EVALUATION OF *MSTN* GENE POLYMORPHISMS IN TWO ANATOLIAN CATTLE BREEDS BY AS-PCR

İKİ FARKLI ANADOLU SIĞIR IRKINDA *MSTN* GEN POLİMORFİZMLERİNİN AS-PZR İLE DEĞERLENDİRİLMESİ

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ÖZET

Miyostatin (*MSTN*) iskelet kası gelişiminde oynadığı kilit rolden dolayı et üretiminin iyileştirilmesinde kullanılabilecek umut vaat eden genlerden birisidir. Farklı moleküler genotipleme yöntemleriyle belirlenebilen *MSTN* genindeki mutasyonlar sığır populasyonlarında çift kaslılık fenotipinin görülmesinde ve et veriminin arttırılmasında önemli bir potansiyele sahiptir. Bu çalışmada, 11 baz çiftlik delesyon olarak ifade edilen *MSTN* genindeki nt821(del11) polimorfizmi Yerli Kara (YK) ve Doğu Anadolu Kırmızısı (DAK) olarak bilinen iki farklı Türkiye yerli sığı ırkında incelenmiştir. Toplam 72 hayvandan toplanan kan örneklerinden tuzla çöktürme yöntemi kullanılarak DNA izole edilmiştir. DNA izolasyonu aşamasının başarısı %1'lik agaroz jel elektroforezi ile teyit edilmiştir. Her bir hayvanın *MSTN* geni bakımından genotiplendirilmesinde allele-spesifik polimeraz zincir rekasiyonu (AS-PZR) yöntemi tercih edilmiştir. Bu moleküler genotipleme yöntemi sayesinde, bir ortak forward ve iki adet reverse primer kullanılarak hem yabani hem de mutant allelin PZR ile çoğaltılması hedeflenmiştir. Her hayvan için yabani ve mutant allelerin görselleştirilmesinde %3'lük agaroz jel elektroforezinden yararlanılmıştır. Bu çalışmada, AS-PZR yöntemi iki farklı Anadolu sığırı ırkında genetik varyasyonun olmadığını ve bütün hayvanların yabani alleli taşıdığını göstermiştir. Genetik varyasyonun eksikliğinden dolayı, *MSTN* geninin Türkiye yerli sığır ırklarında et üretiminin geliştirilmesinde etkili olmadığı belirlenmiştir. Ancak yapay tohumlama uygulamalarından dolayı ülkeye kültür sığır ırklarının getirildiği bilinmektedir. Kullanılan kültür ırklarına ait spermalardan dolayı gelecekte Türkiye yerli sığır populasyonlarında *MSTN* geni bakımından fenotipi etkileyen mutasyonların görülmesi olasılığı bulunmaktadır. *MSTN* genindeki söz konusu mutasyonların sadece et verimini arttırmadığı ayrıca gebelik süresinin uzamasından kaynaklı güç doğumlara da neden olabileceği kayda değerdir. Bu yüzden, Türkiye yerli sığır ırklarının periyodik aralıklarla *MSTN* geni bakımından incelenmesi gerekirken, gelecekte yapılacak olan çalışmaların marker destekli seleksiyon (MDS) çalışmalarının dizayn edilmesinde kullanılabilecek diğer et üretimiyle ilişkili genlere odaklanabileceği önerilmeltedir.

Anahtar kelimeler: Çift kaslılık, genetik varyasyon, et üretimi, miyostatin, PZR.

ABSTRACT

Myostatin (*MSTN*) is one of the promising genes to improve meat production due to playing a key role in skeletal muscle development. Mutations in the *MSTN* gene, which could be detected via different molecular genotyping methods, are the potential to cause double muscling phenotype and to increase meat yield in cattle populations. In this study, nt821(del11) polymorphism of the *MSTN* gene, defining 11 base pair deletion between 821 and 831 nucleotide positions, were investigated in two native Turkish cattle breeds known as Anatolian Black (AB) and East Anatolian Red (EAR). DNA was extracted from blood samples collected from a total of 72 animals via a salting-out isolation method. The success of the DNA extraction stage was confirmed by 1% agarose gel electrophoresis. Allele-specific polymerase chain reaction (AS-PCR) method was preferred to genotype each animal in terms of the *MSTN* gene.

