Journal of Animal Research: v.6 n.3, p. 475-477. June 2016

DOI: 10.5958/2277-940X.2016.00049.8

Study of ABCG2 Gene polymorphism in Sahiwal and Hariana Cattle by *Pst*I/PCR-RFLP Assay

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Received: 01 March, 2016 **Accepted**: 16 March, 2016

ABSTRACT

ATP-binding cassette superfamily G member 2 transporter (ABCG2) is located in membrane of mammary glands alveolar epithelial cells in cows that actively extrudes xenotoxins and drugs into milk from blood. Polymorphism of the ABCG2 gene have been found to be associated with milk yield and composition in cattle. In the present investigation, Sahiwal and Hariana cattle were studied for polymorphism of ABCG2 gene by *Pst*I/PCR-RFLP assay. The RE digestion of the 292 bp PCR product showed the presence of AA genotype with a genotypic frequency of 1.0. AC and CC genotype were not observed in screened samples. The allelic frequency of ABCG2-A allele was calculated as 1.0 and that of ABCG2–C allele was zero. The present study revealed monomorphic nature of ABCG2 gene in the screened samples of Sahiwal and Hariana cattle breed.

Keywords: ABCG2 gene, PCR-RFLP, polymorphism, Sahiwal, Hariana

ATP binding cassette sub family G member 2 (ABCG2) gene, located in membrane of mammary glands alveolar epithelial cells in cows, encodes a transporter protein that facilitates transport of medicines through the cell membrane by binding ATP. The level of expression significantly increases during lactation compared to the dry period. Membrane transport proteins providing with efflux system protect tissues against drugs and xenobiotics. It plays a role in the secretion of xenobiotics and some micro-nutrients such as cholesterol and vitamin K3 into milk from blood (Jonker *et al.* 2005). ABCG2 gene is located on chromosome 6 in cattle and is known to have important effects on the milk yield traits. A single nucleotide polymorphism (SNP) resulted from translocation of adenine/cytosine (SNP A → C) on the 14th exon presents the missense mutation named Y581S as it leads to the replacement of the 581st amino acid tyrosine with cysteine. Mutation in ABCG2 gene is responsible for alterations on milk yield and proportion of milk protein & fat (Cohen-Zinder *et al.* 2005). ABCG2 polymorphism and its effects on milk yield and composition have been studied in the exotic (*Bos taurus*) Holstein cattle (Cohen-Zinder *et al.* 2005), and dairy cattle (Olsen *et al.* 2007) breeds. Considering limited study in Indian cattle (*Bos indicus*) breeds (Tantia *et al.* 2006), the present study was undertaken to investigate the status of *Pst*I polymorphism and allele frequency of ABCG2 gene in Indian Sahiwal and Hariana cattle breeds.

MATERIALS AND METHODS

Location

The study was undertaken at Instructional Livestock Farm Complex (ILFC) and Department of Animal Genetics and Breeding, DUVASU, Mathura (UP).

Animals

A total of 100 animals of Sahiwal (n=50) and Hariana (n=50) cattle were used for the present study. The animals were randomly selected and were reared under proper managemental conditions.

Sampling and Analytical Methods

Five ml of blood was collected form jugular vein of cattle in EDTA containing vacutainer tubes. The genomic DNA was isolated by whole blood DNA isolation kit. The quality of DNA was checked by using 0.7% agarose electrophoresis and the quantity of DNA was estimated by spectrophotometer.

The PCR was performed by using Forward Primer: 5′-AACAGCCTCAGCTCCAGAGAGATA T-3′ and Reverse Primer: 5′- CGGTGAAGATAAGGAGAACATACT -3′ (Cohen-Zinder *et al.* 2005). The PCR reaction was performed in 25µl reaction mixture that included 10µmol of each primer, 100mM dNTP, 1X polymer buffer (2.0mM $MgCl_2$), 0.5 units Taq polymerase and 50ng of cattle genomic DNA as template. The PCR conditions were denaturation at 95ºC for 5 minutes followed by 35 cycles each of 95ºC for 45 seconds, 57.6ºC for 40 seconds, 72ºC for 45 seconds and then a final step at 72ºC for 5 minutes. The products were analyzed by 1.0% agarose gel electrophoresis.

About 10 µl of amplified product was digested with 10 units of *Pst I* enzyme overnight at 37ºC in water bath. The amplified product was digested at 37ºC for 14 h. The digested products were detected by electrophoresis in 2% agarose gel in 1X TBE buffer and eithidium bromide (10mg/µl).

Statistical Analysis

The data was generated by eliminating the frequencies of different amplified products. The allelic frequency and genotypic frequencies of ABCG2 gene was estimated by standard procedure (Falconer and Mackay, 1996).

RESULTS AND DISCUSSION

We have amplified about 292 bp of genomic fragment in Sahiwal and Hariana breeds of Indian cattle. The *Pst*I/PCR-RFLP assay and sequencing of the 292 bp PCR product revealed the presence of AA genotype (uncut product; 292 bp) with a genotypic frequency of 1.00 (Fig. 1). AC and CC genotype were not observed in screened samples and had zero genotypic frequency. CC homozygous genotype was not found in any samples of animals in limited Sahiwal and Hariana population in our study. This result was in agreement with the reports of Cohen-Zinder *et al.* (2005) and Komisarek *et al.* (2009) in exotic cattle.

