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Allelic Variation of MYF5 Gene Detected in the *Camelus bactrianus*

Research Article

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ABSTRACT

The myogenic factors (MYF) 5 gene has been reported to contribute to muscle growth and development, therefore they are considered as candidate genes for growth and meat quality related traits. The MYF5 gene is expressed during proliferation of myoblasts and comprises 3 exons. To ascertain whether there is any variation in the camel MYF5 gene, we have used a polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) method for the analysis. In this study, coding region (exon 1) of the MYF5 gene was investigated. Four unique SSCP patterns were detected in exon 1. Two Single nucleotide polymorphisms (SNPs) were detected - A/G and G/A in 98 and 366 position, respectively that create four haplotype, related to banding patterns. The variations detected in this study leads to a serin/asparagine and tryptophan/stop codon amino acid changes, respectively and could be considered for the development of gene-assisted selection in camel breeding.

KEY WORDS *Camelus bactrianus*, MYF5 genes, PCR-SSCP, SNP.

INTRODUCTION

Breeding of meat-producing animals focuses on growth rate and lean meat production. Skeletal muscles are formed when multi-potential mesodermal cells differentiate into myoblasts, which then proliferate and ultimately differentiate into myotubes and myofibres. These processes are regulated by different extracellular signal molecules and intracellular transcription factors (Buckingham, 2002; Pownall *et al*. 2002). The number of muscle fibers appears to determine the maximal lean meat growth capacity (Francetic and Qiao, 2011). Meat production capability in cattle seems also to be determined by the number of myocytes, since double muscled cattle show a higher number of myofibers than other cattle (Jiyeon *et al*. 2011). Mammalian myofiber formation is strictly an embryonic process, regulated by the MYOD gene family. The MYOD gene family consists of four structurally related genes: MYOD1 (myogenic differentiation 1), MYOG (myogenin), MYF5 (myogenic factor 5) and MYF6 (myogenic factor 6). These genes encode basic helix-loop-helix (bHLH) proteins that are involved in muscle cell determination and differentiation by regulating the expression of muscle differentiation-stage-specific genes (Bhuiyan *et al*. 2009). MYF5 and MYOD1 are expressed during proliferation of myoblasts, myogenin is expressed during terminal differentiation and MYF6 is mainly expressed during postnatal life. The myogenic factors 5 is integral to the initiation and development of skeletal muscle and to the maintenance of its phenotype (Maak *et al*. 2006). During embryogenesis, MYF5 appears to be involved in the development of skeletal muscle cell (Sabourin and Rudnicki, 2000). Subsequently, it has been associated with meat

production and there are significant effects of the MYF5 gene on the content of lean meat, loin weight and intramuscular fat levels in mammals (Te Pas *et al*. 2000; Verner *et al*. 2007). Polymorphism of the MYF5 gene has been reported to be associated with growth rate in pigs (Sajee *et al*. 2009), sheep (Nattrass *et al*. 2006), cattle (Ujan *et al*. 2011a; Ujan *et al*. 2011b; Jiyeon *et al*. 2011), chicken (Yine *et al*. 2011) and fish (Moghadam *et al*. 2007). Recent investigations revealed that SNPs in exon region of MYF5 had significant associations with carcass and meat quality traits in animals. Their grazing behavior is ideally adapted to scarce vegetation and they walk long distances between bites. Camels can go for many days without drinking, graze over areas very far from water resources and therefore can use very remote pastures. Camels' feed intake in relation to their body weight is low. They require only about 5-10 kg of dry matter to perform a day's work of carrying 120 kg over 30 km. Camels need about six to eight times as much salt as other animals, so need to regularly graze on halophytic plants to remain healthy (Köhler-Rollefson, 2005).

In view of the extensive soil salinization caused by the irrigation projects, camels may be a means of alleviating the catastrophic ecological side-effects of such interventions. Even under conditions of extreme drought, camels continue to produce milk. Because camels can minimize water expenditure for cooling and excretion, they yield milk with high water content even if they are dehydrated. Their lactation period of 9-18 months enables pastoralists to subsist on their milk practically all year round. According to some calculations, camels need only 1.9 kg of dry matter to produce a liter of milk, compared with 9.1 kg for cows (Köhler-Rollefson, 2005). In this study, we used a PCR-single-strand conformational polymorphism (PCR-SSCP) method to screen for variation in the part of camel MYF5 gene (exon 1) which might be evaluated in further studies regarding their effects on meat development and growth in this species.

