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Research Article

EcoRI Polymorphism in Intron 6 of the Bovine Lactoferrin Gene in South Anatolian Red and East Anatolian Red Cattle Breeds

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ABSTRACT

Lactoferrin (LF) is an iron-binding glycoprotein that exists in variety of biological fluids such as milk, tear, saliva, bile, mucous secretions and blood. Bovine mastitis is an inflammation of the mammary gland. LF has a vital role in the defense mechanism of the mammary gland and the bovine LF gene is a potential candidate gene for imparting resistance to mastitis in dairy cows. The objective of the study is to determine the genotype and allele frequencies for bovine lactoferrin gene polymorphisms in South Anatolian Red **(**SAR) and East Anatolian Red **(**EAR) cattle. DNA samples of 46 SAR and 46 EAR cattle were selected from DNA collection obtained from previous research projects. Bovine LF gene was amplified by polymerase chain reaction (PCR) and the PCR products were digested by EcoRI restriction enzyme. The frequency of A allele was found higher than B allele in EAR cattle breed and the frequency of B allele was found higher than A allele in SAR cattle breed. We suggest that further studies should be conducted on the bovine LF gene in Bos taurus and Bos indicus cattle to understand the reason of high genotype frequency in SAR and EAR cattle breeds.

Key Words: Bovine Lactoferrin gene, South Anatolian Red cattle, East Anatolian Red cattle

ÖZET

DOĞU ANADOLU KIRMIZISI VE GÜNEY ANADOLU KIRMIZISI IRKI SIĞIRLARDA LAKTOFERRİN GENİNİN 6. İNTRONUNDAKİ ECORI POLİMORFİZMİ

Laktoferrin (LF) süt, gözyaşı, tükürük, safra, mukus salgıları ve kan gibi çeşitli biyolojik sıvılarda bulunan, demir bağlayıcı özelliği olan bir glukoproteindir. LF meme bezi savunma mekanizmasında önemli bir role sahiptir ve sığır laktoferrin geni sütçü ineklerin mastite karşı direnç şekillenmesinde potansiyel aday bir gendir. Bu çalışmada Güney Anadolu Kırmızısı (GAK) ve Doğu Anadolu Kırmızısı (DAK) sığırlarında, laktoferrin genindeki polimorfizmlerin genotip ve allel dağılımlarının belirlenmesi amaçlanmıştır. Çalışmada kullanılan 46 DAK ve 46 GAK DNA örneği, önceki yapılan çalışmalar sonucu elde ettiğimiz DNA koleksiyonundan temin edilmiştir. Hedef bölge polimeraz zincir reaksiyonu (PZR) ile çoğaltılmış ve PZR ürünleri EcoRI restriksiyon enzimi ile kesilmiştir. DAK ırkı sığırlarda A allelinin frekansının B allelinin frekansına oranla yüksek olduğu ve GAK ırkı sığırlarda ise B allelinin frekansının A allelinin frekansına oranla yüksek olduğu tespit edilmiştir. GAK ve DAK sığır ırklarında tespit edilen yüksek genotip

frekansının sebebinin daha iyi anlaşılabilmesi için Bos taurus and Bos indicus ırkı sığırlarında laktoferrin genine yönelik çalışmaların yapılmasını önermekteyiz.

Anahtar Kelimeler: Sığır Laktoferrin geni, Güney Anadolu Kırmızısı, Doğu Anadolu Kırmızısı

Introduction

Lactoferrin (LF), a member of the transferrin family, is an iron-binding glycoprotein with a single polypeptide chain of 708 amino acids that is folded into two lobes each having an iron-binding site (Valenti et al., 2011). LF was found initially in bovine milk and later in other species. LF exists in variety of biological fluids such as tear, saliva, bile, mucous secretions and blood (Lee et al., 2004). It has been known that LF has antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant and immune-modulatory activities (Vogel et al., 2002). The milk LF is secreted mainly from mammary epithelial cells and neutrophils (Pfaffl et al., 2003). The milk LF concentration of healthy cows is ranging between 0.02-0.2 mg/ml and the level is increased approximately 100-fold in response to the infections with sub-clinical and clinical mastitis (Barkema et al., 1998; Hirvonen et al., 1999; Kutila et al., 2003; Schanbacher et al., 1993).

