Characterization and validation of point mutation in exon 19 of CACNA2D1 gene in Karan Fries (Bos taurus x Bos indicus) cattle


Dairy Cattle Breeding Division,
ICAR- National Dairy Research Institute, Karnal-132 001, India.
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ABSTRACT
The objective of this study was to characterize and validate the candidate point mutation in Calcium channel, voltage-dependent, alpha-2/delta subunit 1 (CACNA2D1) gene in Karan Fries (Bos taurus x Bos indicus) cattle. The CACNA2D1 gene reported as one of the potential candidate gene influencing Somatic cell Score and Mastitis. A PCR product of 249 bp amplifying the exon 19 and partial 18 and 19 intronic region of CACNA2D1 gene was digested with Hae III restriction enzyme to screen the reported point mutation. A monomorphic banding pattern with genotype AA was found in Karan Fries cattle. Sequencing was also carried out to characterize and explore insilico screened mutation in the nucleotide sequence of a particular region. The result indicates highly conserved sequence in Karan Fries cattle. The Phylogenetic tree revealed that Karan Fries cattle were closer to Bos taurus cattle, Bos mutus (Yak), and Bison bison (American buffalo) compared to other species.

Key words: CACNA2D1, Karan Fries, Monomorphic, Mutation.

INTRODUCTION
The calcium channel, voltage-dependent, alpha-2/delta subunit 1 (CACNA2D1) gene encodes a member of the alpha-2/delta subunit family, a protein in the voltage gated ion channels found in excitable cells i.e. muscle, glial cells, neurons, etc. with a permeability to the ion Ca^{2+} (Hille and Bertil, 2001; Purves, 2001). It plays an important role in muscle physiology i.e. in contraction of muscles. Thus helps in opening and closing of teat canal during milk let down (Vinci, 1999; Gabashvili et al., 2007). The cattle CACNA2D1 gene contains 39 exons and 38 introns and has been mapped to BTA 4q18 (Buitkamp et al., 2003). It is located within the genomic region of SCS QTL (Zang et al., 1998 and Rupp et al., 2003) and nearby the QTL of Somatic cell count (SCC) (Longeri et al., 2006 and Daetwyler et al., 2008). Therefore, the CACNA2D1 gene is considered to be one of the potential candidate gene influencing Somatic cell score (SCS) and mastitis. Earlier work indicated that the mutation of calcium channel genes would lead to a series of hereditary diseases in human beings and animals (Robinson et al., 2000). Very few works has been carried out in Livestock species. Recently, A526745G point mutation of bovine CACNA2D1 gene was detected and significantly associated with Somatic cell score (SCS), carcass weight, dressing percentage, meat percentage and backfat thickness in Holstein, Sanhe and Simmental cattle (Hou et al., 2010 and Yaun et al., 2011). Keeping all these points in view, the present study was carried with the main objective to characterize and validate reported SNPs of CACNA2D1 gene in Karan Fries cattle and to analyze associations with disease resistance traits (Mastitis).

MATERIALS AND METHODS
The cattle resource population of this study consisted of randomly selected lactating Karan Fries (HF × Tharparker) cows maintained at cattle yard of National Dairy Research Institute, Karnal, India. A total of 100 animals were taken under this study to screen for the presence of polymorphism in genomic region of CACNA2D1. Animals which were not affected up to 3rd lactation/parity will be taken as control. Blood samples were collected and genomic DNA was isolated using standard phenol chloroform method.

Insilico SNP Detection and PCR amplification: The bovine CACNA2D1 gene dbSNP database was also investigated in this study (http://www.ncbi.nlm.nih.gov/SNP/). The resulting sequences were assembled by BioEdit software to screen candidate SNPs. DNA sequencing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) were used to verify the candidate SNPs. Based on reference sequence ENSBTA000000223209 of the bovine CACNA2D1 gene, specific PCR primers were designed using Premier 5.0 software to amplify the specific genomic sequence and validate point mutations. PCR amplification was carried out in a total volume of 25 μl with 100 ng DNA template, Dream
Tag Green PCR Master Mix (2x). Polymerase chain reaction (PCR) was carried out in thermal cycler (T-100 BIO-RAD) with following conditions – initial denaturation at 92°C for 5 min., followed by 35 cycles of 94°C for 30 s, annealing temperature 54 °C for 30 s, 72°C for 30 s and a final extension at 72°C for 5 min. The PCR products were separated on 1.5% agarose gel including 0.5 µg/ml of ethidium bromide, photographed under Ultra Violet light.

