Original Research

Investigation of ABCG2/*Pst*I Polymorphism in Sahiwal Cattle by PCR-RFLP

Manvendra Singh[1]*, A. K. Gupta[2], I. D. Gupta[2], Arun Pratap Singh[3], Ashwani Arya[4] and M. A. Mir[2]

[1]Animal Science, Krishi Vigyan Kendra, Banda – 210001, Uttar Pradesh, INDIA
[2]DCB Division, ICAR-NDRI, Karnal - 132001 Haryana, INDIA
[3]Animal Science, Krishi Vigyan Kendra, Ajmer – 305206, Rajasthan, INDIA
[4]Animal Science, Krishi Vigyan Kendra, Mahoba – 210423, Uttar Pradesh, INDIA

***Corresponding author:** manav31vet@gmail.com

Rec. Date:	May 14, 2019 09:53
Accept Date:	Jun 28, 2019 14:10
DOI	10.5455/ijlr.20190514095350

Abstract

The present study was performed to investigate the polymorphism in the ATP-binding cassette superfamily G member 2 transporter (ABCG2) gene in Sahiwal cattle by PCR-RFLP assay. PCR-RFLP analysis of exon 14 was carried out using restriction enzyme PstI. PstI-RFLP for exon 14 revealed monomorphic pattern showing only one type of genotype, AA (292 bp) with genotypic frequencies of 1.0. AC and CC genotype were not observed in the studied Sahiwal population. The present study revealed that the selected Sahiwal population is monomorphic for ABCG2 gene as only single genotype (AA) was found in all the animals which were screened for studying polymorphism in ABCG2 gene.

Key words: ABCG2 Gene, *Pst*I, Polymorphism, PCR-RFLP, Sahiwal Cattle

How to cite: Singh, M., Gupta, A., Gupta, I., Singh, A., Arya, A., & Mir, M. (2019). Investigation of ABCG2/PstI Polymorphism in Sahiwal Cattle by PCR-RFLP. International Journal of Livestock Research, 9(8), 190-196. doi: 10.5455/ijlr.20190514095350

Introduction

India is a rich reservoir of genetic diversity in cattle with 43 recognized cattle breeds. The total number of cattle populations was 190.90 million, out of which indigenous cattle population comprised of 151.17 million which is 37.28% of the total livestock population (19th Livestock Census, 2012). India is the largest producer of milk in the world with 176.3 million tonnes of total milk production during 2017-18 (BAHS, 2018). The share of indigenous cattle in milk production is around 20% of the total milk produced in the country. Sahiwal is one of the best milch breed of cattle in India known for its higher milk production, disease resistance ability, remarkable power of endurance for hot climate of tropics, higher feed conversion efficiency and low cost of maintenance (Nivsarkar *et al.*, 2000). Selection of superior phenotypes and their selective breeding is a conventional tool which is used for the genetic improvement of livestock. Molecular

genetics tools can be used as an aid to conventional breeding programmes for accurate and effective selection of animals having high production potential.

The key objective of dairy cattle genomics is to identify genes underlying genetic variability of milk production and composition traits that could be implemented in breeding programs (Szyda and Komisarek, 2007). There are number of candidate genes like prolactin, leptin, diacylglycerol acyl-transferase (DGAT1), β-lactoglobulin, ATP-binding cassette subfamily G member 2 (ABCG2), signal transducer and activator of transcription (STAT5A), growth hormone releasing hormone (GHRH), Fibroblast Growth Factor 2 (FGF2) etc. which are associated with milk production traits. (Grisart *et al.,* 2002; Blott *et al.,* 2003; Liefers *et al.,* 2005; He *et al.,* 2006; Olsen *et al.,* 2007; Khatib *et al.,* 2008; Ganai *et al.,* 2009 ; Singh *et al.,* 2019). ABCG2 a member of the ATP binding cassette (ABC) super-family, is a "half-transporter", with only one ATP binding cassette in the N terminus and one C-terminal transmembrane domain (Ejendal and Hrycyna 2002; Gottesman *et al.,* 2002). ABCG2 gene expression is significantly enhanced during lactation and is accountable for the secretion of vitamin K3 or cholesterol into milk (Van Herwaarden *et al.,* 2007; Farke *et al.,* 2008). The ABCG2 gene in cattle is located in the narrow region of chromosome 6 (BTA 6) and spans over 117474 base pairs (bp) long consisting of fifteen introns and sixteen exons encoding 658 amino acids (aa), harbouring the QTL with a large impact on milk production traits (Ron *et al.,* 2006; Olsen *et al.,* 2005). Cohen –Zinder *et al.* (2005) identified a single nucleotide polymorphism (SNP) A to C resulting from translocation of adenine/cytosine on exon 14 of bovine ABCG2 which was a missense mutation named Y581S and it leads to alteration in milk yield and proportion of milk fat and protein. Polymorphism of ABCG2 gene have been studied in different exotic (*Bos taurus)* cattle breeds (Cohen-Zinder *et al.,* 2005; Ron *et al.,* 2006; Soltani-Ghombavani *et al.,* 2016; Fontanesi *et al.,* 2015) while limited work has been done in *Bos indicus* cattle (Tantia *et al.,* 2006; Sharma *et al.,* 2016). The present investigation was undertaken to examine the polymorphism and allele frequency of ABCG2 gene in Sahiwal cattle.