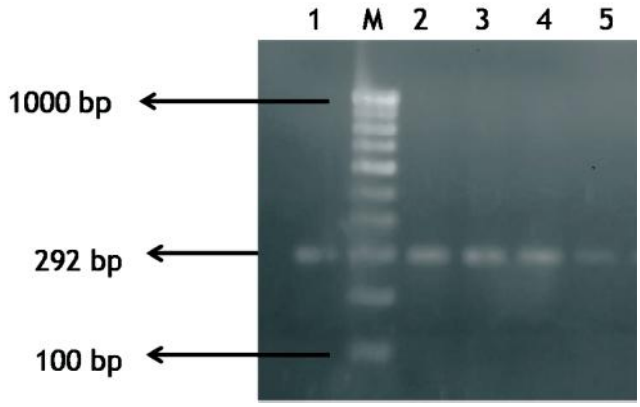

Fig. 1: ABCG2/*PstI* PCR-RFLP assay showing genotype pattern in 2.0% agarose gel; Lane 1: Undigested PCR product, 2: Marker (100bp ladder), 3& 4: AA genotype (292 bp) for Sahiwal cattle, 5: AA genotype (292 bp) for Hariana cattle

In the present study, the allelic frequency of ABCG2-A allele was calculated as 1.0 and that of ABCG2 –C allele was zero. This finding was similar to the reports of Hosseinpour-Mashhadi *et al.* (2012) in Holstein cow (0.98 and 0.02), Kowalehiska-luczak *et al.* (2007) in Jersey cow (0.80 and 0.20) and Cohen-Zinder *et al.* (2005) in Holstein bulls (0.99 and 0.01). Contrarily, Atila *et al.* (2014) found allelic frequency of A and C alleles as about 0.65 and 0.35, respectively in SAR and EAR breed of Turkey cattle. The present study revealed monomorphic nature of ABCG2 gene in the screened samples of Sahiwal and Hariana cattle breed.

The non-conservative Y581S mutation in ABCG2 was found to influence significantly the milk related traits in Israeli Holstein Friesians (Cohen-Zinder *et al.* 2005) and in Norwegian Reds (Olsen *et al.* 2007). Cohen-Zinder *et al.* (2005) reported increase in frequency of ABCG2-A allele with selection for higher milk fat and protein percentage in the Israeli Holstein population. These are several indications implying that ABCG2 because of its

physical role, expression data and chromosomal position may be a strong candidate gene controlling dairy cattle productivity.

In the present study, we observed absence of polymorphism in ABCG2 gene of Hariana and Sahiwal cattle, consequently we could not establish any association between genotype and milk production trait because these cattle were found homozygous for this SNP (A/C: Y581S). Further investigations in large population of these cattle may be useful for studying the status of this allele/SNP in order to exploit it for marker assisted selection for milk traits in cattle.

ACKNOWLEDGEMENTS

The Instructional Livestock Farm Complex (ILFC), DUVASU, Mathura (U.P.) is gratefully acknowledged for providing help during sample and data collection. The authors are thankful to Vice Chancellor, DUVASU, Mathura, (U.P) for providing necessary facilities during entire research work at this esteemed university.

REFERENCES

Atila, A., Gülhan, T.H., Iraz, A., Feraye, E.G., Hasret, Y. and Kemal, O. 2014. Genotype and allele frequencies of polymorphisms in ABCG2, PPARGC1A and OLR1 genes in indigenous cattle breeds in Turkey. *Acta Vet. Beograd.*, **64**(1): 73-80.

Cohen-Zinder, M., Seroussi, E., Larkin, D.M., Loor, J.J., Everts-van der Wind, A., Lee, J.H., Drackley, J.K., Band, M.R., Hernandez, A.G., Shani, M., Lewin, H.A., Weller, J.I. and Ron, M. 2005. Identification of a missense mutation in the bovine ABCG2 gene with a major effect on the QTL on chromosome 6 affecting milk yield and composition in Holstein cattle. *Genome Res.*, **15**(7): 936-44.

Falconer, D.S. and Mackay, T.F.C. 1996. Genotypic frequencies. Introduction to quatitative genetics (4th Edition), pp. 51-55.

Hosseinpour Mashhadi, M., Nassiri, M.R., Mahmoudi, M., Rastin, M., Kashan, N.E.J., Vaez Torshizi, R., Tabasi, N. and Nooraee, S.E. 2012. Polymorphism and Sequencing of DGAT1 Gene in Iranian Holstein Bulls. *Iran. J. Appl. Anim. Sci*., **2**(1): 63-67.

Jonker, J.G., Merino, G., Musters, S., van Herwaarden, A.E., Wagenaar, E., Mesman, E., Dale, T.C. and Schinkel, A.H. 2005. The breast cancer resistance protein *BCRP* (ABCG2) concentrates drugs and carcinogenic xenotoxins into the milk. *Nature Med.*, **11**: 127-129.

Komisarek J. and Dorynek Z. 2009. Effect of ABCG2, PPARGC1A, OLR1 and SCD1 gene polymorphism on estimated breeding values for functional and production traits in Polish Holstein Friesian bulls. *J. Appl. Genet.*, **50**(2): 125-132.

Kowalewska-Łuczak, I., Kulig, H. and Kmieć, M. 2009. Amplification created restriction sites for genotyping SNPs in the bovine ABCG2 and its association with milk production traits. *Arch. Tierz.*, **52**(6): 647-649.

Olsen, H.G., Nilsen, H., Hayes, B., Berg, P.R., Svendsen, M., Lien, S. and Meuwissen, T. 2007. Genetic support for a quantitative trait nucleotide in the *ABCG2* gene affecting milk composition of dairy cattle. *BMC Genet.*, **8**: 32.

Tantia, M.S., Vijh, R.K, Mishra, B.P., Mishra, B., Kumar, S.T.B. and Sodhi, M. 2006. DGAT1 and ABCG2 polymorphism in Indian cattle (*Bos indicus*) and buffalo (*Bubalus bubalis*) breeds. *BMC Vet. Res*., **2**: 32.