MATERIALS AND METHODS

Animal samples and DNA extraction

Forty five *Camelus bactrianus* from Ardabil Province, Iran were considered to investigate MYF5 variation by PCR-SSCP analysis. Blood samples (5 mL) were collected in vacutainer tubes containing EDTA (1 mg/mL) and genomic DNA was purified using Genomic DNA Mini Kit (Real Biotech Corporation, RBC, South Korea).

PCR primers and amplification

Exon 1 of the camel *MYF5* gene was amplified with following primers set: F 5-CTGCCGTTCTCGCCTTC-3 and R 5-TTACCATGCCGTCGGAGC-3 (Shah *et al*. 2007).

Amplification was carried out in myCycler (Bio-Rad Laboratories, Hercules, CA, USA) in a total volume of 25 µl containing 1.2 m*M* $MgCl_2$, 0.2 m*M* dNTP 1.5U Taq DNA polymerase (Genet Bio), 0.15 µ*M* of forward and reverse primer (Metabion) and 100 ng genomic DNA. The thermal profile consisted of denaturation at 94 ˚C for 5 min, followed by 35 cycles of 94 ˚C for 30 s, annealing at 59 ˚C for 30 s and extension at 72 ˚C for 30 s, with a final extension step at 72 ˚C for 5 min.

The PCR amplicons were visualized by electrophoresis in 2% agarose (Sinagene, Iran) gels using TBE buffer (89 m*M* Tris, 89 m*M* boric acid, 2 m*M* Na EDTA). Five microliters (5 µL) aliquot of PCR product was added to 2 µL of loading dye (0.2% bromophenol blue, 0.2% xylene cyanol, 30% (w/v) sucrose) and the gels were run at a constant 10 V/cm for 20 min. Gels were stained with ethidium bromide (10 mg/mL) for 20 min. prior to visualization in UV light at 254 nm.

Single-strand conformational polymorphism (SSCP) analysis

Five microliters (5 µL) aliquot of each PCR product was mixed with 12 µL of loading dye (98% formamide, 10 m*M* EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol), then heated at 95 ˚C for 10 min and rapidly cooled on wet ice. The samples were loaded on 16 cm, 11% acrylamide:bisacrylamide (37.5:1) gels. Electrophoresis was performed using Protean II xi cell (Bio-Rad), at 260 V for 18 h at 4 ˚C in 0.5 TBE buffer. SSCP gels were silver-stained according to the method of Sanguinetti *et al.* (1994).

DNA sequencing and sequence analysis

The fragments of different patterns in SSCP were sequenced in both directions in Animal Science Research Institute of Iran (ASRI) by using BigDye Terminator v3.1 Cycle Sequencing chemistry on an ABI Prism 3130 Genetic Analyzer (USA). All sequence alignments and distance calculations were made by Lasergene software (DNAStar, USA).

Bioinformatics analysis with others species

A total of 6 sequences with the complete CDS of the MYF5 gene belonging to different species were obtained from GenBank (Table 1). The alignments of 7 sequences within a region of 420 bp were carried out using BioEdit. DnaSP (version 5.10.01) software was used to analyze the haplotype diversity (Hd), average number of nucleotide differences (Tajima, 1983), nucleotide diversity (π), synonymous nucleotide diversity (πs), nonsynonymous nucleotide diversity (πa) with Jukes and Cantor corrections, polymorphic site (S), singleton variable sites (SP) and the parsimony informative sites (PIP) for each species, and the average

number of nucleotide substitutions per site between species (D_{xy}) (Lynch and Crease, 1990).

Table 1 MYF5 gene sequences of 6 species

Species	Length (bp)	GenBank accession number
Bos grunniens	768	EF197849
Bos taurus	768	AB257294
Bubalus bubalis	768	EF128444
Ovis aries	583	AF434668
Equus caballus	642	AF411602
Copra hircus	420	Jf829004

The phylogenetic tree among species based on the D_{xy} was constructed using the unweighted pair group method by the arithmetic mean (UPGMA) implemented in Mega 4 software.

RESULTS AND DISCUSSION

PCR amplicons with the expected size (approximately 420 bp) were obtained from camel DNA using a set of the MYF5 primers. Four unique PCR-SSCP banding patterns were detected for exon 1 (Figure 1). These banding patterns represent two different polymorphic sites in exon 1 of MYF5 gene in camel.

The sequence of *Camelus bactrianus*, used in bioinformatics analysis. After sequence and analysis with DNA star we observed relation between variable nucleotide with banding patterns.

