DGAT1 POLYMORPHISM K232A IN SAHIWAL (INDIAN ZEBU) AND FRIESWAL (HOLSTEIN FRIESIAN X SAHIWAL CROSSBRED) CATTLE

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ABSTRACT

The positional candidate gene DGAT1, within QTL of BTA14, harboring a lysine to alanine substitution (K232A) is significantly associated with milk production traits. The present investigation was undertaken to estimate the frequency of the DGAT1 K232A polymorphism in Indian zebu breed, Sahiwal (B. indicus; N=51) and crossbred, Frieswal (HF X Sahiwal; N=126) cattle using PCR-RFLP technique. Detection of allelic variation at nucleotide positions 10433–10434 of the DGAT1 gene was performed by restriction digestion of a 413 bp PCR product with CfrI. The frequency of A allele in Frieswal and Sahiwal cattle was 0.36 and 0.04, respectively. Although all the three genotypes were observed in Frieswal cattle, however, no animal of AA genotype was found in Sahiwal cattle and only four animals were of heterozygous genotype (AK). The sequences, specific to K and A allele, were submitted to GenBank (JF345253 and JF345254). Because of very high frequency to almost fixed nature of K allele in Sahiwal and other well defined Indian cattle breeds, DGAT1 K232A polymorphism may not be a suitable candidate for selection purpose. Nonetheless, the results could be used to guide association studies between this locus and milk production traits especially in Frieswal.

Key words: Quantitative trait locus, DGAT1, PCR-RFLP, Candidate gene.

INTRODUCTION

Major development of multiple QTL-mapping projects in recent years has led to the identification of a quantitative trait locus (QTL) near centromeric region of bovine chromosome14 (BTA14) having major influence on several milk production traits (Coppieters et al., 1998; Grisart et al., 2002). Furthermore, a comparative positional candidate gene approach identified a strong candidate in this region coding acylcoA: diacylglycerol – acyltransferase 1 (DGAT1) that plays a fundamental role in the metabolism of cellular diacylglycerol in physiological processes, such as intestinal fat absorption, lipoprotein assembly, adipose tissue formation and lactation, including metabolism of triacylglycerol in higher eukaryotes (Cases et al., 1998). Numerous mutations in the bovine DGAT1 gene have been reported. However, a nonconservative substitution of lysine by alanine (DGAT1 K232A) with AA → GC exchange identified at position 10433 and 10434 of exon VIII (Grisart et al., 2002) has been shown to be significantly associated with milk production and composition traits. Moreover, the K allele has always been reported to be linked with an increase in fat content, fat yield and protein content and a decrease in protein and milk yields. (Coppieters et al., 1998; Grisart et al., 2002; Winter et al., 2002).

DGAT1K appears to be the ancestral allele and the K232A substitution probably occurred early in the history of cattle domestication or even before domestication after the divergence of the Bos indicus and Bos taurus lineages. (Winter et al., 2002; Kaupe et al., 2004). The two alleles at the K232A polymorphism segregate in several breeds from different countries (Kaupe et al., 2004). Fixation of DGAT1A has been observed in some Bos taurus breeds, whereas the reverse i.e., fixation of DGAT1K has been noticed in some Bos indicus breeds (Kaupe et al., 2004; Tantia et al., 2006).

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Sahiwal is an important cattle breed of India famous for high milk production (2000-2500 kg per lactation) and adaptability. Frieswal, a crossbred population is being evolved by crossing Holstein Friesian and Sahiwal breeds at Military Farms of the country in order to evolve a national milch breed capable of producing 4000 kg milk in a mature lactation of 300 days with 4% butter fat. The population has undergone seven generations of interbreeding and reached to the level of 3550 kg in 4th lactation. Currently, more than 17,000 Frieswal females are available at all the Military farms.

The present study was aimed to estimate the frequency of 2 alternative alleles, K and A, of the DGAT1 K232A polymorphism in Indian zebu breed, Sahiwal (B. indicus; N= 51) and crossbred, Frieswal (HF X Sahiwal; N=126) cattle by PCR-RFLP and nucleotide sequencing.

**MATERIALS AND METHODS**

A total of 51 Sahiwal and 126 Frieswal animals were included in the study. Genomic DNA was isolated from blood samples using standard phenol chloroform extraction method. A fragment of 413 bp of the DGAT1 gene containing K232A substitution was amplified. PCR was carried out from a starting template of approximately 50 ng of genomic DNA in a final reaction volume of 25 μl containing 1X PCR buffer (Sigma), 1.5 mM MgCl₂ (Sigma), 5% DMSO, 200 μM dNTPs (Sigma), 0.5 μM of each primer and 1U Taq DNA polymerase (Sigma). Primer sequences were as follows: forward - 5’-GCACCATCCTCTTCAG-3’ and reverse-5’-GGAAGCGCTTTCGGATG-3’. The PCR profile included an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C (30 s), 59°C (30 s) and 72°C (45 s) and a final extension at 72°C for 10 min.

