Variability of *CAPN1* g.5709 C>G and *MYF5* g.1911 A>G Polymorphisms in Beef Cattle Imported from Brazil to Turkey

Sena Ardıçlı[1], Hakan Ustüner[2], Öznur Arslan[2], Oğuz Kandazoğlu[3]

[1]Department of Genetics, Faculty of Veterinary Medicine, Bursa Uludag University, Gorukle/Bursa, Turkey
[2]Department of Animal Science, Faculty of Veterinary Medicine, Bursa Uludag University, Gorukle/Bursa, Turkey
[3]Tabiat Agriculture Limited Company, Yenisehir/Bursa, Turkey

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Abstract: The objective of the present study was to determine genotypic/allelic frequencies and population genetic indices of *CAPN1* g.5709 C>G and *MYF5* g.1911 A>G polymorphisms in beef cattle imported from Brazil to Turkey. Single nucleotide polymorphisms were carried out using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis. A total of 108 bulls, including Angus, Angus×Hereford×Nellore, Brahman, Hereford, Limousine, and Charolais breeds, were genotyped. Concerning the *CAPN1*, CC genotype was not found in this study. Besides, the G allele frequency was quite high (0.75). Regarding the *MYF5*, frequency of AA genotype was rather low (4.63%) compared to the other two genotypes, AG and GG. Therefore, the frequency of A allele was quite low (0.21). On the basis of breed-specific evaluation, the highest frequency of GG genotype of the *CAPN1* was found in Brahman breed (88.24%) whereas the highest frequency of heterozygous genotype was determined in Charolais breed (85.71%). The frequency of *MYF5* GG genotype was found to be very high in Limousine breed (75.00%). Moreover, *MYF5* AA genotype was absent in Angus, Brahman, Limousine, and Charolais breeds. The present study may be useful for further genetic analyses conducted on beef cattle imported into Turkey.

Key words: Beef cattle, *CAPN1*, *MYF5*, single nucleotide polymorphism

Brezilya'dan Türkiye'ye İthal Edilen Besi Sığırlarında *CAPN1* g.5709 C>G ve *MYF5* g.1911 A>G Polimorfizmlerine Ait Varyasyonlar

Özet: Sunulan bu çalışmanın amacı Brezilya'dan Türkiye'ye ithal edilmiş olan ticari besi sığırlarında, *CAPN1* g.5709 C>G ve *MYF5* g.1911 A>G polimorfizmlerine ait genotipik/allelik frekansların ve populasyon genetiği parametrelerinin belirlenmesidir. Tek nükleotit polimorfizmlerine ait genotiplendirme işlemi, Polimeraz Zincir Reaksiyonu-Restriksiyon Parça Uzunluğu Polimorfizmi (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism: PCR–RFLP) analizi kullanılarak gerçekleştirilmiştir. Bu bağlamda, Angus, Angus×Hereford×Nellore, Brahman, Hereford, Limuzin ve Şarole ırklarını içeren toplam 108 baş erkek sığır genotiplendirilmiştir. Bu çalışmada, *CAPN1* ile ilgili olarak CC genotipinin bulunmadığı görülmüştür. Ayrıca, G allel frekansının oldukça yüksek olduğu belirlenmiştir (0,75). *MYF5* için AA genotip frekansının (%4,63) AG ve GG olan diğer iki genotip ile karşılaştırıldığında oldukça düşük olduğu ve dolayısıyla A allel frekansının (0,21) da düşük olduğu görülmüştür. Irk özelinde yapılan değerlendirmede, en yüksek *CAPN1* GG genotip frekansı Brahman ırkında (%88,24) bulunurken; en yüksek heterozigot genotip frekansı ise Şarole ırkı sığırlarda (%85,71) belirlenmiştir. *MYF5* GG genotip frekansının Limuzin ırkında çok yüksek olduğu (%75,00) görülmüştür. Buna ek olarak, *MYF5* AA genotipinin Angus, Brahman, Limuzin ve Şarole ırklarında bulunmadığı belirlenmiştir. Bu çalışma, Türkiye'ye ithal edilen besi sığırları üzerinde ileride yapılacak genetik analizlere faydalı olabilecektir.

Anahtar kelimeler: Etçi sığır, *CAPN1*, *MYF5*, tek nükleotit polimorfizmi

Introduction

ln recent years, molecular genetics and biotechnological innovations have provided new insights into the identification of genes and gene markers associated with quantitative traits, and hence, investigation of molecular markers and evaluation of their influences on phenotypic measurements have become widely used applications in animal breeding [11].

