenin (*MYOG*) gene plays a significant role in regulating the number of skeletal muscle fibres, maintaining growth and homeostasis. The complete coding sequence of *MYOG* gene of Malabari and Attapady black goats were sequenced. The sequence analysis revealed a highly conserved nature of the gene across different species.

Keywords: *Myogenin* gene, complete coding sequence, Malabari goat, Attapady black goat

INTRODUCTION

Malabari and Attapady black goats are the two native goat breeds of Kerala. Malabari is a dual purpose breed known for its quality of meat, prolificacy and milk production. Attapady black goats are medium sized animal meant for meat purpose. The meat production traits are quantitative and polygenic in inheritance. The meat production trait is also influenced by number of muscle fibre and growth rate. One of myogenic lineage genes, *Myogenin* (*MYOG*) controls the formation of muscle fibre and regulates the expression of various muscle specific genes (Olson, 1990). Variation in *MYOG* genotype affects the muscle fibre number leading to change in muscle mass, body measurements and growth rate (TePas *et al.*, 1999 and Soumillion e*t al.,* 1997). Studies on *MYOG* gene in goat breeds are limited. Hence the present study was undertaken with the objective of characterisation of *MYOG* gene in Malabari and Attapady black goats.

MATERIALS AND METHODS

The total RNA was isolated from muscle tissues of Malabari and Attapdy Black goats using TRI reagent (Sigma) (Chomczynski and Sacchi, 1987). The RNA was subsequently converted into cDNA using Revert Aid first strand cDNA synthesis kit (Thermo Scientific). The primers were designed using the mRNA sequence of caprine *MYOG* gene obtained from NCBI (Accession No. JF829006.1).

PCR amplification of *MYOG* genes of Malabari and Attapady Black goats were carried out in thermal cycler (Bio-Rad T®100) pre-programmed for initial denaturation for 5 minutes at 95°C followed by 34 cycles of denaturation at 95°C for 30 seconds, annealing at 61.2°C for 20 seconds, extension at 72°C for 30 seconds and final extension at 72°C

for 7 minutes. The amplified products were verified on 2% (w/v) agarose gel with DNA loading dye in 1X Tris Borate EDTA (TBE) buffer using a 100 bp ladder as molecular weight marker.

The PCR products were sequenced by Sanger's dideoxy chain termination method at Scigenom Labs Pvt. Ltd., Cochin. The obtained forward and reverse sequences were merged using emboss DNA merger (v6.6.0.0.) (www.bioinformatics.nl/cgi-bin/emboss/merger). The complete coding sequence of *MYOG* gene of Malabari and Attapady Black goats was obtained.

The complete coding sequence of *MYOG* gene was analysed using NCBI Blast to identify the sequence homology with other species. The nucleotide sequences were analysed by MegAlign software (DNASTAR Inc, USA) to construct phylogenetic tree, sequence identity and divergent chart. The amino acids sequences were predicted using translate tool of ExPASy (web.expasy.org/translate/). The secondary structures of proteins were predicted using Self-Optimized Prediction Method (SOPMA) (https://npsa-prabi.ibcp.fr/cgibin/npsaautomatpl?page=/NPSA/npsa_sopma.html). Protean software (DNASTAR Inc, USA) was used to predict the physicochemical properties of protein sequences.

RESULTS AND DISCUSSION

In the present study, the 726 bp sequence obtained by amplifying *MYOG* gene of Malabari and Attapady Black goats constituting an open reading frame of 675 bp was submitted to NCBI GenBank (Accession No. KX17120 and KX056119, respectively).

The BLAST analysis of the *MYOG* gene sequences of Malabari and Attapady Black goats revealed 99, 98 and 97 per cent homology with *Ovis aries, Bubalus bubalis and Bos taurus,* respectively. The analysis of *MYOG* gene sequence using MegAlign programme of lasergene software (DNASTAR Inc, USA) showed 100 per cent similarity with Boer goat breed. (Accession No. JF829006.1) and 95 per cent above homology with cattle, sheep, buffalo, yak and pig (Fig. 1). Similar results were reported by Zi-gui *et al*. (2012) in the case of Longlin goat breed of China. Zheng-Zhu *et al*, (2012) also reported that the 52 flanking sequence of *MYOG* gene in Boer goat showed similarity between 37.22 per cent and 96.85 per cent with cattle, human, mouse and chicken.

