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Detection of New Silent Mutation at 348 bp Position in a CD18 Gene in Holstein Cattle Normal and Heterozygous for Bovine Leukocyte Adhesion Deficiency Syndrome

Research Article

R.K. Patel[1*], R. Kotikalapudi[2] and P.S.S. Sunkara[2]

[1] Sandor Animal Biogenics Pvt. Ltd, Banjara Hills, Hyderabad, India
[2] Sandor Life Science Pvt. Ltd, Banjara Hills, Hyderabad, India

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*Correspondence E-mail: rk_patel@sandor.co.in
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ABSTRACT

In India, Holstein and its crosses are being used extensively in breeding programmes and all these breeding bulls are screened for autosomal recessive genes. Blood samples are collected in ethylenediaminetetraacetic acid (EDTA) coated tubes and DNA was isolated by using phenol-chloroform method. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) were performed by using bovine leukocyte adhesion deficiency (BLAD) specific primers and *TaqI* restriction enzyme for diagnosis of bovine leukocyte adhesion deficiency (BLAD) syndrome. The bull was found to be heterozygous for BLAD allele. The PCR product was sequenced by automated sequencer using the ABI big dye Ver 3.1 for detection of mutation at position 383 bp (A/G). Sequence analysis comparison was performed using the codon code aligner 4.0.4 software. The sequence of carrier animal confirmed polymorphism at 383 bp position. The sequence was also compared with sequence of normal Holstein as a control and the sequence available with NCBI (accession No. NC-007299). The comparison of sequences revealed a heterozygous polymorphism at 348 position (T>C) in a carrier animal whereas in homozygous in a control Holstein which was normal for BLAD. The new polymorphism at 348 position was found to be silent as it does not change amino acid (asparagine, AAT>AAC) within exon 4 of CD18 gene. The partial sequence of new polymorphism / silent mutation has already been submitted to NCBI (accession No. KF840683). Further studies have to be carried out to elucidate the possible association of the CD18 silent point mutation at 348 bp position as a potential molecular marker for milk production traits.

KEY WORDS BLAD, CD18 gene, Holstein, PCR-RFLP, silent mutation.

INTRODUCTION

In India, the Holstein cattle are being used for animal improvement programmes especially for crossbreeding with indigenous cattle. Holstein cattle are carrier of autosomal recessive lethal genes and one of them is bovine leukocyte adhesion deficiency (BLAD). Therefore, it has become mandate to screen Holstein and Holstein crossbreds for genetic disorders before they are inducted in breeding programmes. Incidence of BLAD is high in Holstein cattle compared to other cattle breeds (Patel *et al.* 2007). BLAD causes neutrophil dysfunction which is important in host defence against invading pathogens (Malech and Gallin, 1987). A CD11 / CD18 is surface glycoprotein of neutrophils that is also called β_2 integrin molecule that helps the neutrophils to migrate to the site of inflammation (Hynes, 1987; Larson and Springer, 1990). The impaired surface protein on neutrophils cannot help in migration to the site of infection and inflammation. As a result, affected animals can be with various health problems; recurrent pneumonia,

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ulcerative and granulomatous stomatitis, enteritis with bacterial overgrowth, periodontitis, loss of teeth, delayed wound healing, persistent neutrophilia and death at an early age (Nagahata *et al.* 1987). BLAD is caused by single base pair mutation (adenine→guanine) at 383 bp position in a CD18 candidate gene that alters amino acid from aspartic acid to Glycine (D128G) in a glycoprotein which becomes impaired in function. The cDNA sequence of the bovine β_2 integrin gene comprises 2833 bp (Shuster *et al.* 1992) and is located on BTA 1. The other mutation replaces the cytosine to thymine at position 775 which is silent, as it does not change the deduced amino acid (Shuster *et al.* 1992). The silent mutation was initially localized at 775 bp cDNA position and subsequently at 880 bp cDNA position, due to a 105 bp deletion within exon 4 of the modified CD18 gene structure (gene bank Acc. No. M81233). The structure of gene encoding the CD18 subunit and single point mutation was well defined (Czarnik and Kaminski, 1997; Kriegesmann *et al.* 1997). A large number of cases especially heterozygous (carriers) reported worldwide where Holstein cattle are being used as pure and crossbreeding purpose (Shuster *et al.* 1992; Andrews *et al.* 1996; Nagahata *et al.* 1997; Padeeri *et al.* 1999; Huang *et al.* 2000; Ribeiro *et al.* 2000; Norouzy *et al.* 2005; Citek *et al.* 2006; Patel *et al.* 2007; Yathish *et al.* 2010; Li *et al.* 2011; Patel *et al*. 2011; Roy *et al*. 2012).