Bovine mastitis is an inflammation of the mammary gland and predominantly caused by bacteria, then mycoplasma, yeasts and algae (Braedley, 2002). Mastitis causes financial losses of dairy farmers through lowered milk production as well as adverse effects on cattle welfare and potential effects on public health (Galal Abdel Hameed et al., 2006). Therefore, cattle that are genetically resistant or less susceptible to the disease must be selected for the breeding programs (Youngerman et al., 2004). LF uses two main mechanisms to show its antimicrobial effect. LF acts as bacteriostatic agent due to its Fe^{+3}- ion binding capacity depriving growing microorganisms of their demands for ferric ions in one of the mechanisms and the second mechanism involves that positively charged amino acids of LF can interact with anionic molecules of the bacteria, virus, fungi and parasites for causing cell lysis (Garcia-Montoya et al., 2012; Seyfert et al., 1996). These mechanisms provides LF a vital role in the defense of the mammary gland and the bovine lactoferrin gene is a potential candidate gene for imparting resistance to mastitis in dairy cows (Kawai et al., 1999; Zheng et al., 2005).

The bovine LF gene is located on chromosome 22q24. It contains 17 exons and spans about 34.5 Kbp of a genomic DNA (Schwerin et al., 1994). In intron 6 of LF gene, there is a polymorphism which can be recognized by the restriction enzyme EcoRI. Two alleles were named as A and B and they encode three possible genotypes; AA, AB and BB (Seyfert and Kuhn, 1994).

This study is designed to determine allele and genotype distribution of LF gene polymorphisms that is suggested to be related to mastitis in South Anatolian Red (SAR) and East Anatolian Red (EAR) cattle.

Materials and Methods

In this study, DNA samples of 46 SAR and 46 EAR cattle were selected from DNA collection obtained from previous research projects. SAR breed cattle were selected from South Anatolia (Diyarbakır and Hatay) whereas EAR cattle were selected from East Anatolian part of Turkey (Kars). The animals were not relatives and had phenotypic characteristics of their breed. Genomic DNA samples had been isolated using standard salt-out method (Miller et al., 1998).

The polymerase chain reaction (PCR) was carried out in a final volume of 25 µl containing 1 U Taq DNA polymerase (Fermentas Life Sciences, Canada), 2 µl 10X PCR buffer (750mM Tris-HCl (pH 8.0), 200 mM $(NH4)_2SO_4$, 0.1% Tween 20), 1.5 mM $MgCl_2$, 50-100 ng genomic DNA, 100 µM dNTP (Takara, Biotechnology Co, Ltd, Japan) and 10 pmol of each primer.

Primers used to amplify 301 bp product of bovine LF gene were; F: 5'- GCC TCA TGA CAA CTC CCA CAC -3' and R: 5'- CAG GTT GAC ACA TCG GTT GAC -3' (Wodjak-Maksymiec et al., 2006). Amplification conditions were 94 °C for 5 minutes (m); 20 cycles of 94 °C for 1 m, touchdown annealing from 70 °C to 60 °C for 1 m, 15 cycles of 94 °C for 1 m, 60 °C for 1 m, 72 °C for 1 m and a final extension at 72 °C for 5 minutes. For restriction fragment length polymorphism (RFLP) analysis 10 µl of the PCR products were digested with 10 units of EcoRI (Fermentas Life Sciences, Canada) restriction enzyme at 37 °C for overnight. The digested DNA fragments were separated by electrophoresis in 2% agarose gel in TBE buffer with ethidium bromide to differentiate the alleles A (301 bp) and B (201 and 100 bp). Genotype and allele frequencies of bovine LF gene were calculated by using PopGene v.32 software (Yeh et al., 2000).

Results

Genotype and allele frequencies of bovine LF gene determined in SAR and EAR cattle are shown in the Table 1.

Digestion of 301 bp fragment of bovine LF gene with EcoRI restriction enzyme revealed a polymorphism with the alleles A and B. The allele A was characterized by a single band of 301 bp and the allele B was characterized by 201 bp and 100 bp bands. The distribution of genotypes and alleles of bovine LF were found to be in Hardy-Weinberg equilibrium and differences between expected and observed values showed no significance in both breeds. In EAR cattle, frequency of A allele was found higher than B allele, whereas frequency of B allele was found higher in SAR cattle. Only 11 animals with AA genotype in SAR cattle and only 6 animals with BB genotype in EAR cattle were observed.

Table 1. Distribution of genotypes and allele frequencies of bovine lactoferrin polymorphism in SAR and EAR cattle breeds.

Tablo 1. Sığır laktoferrin geninin GAK ve DAK sığır ırklarında genotip ve allel frekanslarının dağılımı.