The predicted MYOG protein encoded 224 amino acids which showed higher number of hydrophobic amino acids consisting of 22 residues of proline and 25 residues of leucine. The proline residues provide rigidity to the proteins. Leucine and glutamate residues are capable of forming strong helices (Nick and Martin, 1998).The predicted protein contains 41.64 per cent of charged amino acid which included 31 acidic amino acids and 25 basic amino acids. The results also indicated the presence of 57 polar amino acids that participate in the formation of hydrogen bond by interacting with each other.

Secondary structure prediction of MYOG proteins was carried out by SOPMA. The results revealed that the MYOG protein had 81 alpha helices, 25 beta turns, 26 extended strands and 92 random coils. Predicted secondary structure composition of MYOG proteins exhibited 36.16 per cent helix, 11.16 per cent turns, 11.61 per cent strands and 41.07

per cent coils.

The phylogenetic tree was constructed to determine the evolutionary relationship between various biological species based on nucleotide similarities. The nucleotide sequences of *MYOG* gene in Malabari and Attapady Black goats were compared with 17 different species. The 17 species included three primates (human, gorilla and rhesus monkey) six artiodactyls (cattle, goat, sheep, buffalo, pig and yak), two perissodactyls (horse and donkey), two rodents (mouse and rat), two carnivores (dog and cat), rabbit and chicken. The tree depicted all domestic ruminants under same ancestral node. Among ungulates, artiodactyls and perissodactyls formed a separate branch. Primates and rodents were present as sub branch under the same node. Carnivores and rabbit were placed in separate groups. (Fig. 2). The branching pattern of phylogenetic tree confirmed that the *MYOG* gene is highly conserved with very few mutation sites among different species.The conserved nature of *MYOG* gene was also reported by Zhu *et al.* (2010) in different pig breeds.

SUMMARY

The *MYOG* gene in Malabari and Attapady black goats showed 98, 97 and 99 per cent similarities with buffalo, cattle and sheep, respectively. The predicted MYOG protein encoded 224 amino acids. The proteins had higher number of hydrophobic amino acids that provided stability and rigidity to the protein. The phylogenetic analysis of *MYOG* gene revealed a close relationship of Malabari and Attapady Black goats with that of other ruminants.Thus *MYOG* gene in Malabari and Attapady Black goats was found to be highly conserved among different species.

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Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
1		98.8	87.5	97.5	90.7	90.7	90.5	72.3	90.4	90.5	90.4	88.0	87.9	92.4	89.1	97.5	97.5	99.3	97.0	1
2	1.2		87.6	97.7	90.8	90.8	91.2	72.3	90.7	90.5	91.1	88.3	88.4	92.1	89.2	97.8	97.8	98.2	97.3	2
3	13.9	13.7		87.8	91.2	91.2	92.1	72.8	88.7	88.5	89.4	87.3	86.4	88.2	90.8	87.8	87.8	86.9	87.6	3
4	2.6	2.3	13.4		91.2	89.4	90.8	72.1	91.1	91.3	91.0	88.1	88.1	92.3	89.1	100.0	100.0	96.9	99.3	4
5	10.0	9.9	9.4	9.4		99.5	92.3	74.2	93.2	93.2	93.1	89.6	89.2	92.9	92.3	91.5	91.6	90.1	90.8	5
6	10.0	9.9	9.4	11.5	0.5		92.1	73.8	93.0	93.0	92.9	89.8	89.3	92.7	92.1	91.5	91.6	90.1	90.8	6
7	10.2	9.4	8.4	10.0	8.2	8.4		73.3	93.0	93.0	93.1	88.9	88.6	92.6	90.8	90.7	90.7	89.9	90.5	7
8	34.7	34.7	34.0	34.9	31.6	32.3	33.0		73.9	74.1	74.1	71.4	71.7	73.8	75.3	72.1	72.1	71.7	72.0	8
9	10.4	10.0	12.4	9.5	7.2	7.3	7.4	32.1		99.4	97.8	90.8	90.8	94.1	90.8	91.1	91.1	89.8	90.5	9
10	10.2	10.2	12.6	9.4	7.2	7.4	7.4	31.9	0.6		97.5	90.5	90.8	94.1	90.8	91.2	91.3	89.9	90.8	10
11	10.4	9.6	11.5	9.6	7.3	7.5	7.3	31.9	2.3	2.6		89.9	89.9	93.5	90.8	91.1	91.1	89.8	90.7	11
12	13.2	12.8	14.2	13.0	11.3	11.1	12.2	36.1	9.9	10.3	10.9		96.1	90.7	89.7	88.1	88.1	87.4	87.6	12
13	13.4	12.7	15.3	13.0	11.8	11.7	12.6	35.7	9.9	9.9	11.0	4.0		90.2	88.6	88.1	88.1	87.3	87.6	13
14	8.0	8.3	13.0	8.2	7.5	7.7	7.9	32.4	6.2	6.2	6.8	10.1	10.6		90.9	92.3	92.3	91.9	92.0	14
15	11.9	11.7	9.9	11.9	8.2	8.4	10.0	30.0	10.0	10.0	10.0	11.2	12.6	9.8		89.0	89.1	88.4	88.6	15
16	2.6	2.3	13.5	0.0	9.0	9.0	10.1	34.9	9.5	9.4	9.5	13.0	13.1	8.2	11.9		100.0	96.9	99.3	16
17	2.6	2.3	13.4	0.0	9.0	9.0	10.1	34.9	9.5	9.4	9.5	13.0	13.0	8.2	11.9	0.0		96.9	99.3	17
18	0.7	1.8	14.5	3.2	10.7	10.7	10.9	35.6	11.1	10.9	11.0	13.9	14.1	8.7	12.6	3.2	3.2		96.4	18
19	3.0	2.7	13.6	0.7	9.8	9.8	10.2	35.1	10.2	9.9	10.0	13.7	13.8	8.5	12.5	0.7	0.7	3.6		19
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	

