ABCG2 Gene Polymorphism in Holstein Cows of Turkey [1]

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Summary

ABCG2 protein or Breast Cancer Resistant Protein (Bcrp), located in membrane of mammary glands alveolar epithelial cells, is one of the most important active transport proteins and responsible for passing of drugs from blood to milk. It is encoded by ABCG2 (Bcrp). These transport proteins are very important for veterinary medicine. Single nucleotide polymorphism, which is caused by changing amino acid (Y581S), in ABCG2 gene that encodes Bcrp, affect milk yield and milk properties in Israeli Holstein. It is known that fat and protein proportion in milk were increased, but milk yield was decreased in Israeli Holstein which have ABCG2A allele. In this study, allele frequency of ABCG2 was investigated in Holstein cows of Turkey and for this purpose 207 Holstein cows from Trakya region were genotyped. A allele (0.9807) had been found dominant in all investigated animals. C allele (0.0193) had been determined at only 8 animals. On the other hand CC homozygous had not been found in investigated animals.

Keywords: ABCG2 gene, Polymorphism, Turkish Holstein cows

INTRODUCTION

It is known that genetic factors act on drug efficiency and toxicity among individuals. Pharmacogenetic/Toxicogenetic is also important for the successful individual therapy in human and veterinary medicine. The most common topic for pharmacogenetic in veterinary medicine is the ivermectin sensitivity in some Collie dogs. P-glycoprotein (P-gp), the product of the MDR1 (multidrug resistance) gene, is a functional unit of the blood-brain barrier. P-gp actively extrudes drugs from brain tissue back into capillaries, resulting in lower brain concentrations of drugs such as ivermectin. Because of the mutation in the canine MDR1 gene, the synthesized P-gp is a non-

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Membrane transport proteins providing with efflux system protect tissues against drugs and xenobiotics. ABCG2 protein is a membrane transport protein like P-gp and firstly it was isolated from human breast cancer cell so called breast cancer resistant protein (Bcrp). Then it is declared that this protein can be responsible for the development of resistance against the antineoplastic drugs in cancer cells. So it is pointed out that this resistance can be the possible reason of the failure of chemotherapy. BCRP is a product of ABCG2 gene and it consists of 658 amino acids (NCBI no: CAI38796). It is remarked that ABCG2 gene sequence is generally conservative in Holstein cows. Gene, which has non-conservative amino acid (Y581S; S, serine → Y, tyrosine), is called ABCG2. Alterations on milk yield and proportion of milk protein and fat had been observed on cows that have Bcrp which is the product of ABCG2. The animals having A allele and C allele on gene region with the amino acid alteration on exon 14 at 62569 nucleotide (NCBI, AJ871176), compared with each other, it was found that milk yield was decreased and proportion of milk fat and protein were increased in animals who have A allele. Synthesized amino acid is called tyrosine (TAT) when both of two alleles are adenine on 62569 nucleotide but when nucleotide is cytosine (TCT), synthesized amino acid is serine. But, it is still not known whether this alteration (Y581S) effects the activity of Bcrp or not.

In a study on the Bcrp1/- knock out mice which is the analogue of BCRP gene, it was reported that after IV administration of AFB1, milk to plasma ratio of aflatoxin in Bcrp1/- mice was found to be less (3.8 fold) than wild type mice and it was pointed out that BCRP is responsible for the active transport of various nutrient xenotoxins to milk. It has been shown that AFB1 is an important substrate of BCRP1 which can be responsible for active transport of AFB1 on wild type mice. However, it has still not known the effect of the gene polymorphisms in ABCG2 gene on the elimination of AFB1 with milk.

The study was designed to investigate the presence of ABCG2 gene polymorphism (Y581S) and allele frequency in a limited Turkish Holstein population.

MATERIAL and METHODS

Animals

Venous blood samples of 207 Holstein cows (48 of them from Research Farm of Veterinary Faculty at Istanbul University, 103 of them from Farms at Trakya region and 54 of them from Farm at Çatalca and its villages) were collected in tubes containing EDTA. The animals were treated according to the Animal Care and Use Regulation (European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purpose 1996).

PCR Conditions

Roche High Pure PCR Template preparation kit was used for the extraction genomic DNA from blood samples (Roche Diagnostic GmbH, Mannheim, Germany). The PCR for ABCG2 was carried out in a final volume of 50 µl containing 1.25 U GoTaq (Promega), 10 µl 5×Green buffer (750 mM Tris-HCl (pH 8.0), 200 mM (NH4)2SO4, 0.1% Tween 20), 1.5 mM MgCl2, 50-100 ng gDNA, 100 µM dNTP and 10 pmol of each primer. The Primer sequence used for ABCG2 forward: 5’- CCTAAACAGCCTCAGCTCC-3’ (62301-62321) and reverse: 5’- TCTCCCAATCCTTCAGCTCC-3’ (62779-72760) from NCBI database sequence AJ871176.

For ABCG2 touch down PCR profile included 5 min at 95°C; 5 cycles 45 s at 95°C, 45 s at 57°C, 45 s at 72°C; 15 cycles 45 s at 95°C, 45 s at 56°C, 45 s at 72°C; 15 cycles 45 s at 95°C, 45 s at 55°C, 45 s at 72°C; and a final 10 min extension at 72°C.