		Genotype						Allele Frequency (%)		Chi-square test	
		AA		AB		BB		A	B	χ^2	P
Breed	n[1]	Ob[2]	Ex[3]	Ob	Ex	Ob	Ex				
SAR	46	11	9.9231	21	23.1538	14	12.9231	46.74	53.26	0.406977	0.523508
EAR	46	18	18.1648	22	21.6748	6	6.1648	63.04	36.96	0.010918	0.916780

[1]number of animals, [2]observed values, [3]expected values

Discussion

Bovine LF gene were studied in Polish Black and White Holstein, Polish Holstein and Polish Friesian cattle breeds among the European cattle breeds (Kaminsky et al., 2006a and b; Sender et al., 2010; Wojdak-Maksymiec et al., 2006). The genotype frequencies belonging to the previous studies are shown in Table 2. AA and AB genotype frequencies in SAR and EAR cattle breeds were found similar with the genotype frequencies in Polish Black and White Holstein and Polish Friesian cattle breeds (Sender et al., 2010; Wojdak-Maksymiec et al., 2006). No significant difference was detected between the expected and the observed genotype frequencies of bovine LF polymorphism for both two breeds and the distribution of genotypes and alleles followed the Hardy-Weinberg equilibrium.

Several studies have been made to investigate the relation between somatic cell count (SCC) and lactoferrin genotypes (Li et al., 2004; Sender et al., 2010; Wojdak-Maksymiec et al., 2006). Wojdak-Maksymiec et al. (2006) determined that the highest SCC was found in the milk of AB genotyped cattle and the lowest SCC in AA genotypes. Also, the association between SCC and the lactation

parity was found statistically important in the same study (Wojdak-Maksymiec et al., 2006). In our study, it was observed that AB genotype is the most frequent genotype of all animals; frequency of AB genotype in SAR cattle were 46% and frequency of AB genotype in EAR cattle 48%. Li et al. (2004) observed that there is not a significant relation between different types of mastitis and genotypes; but, Sender et al. (2010) found that there was a decrease in the prevalence of sub-clinical mastitis in animals with BB genotype. Higher frequency of BB genotype found in SAR breed, in comparison with Polish dairy cattle.

Table 2. Genotype frequencies calculated in previous studies.

Tablo 2. Önceki çalışmalarda hesaplanmış olan genotip frekansları.

Animal Breed	Genotype Frequency	Reference
Polish Black and White Holstein	AA: 0.63 AB: 0.31 BB: 0.04	Sender et al., 2010
Polish Black and White	AA: 0.37 AB: 0.59 BB: 0.02	Wodjak-Maksymiec et al., 2006

BB genotype frequencies in Polish cattle breeds were found to be very low, ranging between 0.005 and 0.1 (Table 2), whereas higher frequencies in SAR and EAR cattle were found, 0.30 and 0.13 respectively.

Sender et al. (2010) suggested that the low frequency of BB genotype might occur due to prior selection which might be associated with milk yield, led to B allele elimination. In this study, higher BB genotype frequencies found in two different Anatolian cattle breeds, which might be interpreted with the non-prior selection of SAR and EAR cattle breeds related with the milk yield.

Near Eastern cattle breeds display an asymmetrical genome composition with very few Zebu mtDNA haplotypes, sparse Zebu Y-chromosomal markers and significant autosomal Zebu levels. It has been suggested that original gene pool were taurine and then subjected to introgression from Zebu population over time (Edwards et al., 2007). Genotype and allele frequencies of autosomal genes like PRL, GHRH-R, IGF-1 and DGAT-1 genes in SAR and EAR cattle breeds were found closer to Zebu cattle than European cattle breeds (Akis et al., 2010; Eken, 2010; Kaupe et al., 2004; Öztabak et al., 2008). Presence of Zebu-associated alleles in SAR and EAR might be explained by Zebu introgression to the Anatolian cattle breeds (Edwards et al., 2007; Loftus et al., 1999). The relatively higher frequency of BB genotype may also be a result of Zebu introgression. Studies on polymorphisms of Zebu lactoferrin gene must be conducted to support this suggestion.

As a result, AA homozygote and AB heterozygote genotype frequencies in SAR and EAR cattle breeds were found similar with the genotype frequencies in Polish Black and White Holstein, Polish Holstein and Polish Friesian cattle breeds. BB genotype frequency was found higher in SAR and EAR cattle breeds than the Polish cattle. We suggest that further studies should be conducted on the bovine LF gene in Bos taurus and Bos indicus cattle breeds to understand the reason of high genotype frequency in SAR and EAR cattle breeds.

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