Many candidate genes associated with quantitative traits have been identified in livestock. Among these, one of the most favourable gene markers for meat production traits is bovine micromolar calcium-activated neutral protease 1 (*CAPN1*). This gene has been mapped to chromosome 29 and it encodes a cysteine protease (μ-calpain) that seems to be the primary enzyme in the postmortem tenderization process [7]. Thus, it has been suggested that *CAPN1*

Yazışma adresi / Correspondence: *Doç.Dr. Sena Ardıçlı (ORCID: 0000-0003-2758-5945), Department of Genetics, Faculty of Veterinary Medicine, Bursa Uludag University, Gorukle/Bursa, Turkey E-mail: sardicli@uludag.edu.tr*

(GenBank accession number: AF252504) acts as a major gene affecting meat quality [7, 12, 25]. On the other hand, there is evidence to indicate that this gene may influence growth, fattening, and carcass traits [2, 19, 22]. Myogenic factor 5 (*MYF5*), which is a member of the *MRF* gene family (muscle regulatory factors), has been shown to be an important muscle-specific factor which is associated with determination of the myoblast lineage [13]. Previous studies have indicated that *MYF5* (GenBank accession number: M95684) is a strong candidate gene that influence growth traits in cattle [16, 28].

Recently, importation of living animals and/or carcasses has become one of the most frequent applications to regulate meat supply and demand in Turkey. In addition, farms generally do not perform genotypic analyses on animals to be imported. There is limited information about the genotypic structure of the cattle imported to Turkey with respect to candidate genes associated with economically important traits. Evaluations for imported cattle with genotypic information will provide significant insights into the variation in major genes influencing the economically important traits in Turkey's livestock. Moreover, these genomic evaluations may affect the importation strategies in order to achieve more profitable breeding management in Turkey. Therefore the aim of this study was to evaluate the genotypic/allelic frequencies and population genetic indexes of *CAPN1* g.5709 C>G and *MYF5* g.1911 A>G polymorphisms in commercial beef cattle imported from Brazil to Turkey.

Material and Method

Animals

A total of 108 bulls, including Angus (n=27), Angus×Hereford×Nellore (n=12), Brahman (n=17), Hereford (n=29), Limousine (n=16), and Charolais (n=7) breeds, were genotyped. The cattle were transported from Brazil to Tabiat Agricultural Farm located in Bursa province (40°15'28.04"N 29°30'50.18"E), Turkey. All animals were housed for fattening in semi-open pens for approximately nine months. Cattle were slaughtered by means of exsanguination and dressed using standard commercial practices. At the same time, 4 mL of blood sample was obtained from flowing blood.

Genomic DNA extraction and genotyping

Genomic DNA was isolated from peripheral blood, obtained from slaughterhouse, using the phenol-chloroform method as described by Sambrook and Russell [23]. The amount (ng/µL) and purity (the ratio 260/280) were measured with a spectrophotometer (NanoDrop 2000c, Thermo Scientific, Wilmington, DE, USA). Genotyping of the SNP markers was carried out using PCR–RFLP analysis. All PCR reactions were performed in 200 µL PCR tubes (Axygen Scientific, USA) on a thermal cycler (Bioneer, MyGenie 96 Thermal Block, Korea). Reagents were purchased from Biomatik (Cambridge, Canada). Reactions were performed in a total volume of 50 µL containing 2 µL of genomic DNA (approximately 100 ng), 5 µL of 10×PCR buffer, 3 µL of $MgCl_2$ (25 mM), 1 µL of Taq polymerase (2.50 U), 1 µL of dNTPs (2.50 mM), and 1 µL of each primer (0.025 µM). The primer sequences (5' to 3') used for a 415 bp fragment of the *CAPN1* gene were as follows:

Forward primer:
5'-GACTGGGGTCTCTGGACTT-3'

Reverse primer:
5'-GGAACCTCTGGCTCTTGA-3'

Concerning *CAPN1* g.5709 C>G polymorphism, thermal cycling conditions were as follows: initial denaturation step at 95°C for 5 min, 35 cycles of denaturation at 95°C for 45 sec, annealing at 63°C for 45 sec and extension at 72°C for 45 sec, followed by a final extension step at 72°C for 5 min. The reaction was held at 4°C [18]. The specific primer sequences (5' to 3') for a 445 bp of the *MYF5* locus were as follows:

Forward primer:
5'-ACAGCGTCTACTGTCCTGATG-3'