1 Bos grunniens KC184120.1.seq
2 Bubalus bubalis .seq
3 Canis lupus var 2 XM_005622161.1.seq
4 Capra hircus JF829006.1.seq
5 Equus asinus XM_014855253.1.seq
6 equus caballus AB608013.1.seq
7 Felis catus XM003999381.seq
8 gallus gallus KJ130962.1.seq
9 Gorilla gorilla XM_004028184.1.seq
10 homo sapiens NM_002479.5.seq
11 Macaca mulatta XM_015119869.1.seq
12 Mus musculus D90156.1.seq
13 Rattus norvegicus NM_017115.2.seq
14 sus scrofa NM_001012406.1.seq
15 Oryctolagus cuniculus NM_001177749.1.se
16 Malabari.seq
17 Attapady Black.seq
18 Bos taurus AB110600.1.seq
19 Ovis aries NM_001174109.seq

Fig. 1. Per cent identity and divergence chart of *MYOG* nucleotide sequence

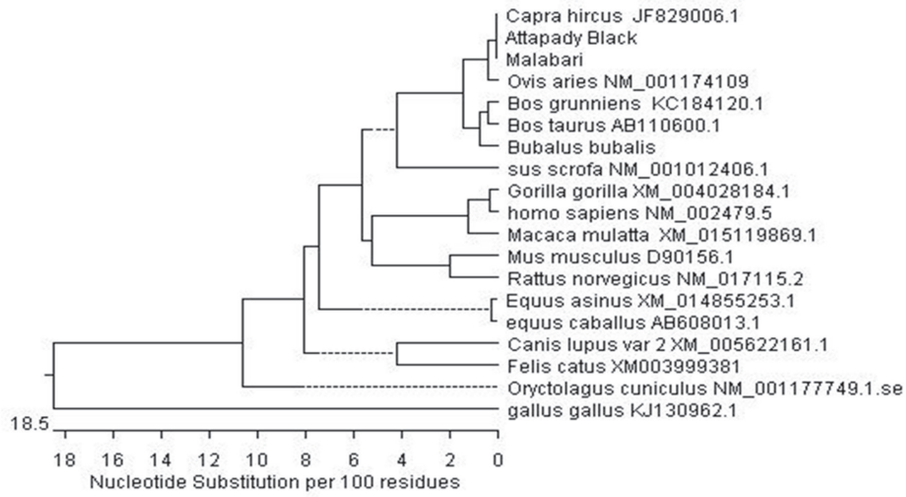

Fig. 2. Phylogenetic tree based on the nucleotide sequence of *MYOG* gene

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