MATERIALS AND METHODS

Blood sample was collected into the EDTA coated tubes from a Holstein bull for routine screening for various autosomal recessive genetic disorders. Genomic DNA was extracted by phenol-chloroform method (Sambrook *et al.* 1989) with little modifications. The quality and quantity of DNA was determined by using agarose gel electrophoresis and UV spectrophotometry. The PCR mix contained 1X PCR buffer, 1.5 m*M* $MgCl_2$, 10 m*M* dNTPs, 5 pM each of forward and reverse primer, 5 unit *Taq* DNA polymerase, 50 ng genomic DNA and distilled water to make a final volume of 18 µL.

DNA fragment of 343 bp was amplified by using forward primer (5'-CCTGCATCATATCCACCAG-3') and reverse primer (5'GTTTCAGGGGAAGATGGAG-3') (Kriegesmann *et al.* 1997). The PCR reaction included the following steps: predenaturation for 3 minutes at 94 ˚C followed by 35 cycles of 30 seconds at 94 ˚C, 30 seconds at 60 ˚C, 30 seconds at 72 ˚C and final extension for 10 minutes at 72 ˚C for utilization of extra dNTPs in mixture. The amplified PCR product was digested by using *TaqI* at 65 ˚C for overnight. The digested product was visualized on 3% agarose gel which exhibited polymorphism in exon 4 of CD18 gene. The PCR product was sequenced along with DNA of normal Holstein as a control in order to confirm the polymorphism. ExoSAP-IT was used to treat PCR products prior to sequencing (Bell, 2008). After purification, PCR product was sequenced by applied bio systems 3130 XL Automated sequencer using the ABI big dye Ver 3.1. Sequence analysis comparison was performed using the codon code aligner 4.0.4 software.

RESULTS AND DISCUSSION

In present investigation, a polymorphism was detected at 383bp position (A/G) in a Holstein bull as shown in the Figure 1.

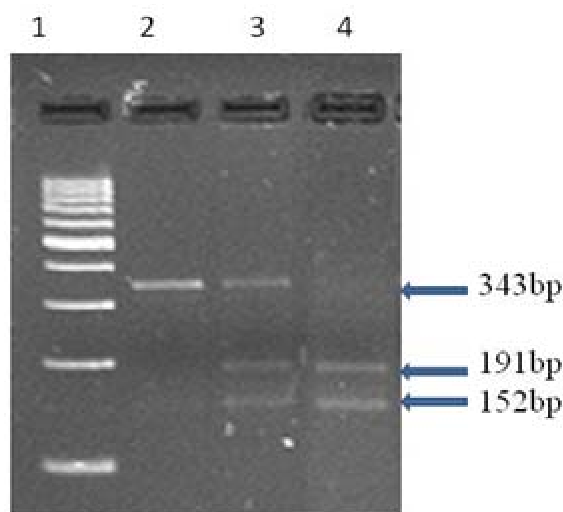

Figure 1 Electrophoretogram (3% agarose gel) of *TaqI* digested PCR product generated by amplification of genomic DNA using BLAD specific primers. Lane # 1: Gene Ruler 100bp DNA ladder (thermo scientific), lane # 2: PCR product of 343bp. Lane # 3: 343, 191 and 152 bp bands of carrier (heterozygous) Holstein and lane # 4: 191 and 152 bp bands for normal Holstein

The size of PCR product was 343 bp and it was subjected to RFLP analysis using *TaqI* restriction enzyme. In normal bulls, the PCR products yielded two fragments of 191 bp and 152 bp, whereas one Holstein bull exhibiting three fragments of 343 bp, 191 bp and 152 bp, indicating that bull is carrier (heterozygous) for BLAD.

The PCR product was sequenced to detect mutation in CD18 gene which confirms polymorphism at 383 bp position. The sequence also revealed a heterozygosity (polymorphism) at 348 position (T>C) in a carrier animal whereas in homozygosity in a controlled Holstein which was normal for BLAD (Figure 2). The new polymorphism at 348 position was found to be silent as it does not change amino acid (asparagine, AAT>AAC) within exon 4 of CD18 gene.

However, the silent mutation at 348 bp position was found to be a new as none of cases of polymorphism reported the same.

