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SINGLE NUCLEOTIDE POLYMORPHISM IN THE CODING REGION OF MYF5 GENE OF THE CAMEL *(CAMELUS DROMEDARIUS)*

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ABSTRACT

The myogenic factors (MYF) 5 and 6 are integral to the initiation and development of skeletal muscles and to the maintenance of their phenotypes. Thus, they are candidate genes for growth and meat quality-related traits. The MYF5 gene is expressed during proliferation of myoblasts and comprises 3 exons: 500, 76 and 191 bp long. Genomic DNA was isolated from the camel hair using NucleoSpin Tissue kit. Two animals of each of the six breeds namely, Marecha, Dhatti, Larri, Kohi, Sakrai and Cambelpuri were used for sequencing. For PCR amplification of the gene, a primer pair was designed from homolog regions of already published sequences of farm animals from GenBank. Results showed that exon 1 comprising of 422 bp of the dromedary MYF5 gene was more homologous (94%) to the cattle than the dog and human. However, phylogram showed that a small number of mutations had been experienced by dromedary camels at their MYF5 gene and was more near to human than other farm animals.

Key words: MYF5 gene, sequencing, camel.

INTRODUCTION

The formation of skeletal muscles involves a series of events including specification, proliferation and differentiation, in which multi-potential mesodermal cells are differentiated into myoblasts. The latter proliferate and ultimately differentiate into myotubes and myofibers. Many extracellular signal molecules and intracellular transcription factors regulate this specification, proliferation and differentiation of muscle cells (Buckingham, 2001; Brent and Tabin, 2002; Buckingham, 2002; Pownall *et al*., 2002). These signaling molecules act on myogenic transcription factors of the MyoD and Mef2 families that directly regulate muscle-specific gene expression and muscle cell differentiation (Black and Olson, 1998; Pownall *et al*., 2002; Martin and Johnston, 2005; Johansen *et al*., 2006). The onset of skeletal myogenesis is characterized by expression of myogenic factors (MYF), notably MYF5 and MyoD, members of the superfamily of helix-loop-helix transcription factors. The MyoD gene family consists of four structurally related genes: MYOD1 (MYF3), MYF5, MYF6 (MRF4) and MYOG (myogenin) (TePas and Visscher, 1994). The myogenic factors 5 and 6 are integral to the initiation and development of skeletal muscle and to the maintenance of its phenotype. Thus, they are candidate genes for growth and meat quality-related traits (Maak *et al*., 2006). MyoD and MYF5 double knockout mice display complete absence of skeletal myocytes or myofibers, while either MyoD or MYF5 null mutants exhibit no significant abnormities in skeletal muscles (Rudnicki *et al*., 1993). But mice expressed about four-fold higher levels of MYF5 when a functional MyoD gene was mutated (Rudnicki *et al*., 1992). On the other hand, mice lacking a functional MYF5 died from severe rib abnormalities although there were no significant abnormalities in skeletal muscle (Braun *et al*., 1992, 1994). The MYF5 gene is expressed during proliferation of myoblasts (TePas and Visscher, 1994) and comprises 3 exons: 500, 76 and 191 bp long (TePas *et al*., 1999). A recent study showed that different growth rates were related to the expression level of myogenic factors which affect muscle growth rates in turkey (Liu *et al*., 2005). The objective of the present study was to obtain sequence and to identify single nucleotide polymorphism in the coding region of MYF5 gene in camels that could be evaluated in a further study with regard to its effects on meat quality traits.

MATERIALS AND METHODS

This study involved 12 dromedary camels of six different Pakistani breeds viz. Marecha, Dhatti, Larri, Kohi, Campbelpuri and Sakrai. These camels belonged to different ecological zones of Pakistan. Genomic DNA was isolated from camel hair using NucleoSpin

Tissue kit (Macherey-Nagel, Germany) according to the manufacturer's protocol. Two animals of each breed were used for sequencing, as demonstrated in our early studies (Shah *et al*., 2006).

S equencing and SNP analysis

The primer pair was designed for a part of the coding sequence of dromedary MYF5 from homolog regions of mouse, horse and human (GenBank accession numbers NM008656, AF411602 and NM005593, respectively) sequences for PCR amplification. The sequence of the resulting fragment was used for primer design in 5' and 3' direction. The primer pair MYF 512 up 5' > TGC CAG TTC TCG CCC TCT GAF T <3' and MYF 511 low 5' > TAT AGT AGT TTT CCA CCT GTT CC <3' were used to amplify axon 1 of camel MYF5 gene.

PCR reactions were carried out using UNO thermo cycler (Biometra, Germany) in a total volume of 25 µl containing 2.5 mM $MgCl_2$, 0.2 mM dNTP 1U Taq DNA polymerase (Genaxxon, Germany), 0.2 µM of forward and reverse primer and 100 ng genomic DNA. After an initial denaturation at 94°C for 2 minutes, 35 cycles were done each consisting with 94°C for 1 minute, 56°C (primer pairs MN-1B up and MN-1B low) for 30 seconds and 72°C for 40 seconds. The final step lasted for 10 minutes at 72°C. PCR amplified fragments were excided from 2% agarose gel and purified using Gene Clean II Kit (Q BIO gene, Canada). The fragment was sequenced in both directions using BigDye Terminator v1.1 Cycle Sequencing chemistry on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA). All sequence alignments and distance calculations were made by Lasergene software (DNAStar, USA).