The PCR performance was verified by electrophoresis of 5 μl amplified product in 1.5% agarose gel stained with ethidium bromide for 60 min and photographed under UV transilluminator and Gel Documentation System (Alphaimager EP, USA). The restriction digestion was carried out following manufacturer instructions. Briefly, 5 μl amplicon was digested with 2.5 U of Cfi restriction enzyme (MBI Fermentas) in 15 μl final volume overnight at 37°C. Digested products were seperated in 2.5% agarose gels. Allele sizes were estimated by comparison to a DNA ladder (2-log DNA ladder, NEB). Further, to validate the genotyping made through PCR-RFLP representative amplicons were sequenced directly using automated DNA sequencer by Sanger’s dyeoxy chain termination method. Gene (allele) and genotype frequencies were calculated as per standard procedure.

**RESULTS AND DISCUSSION**

Agarose gel electrophoresis revealed a fragment of approximately 413 bp as expected from PCR amplification. The addition of DMSO to the PCR reactions allowed an equal amplification of both alleles. The size of the amplicon was further confirmed by nucleotide sequencing and the sequences (specific to K and A allele) were submitted to GenBank (JF345253 and JF345254). Restriction fragment length polymorphisms (RFLP) were identified by restriction digestion of the amplicon with Cfi. Cleavage by Cfi was diagnostic for the alanine-bearing allele. An undigested fragment of 413 bp indicated the K allele and two co-migrated fragments (210 and 203 bp) indicated the A allele (Fig.1).

A total of 48 KK, 66 AK and 12 AA animals were identified in Frieswal crossbred whereas, 47 KK, 4 AK and no animal of AA genotype were observed in Sahiwal. The frequencies of K allele were estimated as 0.64 and 0.96 in Frieswal and Sahiwal cattle, respectively (Table 1). The frequency of K allele (0.64) in Frieswal cattle found to be higher than reported (0.54) in German Holstein (Kaupe et al., 2007) and Polish Holstein-Friesian (Nowacka-Woszuk et al., 2008). The K232A polymorphism in the Holstein-Friesian breed has been reported in several studies and the frequency of the K allele ranged from 0.27 to 0.65 as summarized by Nowacka-Woszuk et al. (2008). A wide range of distribution of the K232A polymorphism in 38 Bos indicus and Bos taurus breeds has also been observed by Kaupe et al. (2004). They did not detect the K allele in Belgian Blue (beef), Gelbvieh, Hereford, Pinzgauer and Slavonian Syrmian breeds, whereas in Nellore and White Fulani breeds the frequency reached to 0.99 and 0.92, respectively. Recently, Tantia et al. (2006) reported fixed DGAT1K allele in six cattle (Bos indicus) and five buffalo (Bubalus bubalis) breeds of India. In the present investigation, similar trend of very high frequency
FIG. 1 Results of PCR-RFLP and sequencing confirmation of genotypes. (A) Animals with genotypes KK, AK and AA are shown in the electrophoretogram. M– length marker: 2-log DNA ladder (NEB), UC-Uncut. (B) Chromatograms of DGAT1 genotypes AA, AK, and KK. Presence of two peaks at SNP site (arrow head) indicates heterozygosity of the sample.

(0.96) of K allele towards fixation was observed in Sahiwal (Bos indicus) breed.

In particular, the allele K is associated with increased fat content of milk compared to allele A. Recently, Nowacka-Woszuk et al. (2008) reported higher BVs of sires for fat content and lower for milk and protein yields association with DGAT1 KK genotype. Szyda and Komisarek (2007) analysed an effect of nine SNPs in BTN1A1, LEP, LEPR and DGAT1 genes on milk production traits and observed that DGAT1 K232A polymorphism had a much larger additive effect on milk, fat and protein yields than the other polymorphisms considered. Moreover, it has been shown that the K allele is related to an increase of saturated and decrease of unsaturated fatty acids in milk which may impose a

<table>
<thead>
<tr>
<th>Nucleotide change</th>
<th>Amino acid change</th>
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<th>Amplicon length</th>
<th>Genotype</th>
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<tr>
<td>AA→GC</td>
<td>Lys-Ala</td>
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<table>
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<tr>
<th>Breeds</th>
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<th>Genotype frequency</th>
<th>Gene frequency</th>
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<tr>
<td></td>
<td>KK</td>
<td>AK</td>
<td>AA</td>
</tr>
<tr>
<td>Frieswal</td>
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<td>0.52</td>
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<td></td>
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<td>(n = 66)</td>
<td>(n = 12)</td>
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<tr>
<td>Sahiwal</td>
<td>51</td>
<td>0.92</td>
<td>0.08</td>
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<tr>
<td></td>
<td>(n = 47)</td>
<td>(n = 4)</td>
<td>(n = 0)</td>
</tr>
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TABLE 1. Gene and genotype frequencies of Cfr I PCR-RFLP.
negative effect on human health (Schennink et al., 2007).

Because of very high frequency to almost fixed nature of K allele in Sahiwal and other well defined Indian cattle breeds, DGAT1 K232A polymorphism may not be a suitable candidate for selection purpose. However, considering the increasing trends of crossbred cattle population in India, elimination of the K allele or selection against it may be suggested to increase milk and protein yield in crossbred like Frieswal once association of K232A polymorphism with production traits is established. Moreover, consideration of selection pressure will be very important to counterbalance the negative effect on fat yield.

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REFERENCES