Reverse primer:
5'-CGTGGTATATACTAAGGACAC-3'

The amplification reactions with respect to *MYF5* g.1911 A>G polymorphism were as follows: initial denaturation step at 94°C for 4 min, 38 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 1 min, and extension at 72°C for 1 min, followed by a final extension step at 72°C for 4 min. Finally, the reaction was kept at 4°C [13]. The PCR products for *CAPN1* and *MYF5*, respectively, were then digested using *Btg*I and *Taq*I restriction enzymes

(New England Biolabs, Beverly, MA, USA) by incubation at 37°C for 16 h. For gel electrophoresis, 8 µL of the amplified product was mixed with 4 µL of 6×gel loading dye (analytical grade water containing 30% glycerol, 0.25% bromophenol blue, 0.25% xylene cynole) and resolved on 3% agarose gel in 1×TBE buffer, stained using ethidium bromide (2 µg/mL) as an intercalated reagent, at 90 volts for 1 h. The genotype of each animal was determined based on the fragment profile. In this sense, 100 bp DNA ladder (New England Biolabs, Beverly, MA, USA) was run with the amplified products as reference. Visualisation of the gels was performed by a gel imaging system (DNr-Minilumi, DNR Bio-Imaging Systems, Israel).

Evaluation of the genotypic data

The allelic and genotypic distributions were determined by equations stated by Falconer [10]. The Hardy–Weinberg equilibrium (HWE) test were performed using Popgene v1.32 [27]. Formulas described by Nei and Roychoudhury [20] and Botstein et al [5] were used to calculate population genetic indexes, including gene heterozygosity-homozygosity (He-Ho), polymorphism information content (PIC), the effective allele numbers (Ne).

Results

The electrophoresis patterns of PCR amplifications for the *CAPN1* and MYF5 genes are shown in Figures 1 and 3. The cleavage of a 415 bp PCR product by *Btg*I indicated that genotype GG of the *CAPN1* polymorphism g.5709 C>G was identified by the presence of one restriction fragment of 415 bp (undigested). The heterozygous (GC) genotype had three fragments (415, 272, and 143 bp). The CC genotype is characterized by two fragments including 272 and 143 bp. However, the CC genotype was not present in this study (Figure 2). Concerning the *MYF5* polymorphism, g.1911A>G genotype AA of was identified by the presence of one fragment with 445 bp. The genotype GG showed two fragments (352 and 93 bp), whereas heterozygous (AG) genotypes showed three (445, 352, and 93 bp) when digested with the restriction enzyme *Taq*I (Figure 4).

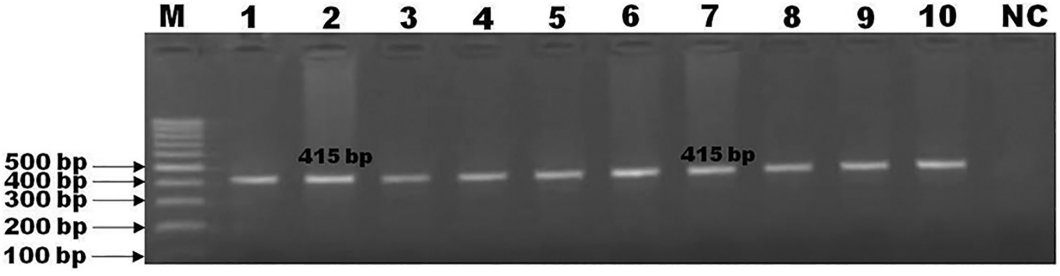

Figure 1. Agarose gel electrophoresis (2%) pattern of g.5709 C>G polymorphism within the bovine *CAPN1* gene for PCR amplification (M: marker 100–1000 bp, NC: negative control).

