Analysis on the effect of genotype encoding a bovine non cytokine candidate gene for the occurrence of mastitis among Frieswal (HFX Sahiwal) cattle breed of Indian origin

Rajib Deb, Umesh Singh, Sushil Kumar, Sandeep Mann, Rani Singh, Gyanendra Sengar, Arunkumar and Arjava Sharma
Indian Council of Agricultural Research, India

Mastitis is one of the most important disease occurring among cross-bred cows, which is one of the important cause of declining milk production. The candidate gene approach may provide a more direct understanding of the genetic basis for the expression of quantitative differences between individuals and revealing genomic regions and specific markers that are associated with mastitis traits. Although various reports are available that cytokine genes like TLR4, TLR2 etc. are strongly associated with mastitis occurring among cattle but there is scanty of information related to non cytokine genes and their association with bovine mastitis. In the present study, A calcium channel, voltage-dependent, alpha-2/delta subunit 1 (CANA2D1) gene was considered to be an important non cytokine candidate gene influencing mastitis, because the cattle CACNA2D1 gene has been mapped to BTA 4q 18 and located within the genomic region of QTL for SCS and near by SCC. Primers were designed from the flanking regions of the SNP (G519663A) of bovine CACNA2D1 gene. The amplified products were digested with HpaII restriction enzyme. PCR RFLP products were analyzed in 2.5% agarose gel. Further SSCP was also conducted for analysis of each genotype. We have also sequenced the PCR products of each genotype and the accession numbers were obtained (JX524782 for GG genotype and JX524783 for AA genotype). Genotype frequency of AA (0.51) was significantly higher than AG (0.34) and GG (0.15). The effects of CACNA2D1 polymorphism on somatic cell score (SCS) were analyzed. Our result shows that AG (3.76 ±0.56) and AA (3.21±0.47) genotypes were associated with higher SCC compared to GG (2.64±0.33). We have also screened some clinical mastitis case and in all the cases were associated with AA and AG genotypes, no GG genotypes were identified. On the basis of SCC association with genotypes come to the conclusion that animals with GG genotypes are resistant to mastitis whereas AA and AG are susceptible to the disease. Further on the basis of genotypes we have classified the animals and the milk samples were collected and mRNA expression was analyzed by Real Time PCR in each group. Our results revealed that GG genotypes were significantly showing higher expression than AG and AA genotypes. Further we have isolated blood sample from all the groups and (Peripheral blood mononuclear cells) PBMCs cells were cultured from each blood sample as per the standard protocol. They were treated with Calcium channel blocker and the expression level of the CACNA2D1 gene was evaluated by Real Time PCR. Results show that expression level decline in each genotypic group after treatment and expression level of GG are again significantly higher than AA and AG.

Biography
Rajib Deb has completed his post graduation and PhD from Indian Veterinary Research Institute, India. Presently he is a Scientist at Project Directorate on Cattle, Indian Council of Agricultural Research, India. He has published few research papers, review papers in reputed international and national journals. He is also published few books and monographs. Presently he is handling two research projects funded by ICAR. He is also serving as an editorial board member of Journal of Veterinary Science & Technology under OMICS Publishing Group.

drrajibdeb@gmail.com