Characterization and validation of point mutation in Exon 19 of Calcium channel, voltage-dependent, Alpha-2/Delta subunit 1 (CACNA2D1) gene and its relationship with mastitis traits in Sahiwal

Ankit Magotra*1, I.D.Gupta, Archana Verma, Rani Alex2, Ashwani Arya, Vineeth M.R and Vijay Kumar3

Dairy Cattle Breeding Division, ICAR- National Dairy Research Institute, Karnal-132 001, Haryana, India.

Received: 03-02-2016 Accepted: 17-06-2016 DOI: 10.18805/ijar.10990

ABSTRACT
Calcium channel, voltage-dependent, alpha-2/delta subunit 1 (CACNA2D1) gene plays an important role in excitation-contraction coupling in muscle-cells, glial cells and neurons. The CACNA2D1 gene may be one of the candidate genes related with some phenotypic traits due to its location in QTLs associated with Somatic cell score (SCS) and mastitis. A total of 120 Sahiwal cattle were selected to characterize exon 19 of CACNA2D1 gene to identify polymorphism and its association with mastitis susceptibility/resistance. A 249 bp PCR fragment of CACNA2D1 gene encompassing the exon 19 and partial intronic region was amplified and digested with Hae III to screen the SNP which was significantly associated with SCS. Genotype analysis using PCR-RFLP revealed a monomorphic banding pattern. Sequencing was also carried out to explore other SNPs which are deposited in dbSNP in the nucleotide sequence of a particular region. This study provides preliminary information that the targeted region of CACNA2D1 gene in indigenous cattle has no significant association with mastitis resistance which may be a breed specific characteristic.

Key words: CACNA2D1, Exon 19, Mastitis, PCR-RFLP, Polymorphism, Sahiwal.

INTRODUCTION
India possesses world’s largest bovine population with 210.2 million cattle’s (Anonymous, 2012), which constitutes approximately 14.7% of the world cattle population. The major concern of the country is the low productivity of indigenous animals with large economic consequences due to production related diseases in high yielding cattle. Among the several bottlenecks in achieving the milk production targets, mastitis continues to remain as the most exigent impediment. Mastitis is an inflammation of the mammary gland attributable to a wide spectrum of etiological agents such as bacteria, viruses, mycoplasma, yeasts and algae (Henna et al., 2013). Mastitis is a global quandary which affects both developing and industrialized nations. Total losses from mastitis amounting to $1.294 billion in 1976 increased to $2 billion in 2012 in United States (Anonymous, 2012). Understanding the genetic traits underlying milk production and mastitis resistance is one of the major goals for genetic improvement in cattle. Mastitis tolerance is a threshold trait which impedes the conventional breeding strategies. To incorporate disease tolerance traits, it is essential to gain insights into the genetic traits underlying such diseases. The calcium channel, voltage-dependent, alpha-2/delta subunit 1 (CACNA2D1) gene encodes for a member of the alpha-2/delta subunit family which constitutes a part of the voltage-dependent calcium channel complex. CACNA2D1 is of 164.28 kb mapped on chromosome 4 (BTA 4) and consists of 33 exons and 33 introns (Ensembl Id: ENSBTA00000020569). CACNA2D1 gene is considered as a potential candidate gene influencing SCS and mastitis owing to the fact that it is present within the genomic region of SCS QTL (Zhang et al., 1998). The present study was undertaken with an aim to explore experimentally verified candidate SNPs in genomic region of CACNA2D1 gene in indigenous cattle (Sahiwal) and further predict association of different genotypes with mastitis susceptibility/resistance.