Materials and Methods

Location and Climatic Conditions of the Study Area

The present study was conducted at Livestock Research Centre and Dairy Cattle Breeding Division, ICAR-NDRI, Karnal situated at an altitude of 235 to 252 meters above the mean sea level at 29.68°N latitude and 76.98°E longitude in eastern zone of Haryana which comes under the Trans-Gangetic plain agro-climatic zone of India.

Experimental Animals

A total of 130 animals of Sahiwal cattle maintained at Livestock Research Centre (LRC) were used for the present study. The selection of animals was done randomly and all animals were maintained under proper and uniform managemental conditions.

 DOI 10.5455/ijlr.20190514095350

Isolation and Extraction of DNA from Blood

Blood samples were collected from randomly selected Sahiwal cattle after obtaining permission from Institute Animal Ethics Committee. Genomic DNA was isolated by Phenol-Chloroform method (Sambrook and Russel, 2001) with minor modifications. The quality and quantity of DNA was checked by agarose gel electrophoresis and UV spectrophotometer. The stock solutions were store at -20°C and used for further analysis. The working solution was prepared by diluting the stock to 100ng/µL for utilizing as DNA template in PCR.

Determination of ABCG2/PstI Polymorphism

For the amplification of 292 bp fragment of exon 14 in PCR the following forward and reverse primers were used; Forward Primer: 5'-AACAGCCTCAGCTCCAGAGAGATAT-3' and Reverse Primer: 5'-CGGTGACAGATAAGGAGAACATACT-3' (Cohen-Zinder *et al.*, 2005). PCR reaction was performed in a final volume of 25 µl containing 100 ng of template DNA, 10 pmol of each primer, 10X PCR buffer (20mM Tris-HCL pH 8.4, 50mM KCl), 1mM $MgCl_2$, 2.0 mM of dNTPs and 1 ul of Taq DNA polymerase (M/s Genetix Biotech Asia Pvt. Ltd). This solution was initially denatured at 95°C for 5 min, followed by 35 cycles of denaturation (95°C for 1 min), annealing (58°C for 1 min), elongation (72°C for 1 min) and a final extension at 72°C for 5 min. The amplified products were detected in 1.5% agarose gel electrophoresis. Aliquots of 5 µl of PCR products were applied to the gel. About 10 µl of amplified product was digested with 10 units of *Pst* I enzyme overnight at 37°C in water bath. The amplified product was digested at 37°C for 14 hours. The digested products were detected by electrophoresis in 2% agarose gel in 1X TBE buffer and ethidium bromide (10 mg/µl).

Statistical Analysis

The allelic frequencies of ABCG2 gene were estimated by simple allele counting according to Hardy-Weinberg equilibrium (Falconer and Mackay, 1996).

Results and Discussion

In the present study a 292 bp fragment of genomic DNA was amplified (Fig. 1). PCR-RFLP analysis of exon 14 was carried out using *Pst*I. *Pst*I-RFLP for exon 14 revealed monomorphic pattern (Fig. 2) showing only one type of genotype, AA (292 bp) with genotypic frequencie of 1.0. AC and CC genotype were not observed in the studied Sahiwal population. Similar findings were reported by Sharma *et al.* (2016) in Sahiwal and Hariana cattle and Tantia *et al.* (2006) in different *Bos indicus* breeds. The allelic frequencies for ABCG2-A and ABCG2-C allele were 1.0 and 0, respectively. This is in agreement with the results presented by Soltani-Ghombavani *et al.* (2016) in Iranian Holstein cows (0.97 and 0.03), Fontanesi *et al.* (2015) in Reggiana cattle breed of Italy (1.0 and 0), Hosseinpour-Mashhadi *et al.* (2012) in Holstein cows

 DOI 10.5455/ijlr.20190514095350

(0.98 and 0.02), Kowalehiska-luczak *et al.* (2007) in Jersey cows (0.80 and 0.20) and Cohen-Zinder *et al.* (2005) in Holstein bulls (0.99 and 0.01).

