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Genetic variation in the bovine myogenic determination factor 1 (g.782G>A polymorphism) and its influence on carcass traits in Turkish Grey Steppe cattle

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Abstract: *MyoD1* gene is a member of the myogenic differentiation gene family, which plays a crucial role in growth and development. There is a lack of information about the *MyoD1*variants and their phenotypic influences in Turkish Grey Steppe cattle. Therefore the present study aimed to determine the genetic variability of *MyoD1* g.782G>A polymorphism and to evaluate its effects on carcass characteristics in Turkish Grey Steppe cattle. Analyses were conducted on a total of 142 Turkish Grey Steppe bulls. The PCR-RFLP technique was used for genotyping of the g.782G>A polymorphism in exon 1 of the *MyoD1* gene. The population genetic parameters including compatibility with Hardy–Weinberg equilibrium, heterozygosity, number of effective alleles, the polymorphism information content, and the fixation index were calculated. Statistical analysis was carried out using the least square methods of the general linear model procedure. Results revealed that BB was the preponderant genotype (41.55%) but the AA genotype exhibited a remarkable close frequency (39.44%). Accordingly, allelic frequencies were very close to each other (A:0.49; B:0.51). The $\chi 2$ test revealed that the corresponding *MyoD1* locus did not conform to Hardy–Weinberg equilibrium ($p < 0.001$). Heterozygosity, number of effective alleles, and the polymorphism information content values were 0.4998, 1.9992, and 0.3749, respectively. Moreover, the fixation index value was found to be 0.6195. These results indicated that *MyoD1* g.782G>A polymorphism is a mildly informative genetic marker for Turkish Grey Steppe cattle. Statistical analysis indicated that the studied *MyoD1* locus was significantly associated with chilling loss, carcass bone content, and carcass length ($p < 0.05$). The AA genotype was characterized by a higher chilling loss percentage but lower carcass length and bone content compared to BB and heterozygous genotypes. This study may provide valuable information regarding cattle carcass assessment and improvement through marker-assisted selection.

Key words: Cattle, *MyoD1*, carcass characteristics, SNP

1. Introduction

In cattle breeding, one of the most important goals is to increase productivity. Conventional breeding methods may be insufficient, especially in traits with low heritability. Molecular techniques have enabled the genetic background of economically important quantitative traits to be evaluated effectively and reliably in animal breeding. The use of these techniques in the field is increasing day by day. Thus, exploiting the genetic variations in the particular phenotypes is one of the most prevalent targets of animal breeders [1,2].

Improvements in quantitative traits in cattle depend on the identification of novel genetic variations as well as the investigation of significant associations within the genes related to variability in the performance traits [2]. For instance, the evaluation of genotypic variation associated with myogenesis provides a more direct understanding of meat production, meat quality, and growth differences between individuals. These differences are highly associated with muscle fiber formation throughout embryonic development, maturation, and differentiation, which is regulated by the *MYOD* gene family [3–5]. This gene family contains four structurally and functionally related genes including myogenic differentiation 1 (*MyoD1*), myogenic factor 5 (*Myf5*), myogenic factor 6 (*Myf6*; also known as myogenic regulatory factor 4: *MRF4* or herculin), myogenin (*MyoG*) [3,6]. Among them, *MyoG* and *Myf6* are denoted as the myogenic differentiation factors whereas *Myf5* and *MyoD1* are known to be myogenic determination factors [6]. All of these genes have three exons and possess a conserved basic helix-loop-helix (bHLH). This domain is essential for DNA-binding and protein dimerization [3,6,7]. *MyoD1* gene is located on BTA15 (37 to 40 cM interval). This location is related to meat quality and carcass traits QTLs [8]. The *MyoD1* g.782G>A is located in exon 1 and it is a missense mutation that causes a glycine, GGC, to serine, AGC, substitution [3,7]. *MyoD1* provides important regulatory functions

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involved in controlling myogenic processes concerning the transcriptional activation and the intensity, specificity, and expression of transcriptional muscle-specific genes. It also plays a key role in stem cell-myoblast-differentiation and the formation of muscle cells. Thus *MyoD1* has been designated as a prevalent marker of myogenesis regulation [6,9,10]. Taken together, the polymorphisms in this gene are the decisive indicators for the carcass characteristics based on a molecular aspect.