MATERIALS AND METHODS
Animals and DNA preparation: The analysis was performed on randomly selected 120 Sahiwal cattle maintained at the cattle yard of National Dairy Research Institute, Karnal, India. Animals which were not affected up to third lactation were taken as control i.e 60 animals in control group and 60 animals in affected group. Genomic

*Corresponding author’s e-mail: ankitoms@gmail.com. 1Department of Animal Genetics and Breeding, LUVAS, Hisar-125001, Haryana, India. 2ICAR- Central Institute For Research On Cattle, Meerut-250001, Uttar Pradesh, India. 3Department of AGB, DUVASU, Mathura-281001, Uttar Pradesh.
DNA was extracted from blood by standard protocol (Sambrook and Russell 2001). Quality and quantity of the isolated genomic DNA was evaluated using UV spectrophotometer (Biophotometer Plus, Eppendorf).

**Candidate SNPs screening:** The dbSNP (build 144) database (http://www.ncbi.nlm.nih.gov/SNP/), a public repository for genetic variation within and across different species, was screened for reported polymorphisms in exon 19 of the CACNA2D1 gene. The bovine CACNA2D1 gene sequence was retrieved from Ensemble Genome Brower (URL: http://asia.ensembl.org/Bos_taurus/Location/View?db=core;g=ENSBTAG00000020569;r=4:38826870-38827067;tv=ENSBTAT0000027409). The resulting sequence was assembled in DNAstar software to screen for candidate SNPs.

**Primer design and PCR amplification:** Reported set of primers (Yaun et al., 2011) were used to amplify partial intron 18, exon 19 and intron 19 region. PCR amplification was carried out in a total volume of 25 ml with 100 ng DNA template, 1x PCR buffer, 1.5 mM MgCl₂, 200 mM of each dNTPs, 20 pmol of each primer and 1 unit of Taq DNA polymerase. PCR was carried out in Thermal cycler (T-100 Bio Radd) with following layout: initial denaturation at 92°C for 5 min., followed by 35 cycles of 94°C for 30 s., annealing at 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 UV light.

**Sequencing and PCR-RFLP:** According to the Yaun et al., (2011) genotyping of the SNP polymorphism at position 526745 of the CACNA2D1 gene was carried out using PCR restriction fragment length polymorphism (PCR–RFLP). 120 amplified PCR products were digested with 2 U HaeIII restriction enzyme (New England Biologicals) at 37°C for 10 h. The digested product was separated through 2.5% agarose gel and the gel was stained with ethidium bromide. Further in order to characterize and validate *insilico* screened SNPs in the targeted region, the PCR amplified products of 20 samples (10 individuals from both affected and non-affected groups selected randomly) were sent to the 1st Base Molecular Biology Services (Malaysia) for purification and sequencing in both directions.

**RESULTS AND DISCUSSION**

*insilico screened SNPs:* In this study, ten SNPs from dbSNP were identified in exon 19 of the bovine CACNA2D1 gene. Table 1 lists the SNPs which were screened from dbSNP along with their mutation type and their effect on translated protein. The PCR amplification generated a 249 bp segment encasing exon 19 and partial intronic regions of CACNA2D1 gene (Figure 1). The PCR products of animals from both groups were digested with *HaeIII* enzyme. The results of the RFLP analysis for endonuclease demonstrated the existence of one allele ‘A’, showing single band consisting of 249 bp which was assigned the AA genotype.

![Fig 1](image.png)

