Bovine SMO gene polymorphism in Anatolian Black Cattle and Anatolian Water Buffaloes

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Abstract: The purpose of this study was to determine the G>C mutation on exon 9 (G21234C) of Bovine Smoothened (SMO) gene polymorphism by using *Cfr*13I restriction enzyme with PCR-RFLP method in both Anatolian Black cattle and Anatolian Water Buffaloes. According to the G>C mutation on exon 9 (G21234C) of SMO gene, the results showed that there were three genotypes such as GG, GC and CC in Anatolian Black cattle, but there was only one genotype GG in Anatolian Water Buffaloes. Allele and genotype frequencies in Anatolian Black cattle were estimated as 0.35 and 0.65 for G and C alleles and 0.22, 026 and 0.52 for GG, GC and CC genotypes, respectively. The Chi-square test showed that the Anatolian Black cattle population was not in Hardy-Weinberg equilibrium with respect to G21234C substitution (P<0.05).
Because G>C mutation on exon 9 (G21234C) of SMO gene is polymorphic in cattle, it can be concluded that SMO gene is a potential candidate gene. However, such a statement cannot be expressed for buffaloes since it is monomorphic in Anatolian buffaloes.

Keywords: Bovine, Smoothened, livestock, *Cfr*13I, restriction enzyme

Yerli Kara sığır ırkı ve Anadolu mandalarında SMO gen polimorfizmi

Öz: *Bu çalışmanın amacı hem Yerli Kara sığırlarında hem de Anadolu mandalarında PCR-RFLP methodu ile Cfr13I restriksiyon enzimi kullanılarak Bovine Smoothened (SMO) gen polimorfizminin 9. ekzonundaki (G21234C) G>C mutasyonunu belirlemektir. SMO geninin 9. ekzonundaki G>C mutasyonuna göre sonuçlar Yerli Kara sığırlarda GG, GC ve CC genotiplerinin olduğunu, fakat Anadolu mandalarında yalnızca GG genotipinin olduğunu göstermiştir. Yerli Kara sığırlarda allel ve genotip frekansları sırasıyla G ve C allelleri için 0.35 ve 0.65, GG, GC ve CC genotipleri için 0.22, 026 ve 0.52 olarak tahmin edilmiştir. Ki-kare testi Yerli Kara sığır populasyonunun G21234C değişikliği ile ilgili olarak Hardy-Weinberg dengesinde olmadığını göstermiştir (P <0.05).*
SMO geninin 9. ekzon (G21234C) üzerindeki G> C mutasyonu sığırlarda polimorfik olduğu için SMO geninin potansiyel bir aday gen olduğu sonucuna varılabilir. Fakat, Anadolu mandalarında monomorfik olduğu için mandalar için böyle bir ifade söylenemez.

Anahtar kelimeler: *Bovine, Smoothened, çiftlik hayvanları, Cfr13I, restriksiyon enzimi*

INTRODUCTION

Buffaloes are reared mostly in the half northwestern and also north part of the middle Anatolia of Turkey. It is more common along the coast of the Black sea and is used for the purpose of producing milk and meat (Soysal et al., 2013). Another species, the Anatolian Black cattle is a *Bos brachyceros* type with a long narrow head (Yilmaz et al., 2012). Also, this cattle breed is black, small-bodied, small horns and is used for the purpose of producing milk and meat.

Along with its widespread use in molecular biology and in many various areas, molecular markers have also begun to take place in animal sciences. Many genes have been identified as having an association economically traits such as productivity and health in livestock until today. Even, some potential genes concerning with economic characteristics of farm animals such as qualitative or quantitative traits have been studied for marker assist selection (MAS) by now (Aytekin et al., 2011; Fadhil and Zülkadir, 2017). One of these genes is the Smoothened (SMO) gene. The SMO gene was first identified in the fly *Drosophila melanogaster* and later in vertebrates (Murone et al., 1999; Quijada et al., 2007). The development of a multicellular organism depends on mechanisms that initially specify and then maintain positional information (Alcedo et al., 1996). Hh (Hedgehog) pathway is one of these mechanisms. Smoothened (SMO) is the seven-pass transmembrane signal transducer of the Hh pathway and a member of the G-protein-coupled receptor superfamily. Also, SMO is encoded by the SMO gene (Alcedo et al., 1996; Murone et al., 1999). Hh signaling plays many important developmental roles in animals. The Hh signaling pathway is a signaling pathway that transmits information to embryonic cells required for proper cell differentiation and it is one of the key regulators of animal development and is present in all bilaterians (Ingham et al., 2011). SMO takes part in the regulation of either osteogenesis or adipogenesis with Hh pathway (James, 2013; Liang et al., 2015; Wu et al., 2004). Being implicated in the development of some cancers or tumors, Hh path is studied by researchers in human health (Avci, 2012; Steg et al., 2012). So, SMO, as a central regulator of the pathway and an accessible cell membrane component, has been the primary focus in the development of small molecule Hh