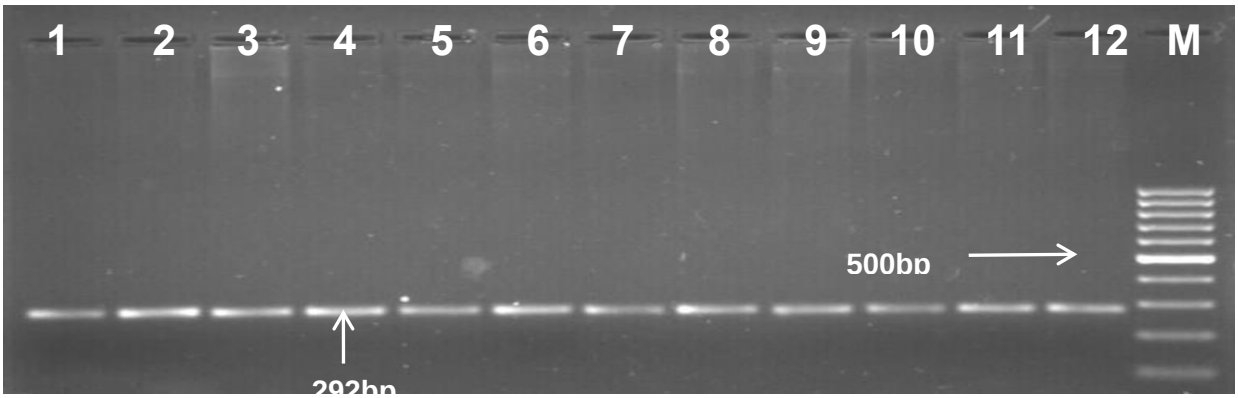

Fig. 1: Resolution of PCR products of ABCG2 gene in Sahiwal cattle
Lane 1-12: PCR product (292 bp)
Lane M: Marker (100 bp ladder)

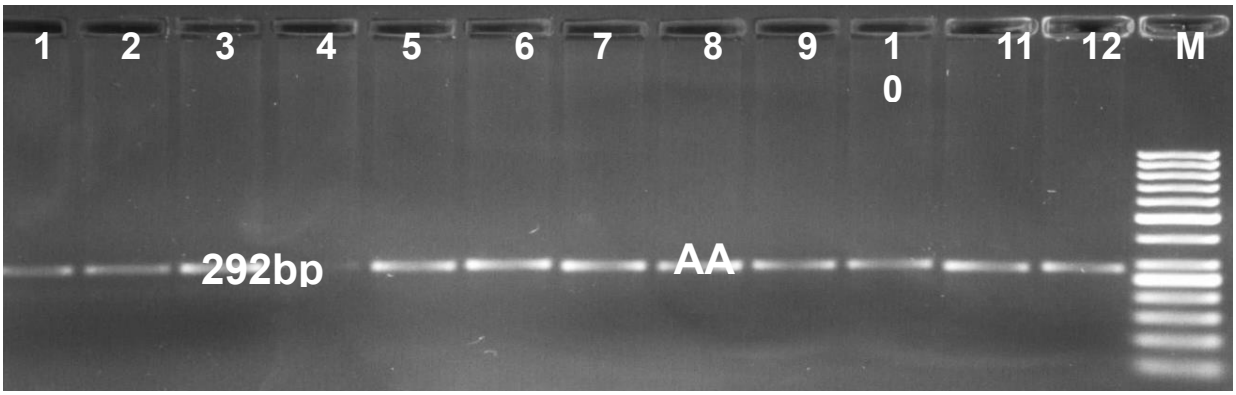

Fig. 2: PstI PCR-RFLP pattern of ABCG2 gene showing genotype pattern.
Lane 1-12: AA genotype (292 bp)
Lane M: Marker (50 bp ladder)

Atila *et al.* (2014) carried out a study to determine polymorphisms present in ABCG2 gene in South Anatolian Red (SAR) and East Anatolian Red (EAR) indigenous cattle breeds in Turkey. They amplified target region of ABCG2 (SNP. Y581S) and found that genotype AA (SAR: 0.50; EAR: 0.62) allele A (SAR: 0.63; EAR: 0.64) were high in both SAR and EAR cattle breeds. Ron *et al.* (2006) studied the polymorphism in ABCG2 gene in 32 *Bos taurus* and 3 *Bos indicus* breeds and observed that allele - A was most frequent in all the populations and is responsible for less milk yield and more fat and protein concentration. The ABCG2- C allele was present only in *Bos taurus* breed suggesting that allele - A is the ancestral allele. Milk composition traits are significantly influenced by the non-conservative Y518S mutation in ABCG2 gene as reported by Cohen-Zinder *et al.* (2005) in Israeli Holstein cattle and Olsen *et al.* (2007) in Norwegian Red