**Sequencing and PCR-RFLP:** The PCR product of 20 animals (10 samples were selected randomly from affected and not affected animal group, respectively) were sent to the 1st Base Molecular biological services (Malaysia) for purification and sequencing in both directions. PCR-RFLP was also performed to genotype animal for reported A526745G candidate SNP (Yaun et al., 2011b), amplified PCR products (10 µl) of all animals were digested with 2 U HaeIII restriction enzyme (New England Biologicals) at 37°C for 10 h and were subsequently resolved in 2.5% agarose gel stained with ethidium bromide.

**Phylogenetic and Sequence Analysis:** A phylogenetic tree analysis was performed to understand the biological diversity and seeking insights into events which transpired during evolution. To understand the evolutionary history of Exon 19 coding sequence from CACNA2D1 gene of Karan Fries cattle, we have constructed a phylogenetic tree based on Exon 19 coding sequence data from Karan Fries cattle and related organisms using MEGA 5.2 software (Tamura et al., 2011). Multiple Sequence Alignment (MSA) was performed using MUSCLE program embedded in MEGA with default parameter settings. For phylogenetic tree construction, Maximum Likelihood (ML) method was employed using substitution model which uses different rates of mutation among sites. To assess the accuracy of the tree generated, we performed a bootstrap search with 500 number of replications and constructing a consensus phylogenetic tree.

**RESULTS AND DISCUSSION**

In present study, ten SNPs i.e C38826926G, C38826959T, T38826989C, G38826983C, T38826985A, T38826986G, A38826991G, T38826992A, A38826997C, A38827011C were screened in Exon 19 of the *Bos taurus* CACNA2D1 gene in various databases by using bioinformatics tools. The PCR amplification generated a 249 bp segment covering exon 19 and partial intronic regions of CACNA2D1 gene (Fig.1). The PCR- RFLP demonstrated the existence of only one allele A, showing single band consisting of 249 bp, was assigned as the AA genotype corresponding to Aspartic homozygote for Karan Fries cattle. Sequencing result confirmed the monomorphism of the genotype of CACNA2D1 gene. The clustal W analysis revealed that the nucleotide sequence as well as the derived amino acid sequence of CACNA2D1 gene of Karan Fries cattle was in concord with *Bos taurus* sequence (Gene id ENSBTA00000020569). Thus, the animals under study were found to be monomorphic, which was reported first time in Karan Fries cattle. Whereas, in same region (Yaun et al., 2011ab) detected a candidate SNP significantly associated with Somatic cell score (SCS), carcass weight, dressing percentage, meat percentage and backfat thickness in Holstein, Sanhe and Simmental cattle.

**Sequence Analysis and Phylogenetic Tree**

Karan Fries cattle amplified CACNA2D1 gene sequence was 100% identical to *Bos taurus, Bos mutus* and Bison. *Ovis aries* and *Capra hircus* sequences was 99% identical and 98% to *Bubalus bubalis, 96% identical to Sus scrofa* and more divergent to camel and *Homo sapiens* with 37 and 35% similarity in sequence respectively (Table 1). The phylogenetic tree revealed that Karan Fries cattle was closer to bovine species compared to other species and are emerging from the same node. Thus bovines were clustered together. Next in the evolution closer to bovines was the phylogenetic clade of other ruminants involving caprines, and genus bubalus and camelus. Similar pattern was observed by (Pal et al., 2009) when a phylogenetic tree was constructed for predicting the evolutionary relationship between different species based on the nucleotide sequence of CD14 gene.
Table 1: Identity of CACNA2D1 gene of Karan Fries cattle with other species.

<table>
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*Pair Distances of Karan fries cattle, Similarity in upper triangle; Divergence in lower triangle in proportion.

CONCLUSION

This study insight an evidence that Karan Fries cattle have no variability in amplified CACNA2D1 Locus. All animals were found to be monomorphic with respect to insilico and reported SNPs. So, in Karan Fries cattle particular amplified region is highly conserved. This monomorphism may be a breed specific characteristic. Since present study has formulated the results based on a relatively small sample, further studies are required to study these SNPs in large samples to establish the role of these SNPs in CACNA2D1 gene in conferring resistance against mastitis. Therefore, reported as well as, insilico SNPs was not consider as a universal marker for particular trait in all breeds. Thus, need to be warrantee explore before implementation in selection criteria.

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REFERENCES