Turkish Grey Steppe cattle is an important indigenous animal genetic resource of Turkey. Moreover, this native breed is the relatives of European Grey cattle [11,12]. Previous studies showed that the ancestors of Grey cattle were living in Ukraine steppes. Thereafter, they moved west and south into Italy, Hungary, the Balkans, and Turkey. These cattle contributed to the development of many different local breeds such as Croatian Dalmatian Grey, Slovenian Podolian, Hungarian Steppe Grey (crossed with Italian Maremmana, and Yugoslavian Podolian) [12]. Not surprisingly, Turkish Greys have low production performance but they are characterized by remarkably high resistance to diseases or parasites and very high adaptability for surviving under challenging environmental conditions and low-quality feed opportunities. The genetic studies conducted on such native breeds may provide a wider aspect of the variability of specific genomic locations compared to high-yielding breeds which can have extremely high inbreeding levels, and accordingly, high homozygosity rates.

The candidate gene applications allow the investigation of SNPs in genes that are possibly influential on the phenotypic traits analyzed [3]. The effects of the *MyoD1* genotypes on these traits have been reported in sheep [13], poultry [14,15], and particularly, in pigs [16–19]. Some previous studies have described the relationship of the bovine *MyoD1* gene with carcass and meat quality traits [3,7,20]. However, there is very limited information on the association between the g.782G>A polymorphism in the *MyoD1* gene and cattle carcass characteristics. Moreover, there are no published data related to this gene marker in the Turkish Grey Steppe cattle breed. Therefore, this study aimed to determine the genetic variability of *MyoD1* g.782G>A polymorphism and to evaluate its association with carcass characteristics in Turkish Grey Steppe cattle.

2. Materials and methods

2.1. Animals and sampling

A total of 142 male Turkish Grey Steppe cattle were blood sampled and used to evaluate the distributions of genotypes and alleles of *MyoD1* g.782G>A polymorphism in the South Marmara region of Turkey. For all animals, housing and management procedures were the same. Blood sampling performed complied with ethical considerations (Approval Number: 2010/6-05). The cattle were slaughtered at a commercial slaughterhouse regarding routine practices. Blood samples (approx. 4 mL) were obtained in sterile K_3EDTA vacutainers (Vacutest Kima, SRL, Piove di Sacco, Italy) for subsequent genetic analyses. Before slaughter, all cattle fasted for 12 h, and preslaughter live weights (LW) were recorded.

2.2. Determination of carcass characteristics

In the slaughterhouse, following the removal of noncarcass components, hot carcass weight (HCW) was measured. HCW was determined without including the perinephric/pelvic fat and the kidneys. Then carcasses were chilled for 24 h in a ventilated room at 4 °C, and thus, chilled carcass weight (CCW) was measured. The difference between HCW and CCW was used to determine chilling loss (CL). CL was considered as the weight loss during the 24 h chilling period of the carcasses. The dressing percentage (DP) was calculated based on HCW [21]. The backfat thickness (BFT) was the thickness of the fat covering the outer surface of the *musculus longissimus dorsi* at the 12th rib. Carcass length (CAL) was measured as the distance from the *os pubis* to the tip of the first rib. Carcass pH value (between the 12th and 13th ribs) was measured at 24 h postmortem using Testo 205 digital pH meter (Testo AG, Lenzkirch, Germany). Furthermore, weight means (kg) of bone content (BC) and the yield of valuable cuts (VCY) were determined. Sirloin, strip loin, rib, roast, and cutlet were evaluated as valuable cuts of the carcasses. The percentages (%) of bone (BP) and valuable retail cuts (VCP) were determined based on CCW [22]. The evaluation of carcass characteristics was performed in 53 randomly selected bulls.