Sequence length variation within species

There are length variations in MYF5 gene of *Camelus bactrianus* with two variant regions, and including two substitutions. A to G and G to A substitutions in positions 99 and 367 respectively created four haplotypes, including GG, AA, GA and AG. These haplotypes were related with banding patterns; AA, BB, AB and BA respectively. The frequencies of banding patterns and haplotypes were shown in Table 2. Analysis of allele frequency showed that alleles G (0.68) and A (0.6) in positions 99 and 367, respectively were most common. Detected polymorphism causes a change of serine to asparagine (position 99) and tryptophan to stop codon (positions 367).

In literature, several polymorphism in swine and cattle have been detected; three mutations have been detected that changed A to C, C to T and C to t in 65, 580 and 613 position, respectively (Urbanski *et al.* 2006). A mutation in 2931 position caused C to change to T in exon 3 of gene MYF5 that leads to leucine/proline amino acid changes. Three genotypes BB, (580 bp+400 bp), AB (980 bp+580 bp+400 bp) and AA (980 bp) with frequencies 0.62, 0.32, and 0.06, respectively detected in Barbari goat (Saikia *et al.* 2014). The homozygous BB genotype (0.84) was predominant in high growth animals while heterozygous AB genotype (0.52) was predominant in animals with low growth.

Polymorphism and genetic diversity

DNASP analysis indicated that the selected region (1-420) within sequences from 8 different species have 420 sites, excluding gaps (280). There are 383 invariable sites and 37 variable sites that include 17 singleton variable sites and 20 parsimony informative sites. The nucleotide diversity (p=0.044) and the average number of nucleotide differences (K=14.536) for all sequences were higher than the values in *Camelus bactrianus* (p=0.00622, K=2.250). Distinct differentiation of the species could be concluded based on the high genetic diversity of the MYF5 gene. The polymorphic information and haplotype diversity of the MYF5 gene for *Camelus bactrianus* are listed in Table 4.

Nonsynonymous nucleotide diversity was higher than synonymous nucleotide diversity, possibly due to direct selection, in *Camelus bactrianus* (Table 4). The haplotype diversity between species was equal 1.00, indicating most abundant genetic diversity between species.

DNA divergence and clustering analysis

The average number of nucleotide substitutions per site (D_{xy}) of the MYF5 gene between species is shown in Table 5. D_{xy} is the index of DNA divergence between or among the sequences. When the D_{xy} value increases the genetic distance decreases. Based on D_{xy}, a phylogenetic tree was constructed for all species using the UPGMA method (Figure 2). The divergence time among different species was also labeled on the scale bar calculated from the average nonsynonymous nucleotide rate (0.85* 10-9 per year) (Li and Dan, 1991). The dendrogram of different species based on the differentiation of the MYF5 gene agreed with the taxonomy of NCBI. The smallest D_{xy} of 0.010 and divergence time about 0.5 milion year (Mya) showed the closest relationship between *Bos taurus* and *Bos grunniens* and closest species to Bactrian camels is *Copra hircus* with D_{xy} of 0.17 and divergence time of 1.9 Mya.

Kumar and Hedges (1998) estimated the divergence time between *Bos taurus* and *Capra hircus* (19.6 Mya) from an analysis of 16 nuclear genes. Arnason *et al.* (2000) and Nikaido *et al.* (2001) separately pointed out that the divergence time between *Bos taurus* and *Ovis aries* was 39 Mya and 30.9 Mya, based on an analysis of mtDNA. We observed closer relationship between *Camelus bactrianus* and *Sus scrofa*, which was similar to that of Jing *et al.* (2008) which showed comparability of cDNA sequence by alignment of lactoferrin gene. Based on the full-length cDNA sequences of caBD21 gene alignment in camel, pig, cattle, and sheep they showed that the similarity of sequences was highest between pig and camel.

Table 2 Sequence variation detected in the exon 1 of camels MYF5

Nucleotide change position	AA	BB	AB	BA	Amino acid change
99	AGC	AAC	AGC	AAC	Ser/Asn
367	UGG	UGA	UGA	UGG	Trp/stop
Frequency	0.31	0.44	0.17	0.08	-

Table 3 Frequencies of alleles in positions 99 and 367 of camels MYF5 gene

	Position 99		Position 367	
Allele	G	A	G	A
Frequency	0.68	0.32	0.40	0.60

Table 4 Genetic diversity of the MYF5 gene in *Camelus bactrianus*

Species	Diversity parameter								
	H	H_d	K	Π	π_s	π_a	S	SP	PIP
Camelus bactrianus	7	0.911	2.689	0.007	0.074	0.007	6	1	5

H: number of haplotypes; H_d haplotype diversity; K: average number of nucleotide differences; π_s: synonymous nucleotide diversity; π_a: nonsynonymous nucleotide diversity; S: number of polymorphic sites; SP: singleton variable sites and PIP: parsimony informative sites.

