Research Article

Association of *Calpain* Gene Polymorphism and Type Traits in Iranian One-hump Camels

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Abstract

The present study investigated the calpain gene diversity in Iranian one-hump camels (120 camels) using PCR-RFLP method. Three genotypes AA, AG and GG were observed with the frequencies of 0.02, 0.19 and 0.78, respectively. The frequencies of A and G alleles were 0.12 and 0.88, respectively. The Chi-squared test didn't show significant deviations from Hardy-Weinberg equilibrium ($P<0.05$). Association between the genotypes and type traits including body length, heart girth, height at withers, height at back and leg girth was modeled using the GLM procedure of the SAS program. There was a significant association between calpain genotypes and biometric traits, except for the body length ($P<0.05$). The GG genotype had a greater height at back (ones of type traits) compared to other genotypes. The type traits including height at withers, heart girth and abdomen girth for GG genotype were significantly higher than the AA genotype but had no significant difference with the GA genotype ($P<0.05$). The results suggested that calpain gene polymorphism may be a potential genetic marker for improving the growth-related traits in camels. More studies are required to confirm the effects of calpain gene on growth traits in camels.

Key words: Biometric trait; Calpain gene; Camel; PCR-RFLP; Polymorphism

Introduction

Since ancient times, domestic even-toed ungulates have played pivotal roles for man, being exploited for meat and milk, for fiber production, as beasts of burden for transport in agricultural/ rural oriented community; they were even worshipped. These animals have served for traditional technologies since the very early era of domestication (1).

Camel belongs to the order Artiodactyla, sub-order Tylopod, and family of Camelidae. The genus Camelus consists of Camelus dromedarius (one hump) and Camelus bactrianus (two humps). Both species have been domesticated and reared for producing milk and meat. The meat of camels has been used as food in various parts of world for many centuries (2).

Genetic improvement has been considered as an important factor in the devel-

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opment of domestic animal productions. Identifying the associated genes with economic traits as well as understanding their interactions with the environment or the other genes might be useful for applications of marker-assisted selection in the commercial animal population (3). Association of several polymorphic regions in candidate genes with economic traits has been considerably investigated in different animal species as cattle (4-7), goat (8,9), sheep (10,11), poultry (12,13) and camel (14).

The calpain-calpastatin system (CCS) includes a family of calcium-dependent neutral proteases. It is more likely to be found in most of the animal tissues (15). The first protein of CCS (m-calpain) was found in 1976 and μ calpain and calpastatin were recognized several years later. The activity of calpain depends on Ca2+, as this enzyme plays a significant role in the growth of muscle and slaughtered meat tenderness through proteolysis of myofibrils (16). So far, the sequencing of regulatory subunit of calpain gene has been done by many researchers, all emphasizing the polymorphism of this region. In addition, other studies investigated the effects of this polymorphism on growth traits.

Chung et al. (17) amplified some parts of calpain III gene in sheep genome and identified the point mutations in this region using PCR-SSCP method. Furthermore, Chung et al. (18) has found that there is a meaningful difference between fat around the hips, kidneys and heart of the different genotypes for calpain gene. They also studied this region in Angus breed cattle and its relationship with slaughtered meat tenderness and carcass traits. In addition, the effects of this gene on beef quality traits have been discussed by Esmailizadeh et al. (19). Human studies have associated calpain-10 variants with type 2 diabetes and metabolic traits (20).

The calpain alleles are easily detected by PCR amplification (17). Therefore, calpain gene was investigated as a candidate gene to identify quantitative trait loci (QTL) affecting slaughtered meat tenderness and body weight traits in many species of animals.

Determination of gene polymorphism is important in breeding of farm animals (21,22). GhasemiMeymandi et al. (23) investigated genetic variation within Iranian indigenous Camelus dromedarius in northern Kerman province using five microsatellite markers. The results of the study indicated that the Camelus dromedarius in the North of Kerman province populations had a relativity high genetic variation resulting in adaptation with drastic environmental changes of Kerman province. In another study, Ghasemi Meymandi et al. (24) investigated individual's assignment of the Camel Populations of North of Kerman Province using 7 different methods. Among the methods base on the likelihood, the Rannala and Mountain method and among the methods base on the genetic distance, Nei DA method showed the highest accuracy. Furthermore, Ghasemi Meymandi et al. (25) analyzed genetic structure of Camelus dromedarius using PCA and Hierarchical clustering methods and concluded that all samples could be mainly regrouped into three main clusters and most suitable number of genetic

groups in dataset was explained and a similarity of geographical distribution was in good accordance with founded genetic relationships in this study.