2.3. Genetic analysis

Purification of genomic DNA from whole blood was performed using an appropriate phenol-chloroform method [23]. Spectrophotometric measurements of DNA concentration (ng/μL) and the purity (260/280 nm ratios) were applied by using a spectrophotometer (NanoDrop 2000c, Thermo Scientific, Wilmington, DE, USA).

Genotyping of the *MyoD1* g.782G>A polymorphism was performed by PCR-RFLP. PCR amplification was performed in a total volume of 25 μL by using MyGenie 96 thermal cycler (Bioneer Corporation, South Korea). Reactions contained 2.50 μL DNA sample (approximately 65 ng genomic DNA) as a template, 12.50 μL PCR master mix (OneTaq Quick-Load 2x MM, New England BioLabs Inc., Ipswich, MA, USA,Cat# M0486S), 1 μL of forward and reverse primers (0.5 μM), and 8 μL of distilled deionized water (ddH_2O). The primers for the *MyoD1*gene (GenBank Accession Number: NW_001493305) were:

MyoD1-F: 5' GTCACCCAGGAGCACAAAT 3'

MyoD1-R: 5' CCTGAGCAAAGTCAACGAG 3'

PCR was conducted to amplify a 633 bp fragment

of exon 1 in the *MyoD1* gene [7] and the corresponding protocol was as follows: initial denaturation at 94 °C for 5 min, 35 cycles of denaturing at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s, with a final extension at 72 °C for 10 min. The amplification products were controlled by electrophoresis (migration for 1 h at 100 V) using 2% agarose gel (Sigma-Aldrich, Steinheim, Germany) with 10 µL of PCR product and 3 µL of 6x-gel loading dye, Purple (New England BioLabs Inc., Cat# B7024S).

*Bgl*I restriction enzyme (New England BioLabs Inc.) was used for *MyoD1* g.782G>A genotyping. PCR products (15 µL) were mixed with the enzyme (1 µL), buffer, and ddH_2O. The mixture was incubated for 16 h at 37 °C, and afterward, was applied to the 3% agarose gel (Sigma-Aldrich). A 100-bp ladder (Biomatik Cat No. M7123) was added to each gel for calculating the size of the fragments produced. The gels (for both PCR amplification and enzyme digestion) were photographed under UV illumination using theDNR-MiniLumi gel documentation and analysis system (DNR Bio-Imaging Systems, Israel). Ultimately, the genotypes were determined individually by analyzing the corresponding fragment sizes as demonstrated by Du et al. [7].

2.4. Statistical analysis

Genotypic/allelic frequencies and the compatibility with Hardy–Weinberg equilibrium (HWE) were estimated by using Cervus v3.0 software [24]. In this context, a test of goodness of fit was used to evaluate HWE based on the expected and observed genotype frequencies ($\alpha = 0.05$). Thus, genotype distributions were tested for concordance with HWE. Genetic indices, including heterozygosity (He), Number of effective alleles (Ne), and the polymorphism information content (PIC), were calculated using the following formulas based on the relevant studies [25,26]:

$He=1-\sum^{n}_{i=1}P_i^2$,

$Ne=1/\sum^{n}_{i=1}P_i^2$,

$PIC=1-(\sum^{n}_{i=1}P_i^2)-\sum^{n-1}_{i=1}\sum^{n}_{j=i+1}2P_i^2P_j^2$,

where P_i was the ith allele frequency, *n* was the allele number.

The fixation index (F_{IS}) was estimated from the values of theoretical (H_{the}) and experimental (H_{exp}) heterozygosities using the following formula:

$F_{IS} = (H_{the} - H_{exp}) / H_{the}$

Analysis of variance (ANOVA) was carried out using general linear models (GLM) procedure in Minitab v19 software (Minitab Inc., State College, PA, USA). In this context, the following statistical models were used:

Model-1 was used to test the effects of *MyoD1* genotypes on LW, HCW, CCW, CL, DP, and BFT:

$Y_{ijklm} = \mu + A_i + S_j + G_k + I_l + e_{ijklm}$,

where

Y_{ijklm} = the studied trait,

µ = the mean,

A_i = the fixed effect of slaughter age (i = 15–17 months),

S_j = the fixed effect of slaughter season (j = autumn and winter),

G_k = *MyoD1* genotypes (k = AA, AB, and BB),

I_l = two-way interactions,

e_{ijklm} = random error.