PCR products (~478 bp) were purified with commercial purification kit (Roche Diagnostic GmbH, Mannheim, Germany). Purified DNA samples were shown in Fig. 1. Sequences were performed by REFGEN Biothecnology (ODTÜ, Teknokent, Ankara). Presence of nucleotide alteration in each chromatograms were controlled.

Allele (gene) Count Method was used for the calculation of gene frequencies.
RESULTS

Both of two alleles were found A (AA homozygous) in the samples from Çerkezköy-Tekirdağ, Lüleburgaz-Kirklareli and Uzunköprü-Edirne that were located in Trakya region. We found that 50 animals from Çatalca-Istanbul, 47 animals from Farm of Veterinary Faculty at Istanbul University, 18 animals from Saray-Tekirdağ and 16 animals from Malkara-Tekirdağ have AA homozygous and 4 animals from Çatalca-Istanbul, one animal from Farm of Veterinary Faculty at Istanbul University, 2 animals from Saray-Tekirdağ and only one animal from Malkara-Tekirdağ have CA heterozygous. CC homozygous was not found in 207 investigated animal. Samples, number of animals and results of statistical analysis for allele frequency were shown in Table 1.

According to the sequence chromatograms, the animals that have CA heterozygous, was shown in Fig. 2.

Results of sequences were analysed with DNASTAR analysis software, mode of “MegAlign”.

DISCUSSION

CC homozygous genotype was not found in any samples of animals in limited Holstein population from Trakya region in our study which is purposed to exhibit single nucleotide polymorphism that is caused by the alteration on 581st amino acid (tyrosine → serine) in ABCG2 gene in Israeli Holstein cows.

Komisarek et al. reported that CC genotype in ABCG2 was not detected in 453 genotyped animals. It was pointed out that ABCG2 A allele was found in Belgian Blue, Belgian Blue mix, Bohemian Red, British Friesian, East Anatolian Red, German Angus, German Black Pied, German Brown, German Simmental, Israeli and German Holstein, Menorguina.

Our study is the first investigation associated with the ABCG2 gene polymorphism in Holstein cows in Turkey. We found that A allele frequency is 98.07% and C allele frequency is 1.93% in genotyped 207 animals.

Table 1. Allele frequency of genotyped animals in relation to region of Trakya

<table>
<thead>
<tr>
<th>Region of Trakya</th>
<th>The Number of Genotyped Animal</th>
<th>Allele Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>ÇERKEZKÖY Çerkezköy</td>
<td>9</td>
<td>1.000</td>
</tr>
<tr>
<td>LÜLEBURGAZ</td>
<td>18</td>
<td>1.000</td>
</tr>
<tr>
<td>EDİRNE Uzunköprü</td>
<td>41</td>
<td>1.000</td>
</tr>
<tr>
<td>EDİRNE Lüleburgaz</td>
<td>54</td>
<td>0.9630</td>
</tr>
<tr>
<td>ISTANBUL Çatalca</td>
<td>54</td>
<td>0.9630</td>
</tr>
<tr>
<td>ISTANBUL Res. Farm of Vet Fac</td>
<td>48</td>
<td>0.9896</td>
</tr>
<tr>
<td>TEKİRDAĞ Malkara</td>
<td>17</td>
<td>0.9706</td>
</tr>
<tr>
<td>TEKİRDAĞ Saray</td>
<td>20</td>
<td>0.9500</td>
</tr>
<tr>
<td>Total</td>
<td>207</td>
<td>0.9807</td>
</tr>
</tbody>
</table>

Fig 2. Chromatogram shows CA heterozygous

Şekil 2. CA heterozigotluğunu gösteren kromatogram
DNA sequences of ABCG2 polymorphism in Turkish Holstein cows were submitted by GENBANK with EU672817 number.

Ron et al.\textsuperscript{10} and Schennink et al.\textsuperscript{14} who had investigated the ABCG2 allele frequency in 32 cow breeds (3 of them are Holstein) and 72 Holstein-Friesian bulls respectively, shown that A allele is predominant in all of the breeds. Similar to these findings we have found that, A allele is also predominant in Holstein cows in Trakya region of Turkey. This finding can be an evidence that our study population is similar to German Holstein cows. Ron et al.\textsuperscript{10} also reported that A allele was found in all of the Anatolian Black, South Anatolian Red, Turkish Gray Steppe and C allele was found only in East Anatolian Red.

As reported earlier the presence of high frequency of ABCG2 A allele can be explained that it causes a decrease in milk yield and increases protein and fat percentage which are economically favourable for most selection indexes\textsuperscript{14}.

The aim of our study was to exhibit genotype diversity of ABCG2 gene. It was observed that AA homozygous and CA heterozygous genotype are available however CC homozygous genotype is not available in studied Turkish Holstein cows. If all genotype were at significant ratio in the genotyped population, we would search the effectiveness of ABCG2 gene polymorphism on the elimination of aflatoxin with milk. For this purpose, much more animal have to be genotyped to find CC homozygous allele.

REFERENCES

9. Tantia MS, Vijh RK, Mishra BP, Mishra B, Kumar S., Sodhi M: DGAT1 and ABCG2 polymorphism in Indian cattle (Bos indicus) and buffalo (Bubalus bubalis) breeds. BMC Vet Res, 2, 1-5, 2006.