However, no study concerning the polymorphism of calpain gene in Iranian camel breed (one hump) and its association with body measurements has been conducted yet. Thus, this study aimed to genotype the calpain gene in Iranian camel breed (one hump) for investigating the possibility of its correlation with body measurements.

Materials and Methods

Animals and phenotypic traits

One hundred and twenty camels were randomly selected from different herds in Hormozgan province, Iran. Blood samples (approximately 8 ml) were collected from jugular veins of the camels in EDTA-coated tubes and stored at -20°C until DNA extraction.

In Table 1, the measured type traits including length of body, heart girth, abdomen girth, height at withers and height at back are shown. Also, sex, age, type of birth and pregnancy state of animals were recorded.

Table 1: Overall mean, number of observations and phenotypic standard deviation for measured traits

trait	Number	Mean (cm)	Standard deviation
length of body	120	148.25	0.10
heart girth	120	172.43	0.13
abdomen girth	120	165.56	0.15
height at back	120	179.21	0.09
height at withers	120	219.75	0.13

Genomic DNA extraction

Total genomic DNA was extracted from blood samples using Accuprep Genomic DNA Extraction Kit, Cat No: K-3032 according to the manufacturer protocol. Quantity of the extracted DNA was measured according to spectrometry method using Nano-drop and 2000 spectrophotometer of USA Thermo Company and its quality was checked on 1% Agarose gel.

PCR Primers and Amplification

Primers for PCR amplification of calpain were designed with regard to the reported bovine CAPN1 sequences (Gen Bank AF252504), because of unavailability of sequence of this gene in camels and also Camelidae family. 120 camels were genotyped using the PCR-RFLP method. A 787 bp fragment was amplified with the primers of 5'-AGCGCAGGGACCCAGTGA-3' (forward) and 5'-TCCCCTGCCAGTTGTCTGAAG-3' (reverse) using the following thermal cycle: an initial hot start at 95°C for 5 min followed by 35 cycles, denaturation at 95°C for 45s, annealing at 62°C for 45s, extension at 72°C for 50s and a final extension for 7 min at 72°C after 35 cycles. PCR reactions were performed in a total volume of 25µl containing 2.5µl of standard PCR buffer, 1.5mM MgCl2, and 0.5µM of each primer, 2µM dNTP, 0.2 U Taq DNA polymerase and 120ng of template DNA. The PCR products were analyzed by electrophoresis on a 1% agarose gel and the gel was stained with ethidium bromide. In addition, the marker size was used for confirming the accuracy of desired fragment. 16 ng of PCR products were digested using the restriction enzyme AvaII (2U). Restriction site of this enzyme is 5'---G/GWCC---3' and 3'---CCWG/G---5'. The digested fragments were analyzed by electrophoresis in

2% agarose gel, stained with ethidium bromide and photographed under UV light.

Statistical analyses

The observed allele frequency and the Hardy-Weinberg equilibrium were analyzed using the PopGene32 software. For the association study, the traits were analyzed using general linear model (GLM) of Statistical Analysis System (SAS Institute, 2004) program least squares means of genotypes were compared by the Duncan test. The applied linear model was as follow:

Yijklns=μ + Sexi + Agej + Typek + Pregnancyl + genotypen+ eijklns

Where Yijklns is the trait of interest, μ is the general mean, Sexi is the fixed effect of ith sex (i=1,2), Agej is the fixed effect of jth age (j=1,…,4), Typek is the fixed effect of kth type of birth (k=1,2), Pregnancyl is the fixed effect of lth Pregnancy state (l=1,2), Genotypen is the fixed effect of nth genotype (n=1,2,3), and eijklns is the random error.

Results

Genotype and allele frequencies

A 787bp amplicon of exon 14 comparable to that of the bovine amplicon was amplified by PCR for camel calpain gene. The PCR products were electrophoresed on 1 % agarose gel shown in Figure1 . The PCR-RFLP analysis displayed three genotypes including AA, AG and GG.