Model-2 was used to test the effects of *MyoD1* genotypes on BC, BP, VCY, and VCP:

$Y_{ijklmn} = \mu + A_i + S_j + G_k + \beta W_l + I_m + e_{ijklmn}$,

where

Y_{ijklmn} = the studied trait,

µ = the mean,

A_i = the fixed effect of slaughter age (i = 15–17 months),

S_j = the fixed effect of slaughter season (j = autumn and winter),

G_k = *MyoD1* genotypes (k = AA, AB, and BB),

βW_l = regression effect of chilled carcass weight

I_m = two-way interactions,

e_{ijklmn} = random error.

Concerning statistical analyses, statistical significance was set at a *P*-value of less than 0.05. Tukey's test was used as a post hoc evaluation.

Additive and dominance effects were calculated based on the following reparameterized model as indicated by Falconer and Mackay [27]:

The degree of dominance=d/a

where

Additive effect (a) = the difference between the means of two homozygous divided by two

Dominance effect (d) = the heterozygote's deviation from the mean of the homozygotes

If there is overdominance, d is greater than + a or less than – a [27].

3. Results

3.1. PCR-RFLP patterns

The amplification of the *MyoD1* gene using the appropriate primers yielded a 633-bp amplicon (Figure 1). The cleavage of the PCR product with the *Bgl*I restriction enzyme resulted in three bands (633bp, 447 bp, and 186 bp) for heterozygous genotype (AB). The digestion resulted in two bands (447 bp and 186 bp) and was diagnostic for the homozygote BB genotype. Concerning the AA genotype, the 633 bp product remained undigested in the *MyoD1* assay (Figure 2).

3.2. Genetic variation

Genotypic distributions, population genetic indices, and concordance with the HWE are shown in Table 1. Results revealed that the selected cattle population do not fit the HWE predictions ($p < 0.001$), regarding the observed genotypic frequencies. The most frequent genotype for *MyoD1* was BB (41.55%). But the AA genotype exhibited a

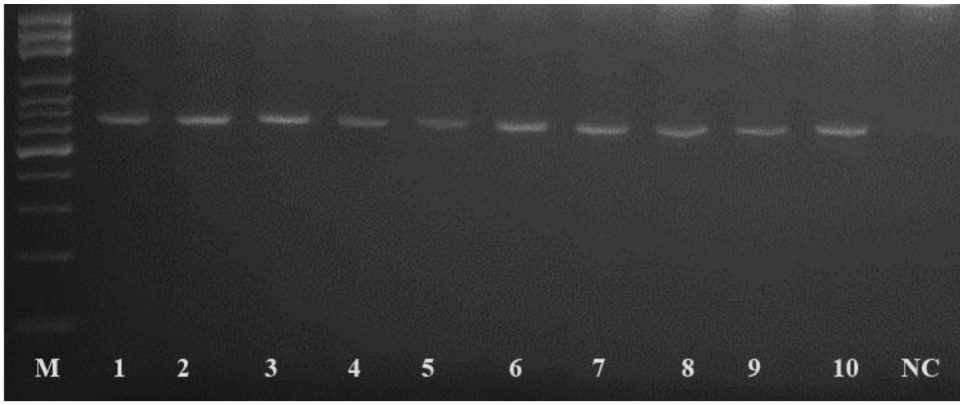

Figure 1. The electrophoresis pattern PCR amplification (633 bp) for g.782G>A polymorphism within the bovine MyoD1 gene in Turkish Grey Steppe cattle.
M: marker; NC: negative control.