RESULTS

A part of exon 1 sequence (422 bp) of the dromedary MYF5 gene was amplified with MYF 512 up and MYF 511 low primer pair. Coding sequence of dromedary MYF5 with primers positions and single nucleotide polymorphism (SNP) detected is shown in Fig. 1.

Single nucleotide polymorphism was found in the coding sequence of MYF5 gene of the camel at 110 bp after ATG (Fig. 1). The frequency of different genotypes among 12 camels (two from each breed) was CT = 0.42, CC = 0.33 and TT = 0.25. The SNP caused amino acid change from Ala (C allele) to Val (T allele). Multiple sequence alignment of dromedary MYF5 and that of human, cattle and dog showed good homology (Table 1). Phylogram showed camel formed separate cluster and was more near to human and cattle than dog at this gene (Fig. 2).

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1   atg-------  -------tt  gccagttctc
gccctctgag tacttctacg acggctcctg
61  catcccgtcc  cctgagggcg  agttcgggga
cgagtttgag ccgcgagtgg ntgccttcgg
121  ggcgcacaaa  gcagacttgc  aaggctcaga
cgaggatgag cacgtgagag cacctatggg
181  ccaccaccag  gccggccact  gcctcatgtg
ggcctgcaaa gcatgcaaga ggaagtccac
241  caccatggat  cggcggaagg  cggccaccat
gcgcgagcga agacgcctga agaaggtcaa
301  ccaggctttc  gagacgctca  agagatgcac
cacgaccaac cccaaccaga ggctgcccaa
361  ggtggagatc  ctcaggaatg  ccatccgcta
cattgagagc ctgcaggagc tgttgaggga
421 acaggtggaa aactactata
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Fig. 1: Camel myogenic factor 5 (MYF5) gene sequence with primers positions (MYF 512 up (desh) and MYF 511 low (box) and SNP n = t/c (oval).

Table 1: Comparisons of nucleotide sequences of myogenic factor 5 (MYF5) gene in farm animals (%)

	Camel	Human	Cattle	Dog
Camel	--	91	94	86
Human		--	86	73
Cattle			--	75
Dog				--

GenBank accession numbers for human, cattle and dog were X14894, NM174116 and XM539699, respectively.

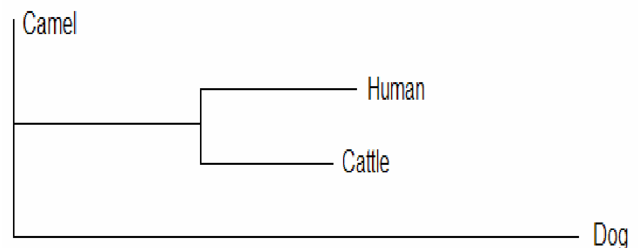

Fig. 2: Phylogenetic tree showing the common ancestry at MYF5 gene.

DISCUSSION

Exon 1 comprising of 422 bp of the dromedary MYF5 gene was observed to be more homologous to that of the cattle (94%) than that of the dog (86%) and human (91%). However, phylogram deduced from the

available dromedary MYF5 sequence and sequences of other species from the GenBank showed that a little number of mutations had been experienced by dromedaries at their MYF5 gene and was more near to human than other farm animals. Variability in the frequency of the SNP was also observed in different camel breeds.

In the literature, extensive investigations lead to the detection of several polymorphisms in swine and cattle. In the promoter region of MYF5 gene, three mutations have been identified at positions A65C (PCR-RFLP/AciI), C580T (PCR-RFLP/FokI) and C613T (PCR-RFLP/HinPI). Mutations C580T and C613T were characteristic for Pietrain × (Polish Large White X Polish Landrace) crossbred pigs named Torhyb (Urbanski *et al*., 2006). The C2931T transition, which is not recognized by any restriction enzyme, was identified in exon 3 of gene MYF5. This mutation resulted in a change of the amino acid sequence (Leu_Pro). Frequency of particular genotypes at the MYOD1 and MYF5 loci have been shown to be dependent on particular breed (Urbanski and Kury, 2004).

Prenatal expression patterns of genes were studied in muscles, including MyoD family genes, by a microarray technique (TePas, 2004). A number of genes in several pathways including myogenesis, energy metabolism and structural muscle genes show differences in expression between porcine embryos/ fetuses (pig breeds extreme for meat quality) at several different prenatal ages. Other studies performed in the postnatal period on pigs of different genotypes, regarding polymorphisms in regulatory sequences of gene, could help to establish the gene expression pattern during muscle growth (Urbanski *et al*., 2006). These SNPs might be analyzed in a further study as probably influencing carcass meatiness.

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