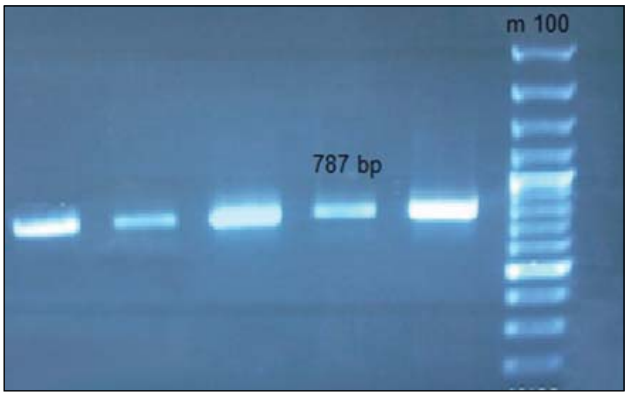

Figure 1: Electrophoresis of PCR products for 14 exon region of calpain gene

The observed number, observed genotype frequencies, the expected frequency, the expected number, Chi-square (χ2) and observed allele frequencies in the exon 14 of calpain gene in Iranian camel breed (one hump) have been shown in Table 2. The allelic frequencies were 0.878 and 0.122 for G and A, respectively. Genotype GG showed maximum observed frequency (78.33%) in the herd. Heterozygous genotype AG showed lower frequency (19.17%) than the genotype GG while the genotype AA had lowest frequency (2.5%) in the testing herd. To evaluate H ardy-Weinberg equilibrium, Chi-squared test was applied and the results have been shown in Table 2. Differences of observed genotypes and expected genotypes were not significant according to the calculation of χ2 =1.247, suggesting that the studied population for calpain gene is in the Hardy - Weinberg equilibrium.

Table 2: Observed number, observed genotype frequencies, expected frequency, expected number, Chi-square (χ2) and observed allele frequencies of exon 14 of calpain gene in camel

genotype	Observed genotype number	observed genotype frequencies	expected genotype frequency	expected genotype number	Chi-squareχ2 (O-E)2/E	observed allele frequencies G	A
AA	3	2.5	1.425	1.71	0.97		
AG	23	19.17	21.3	25.57	0.26	0.878	0.122
GG	94	78.33	77.275	92.72	0.0179		
total	120	100	100	120			

Having observed and expected heterozygosity, Nei expected heterozygosity and the average heterozygosity of calpain gene in the studied herd are given in Table 3. Heterozygous level is considered as one of the indicators of introducing the genetic variations in a population. Average heterozygosity, Nei heterozygous and observed heterozygosity were 0.193, 0.214 and 0.214, respectively.

Table 3: Observed and expected heterozygosity, Nei expected heterozygosity and the average heterozygosity of exon 14 of calpain gene in camels

gene	Observed heterozygosity	expected heterozygosity	Nei expected heterozygosity	average heterozygosity
calpain	0.193	0.215	0.214	0.214

Association between genotypes and type traits

There were significant detectable effects of calpain genotypes on the measured type traits except length of body in camels ($P<0.05$) (Table 4) .The height at withers, heart girth and abdomen girth traits for GG genotype were higher than those for the AA genotype, but there was no significant difference in these traits between GG and AG genotypes ($P>0.05$). Also, the height at back for GG genotype was significantly higher than that of the AG and AA genotypes ($P<0.05$).

Discussion

Calpain has been considered as a candidate gene for carcass performance and meat quality traits in farm animals. In livestock, variations in the calpain gene have been detected in the cattle, sheep and pigs, but not in camels. Page et al. (26) recognized marker calpain1 (an adenosine/guanosine (A/G) substitution) in the exon 14 of bovine calpain1 gene. This marker has been suggested to be associated with meat tenderness (26-30).

In the present study, three different genotypes (AA, AG and GG) were found in calpain1 locus. Similar result for calpastatin locus was observed in Sistani cattle by FakhrKazemi et al. (31). FakhrKazemi et al. (31) carried out a study on polymorphism of calpastatin gene for Sistani cattle and MM, MN, NN genotypes with frequencies of 51.86, 32.27 and 4.87 with respect to the calpastatin gene of cattle were reported. M and N allele frequencies were given as 76.4 and 23.6%, respectively. Low heterozygosity of calpastatin gene of cattle was obtained from a closed herd.

A high degree of calpain polymorphism has also been reported by Azari et al. (32), Bahrampour et al. (15), Mohammadabadi (33) and Arora et al. (34) in different breeds of sheep. Azari et al. (32) observed 3 different genotypic patterns (G1, G2 and G3)

Table 4: Least square means and standard errors of the length of body, heart girth, abdomen girth, height at withers and height at back determined for genotypes of calpain polymorphism