Via this molecular genotyping method, both wild and mutant alleles were expected to amplify in PCR stage by using a common forward and two reverse primers. 3% agarose gel electrophoresis was utilized to visualize wild and mutant alleles per each animal. In this study, the AS-PCR method showed that no genetic variation was present in two Anatolian cattle breeds in which all animals were detected to carry wild alleles. Due to a lack of genetic variation, *MSTN* was found to be inefficient in improving meat production in native Turkish cattle breeds. However, it is known that exotic cattle breeds are introduced into the country due to artificial insemination practices. Depending on the used sperms of the exotic breeds, causative mutations in the *MSTN* gene may be observed in cattle populations raised in Türkiye in the future. It is noteworthy that mutations in the *MSTN* gene may not only increase meat yield but also it may cause calving difficulties due to extending the gestation period. Therefore, native Turkish cattle breeds should be periodically screened in terms of the MSTN genes, whereas further studies may focus on other meat production-related genes to design marker-assisted selection (MAS) studies.

Keywords: Double muscling, genetic variation, meat production, myostatin, PCR.

INTRODUCTION

Depending on production type, cattle rearing in Türkiye is practiced at different scales to produce animal-derived food resources such as meat and milk. Large-scale production practiced by commercial companies is based on cosmopolitan cattle breeds. In contrast, native cattle breeds including AB and EAR are reared by smallholders at a small scale to increase incomes in rural areas. As reported by Demir et al., (2021), AB the coat color of which is completely black is the most geographically distributed breed, whereas EAR is raised in a limited area of east Anatolia including Erzurum, Kars, and Ardahan provinces.

Meat is one of the essential animal-derived products to supply society with a healthy diet. The fact that the human population continues to grow (Gu et al., 2021) forces farmers to improve meat yield (Brameld and Parr, 2016). However, meat yield shows a quantitative inheritance indicating that it is determined by the interaction of numerous environmental factors and genes (Demir et al.2020). Fortunately, previous studies reported several major genes such as calpain 1 (*CAPN1*), calpastatin (*CAST*), growth hormone receptor (*GHR*), paraoxonase 3 (*PON3*), DNA Primase subunit 2 (*PRIM2*), myotrophin (*MTPN*), and *MSTN* affecting meat production in different cattle breeds (Ardicli et al., 2017; Bordbar et al., 2020; Konovalova et al., 2021; Wang et al., 2022). Of these genes, *MSTN* including three exons and two introns is reported to be highly conserved throughout the evolution in mammals (Aiello et al., 2018). Also called growth differentiation factor 8 (GDF8), *MSTN* is a member of the transforming growth factor-beta superfamily and is mainly expressed in skeletal muscle (Sheng et al., 2021). As known, muscle development is negatively controlled by the expression of *MSTN* to limit muscle mass. Indeed, several studies confirmed that larger skeletal muscle phenotype was observed in *MSTN*-knocked-out animals such as chickens (Kim et al., 2020) and pigs (Wang et al., 2017) via genome editing technologies. Naturally, some causative mutations also lead to the same phenotype called double muscling in numerous cattle breeds including Belgian Blue, Piedmontese, and Marchigiana (Aiello et al., 2018). Thanks to advances in molecular genotyping methods such as polymerase chain reaction (PCR), real-time PCR (RT-PCR), AS-PCR, and restriction fragment length polymorphism (RFLP) (Pozzi et al., 2009; Konovalova et al., 2021), genetic variations in the *MSTN* gene could be easily identified to shape selection practices for maintaining current and future demand for animal-derived products. Indeed, improving meat yield is of potential to face future challenges such as the increased demand for meat consumption caused by the increasing human population. Hence, this study aims to monitor genetic variations in the *MSTN* gene in two Anatolian cattle breeds (AB and EAR) by using the AS-PCR technique.