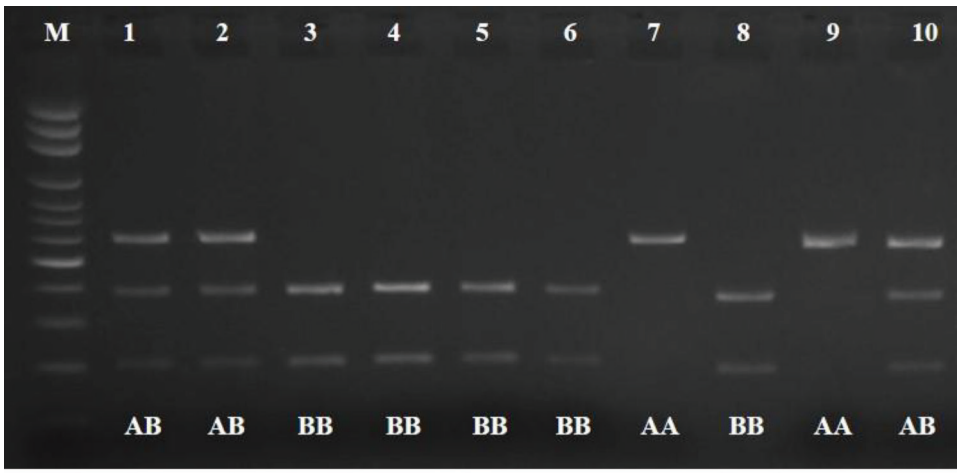

Figure 2. The electrophoresis pattern of restriction enzyme digestion with BglI for g.782G>A polymorphism within the bovine MyoD1 gene in Turkish Grey Steppe cattle.
Lines 7 and 9: AA genotype; Lines 1, 2, and 10: heterozygote genotype; Lines 3–6 and 8: BB genotype.
M: marker; NC: negative control.

remarkable close frequency (39.44%). Accordingly, allelic frequencies of A and B were very close to each other (0.49 and 0.51, respectively). Moreover, heterozygous genotype frequency was found to be rather low (19.01%) in this study. This resulted in a relatively low value of He (0.4998). Ne value approached 2.00 (1.9992) whereas the PIC value was observed to be at a moderate level (0.3749). Besides, the F_{IS} value was 0.6195 for this locus.

3.3. SNP effects on carcass characteristics

After the genotypes were identified, the association of the *MyoD1* g.782G>A with carcass traits were analyzed. As presented in Table 2, this locus was determined to be significantly associated with CL, CAL, and BC ($p < 0.05$). In this respect, the AA genotype was characterized by higher CL but lower CAL and BC compared to BB and heterozygous genotypes. The data also indicated overdominance effects for the associated traits including CL and BC (Table 3). No significant associations were observed between the genotypes and the remaining traits evaluated in the present study ($p > 0.05$). The two-way interactions were insignificant and will not be discussed further.

4. Discussion

At present, studies investigating the novel associations of particular candidate genes with economically important quantitative traits provide a genetic evaluation of these

Table 1. Genotype and allele frequencies of *MyoD1* g.782G>A in bovine myogenic determination factor 1, population genetic indices, and compatibility with the Hardy–Weinberg equilibrium.

Locus	*MyoD1*		
Genotypes	AA	AB	BB
n	56	27	59
GF (%)	39.44	19.01	41.55
EGF (%)	34	71	37
Alleles	A	B	
AF	0.49	0.51	
Ho	0.5002		
He	0.4998		
Ne	1.9992		
PIC	0.3749		
F_{IS}	0.6195		
χ^2(HWE)	54.5053		
*P****	0.0000		

MyoD1: myogenic determination factor 1; *n*: number of experimental bulls; GF: genotype frequency; EGF: the expected genotype distribution according to HWE; AF: allele frequency; Ho: homozygosity; He: heterozygosity; Ne: effective allele numbers; PIC: polymorphism information content; F_{IS}: fixation index; χ^2(HWE): Hardy–Weinberg equilibrium χ^2 value.
***$p < 0.0001$: not consistent with HWE.

Table 2. Least square means for *MyoD1* g.782G>A genotype effects on live weight and carcass characteristics.