Table 5 Average nucleotide substitutions per site D_{xy}

	Bos grunniens	*Bos taurus*	*Bubalus bubalis*	*Ovis aries*	*Equus caballus*	*Sus scrofa*	*Camelus bactrianus*
Bos grunniens	-	-	-	-	-	-	-
Bos taurus	0.01	-	-	-	-	-	-
Bubalus bubalis	0.02	0.01	-	-	-	-	-
Ovis aries	0.31	0.30	0.31	-	-	-	-
Equus caballus	0.25	0.25	0.25	0.31	-	-	-
Sus scrofa	0.65	0.59	0.60	0.65	0.38	-	-
Camelus bactrianus	1.03	1.02	1.01	0.80	0.74	0.39	-
Copra hircus	0.78	0.77	0.77	0.86	0.53	0.20	0.17

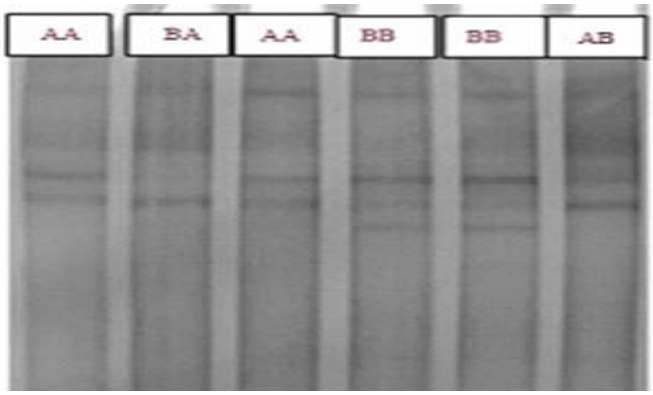

Figure 1 PCR-single-strand conformational polymorphism (PCR-SSCP) of the camel MYF5 gene

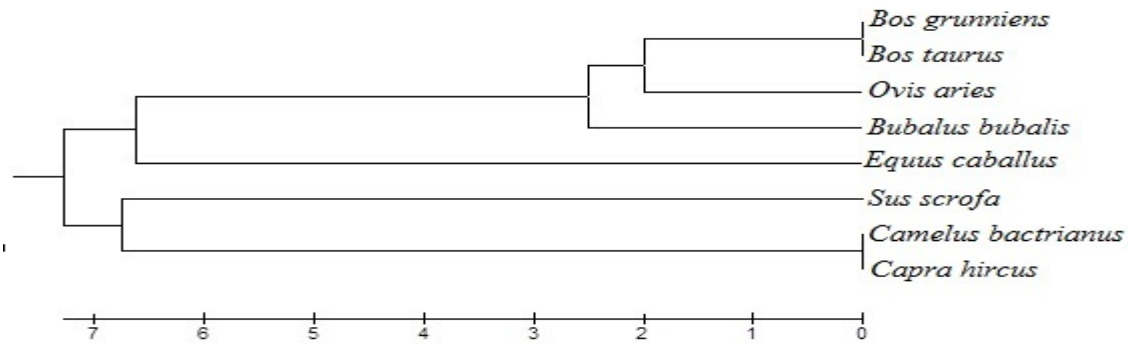

Figure 2 Phylogenetic tree of the MYF5 gene among 8 species

The divergence time between *Camelus dromedarius* and *Sus scrofa* seems to be about 90 Mya (Tang *et al*. 2006). Delsuc *et al*. (2004) compared nuclear genomic data of *Lama glama* and *Sus scrofa* to show the reconstruction of phylogeny and evolutionary time of 62 Mya. The divergence time was 68.2 Mya, based on analysis of mitochondrial genomes of *Lama glama* and *Sus scrofa* (Nikaido *et al*. 2001).

In this study, polymorphisms in MYF5 gene were found. Like other mammals, these polymorphism genes could have played potential roles on muscle development. The SNPs (99G>A and 367A>G) of MYF5 gene are located in the exon 1 suggesting that they might be related with differences in growth traits.

CONCLUSION

In this study two single nucleotide polymorphisms were identified in the camels MYF5 gene. Both of them are functional and cause amino acid change and stop codon introduction. The role of MYF5 in meat quality and carcass related traits in different domestic animals suggested it might be important genetic marker for similar traits in camel. After association analysis these newly identified SNPs could be used as a markers for selection of animals and potentially used for camel breeding using modern methods, such as marker assisted selection or marker assisted introduction.

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