METHOD

Sampling strategy and DNA extraction

A total of 72 animals belonging to AB (n=35) and EAR (n=37) cattle breeds were sampled from representative herds located in Antalya and Erzurum provinces, respectively. Blood samples (5-10 ml) taken from the jugular vein were collected into vacutainer tubes containing EDTA solution and stored at -20 ºC until DNA extraction was performed. A salting-out method reported by Miller et al., (1988) was deployed to isolate DNA. The success of the DNA extraction stage was confirmed by the 1% agarose gel electrophoresis.

AS-PCR Process and Genotyping

Recently, Konovalova et al., (2021) designed a common forward (TGAGGTAGGAGAGTGTTTTGGG) and two reverse primers to amplify wild (CCTCTGGGGTTTGCTTGGT) and mutant (ACAGCATCGAGATTCTGTCACA) allele by AS-PCR protocol allowing for investigating 11 bp deletion between 821 and 831 nucleotide position of bovine *MSTN* gene. In this protocol, wild and mutant alleles are amplified at 310 and 144 bp lengths, respectively, in agarose gel electrophoresis. In this study, the same protocol designed by Konovalova et al., (2021) was utilized to assess *MSTN* polymorphism in AB and EAR breeds via an optimized AS-PCR protocol. PCR mixture containing 11.1 µl H_2O 9 µl 2XPCR Mastermix (EcoTech), 0.3 µl (10 pm) of each primer, and 50 ng template DNA was amplified via thermocycler conditions summarised in Table 1.

Table 1. PCR conditions to amplify nt821(del11) polymorphism of *MSTN* gene

Stage	Temperature (ºC)	Duration (sec)	Cycle
Initial denaturation	95	180	1
Denaturation	95	45	35
Annealing	60	30	
Elongation	72	30	
Final extension	72	300	1

Two separate PCRs were conducted to amplify wild and mutant alleles, respectively, per each animal, whereas both alleles were visualized simultaneously in 3% agarose gel electrophoresis. Based on the electropherogram image, animals were genotypes as free from mutation (310 bp), carrier (144 and 310 bp), and homozygous mutant (144 bp).

RESULTS

In this study, no genetic variation was detected in AB and EAR breeds in terms of nt821(del11) polymorphism of the *MSTN* gene. As illustrated in Figure 1 and Figure 2, all animals turned out to carry only wild alleles, while no mutant allele (144 bp) was identified.