Trait	Genotype			*p*-value
	AA	AB	BB	
Live weight (kg)	449.99 ± 14.44	476.34 ± 12.07	482.64 ± 10.61	0.183
Hot carcass weight (kg)	242.97 ± 8.12	257.46 ± 9.02	259.60 ± 5.97	0.241
Chilled carcass weight (kg)	237.76 ± 7.86	252.13 ± 8.74	255.86 ± 3.78	0.207
Chilling loss (%)	2.32 ± 0.15[a]	2.12 ± 0.16[ab]	1.85 ± 0.10[b]	0.043
Dressing percentage (%)	54.13 ± 0.59	54.11 ± 0.66	53.78 ± 0.44	0.859
Back fat thickness (cm)	3.19 ± 0.27	2.98 ± 0.30	3.23 ± 0.20	0.781
Carcass length (cm)	130.23 ± 1.38[b]	133.87 ± 1.54[ab]	134.85 ± 1.02[a]	0.030
Carcass pH	5.59 ± 0.08	5.76 ± 0.09	5.67 ± 0.06	0.414
Bone content (kg)	46.55 ± 2.02[b]	53.08 ± 2.24[a]	53.35 ± 1.49[a]	0.022
Bone percentage (%)	20.09 ± 0.76	21.12 ± 0.85	21.06 ± 0.56	0.538
Valuable cuts yield (kg)*	73.82 ± 3.55	73.89 ± 3.58	68.14 ± 2.36	0.290
Valuable cuts percentage (%)*	32.09 ± 1.88	30.02 ± 1.90	27.23 ± 1.25	0.106

[a,b] Different superscripts within a row indicate a significant difference.
*Valuable cuts included rib, roast, sirloin, cutlet, striploin.

Table 3. Additive and dominance effects of the markers with significant associations.

Trait	Additive effect	Dominant effect	Overdominance
Chilling loss	0.235	2.085*	+
Carcass length	0.310	–1.330*	-
Bone content	–6.810	–20.145*	+

*$p < 0.05$.

traits for more reliable and effective breeding applications in livestock. Moreover, this candidate gene approach is an appropriate way to elucidate and characterize the corresponding genetic variability in native breeds. In this study, the evaluation of the genotypic distribution and population genetic structure of *MyoD1*g.782G>A polymorphism was performed in Turkish Grey Steppe cattle. It is important to note that, to the best of the author's knowledge, this is the first assessment of this marker in Turkish Greys. On the other hand, results from the association analysis between *MyoD1*g.782G>A and carcass characteristics revealed some novel relationships. Growth rate and muscle development are considered highly important traits in cattle breeding. The *MyoD1* gene encodes for muscle-specific transcription factors, and therefore, plays a key role in meat production in farm animals [3]. Thus, this genomic region has a very high potential for improving meat-related traits.

Concerning the genetic variability in the *MyoD1*g.782G>A, the BB genotype exhibited the highest frequency (41.55%). A similar frequency was observed for the AA genotype (39.44%). This led to the observation of allele frequencies close to each other (Table 1). Based on these frequencies, the population genetic indices were calculated and results revealed that the *MyoD1*g.782G>A marker might be considered moderately polymorphic. In this context, the He value was found to be 0.4998 in the genotyped cattle population. This index indicates inbreeding characteristics and a decrease in He values can be associated with high levels of eventual inbreeding [28]. Ne value, which shows the effectiveness of the allele in the corresponding locus, was 1.99 in this study. Another common indicator of population genetic structure is PIC. According to Botstein et al. [26], the PIC > 0.5 genetic markers can be considered to be very informative while the PIC < 0.25 markers have limited usefulness in the studied population and should be evaluated as not informative. PIC between 0.25 and 0.5 is an indicative value for a mildly informative marker. Regarding the abovementioned criteria, the *MyoD1*g.782G>A marker might be considered mildly informative (PIC = 0.3749). These interpretations were partially confirmed by the estimated F_{IS} value (~0.62) which is a good indicator of diversity based on population heterozygosity dynamics. Population genetic indices are important parameters in evaluating the population structure defined by genetic variation [29]. Taken together, the *MyoD1*g.782G>A can be asserted as a useful marker in Turkish Grey Steppe cattle.