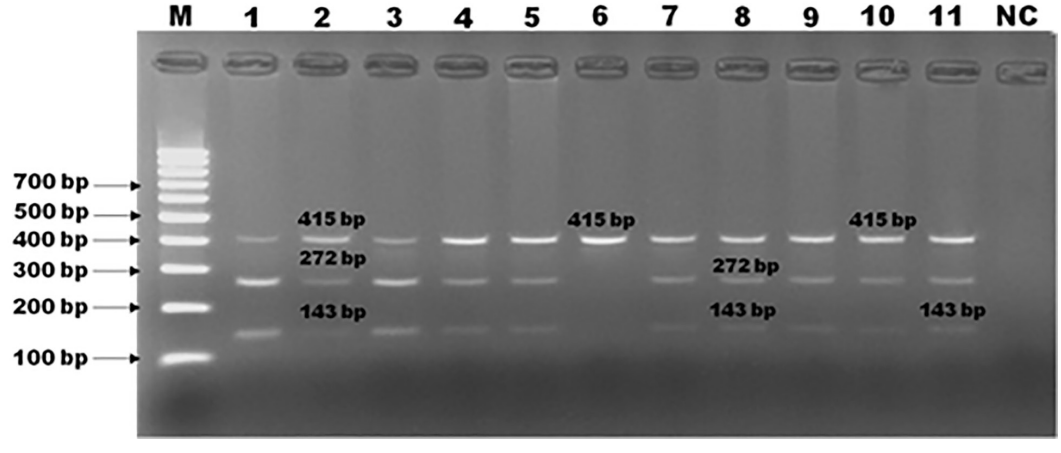

Figure 2. Agarose gel electrophoresis (3%) of PCR fragment (415 bp) of *CAPN1* gene digested with *Btg*I. Genotype GC is in lanes 1-5 and 7-11, genotype GG is in lane 6, genotype CC was not present (M: marker 100–1000 bp, NC: negative control).

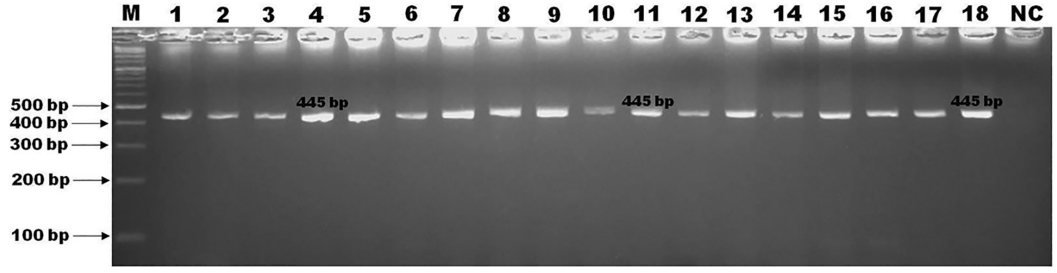

Figure 3. Agarose gel electrophoresis (2%) pattern of g.1911A>G polymorphism within the bovine *MYF5* gene for PCR amplification (M: marker 100–1000 bp, NC: negative control).

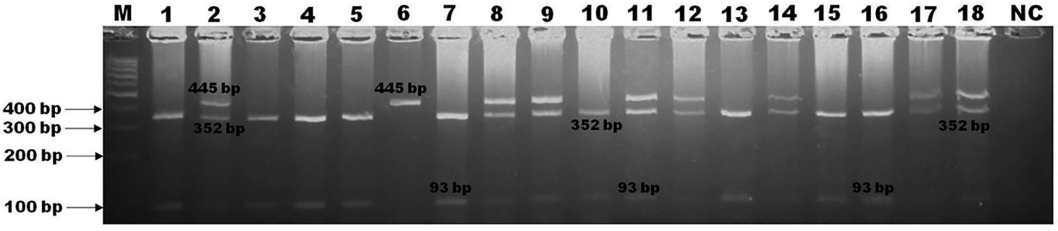

Figure 4. Agarose gel electrophoresis (3%) of PCR fragment (445 bp) of *MYF5* gene digested with *Taq*I. Genotype AG is in lanes 2, 8, 9, 11, 12, 14, 17, and 18, genotype GG is in lanes 1, 3-5, 7, 10, 13, 15, and 16, genotype AA is in lane 6 (M: marker 100–1000 bp, NC: negative control).

The genotypic and allelic frequencies in the different breeds and in the total sample of animals, with respect to *CAPN1* g.5709 C>G polymorphism, are shown in Table 1. Concerning the *CAPN1* g.5709 C>G, the genotype CC was not present in this study. Besides, the G allele frequency was quite high (0.75). Regarding the *MYF5* g.1911 A>G polymorphism, the frequency of AA genotype was rather low (4.63%) compared to the other two genotypes, AG and GG, and accordingly, the frequency of A allele was quite low (0.213). On the basis of breed-specific evaluation, the highest frequency of GG genotype of the *CAPN1* was found in Brahman breed (88.24%) whereas the highest frequency of heterozygous genotype was determined in Charolais bulls (85.71%). The frequency of GG genotype in *MYF5* gene was found to be very high in Limousine breed (75.00%). Moreover, AA genotype was absent in Angus, Brahman, Limousine, and Charolais breeds (Table 2).