‘Lane 1-2: 249 bp PCR Product
M: Marker

**Table 1:** SNPs within exon 19 of CACNA2D1 gene retrieved from dbSNP database

<table>
<thead>
<tr>
<th>SNP</th>
<th>Type of Mutation</th>
<th>Change occur</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.38826926 C&gt;G</td>
<td>Stop gained</td>
<td>A Sequence variant whereby at least one base of the codon is changed, resulting in a premature stop codon which leads to a shortened transcript</td>
</tr>
<tr>
<td>c. 38826959 C&gt;T</td>
<td>Synonymous mutation</td>
<td>A sequence variant where there is no resulting change in the encoding amino acid</td>
</tr>
<tr>
<td>c. 38826989 T&gt;C</td>
<td>Synonymous mutation</td>
<td>A sequence variant where there are changes of one or more bases, resulting in a different amino acid sequence but the length is preserved</td>
</tr>
<tr>
<td>c. 38826983 G&gt;C</td>
<td>Missense mutation</td>
<td></td>
</tr>
<tr>
<td>c. 38826985 T&gt;A</td>
<td>Missense mutation</td>
<td></td>
</tr>
<tr>
<td>c. 38826986 T&gt;G</td>
<td>Missense mutation</td>
<td></td>
</tr>
<tr>
<td>c. 38826991 A&gt;G</td>
<td>Missense mutation</td>
<td></td>
</tr>
<tr>
<td>c. 38826992 T&gt;A</td>
<td>Missense mutation</td>
<td></td>
</tr>
<tr>
<td>c. 38826997 A&gt;C</td>
<td>Missense mutation</td>
<td></td>
</tr>
<tr>
<td>c. 38827011 C&gt;G</td>
<td>Missense mutation</td>
<td></td>
</tr>
</tbody>
</table>

*Position is relative to reference sequence ENSBTAE00000223209*
corresponding to Aspartic homozygote for Sahiwal cattle. Monomorphism of the genotype of CACNA2D1 gene was confirmed by nucleotide sequencing results as shown in Figure 2, Clustal W analysis revealed that the amplified CACNA2D1 nucleotide sequence from Bos indicus (deposited with Gene Bank Accession number KT 152938) as well as the sequence corresponding to amplified region of CACNA2D1 gene from Bos taurus (Gene Id ENSBTA00000020569) are in consonance.

Development of successful vaccines against mastitis remains an intractable problem due to involvement of wide variety of etiological agents, lack of information on the genetic determinants of disease resistance and complications associated with damage to mammary epithelial cell by both the agents and the host factors (Henna et al., 2013). There are complications associated with therapeutic interventions involving resistance to antibiotics, efficacy and cost-effectiveness issues. There has been growing amount of interest in selection for health traits in the dairy industry (Hou et al., 2010). The candidate gene approach may offer us with a more direct and comprehensive understanding of the genetic basis underlying the differences in the quantitative expression between different breeds. This study provides preliminary information that the CACNA2D1 gene in indigenous cattle has no significant association with mastitis resistance which maybe a breed specific characteristic. We characterized exon 19 of CACNA2D1 gene in indigenous cattle (Sahiwal) to explore A526745G polymorphism as well as other SNPs screened from dbSNP in Bos taurus. The amplified PCR product of 249 bp was digested with HaeIII enzyme to genotype the reported SNP A526745G (Yuan, 2011). In Sahiwal animals under study, only “AA” genotype was revealed. Monomorphism of the genotype of CACNA2D1 gene was confirmed by nucleotide sequencing which is contrary to earlier findings. These studies through PCR–RFLP and DNA sequencing methods reported that A526745G allelic variant was corresponding to replacement of aspartic acid (Asp) to glycine (Gly) in bovine CACNA2D1 gene translation. The A526745G mutation was significantly associated with Somatic cell score (SCS), carcass weight, dressing percentage, meat percentage and backfat thickness in various cattle breeds such as Holstein, Sanhe and Simmental (Hou et al., 2010, Yuan et al., 2011). This is the first study which reports monomorphism of CACNA2D1 in indigenous cattle contradicting with previous studies. This particular region is highly conserved in indigenous cattle; whereas a candidate SNP with significant association to Somatic cell score (SCS) was detected in the same region (Yuan et al., 2011). This monomorphism of the indigenous cattle (Sahiwal) may be a breed specific characteristic. So it is strongly suggested to explore the variation in different breeds before implementation in selection criteria. 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.

ACKNOWLEDGEMENT

Facilities provided by Director, NDRI (ICAR), Karnal, India to conduct this research is duly acknowledged.

---

**Figure 2:** Sequence comparison of exon 19 of CACNA2D1 gene in *Bos indicus* (Sahiwal) against *Bos taurus* for primer design. Stars denote identities between two sequences. Both sequences reveal 100% identity.
REFERENCES