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pathway inhibitors (Kim et al., 2013). The Hh pathway is a major regulator for cell differentiation, tissue polarity and cell proliferation (Jia et al., 2015). Hh pathway in various animal tissues and organs is necessary for the patterning, growth, and morphogenesis (Zhang et al., 2014). Briefly, Hh signaling pathway has been recognized as a key regulatory component of many fundamental processes.

In *Bos taurus*, the SMO gene has been localized of chromosome 4, consists of 13 exons with a total length of 24316 bp (GenBank accession No: 539308). Zhang et al. (2014) detected polymorphisms in the bovine SMO gene of Qinchuan cattle, and also researchers stated that associations between this gene and body measurement traits (BMTs) - meat quality traits (MQTs). As a result, authors were stated that the SMO gene could be used as a candidate gene to alter BMTs and MQTs in Qinchuan cattle or for marker-assisted selection to breed cattle with superior BMTs and MQTs. Moreover, in a study made with SNP markers about the molecular characterization of SMO gene and effects of its genetic variations on body size traits in Qinchuan cattle (*Bos taurus*), Zhang et al. (2015) identified the different SNPs and stated that wild-type alleles of some detected SNPs appeared to be more beneficial for selecting cattle with superior body size traits.

The purpose of this study is to determine the G>C mutation at exon 9 (G21234C) of Bovine Smoothened (SMO) gene polymorphism in Anatolian Black Cattle and Anatolian Water Buffaloes.

MATERIAL AND METHODS

A total of 50 Anatolian Black Cattle (ABC) reared in Ankara city and 50 Anatolian Water Buffaloes (AWB) reared in Konya and Kütahya cities were used for SMO gene in this study. Blood samples from each animal were taken into vacutainer tubes containing EDTA from the *vena jugularis* and stored at -20^{o}C until DNA extraction. Genomic DNA from whole blood was extracted according to QuickGene DNA whole blood kit S (DB-S; KURABO, JAPAN). After extraction, the DNA concentration of all samples was assessed with the Nanodrop spectrophotometer (ND1000; NanoDrop Technologies, USA) in order to determine quality and quantity. The RFLP method was used to determine for the G>C polymorphism on exon 9 (G21234C) of SMO gene. A pair of primers with the following nucleotide sequences: F:5'-GCTTCACCCGTCTACTACCC-3' and R:5'-GCTCATGGAAATGCCAGTTC -3' was used to amplify a DNA fragment of 163 bp from exon 9 of SMO gene (Zhang et al., 2014). The PCR reaction was performed in 10 ul reaction volume. The polymerase chain reaction comprised genomic DNA, 5 µmol L^{-1} Dream Taq Green PCR Master Mix (2X; Thermo Scientific), 0.3 µmol L^{-1} of each primer and 2.4 µmol L^{-1} ddH_2O in a total volume of 10 ul. The amplification was performed in a gradient thermal cycler (Techne TC-512) using the following program: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 60.7 °C for 30 s and 72 °C for 30 s. Final extension was at 72 °C for 10 min. The PCR products were digested with 10 U of *Cfr13I* fast digest restriction enzyme in 20 ul volume (Thermo Fisher Scientific). The restriction fragments were subjected to electrophoresis on 2% agarose/ethidium bromide gel in 1X TBE buffer and then visualized under UV light and scored in a gel documentation system. The Chi-square test whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium was carried out via PopGene32 (ver. 1.32).