Table 1. Genotype and allele frequencies of the SNP g.5709 C>G of the bovine *CAPN1* gene in the different genetic groups and in the total sample of animals

Breed	Individuals	Genotype frequencies (%)*			Allele frequencies	
		GG	GC	CC	G	C
Angus	27	62.96 (17)	37.04 (10)	0	0.8148	0.1852
Angus×Hereford×Nellore	12	33.33 (4)	66.67 (8)	0	0.6667	0.3333
Brahman	17	88.24 (15)	11.76 (2)	0	0.9412	0.0588
Hereford	29	27.59 (8)	72.41 (21)	0	0.6379	0.3621
Limousine	16	50.00 (8)	50.00 (8)	0	0.7500	0.2500
Charolais	7	14.29 (1)	85.71 (6)	0	0.5714	0.4286
Total	108	49.07 (53)	50.93 (55)	0	0.7454	0.2546

*The number of animals per genotype is presented in parentheses.

As shown in Table 3, the HWE test evidenced that the analysed population was not in equilibrium at the *CAPN1* locus ($P<0.001$). The values of four population genetic indices including He, Ho, Ne, and PIC to evaluate the genetic diversity in the different genetic groups and in the total sample of animals are also presented in Table 3. Results revealed that, Ho was higher than 0.50 for both *CAPN1* and *MYF5* markers in all breed groups. Regarding the *CAPN1*, He values ranging from 0.1107 (in Brahman bulls) to 0.4898 (in Charolais bulls) and Ne values ranging from 1.1245 (in Brahman bulls)

to 1.9600 (in Charolais bulls) were observed. The PIC values were 0.3075 and 0.2791 for *CAPN1* and *MYF5* markers, respectively, in the total sample of cattle (Table 3).

Table 2. Genotype and allele frequencies of the SNP g.1911 A>G of the bovine *MYF5* gene in the different genetic groups and in the total sample of animals

Breed	Individuals	Genotype frequencies (%)*			Allele frequencies	
		AA	AG	GG	A	G
Angus	27	0	33.33 (9)	66.67 (18)	0.1667	0.8333
Angus×Hereford×Nellore	12	16.67 (2)	16.67 (2)	66.67 (8)	0.2500	0.7500
Brahman	17	0	35.29 (6)	64.71 (11)	0.1765	0.8235
Hereford	29	10.34 (3)	37.93 (11)	51.72 (15)	0.2931	0.7069
Limousine	16	0	25.00 (4)	75.00 (12)	0.1250	0.8750
Charolais	7	0	57.14 (4)	42.86 (3)	0.2857	0.7143
Total	108	4.63 (5)	33.33 (36)	62.04 (67)	0.2130	0.7870

*The number of animals per genotype is presented in parentheses.

Table 3. Genetic diversity at the *CAPN1* and *MYF5* genes in the different genetic groups and in the total sample of animals

Breed	Genes	He	Ho	Ne	PIC	Locus equilibrium HWE test
Angus	*CAPN1*	0.3018	0.6982	1.4323	0.2563	-
	MYF5	0.2778	0.7222	1.3847	0.2392	-
Angus×Hereford×Nellore	*CAPN1*	0.4444	0.5556	1.7999	0.3457	-
	MYF5	0.3750	0.6250	1.6000	0.3047	-
Brahman	*CAPN1*	0.1107	0.8893	1.1245	0.1046	-
	MYF5	0.2907	0.7093	1.4098	0.2484	-
Hereford	*CAPN1*	0.4620	0.5380	1.8586	0.3553	**
	MYF5	0.4144	0.5856	1.7076	0.3285	-
Limousine	*CAPN1*	0.3750	0.6250	1.6000	0.3047	-
	MYF5	0.2188	0.7812	1.2800	0.1948	-
Charolais	*CAPN1*	0.4898	0.5102	1.9600	0.3699	*
	MYF5	0.4082	0.5918	1.6896	0.3249	-
Total	CAPN1	0.3796	0.6204	1.6118	0.3075	***
	MYF5	0.3353	0.6647	1.5044	0.2791	-

HWE: Hardy–Weinberg equilibrium; He: gene heterozygosity; Ho: gene homozygosity; Ne: effective allele number; PIC: polymorphism information content.
*P<0.05; **P<0.01; ***P<0.001.