 DOI 10.5455/ijlr.20190514095350

cattle breed. There is increase in frequency of ABCG2-A allele if selection is done for higher milk fat and protein percentage as reported by Cohen-Zinder *et al.* (2005). Nahas *et al.* (2018) reported higher frequency of AA genotype which is associated with higher lactose percentage and low milk yield in less selected population of Baladi cattle breed of Egypt. These are several findings which indicate that ABCG2 is a strong candidate gene which significantly influence milk composition traits due to its physical role in mammary system and chromosomal positioning.

Conclusion

In the present study, the selected Sahiwal population was found to be monomorphic for ABCG2 gene as only single genotype (AA) was found in all the animals which were screened for studying polymorphism in ABCG2 gene and hence no association could be established between genotype and milk composition traits. The absence of ABCG2-C allele also indicates that ABCG2-A is the ancestral allele, and that the Y518S substitution occurred after the separation of *Bos indicus* and *Bos taurus* lineages. Further investigation on larger population of this breed is required in order to explore the polymorphism (A/C: Y518S) in ABCG2 gene and to exploit it for marker assisted selection for milk composition traits.

Acknowledgments

The authors are thankful to Director ICAR-National Dairy Research Institute, Karnal, Head Dairy Cattle Breeding Division and Head Livestock Research Complex for providing all the necessary facilities required for successful completion of research. Financial assistance in the form of institute fellowship is deeply acknowledged.

References

1. Atila, A., Gulhan, 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 Veterinaria-Beograd*, 64, 73-80.
2. BAHS. 2018. Basic Animal Husbandry Statistics. Department of Animal Husbandry, Dairying & Fisheries. Ministry of Agriculture, Govt. of India.
3. Blott, S., Kim, J. J. and Moisio, S. (2003). Molecular dissection of a quantitative trait locus: a phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. *Genetics*, 163, 253-266.
4. 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 Research*, 15, 936-44.
5. Ejendal, K. F. and Hrycyna, C. A. (2002). Multidrug resistance and cancer: The role of the human ABC transporter ABCG2: Review. *Current Protein & Peptide Science*, 3, 503-511.
6. Falconer, D. S. and Mackay, T. F. C. (1996). Genotypic frequencies. Introduction to quantitative genetics.4th edition, 51-55.

