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SHORT COMMUNICATION

Identification of Alleles for Factor XI (FXID) and Uridine Monophosphate Synthase (DUMPS) Deficiencies in Holstein Cows Reared in Antalya [1]

Taki KARSLI * Emine ŞAHİN * Bahar ARGUN KARSLI *
Sezai ALKAN * Murat Soner BALCIOĞLU *

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* Akdeniz Üniversitesi Ziraat Fakültesi, Zootekni Bölümü, TR-07070 Antalya - TÜRKİYE

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Summary

Deficiencies of Factor XI (FXID) and Uridine Monophosphate Synthase (DUMPS) are autosomal recessive inherited diseases in Holstein cattle breed. The aim of this study was to investigate whether FXI deficiency and DUMPS alleles exist in Holstein cattle reared in Antalya region Turkey or not. 504 Holstein cows were used for this study. PCR (Polymerase Chain Reaction) was used for determination of FXI genotypes and, PCR-RFLP (PCR- Restriction Fragment Length Polymorphism) was used for determination of DUMPS genotypes. As a result, DUMPS allele was not detected while the prevalence of FXID carriers was found as 0.4%.

Keywords: *FXID, DUMPS, PCR-RFLP, Holstein cattle*

Antalya'da Yetiştirilen Siyah Alaca İneklerde Faktör XI (FXID) ve Üridin Monofosfat Senteaz Eksikliği (DUMPS) Allellerinin Belirlenmesi

Özet

Faktör XI (FXID) ve Üridin Monofosfat Senteaz Eksiklikleri (DUMPS) Siyah Alaca sığır ırkında otozomal resesif kalıtım gösteren kalıtsal hastalıklard ır. Bu çalışmanın amacı Antalya bölgesinde yetiştirilen Siyah Alaca ırkı sığırlarda DUMPS ve FXI eksikliği allelinin bulunup bulunmadığının araştırılmasıdır. Bu çalışmada 504 Siyah Alaca inek kullanılmıştır. FXI genotiplerinin belirlenmesi için PCR (Polimeraz Zincir Reaksiyonu), DUMPS genotiplerinin belirlenmesi için PCR-RFLP (PCR- Restriksiyon Parça Uzunluk Polimorfizmi) yöntemi kullanılmıştır. Sonuç olarak DUMPS alleli tespit edilemezken FXI taşıyıcılarının oranı %0.4 olarak bulunmuştur.

Anahtar sözcükler: *FXID, DUMPS, PCR-RFLP, Siyah Alaca*

INTRODUCTION

FXI deficiency is an autosomal recessive disorder and, has been identified in several species of mammals, including humans, dogs and cattle. It is found that the mutation consists of a 76 bp segment (AT(A)28TAAAG (A)26GGAAATAATAATTCA) insertion into exon 12 of FXI gene on chromosome 27 [1]. FXI is a plasma serine protease involved in the early activation of the intrinsic blood coagulation cascade. FXIa along with FVIIIa are responsible for the conversion of FX to its activated form FXa. Subsequently, FXa initiates the conversion of prothrombin to thrombin. This reaction makes possible the formation of the insoluble fibrin clot from soluble fibrinogen [2]. As a result of a mutation in FXID gene, this process cannot be completed and serious health problems such as pro-

İletişim (Correspondence)
+90 242 3102446
msoner@akdeniz.edu.tr

longed bleeding occur in organisms.

Deficiency of Uridine Monophosphate Synthase (DUMPS) is a hereditary autosomal recessive disorder in Holstein cattle breed. UMP synthase is necessary for novo synthesis of pyrimidine nucleotides. UMPS catalyze the conversion of orotic acid into UMP, a precursor for all other pyrimidines and a normal constituent of the milk from cow and other ruminants [3]. Growth and development of the homozygous recessive calves are arrested, leading to embryonic mortality before day 40 of gestation [4]. DUMPS is caused by single point mutation (C→T) at codon 405 within exon 5. The UMP synthase gene was mapped to the bovine chromosome 1 (q31-36) [5].

As the molecular basis of DUMPS disease single nucleotide change (C→T), one of the most commonly DNA marker methods is PCR-RFLP which is used the identification of similar point mutation in livestock [6]. Because FXI deficiency consists of 76 bp segment insertion mutation, it can be detected by PCR only.

The aim of this study is to estimate the prevalence of DUMPS and FXID in Holstein cows reared in Antalya by using DNA based analyses.

MATERIAL and METHODS

Animal Sampling and DNA Isolation

The blood samples were randomly collected from 504 Holstein cows belonging to different parts of Antalya province. Genomic DNA was extracted from blood using the Gen Elute Blood Genomic DNA kit (Sigma Aldrich, St. Louis, MO, USA). DNA quality and quantity were analyzed on agarose gel and measured by spectrophotometric methods.