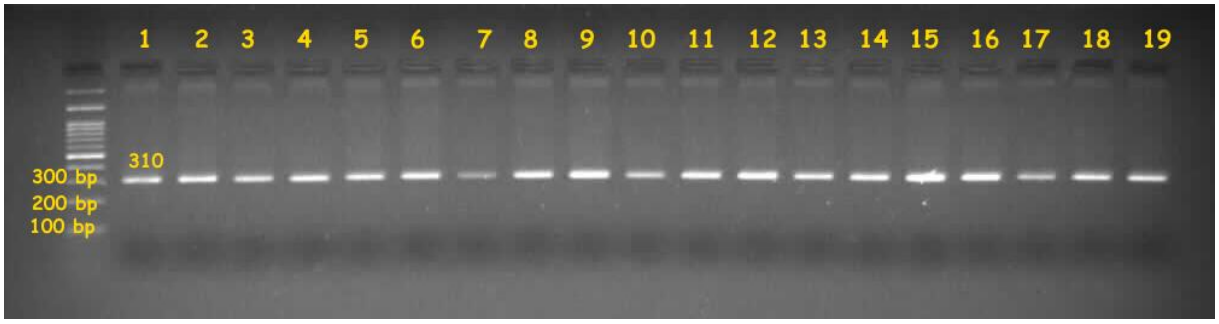

Figure 1. %3 agarose gel image for *MSTN* polymorphism in AB breed

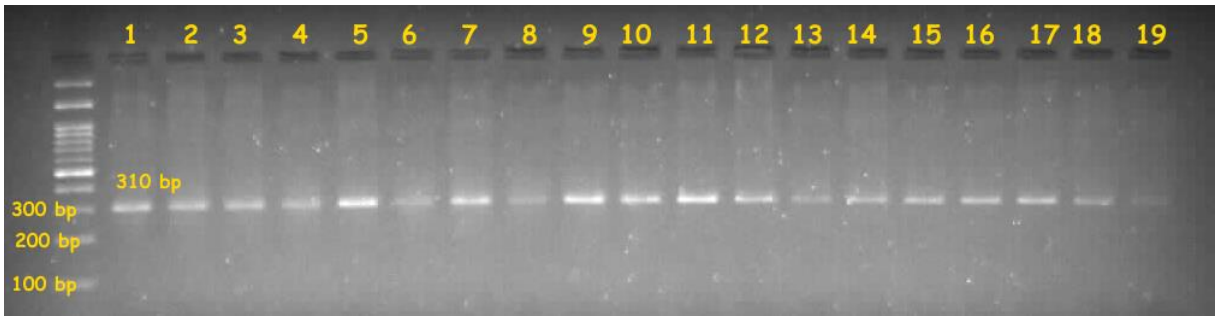

Figure 2. %3 agarose gel image for *MSTN* polymorphism in EAR breed

DISCUSSION AND CONCLUSION

This study demonstrates that the *MSTN* gene is highly conserved in two Anatolian cattle breeds since no genetic variations were detected via the AS-PCR technique. This finding is not surprising due to the fact that no double muscling phenotype has been reported in native Turkish cattle breeds yet. Indeed, all animals were recorded to possess wild-type alleles in terms of nt821(del11) polymorphism of the *MSTN* gene. Similar findings were also reported in native Turkish cattle breeds by numerous previous studies. For example, Sakar and Zülkadir, (2021) screened 104 animals belonging to the AB breed in terms of *MSTN*, *GHR*, and *PIT-1* genes via the PCR-RFLP technique in which *MSTN* gene was reported to be monomorphic. In another study focusing on *CAPN1*, *MSTN*, *CAST*, and *SPP1* gene polymorphisms by PCR-RFLP method among five native Turkish cattle breeds, two alleles were detected for *CAPN1*, *CAST*, and *SPP1* genes, whereas no genetic variation was observed for *MSTN* gene (Ağaoğlu et al., 2015). On the other hand, the double muscling phenotype caused by the mutation in the *MSTN* gene is observed in some beef cattle breeds. For instance, Konovalova et al., (2021) monitored six cattle breeds (Aberdeen Angus, Limousin, Simmental, and Belgian Blue) in terms of two different mutations in the *MSTN* gene in which 2.18% and 100% of animals belonging to Aberdeen Angus and Belgian Blue, respectively, were carriers in terms of nt821(del11) mutation. Variation caused by 11-bp deletion was also reported by Jakaria et al., (2021) in Belgian Blue cattle.

Previous studies emphasize that mutations of the *MSTN* gene leading to double muscling could be observed in beef cattle breeds, particularly in Belgian Blue. Indeed, it appears from the current study that the *MSTN* gene is fixed in Anatolian cattle breeds due to a lack of genetic variation. However, it is possible to identify some causative mutations in *MSTN* and other meat-related genes in cattle breeds farmed in Türkiye in the future because cosmopolitan genotypes are introduced to the country via artificial insemination. This scenario requires that native Turkish cattle breeds should be periodically screened for *MSTN* gene polymorphisms to design efficient mating programs. It will be useful because mutations in the *MSTN* gene may be used in MAS studies to enhance meat production, but it comes with some disadvantages. As highlighted by Widyas et al., (2018), loss of function of the *MSTN* gene may yield animals with the double muscling phenotype in which since the gestation period is larger, calves are born with higher body weight. This disadvantage may negatively affect reproduction such as calving difficulties in farm animals (Vankan et al., 2009).

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