Novel SNP and Unique Sequences in ATP-binding Cassette Super Family-G Member-2 Transporter (ABCG2) Gene of Vechur cattle (Bos indicus)

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INTRODUCTION

According to National Bureau of Animal Genetic Resources, India possesses 160 indigenous breeds of livestock, including 40 cattle breeds. Each native breed has developed in its home tract with its own unique features. Vechur is the smallest cattle breed in the world and is only recognised cattle breed of Kerala, India. The Vechur bull and cow measure an average of 99 and 89 cm in height and 104 and 93 cm in length, respectively (Iype and Venkatachalapathy, 2001) and it appeared as a world record in the Guinness Book. The distinctive features of Vechur cattle include high milk fat percentage (4.7), smaller sized milk fat globule (3.21 microns), low level of feed requirements and high disease resistance and tropical climatic adaptability (Lali and Bindu, 2011).

More than 90% of the cattle population of Kerala is crossbred, developed by decades of crossbreeding to combine high milk yield and early maturity of European dairy breeds with hardiness, disease resistance, and adaptability of local cattle along with selection. Further improvement can be derived only by combining molecular data regarding desirable characteristics of indigenous and exotic breeds in selection process. Genes or chromosomal regions responsible for unique features should be found out to explore the unique features of the indigenous animals.

Bovine chromosome 6 (BTA-6) has highest number of reported quantitative trait loci (QTL) and it is widely studied for milk production traits in cattle (Khatkar et al., 2004). Olsen et al. (2005) mapped PPARGC1A, PKD2, SPP1, OPN and ABCG2 genes in the milk production quantitative trait loci to a 420 Kb region on BTA-6. ABCG2 is located within a linkage region with Quantitative Trait Locus (QTL) for milk production and milk content resulting from it represents a functional candidate gene for association with milk production traits in dairy cattle. Cohen-Zinder et al. (2005) identified 15 exons of ABCG2 gene which span 44.9 Kb in size. It encodes ABCG2 protein that transports various xenobiotics across the plasma membrane as well as cholesterol into milk (Leslie et al., 2005). Thus, the objective of this study was to find out the uniqueness of ABCG2 gene in Vechur cattle.

MATERIALS AND METHODS

DNA was isolated from venous blood collected from Vechur cattle maintained at Vechur conservation unit of Centre for Advanced Studies in Animal Genetics and Breeding, Mannuthy, Kerala Veterinary and Animal Sciences University using phenol chloroform extraction method (Sambrook and Russell, 2001). The concentration and purity of stock DNA samples were assessed by nanodrop spectrophotometry. Template DNA for PCR was prepared by diluting the DNA stock solution with TE buffer to a concentration of 50 ng/µl. The integrity of DNA was also checked on an 0.8% agarose gel. To check single nucleotide polymorphisms two micro litre of each DNA sample was mixed and a uniform template was prepared.

Partial sequence of ABCG2 gene was amplified (Komisarek and Dorynek, 2009) in thermal cycler (Biorad T100TM, USA) with a final concentration of 200 µM dNTPs. Magnesium chloride 1.5 mM, 5 pmol of forward and reverse primers and Taq DNA polymerase0.5 U in 15 µl reaction with initial denaturation at 94 ºC for 4 min, denaturation at 94 ºC for 45 s, primer annealing 61.7 ºC for 30 s, primer
extension 72 °C for 45 s and final extension 72 °C for 4 min. The amplified product was checked by 2% agarose gel in 1X Tris BorateEDTA (TBE) buffer and the product size was confirmed using 50 bp DNA ladder as DNA size marker.

The forward and reverse strands of PCR products were sequenced by Sanger’s Dideoxy sequencing protocol (SciGenom Labs, Cochin) and analyzed with the aid of Sequence Manipulation Suit (SMS) and Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information (NCBI) site (http://www.ncbi.nlm.nih.gov/BLAST).

RESULTS AND DISCUSSION

Good quality DNA samples were obtained from Vechur animals with a stock concentration of 1105.8 ng/µl. The ratios of optical density at 260 and 280 nm was 1.8. Amplicon was near to the expected size of 292 bp.

Trimmed forward and reverse sequences of PCR products in fasta format were aligned using EMBOSS. The actual amplicon size was found to be 303 bp in Vechur cattle instead of expected 292 bp (GenBank: KP866213.1). Further, the ABCG2 gene of Vechur cattle was aligned with available Bos taurus sequences in NCBI using BLAST (Fig. 1).

Chromatogram of thesequences confirmed the addition of nucleotides in intron 13 of ABCG2 gene of Vechur cattle (Fig. 2). A novel SNP (G4104A) was identified at 153rd position of the PCR product (intron 13) in ABCG2 gene of Vechur cattle (Fig. 2) and it is the first report of nucleotide A at 4104th nucleotide of the intron 13. Several SNPs have been identified in the ABCG2 gene having significant associations with milk production and composition traits (Olsen et al., 2005; Olsen et al., 2007; Mousavizadeh et al., 2013).

![Fig-1: Blast analysis of intron-13 and exon-14 of ABCG2 gene of Vechur cattle and Genbank reference sequence AJ871176.1](image)

![Fig-2: Chromatogram of intron-13 and exon-14 of ABCG2 gene of Vechur cattle](image)
The SNPs showing significant association with milk components would afford a main opportunity for Marker-Assisted Selection (MAS) programs in livestock (Khatib et al., 2007). More researches are suggested to reveal the frequency of wild and mutant alleles of the newly identified SNP at 4104th nucleotide of the 13th intron (A/G) of ABCG2 gene. Further research is needed to know whether the SNP or the unique sequences of Vechur cattle is associated with its unique characters.

REFERENCES