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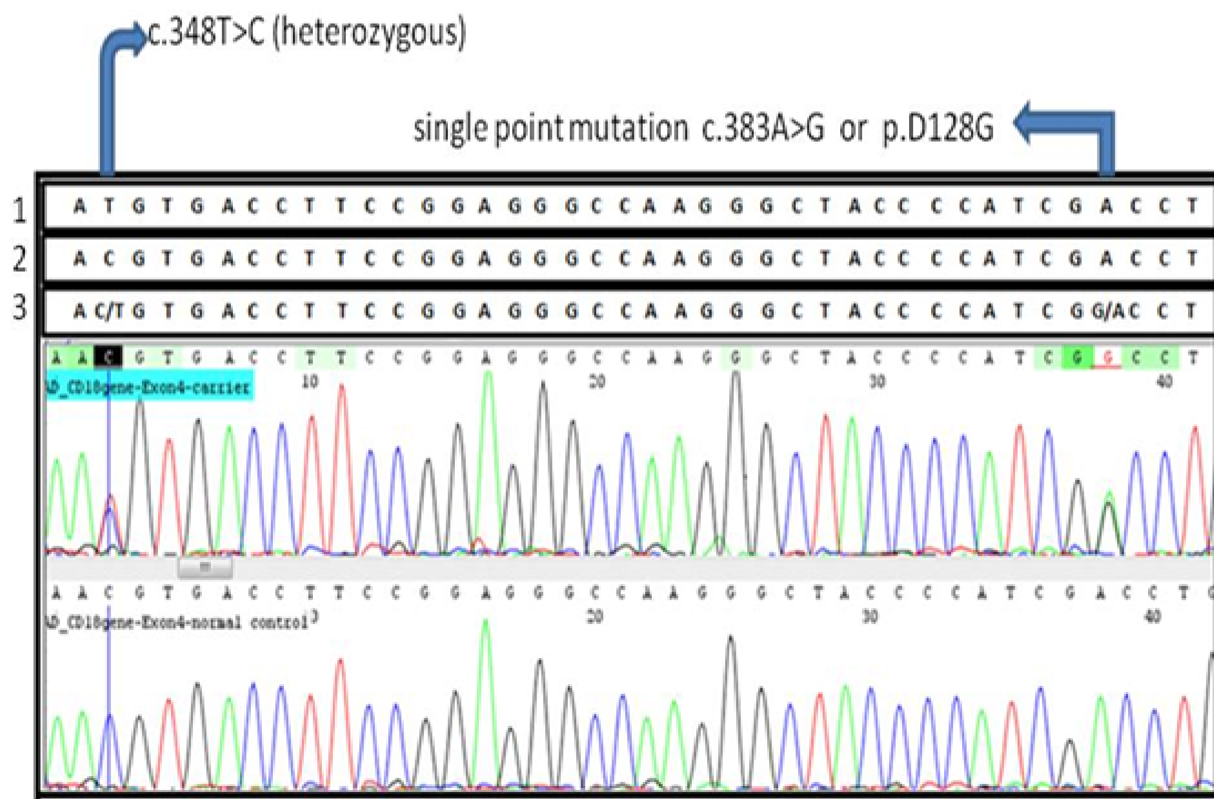

Figure 2 Line 1 indicates partial sequence of exon 4 of CD18 gene mentioned in NCBI (Accession No. NC_007299), lane 2 indicates exon 4 of normal control animal which shows silent homozygous mutation at 348bp position and Lane 3 indicates exon 4 of carrier Holstein

The partial sequence of new polymorphism / silent mutation has already been submitted to NCBI (accession No. KF840683). The earlier silent mutation was reported at 775 positions (Shuster *et al.* 1992). Polymorphism in exon 4 of CD18 gene was correlated with production traits or performance in cattle. The mutation D128 (carrier) was found to be associated with milk production traits (Czarnik, 2000). Similarly, the positive impact of BLAD allele was observed on the contents of fat and protein (Taralik, 1998). The effect of polymorphism on milk production in Hungarian Holstein was also noticed (Janosa *et al.* 1999). Theoretical considerations indicate that the silent mutation T > C at the 348 bp position of the exon 4 of CD18 gene in present study might be potential marker for higher milk yield or production trait as suggested by Shuster *et al.* (1992) for silent mutation at 775 bp position. The silent single nucleotide polymorphism (C→T) at 775 bp position was found more efficient quantitative trait locus (QTL) marker than polymorphism at 383 bp position (Czarnik *et al.* 2007).

CONCLUSION

However, the silent mutation (heterozygous or homozygous) at 348 position (T>C) detected in the present investigation in a BLAD carrier as well as in non-BLAD Holstein was not studied for its association with productive traits as our mandate is to diagnose genetic disorders. Hence, it is necessary to undertake further studies to elucidate the possible applications of the CD18 silent point mutation at 348 bp position as a potential molecular marker for milk production traits to infer valid conclusions.

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