PCR Conditions

The primer sequences were given in *Table 1*. DNA was amplified initial denaturation at 94°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 1 min, annealing (temperatures for each primer pair are shown in *Table 1*) for 30 sec, extension at 72°C for 1 min, with final extension 72°C for 5 min on Mastercycler 5333 (Eppendorf AG, Germany). The PCR reaction was performed in 1X PCR buffer (containing $(NH_4)_2SO_4$, pH: 8.8), 2.5 mM $MgCl_2$, 2.5 mM dNTPs mix, 1 U Taq DNA Polymerase (BIORON), 0.5 µM of each primer and 50 ng/µl of template DNA in 25 µl reaction volume. PCR products resolved by electrophoresis on 1.5% agarose gel. After PCR process for DUMPS, 10 µl PCR products were digested with *Ava*I for 2 h at 37°C. The digestion products were separated on 3% agarose gel.

RESULTS

After digestion of the PCR products with *Ava*I restriction enzymes, the normal DUMPS allele exhibits three fragments of 53, 36 and 19 bp. Actually, DUMPS carrier gives four fragments of 89, 53, 36 and 19 bp but none of the animals gave 4 fragments. So all 504 cows were found to be DUMPS unaffected in this study.

After the PCR, the normal FXI allele produces a single 244 bp fragment and the carrier cow exhibited two fragments of 244 bp and 320 bp. As a result of this study, two cows were found to be carrier for FXI deficiency *(Fig. 1)*.

DISCUSSION

DUMPS allele has been reported in different countries in America and Europe [5]. However, no carriers were found for DUMPS in the previous studies of Turkey [8-10]. DUMPS allele was also not found in this study as in the other studies in Turkey. The results obtained in this study were also similar to the findings in Poland [11], India [12] and Iran [13].

FXI deficiency in Holstein cattle has been reported in different countries [1,2,14] and Turkey [8,10,15]. Meydan et al. (2009 and 2010) [8,15] and Oner et al.[10] reported the prevalence of FXI carrier as 1.8%, 1.2% and 1.2% respectively. In this study, frequency of FXI carrier was found lower (0.4%) than other three studies. These studies showed very low frequencies of FXI deficiency in different region of Turkey. In addition, DUMPS was not found up to the date in Turkey. These diseases have spreaded all over the world with the use of some carrier sires for artificial insemination. In the 1990s, DNA diagnostic techniques were developed and have been used for genetic disorders such as FXI deficiency and DUMPS. Because imported semen was

Table 1. *Primer sequences and annealing temperatures for each genetic disorder*
Tablo 1. *Her bir genetik bozukluk için primer sekansları ve bağlanma sıcaklıkları*

Genetic Disorders	Method	Primer Sequences	RE	Annealing Temp(°C)
DUMPS [7]	PCR-RFLP	F 5′-GCAAATGGCTGAAGAACATTCTG- 3′	AvaI	60
		R 5′-GCTTCTAACTGAACTCCTCGAGT-3′		
FXID [1]	PCR	F 5′-CCCACTGGCTAGGAATCGTT-3′,	-	57
		R 5′-CAAGGCAATGTCATATCCAC-3′		

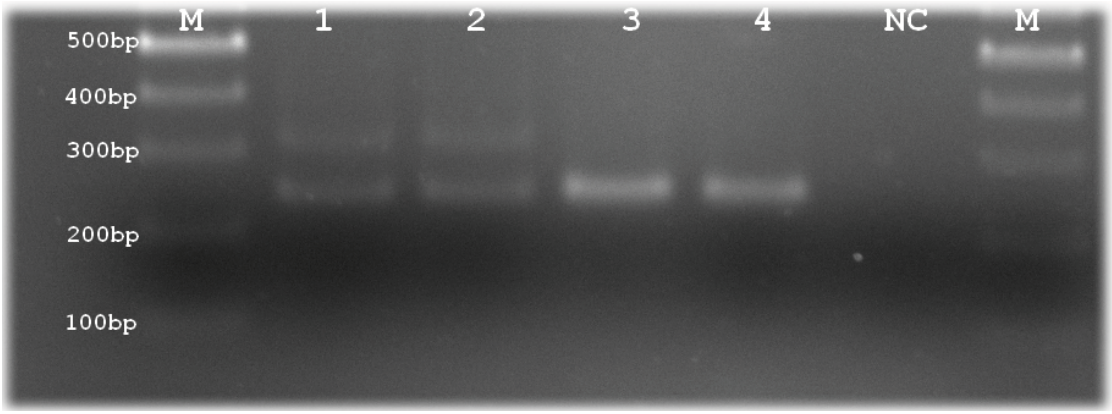

Fig 1. The illustration of FXID genotypes on 1.5% agarose gel. Lane 1 and 2: 244 and 320 bp heterozygous cows, Lane 3 and 4: 244 bp normal cows, Lane 5: negative control consisted of only PCR mix. M: 100 bp DNA Ladder (BIORON-Cat.No:306005)

Şekil 1. FXID genotiplerinin %1.5 agaroz jelde gösterilmesi. Hat 1 ve 2: 244 ve 320 bç heterezigot inekler, Hat 3 ve 4: 244 bç normal inekler, Hat 5: yalnızca PCR karışımı içeren negatif kontrol. M: 100 bç DNA Marker (BIORON-Kat.No:306005)

began to use 1990s, Turkey have less affected cows than America and European countries.

PCR based diagnostic techniques are fast and reliable for determining the hereditary diseases caused by different mutations. With regular use of these methods, frequency of diseases such as FXI deficiency and DUMPS quite decreased in the world. These diseases can be kept under control with the use of molecular diagnostic methods.

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