RESULTS

Figure 1 show that agarose gel electrophoresis of PCR and digested products of SMO gene digested with *Cfr13I* restriction enzyme in Anatolian Water Buffaloes and Anatolian Black Cattle. In this study by using *Cfr13I* restriction enzyme in G>C mutation on exon 9 (G21234C) of SMO gene, the results in Anatolian Black cattle showed that there were three genotypes such as GG, GC and CC, but was only one genotype GG in Anatolian Water Buffaloes (Figure 1). Allele and genotype frequencies were estimated as 0.35 and 0.65 for G and C alleles and 0.22, 0.26 and 0.52 for GG, GC and CC genotypes, respectively (Table 1). Also, H_e (expected) and H_o (observed) heterozygosity values were found to be 0.1 and 0.2, respectively.

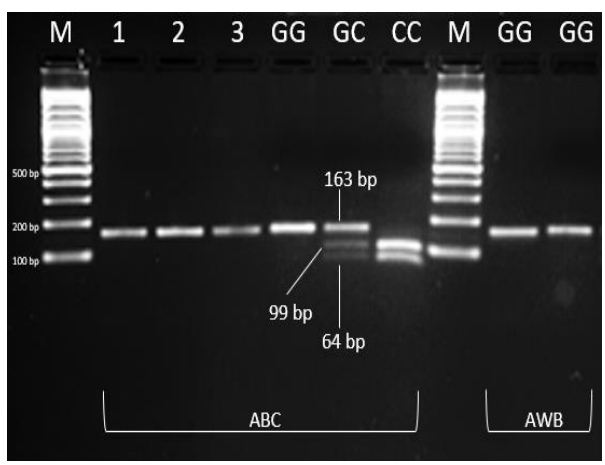

Figure 1 - Agarose gel electrophoresis of PCR and digested products; M: 100bp Plus DNA Ladder (Vivantis Technologies), Line 1-3: PCR products, GG: 163 bp, GC: 163, 99 and 64 bp, CC: 99 bp and 64 bp

Table 1 - Genotype/allele frequencies of SMO gene in ABC and AWB

Breed	N	Genotype frequencies			Allele frequencies		
		GG	GC	CC	G	C	χ^2
ABC	50	0.22	0.26	0.52	0.35	0.65	9.183*
AWB	50	1.00	0.00	0.00	1.00	0.00	-

*P<0.05: not in Hardy-Weinberg equilibrium

DISCUSSION

The genetic diversity within inter or intra breeds is important for breeding programs in livestock. Turkey is a rich country in terms of genetic diversity. It contains many native cattle breeds and a native buffalo breed. Although these native breeds have not high productivity traits, they are adapted to withstand bad environmental conditions. SMO is a member of G protein-coupled receptors (GPCRs) families and an ingredient of the Hh signaling pathway (Ingham and McMahon, 2001; Ruiz-Gómez et al., 2007). There are little studies about the SMO gene in cattle breeds. Where, Zhang et al. (2014) confirmed that SMO gene is a candidate gene that association with the growth traits and meat quality in Chinese cattle breeds. In this study we analyzed SMO gene polymorphism in AWB and ABC. Three genotypes were found in ABC and one genotype in AWB. As far as we know, this study is the first study in the literature on SMO gene polymorphism in buffaloes. (Zhang et al., 2014) reported an association between SMO gene and growth traits and meat qualities in Qinchuan cattle breeds with genotype and allele frequency; GG: 0.3577, GC: 0.5427, CC: 0.0996, G: 0.6290, C: 0.3710. (Zhang et al., 2015) confirmed the influence of SMO gene polymorphism on the growth traits of Qinchuan cattle breeds. Since SMO gene is monomorphic in the Anatolian buffaloes, it is necessary to do sequence analyses to find new or different SNPs in this species. Because genome-wide association studies (GWASs) are possible due to the availability of technology that allows high-throughput genotyping of single-nucleotide polymorphisms (SNPs). These SNPs are variants, or alleles, in the DNA sequence that may be associated with the expression of a trait or characteristic in cattle. The technology allows deciphering the genetics behind the expression of economically important traits (Casas and Kehrli Jr, 2016; Zimin et al., 2009). There are genetic conditions besides environmental conditions that influence productivity in livestock phenotypes. Namely, it is important to carry out studies involving recently found or known genes with effects on the performance and health of livestock. Many molecular markers have proved their ability to improve important economic traits until today. In fact, studying the functions of genes, and also pathways and signaling, will make it easier to understand their functions. As a result, further studies need to be done to understanding genetic diversity, association between productions or health and SMO gene in Anatolian Black Cattle and Anatolian Water Buffaloes.

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