In the present study, the association analysis indicated that polymorphism within the *MyoD1* gene might be effective on certain carcass characteristics including CL, CAL, and BC ($p < 0.05$). The AA genotype was characterized by the highest CL (2.32%) compared to alternative genotypes. In this context, carcasses from the AA genotype carrier animals had +0.47% and +0.20% higher values of CL compared to those with the BB and heterozygous genotypes, respectively. Apart from CL, the genotype AA was significantly associated with lower values of CAL and BC ($p < 0.05$). Bulls with this genotype had –4.62cm and –0.98cm lower values of CAL, and furthermore, –6.80kg and –0.27kg lower BC compared to BB and heterozygous animals, respectively. These results indicated that AA animals may be smaller in size. This interpretation was partially confirmed by the lower LW and carcass weights observed for this genotype in this study (Table 2). However, this result was not substantiated by ANOVA results ($p > 0.05$). These findings agree in part with those reported by Du et al. [7] but these researchers have indicated a significant association only for the loin muscle area. In the literature, there are many papers on the relationship of *MyoD1* genotypes with growth, carcass, and meat quality traits in various livestock animals. For instance, many pieces of evidence have been presented that the SNPs of the *MyoD1* gene were significantly associated with these traits in different pig breeds [16–19]. Concerning cattle, analyses mostly revealed conflicting results in different breeds, and in some cases, failed to show any significant relationship to carcass characteristics or meat quality traits [5]. Bhuiyan et al. [3] reported significant associations of *MyoD1* g.1274A>G marker with LW and carcass weights in Korean cattle. But these researchers did not find any association of g.782G>A polymorphism with phenotypic traits. Tian et al. [20] showed that *MyoD1Taq*I locus in intron 2 had significant effects on LW, carcass weights, and the loin muscle area. Chu et al. [30] reported a significant relationship between the mutation at the 3' untranslated region of the *MyoD1* gene and body weight in yaks. It is important to note that the abovementioned associations are for different polymorphisms of the *MyoD1* gene. There have been very few studies on the g.782G>A polymorphism in cattle. To the best of the author's knowledge, this is the first study indicating a significant relationship between the bovine *MyoD1* g. 782G>A marker and carcass characteristics including CL, CAL, and BC. *MyoD1* encodes for transcription factors

related to skeletal muscle. These factors contain highly conserved basic helix-loop-helix regions that are essential for muscle development and growth [3]. Here, it should be stated that BTA15 harbors QTLs regulating meat quality (especially tenderness) and carcass traits [8,31]. It is thus very well possible to recognize novel genetic associations by focusing on this genomic region.

So far as we are aware, this study provides the results of the first analysis of the *MyoD1* gene in Turkish Grey Steppe cattle which is an important native breed of Turkey. On the other hand, this breed is a relative of many European cattle breeds that have contributed to the formation of newly developed local breeds [11,12]. Thus, the genotypic evaluation of this marker in different grey cattle populations may result in obtaining interesting findings.

5. Conclusion

This study focused on the variability of bovine *MyoD1*g.782G>A polymorphism and the association between the corresponding genotypes and carcass characteristics. Concerning the evaluation of population genetic parameters in Turkish Grey Steppe cattle, the *MyoD1* marker has admissible usefulness in this breed. *MyoD1*g.782G>A significantly affected chilling loss, carcass length, and bone content. The carcasses from AA animals were characterized by higher chilling loss whereas lower carcass length and bone content. Although statistically insignificant these animals had lower values of live weight and carcass weights. The BB genotype seemed to be favorable compared to alternative genotypes, especially the AA. It is important to note at this point that the association between *MYOD1* genotypes and the abovementioned carcass characteristics in cattle represented here is new and maybe supportive for studies that evaluate data to define novel genetic relationships and effective genomic variations involved in meat production traits that are under polygenic inheritance. Hence these results may be useful in modern molecular-based methods, such as marker-assisted selection.

Consequently, this study pointed out the potential novel effects of bovine *MyoD1* g.782G>A polymorphism on carcass traits. The data reported here may provide useful information for genetic improvements in cattle breeding but further studies are needed to discuss the present associations and to assess the use of this SNP in marker-assisted selection applications in larger cattle populations.

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