Trait (cm)	Genotypes		
	AA	AG	GG
length of body	147.33 ± 0.12^{a}	148.46 ± 0.11^{a}	150.74 ± 0.14^{a}
height at withers	164.33 ± 0.17^{b}	174.12 ± 0.13^{a}	174.29 ± 0.11^{a}
height at back	157.00 ± 0.11^{c}	166.33 ± 0.12^{b}	184.97 ± 0.09^{a}
heart girth	155.00 ± 0.09^{b}	182.54 ± 0.09^{a}	180.32 ± 0.08^{a}
abdomen girth	174.33 ± 0.15^{b}	225.29 ± 0.11^{a}	218.92 ± 0.13^{a}

with the frequencies of 0.082, 0.892 and 0.027 in Iranian Dalagh sheep. The results of their research conformed to earlier reports of Tahmoorespour (35) who reported three genotypes (AA, AB and BB) with allele frequencies of 0.56 and 0.44 for A and B alleles respectively in Baluchi sheep. Bahrampour et al. (15) also reported 3 genotypes MM, MN and NN with frequencies of 0.69, 0.20 and 0.11 respectively in Kermani sheep. Mohammadabadi (33) demonstrated that 3 genotypes MM, MN and NN had frequencies of 0.67, 0.25 and 0.08, respectively in Sanjabi sheep. Arora et al. (34) studied 11 Indian sheep breeds and studied the allele frequencies of 0.603 and 0.397 for A and B alleles respectively and frequencies of 0.388, 0.429 and 0.183 for AA, AB and BB genotypes, respectively. Furthermore, the observed and expected heterozygosity were estimated as 43.5 and 56.9%, respectively in this research.

These results were consistent with those obtained in the present study in camel. Accordingly, there is no selective advantage or disadvantage of a particular genotype in this population and Hardy-Weinberg equilibrium is established in this camel herd. Moreover, moderate and high heterozygous levels of calpain gene demonstrated good genetic variations based on obtained results of average heterozygosity, Nei heterozygous and observed heterozygosity in the studied population. This may be explained by the conservation and breeding strategies, which have been carried out.

Many studies have reported a relationship between polymorphism of calpain gene and quality of carcass traits while some researchers have investigated the relationships between polymorphism of this gene and growth traits in cattle and sheep (36-38). Development of skeletal muscle depends on three main factors, these factors involve protein synthesis of muscles and decomposition of muscle proteins and muscle cells. Increased skeletal muscle growth could be associated with decreased protein decomposition of muscle caused by calpain system activity. Calpain gene is introduced as an effective candidate gene in meat production (15,33).

The results of our research on the association between calpain gene and type traits in camel were verified with previous reports in other species on the body weight traits. Cheong et al. (3) examined the association between polymorphism of the micro-calpain gene and carcass traits in Korean cattle. The results revealed that there was a significant association between the 3'UTR and marbling score ($P<0.02$). Furthermore, Chung et al. (39) investigated the association between ovine calpain gene polymorphism and body weight traits. Body weight was recorded at birth, weaning and post-weaning. Calpain 3 genotypes of CAPN31112 segment were associated with birth weight ($P<0.01$) and a dominant gene effect was observed. Also, associations between m-calpain/HhaI polymorphism and growth traits in Chinese indigenous cattle breeds were studied by Zhang and Li (38). The m-calpain/HhaI polymorphism was associated with body weight, withers height, and body length for 6 months ($P< 0.05$ or $P<0.01$), body length for18 months ($P<0.05$), and body length and heart girth for 24 months ($P<0.01$) in Nanyang cattle.

The public combining ability of a gene is caused by additive effects in general, but for a specific combining ability of a gene the dominant effects and epistasis are important (40). In this study a dominant effects was recognized for height at withers, heart girth and abdomen girth traits. The effect may shape the specific combining ability. No dominant effects were found for height at back. The results suggest that type traits may be related to genetic factors as well as environmental factors. Some environmental factors were confirmed to be significant in this study.

Linear type classification is an important tool in decision making as it focuses on the selection of animals that should have a longer herd life while expressing their productive and reproductive potentials based on their morphologic traits (41). Previous researches have indicated the usefulness of linear type traits as predictors of body weight (42,43), health (44) and fertility (44,45) in dairy cattle. The correlations between type traits and performance were estimated as moderate to high (46,47).

Janssens and Vandepitte (48) estimated genetic parameters for seven body measurements and 13 linearly scored traits in three sheep breeds. Heritability of measured traits was in the range of 0.26–0.57 and genetic correlations were high between these traits. Also, Ishag et al. (14) estimated weights of camels using the Boue formula (1949) with regard to type traits. Moreover, knowledge of association between polymorphisms and performance can increase the accuracy of selection.

Results of this research showed that calpain marker had a detectable effect on type traits in camels. However, with regard to high correlation between type traits and body weight traits, calpain gene is considered as a main economic gene due to its relation with productive traits in camels and it may explain variations of either body weight or growth. The polymorphism may also be useful in marker-assisted selection for body weight.

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