Discussion and Conclusion

In the current study, a genotypic evaluation of the SNPs at the *CAPN1* and *MYF5* genes was performed in commercial beef cattle imported from Brazil to Turkey. Bovine *CAPN1* gene has been shown to be a strong candidate for meat production traits. Polymorphism g.5709 C>G is a cytosine/guanine (C/G) substitution in exon 9 of the *CAPN1* gene that produces a glycine/alanine amino acid alteration [7]. Several studies have indicated that C allele in this gene marker is associated with meat quality, especially the tenderness [7, 12, 19]. Bovine *CAPN1* is located on the telomeric end of chromosome 29 and this genomic region involves quantitative trait loci related not only to meat tenderness but also growth and feed efficiency [6, 21, 22]. Thus, determination of the genotypic and allelic distributions corresponding to *CAPN1* g.5709 C>G polymorphism may provide economic benefits in commercial cattle to be raised and in cattle to be imported as well. Previous studies have reported that CC genotype was absent or the frequency was quite low in various cattle populations [1, 8, 17]. This knowledge was substantiated by the current study, indicating that there was no animal with the CC genotype. Moreover, the frequency of C allele (~0.26) was rather low in all breeds included in this study. Present results revealed that there was a deviation from HWE for the *CAPN1* marker with respect to Hereford and Charolais breeds and total sample of animals. Deviations from HWE may be explained by population characteristics such as inbreeding and stratification [14].

The *MYF5* gene influences myogenic lineage determination and myocyte differentiation. Thus, this gene plays a role in the control of carcass and

growth traits [24]. *MYF5* g.1911A>G polymorphism is a adenine to guanine substitution in intron 2 [16]. Although genomic alterations in the intronic regions do not change the amino acid sequence, they have been shown to be associated with gene splicing, regulation of proteins during transcription, mRNA stability, and translation [15]. In the literature, there are contrasting results with respect to genotypic distribution evaluation of the *MYF5* g.1911 A>G polymorphism. Curi et al [9] has suggested that a lower frequency of the A allele of the *MYF5* g.1911A>G was observed in *Bos indicus* (Nelore) cattle when compared to crossbreds (*Bos indicus* × *Bos taurus*), and moreover, the A allele is more common in *Bos taurus* cattle. Bhuiyan et al [4] reported that the A allele was ranging from 0.04 to 0.06 in *Bos indicus* breeds including Brahman and Red Chittagong, and from 0.21 to 0.39 in *Bos taurus* breeds including Angus, Hereford, Hanwoo, Simmental, and Shorthorn. In addition, Kisacova et al [13] suggested that heterozygous genotype was the most frequent in Charolais breed. In the present study, comparisons among groups indicated that the G allele had a high frequency in all breeds but the highest value was observed in Limousine breed (0.875). The A allele was rather low in Angus, Brahman, Limousine, and Charolais, and moreover, there was no animal with the AA genotype in these breeds. However, genotypic evaluations should be conducted on larger cattle populations.

Regarding genetic studies, evaluation of population genetic indices including He/Ho, Ne, and PIC may provide valuable clues about population characteristics. These indices express the breeding properties, such as eventual inbreeding, or the effectiveness and usefulness of loci allele impact [3, 26]. In this study, high Ho values were observed for both *CAPN1* g.5709 C>G and *MYF5* g.1911A>G polymorphisms in Brahman bulls resulting in low genetic variabilities of He, Ne, and PIC. According to Botstein et al [5], PIC values are classified into three categories including very informative (PIC>0.50), mildly or moderately informative (0.25<PIC<0.50), low or not informative (PIC<0.25). In this respect, *CAPN1* g.5709 C>G can be considered as moderately informative in all breeds, except Brahmans. Concerning *MYF5* g.1911A>G, PIC values ranged from 0.1948 (in Limousine breed) to 0.3285 (in Hereford breed). Furthermore, the marker seemed to be low informative for Angus, Brahman, and Limousine, whereas, moderately informative for Angus×Hereford×Nellore, Hereford, and Charolais.

Consequently, the present study focused on the genotypic evaluation regarding *CAPN1* g.5709 C>G and *MYF5* g.1911A>G polymorphisms in beef cattle imported to Turkey. Importation of beef cattle and/or carcasses stands out in relief in Turkey's livestock industry. Molecular biological techniques have rapidly gained pace in recent years and they have enabled us to determine frequencies and population genetic parameters of the genes that are associated with economically imprtant traits in cattle breeding. Selecting animals with the favourable genotypes may result in production of animals with higher performance. This is a crucial point not only for raising farm animals in Turkey conditions but also for selecting the individuals with superior performance before importation. Consequently, the genotypic evaluations on the cattle to be imported may be required to achieve sustainable and profitable production in livestock.

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