DOI 10.5455/ijlr.20190514095350

7. Farke, C., Meyer, H. H., Bruckmaier, R. M. and Albrecht, C. (2008). Differential Expression of ABC Transporters and Their Regulatory Genes during Lactation and Dry Period in Bovine Mammary Tissue. *Journal Dairy Research*, 75, 406-414.
8. Fontanesi, L., Scotti, E., Samore, A. B., Bagnato, A. and Russo, V. (2015). Association of 20 candidate gene markers with milk production and composition traits in Sires of Reggiana breed, a local dairy cattle population. *Livestock Science*, 176, 14-21.
9. Ganai, N. A., Bovenhuis, H. and Van Arendonk, J.A. (2009). Novel polymorphisms in the bovine beta-lactoglobulin gene and their effects on beta-lactoglobulin protein concentration in milk. *Animal Genetics*, 40, 127-133.
10. Gottesman, M. M., Fojo, T. and Bates, S. E. (2002). Multidrug resistance in cancer: Role of ATP-dependent transporters. *Nature Review Cancer*, 2, 48-58.
11. Grisart, B., Coppieters, W. and Farnir, F. (2002). Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Research*, 12, 222-231.
12. He, F., Sun, D. X. and Yu, Y. (2006). Association between SNPs within prolactin gene and milk performance traits in Holstein dairy cattle. *Asian-Australasian Journal of Animal Sciences*, 19, 1384-1389.
13. Hosseinpour, M. M., Nassiri, M. R., Mahmoudi, M., Rastin, M., Kashan, N. E. J., Vaez, T. R., Tabasi, N. and Nooraee, S. E. (2012). Polymorphism and sequencing of DGAT1 gene in Iranian Holstein Bulls. *Iranian Journal of Applied Animal Science*, 2, 63-67.
14. Khatib, H., Monson, R. L. and Schutzkus, V. (2008). Mutations in the STAT5A gene are associated with embryonic survival and milk composition in cattle. *Journal of Dairy Science*, 91, 784-793.
15. Kowalewska-Luczal, I., Kulig, H. and Kmiec, M. (2009). Amplification created restriction sites for genotyping SNPs in the bovine ABCG2 and its association with milk production traits. *Archieve for Tierzucht*, 52, 647-649.
16. Liefers, S. C., Veerkamp, R. F. and Te Pas, M. F. (2005). Genetics and physiology of leptin in periparturient dairy cows. *Domestic Animal Endocrin*ology, 29, 227-238.
17. Livestock Census. (2012). 19th All India Livestock Census. Department of Animal Husbandry, Dairying & Fisheries. Ministry of Agriculture, Govt. of India.
18. Nahas – El, A. F, Basiony, W. M., Kassas-El, S. and Mahmoud, S. (2018). Variation in the Genetic Effects of ABCG2, Growth Hormone and Growth Hormone Receptor Gene Polymorphisms on Milk Production Traits in Egyptian Native, Holstein and Hybrid Cattle Populations. *Pakistan Veterinary Journal*, 38, 371-376.
19. Nivsarkar, A.E., Vij, P.K. and Tantia, M.S. (2000). Animal Genetic Resources of India- Cattle and Buffalo. Indian Council of Agricultural Research, Directorate of information and publications of agriculture, New Delhi. 79-82.
20. Olsen, H. G., Lien, S., Gautier, M., Nilsen, H., Roseth, A., Berg, P. R., Sundsaasen, K. K., Svendsen, M. and Meuwissen, T. H. E. (2005). Mapping of a Milk Production Quantitative Trait Locus to a 420-kb Region on Bovine Chromosome 6. *Genetics*, 169, 275-83.
21. Olsen, H. G., Nilsen, H. and Hayes, B. (2007). Genetic support for a quantitative trait nucleotide in the ABCG2 gene affecting milk composition of dairy cattle. *BMC Genetics*, 8, 32.
22. Ron, M., Cohen-Zinder, M., Peter, C., Weller, J. I. and Erhardt, G. (2006). Short Communication: A Polymorphism in *ABCG2* in *Bos indicus* and *Bos taurus* Cattle Breeds. *Journal of Dairy Science*, 89, 4921-4923.
23. Sambrook, J. and Russell, W.D. (2001). *Molecular cloning* - A laboratory manual. 3rdedn. Vol.1 Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1, 1.32-1.34 and 8.14.
24. Sharma, A., Tiwari, M., Singh, S. P., Sharma, D., Kumar, S., Sharma, A. and Verma, A. K. (2016). Study of ABCG2 gene polymorphism in Sahiwal and Hariana cattle by *Pst*I / PCR-RFLP assay. *Journal of Animal Research*, 6, 475-477.

DOI 10.5455/ijlr.20190514095350

25. Singh, A. P., Chakravarty, A. K., Arya, A., Singh, M. and Mir, M. A. (2019). Effect of Polymorphism in FGF2 Gene on First Lactation Milk Yield and Milk Composition Traits in Karan-Fries Cattle. *International Journal of Livestock Research*, 9(3), 297-304.
26. Soltani Ghombavani, M., Ansari-Mahyari, S., Rostami, M., Ghanbari-Baghenoei, S. and Edriss, M. A. (2016). Effect of polymorphisms in the ABCG2, LEPR and SCD1 genes on milk production traits in Holstein cows. *South African Journal of Animal Science*, 46, 1-8.
27. Szyda, J. and Komisarek, J. (2007). Statistical modeling of candidate gene effects on milk production traits in dairy cattle. *Journal Dairy Science*, 90, 2971-2979.
28. Tantia, M. S., Vijh, R. K., Mishra, B. P., Mishra, B., Kumar, S. T. B. and Sodhi, M. (2006). DGATI and ABCG2 polymorphism in Indian cattle (*Bos indicus*) and buffalo (*Bubalus bubalis*) breeds. *BMC Veterinary Research*, 32, 1-5.
29. Van Herwaarden, A. E., Wagenaar, E., Merino, G., Jonker, J. W., Rosing, H., Beijnen, J. H. and Schinkel, A. H. (2007). Multidrug Transporter *ABCG2*/Breast Cancer Resistance Protein Secretes Riboflavin (Vitamin B2) into Milk. *Molecular Cell Biology*, 27, 1247-1253.

DOI 10.5455/ijlr.20190514095350