

## GENETIC BASIS OF MASTITIS RESISTANCE IN CATTLE

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**Abstract:** Mastitis is one of the most important diseases affecting production in dairy cattle. Control strategies against mastitis such as antibiotic therapy, vaccination, improvement of farm and feeding management have met with little success. Genetic variability of mastitis resistance is well established by several independent studies in different breeds of dairy cattle. Usage of somatic cell count or clinical mastitis as an indicator for selection, to produce genetically mastitis resistant animal have led to limited achievement. This is due to polygenic control of mastitis where many genes are involved. Polygenic nature of this trait projected the need for exploring candidate genes responsible for mastitis resistance. The wisdom about genetic basis of mastitis resistant would aid us in producing future herd with genetically mastitis resistant animals.

**Keywords:** Genetic resistance, Candidate gene, Mastitis, Cattle.

### Introduction

Mastitis is defined as an inflammatory reaction of udder tissue to bacterial, chemical, thermal or mechanical injury. One of the reason for low milk productivity is poor animal health due to diseases particularly mastitis. It is a global problem as it adversely affects animal health, quality of milk and responsible for heavy economic losses due to reduced milk yield (70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%) (Patnaik *et al.*, 2013). In India, an economic loss due to mastitis is Rs.7165.51 crore per annum (Newsletter, NDRI, 2012). Clinical mastitis induces hormonal alterations like decreased pulsatile secretion of luteinizing hormone (LH), significant decrease in the ovulatory LH peak, decreased estradiol production and failure of ovulation, which reveals scientific basis for positive correlation between mastitis and infertility in cattle (Kadarmideen *et al.*, 2000). Many studies have proved genetic variation exist in mastitis resistance and susceptibility among dairy cattle, which is needed to be explored in order to gain knowledge to combat against mastitis.

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### **Candidate gene for mastitis resistance**

Resistance to mastitis is a complex function involving various pathways contributed by numerous candidate genes. Candidate gene approach focuses more on identifying genes that are connected to mastitis through activities such as recognition of pathogens, leukocyte recruitment, migration, pathogen elimination and resolution. For mastitis resistance, it is difficult to select candidate genes that control the trait because of polygenic controls. Complex traits are also determined by many genes with little effect, versus a limited number of genes with large effect, once again limiting the ability of genome-wide association studies to identify these genes (Hayes *et al.*, 2010). For past few years researches were focused on candidate gene for innate immunity in relation with mastitis. *TLR 4 (toll-like receptor 4)* is one such candidate gene responsible for initial recognition of invading organisms. Bovine *TLR4* is highly polymorphic, with 36 single nucleotide polymorphisms (SNP) discovered across 14 breeds of cattle (Deb *et al.*, 2013). The bovine *TLR* family contains members 1–10 and enables recognition of bacterial, viral and danger signals by individual cells (Ingham and Menzies, 2006). Also *NLR (nod-like receptor)*, which represents an intracellular family of viral, bacterial and danger sensors, has been proven to be connected to mastitis resistance (Pant *et al.*, 2007). *Lactoferrin* has received attention as a candidate gene for mastitis resistance due to connection of this protein with innate immunity. *Lactoferrin* could be a promising candidate gene for mastitis resistance because of polymorphisms occurring in the regulatory region of the gene seems to affect expression of that gene (Pawlik *et al.*, 2009). Lysozyme is a ubiquitous bacteriolytic enzyme present in external secretions and in polymorphonuclear leukocytes and macrophages. Studies revealed SNP in encoding region of *Lysozyme* gene correlated significantly with Somatic cell score (SCS) and 305 daymilk yield (Chen *et al.*, 2013). The chemokine IL-8 is released during infection by neutrophils and other cells in response to invading pathogens. Subsequent binding of IL-8 to CXCR1 or CXCR2 induces migration, modifies cytokine production, increases phagocytosis and reactive oxygen species generation (Lahouassa *et al.*, 2008). Several candidate pathways were also found in the study on integrated genomic data from genome-wide association mapping in cattle and transcriptomic data from microarray studies on mastitis pathogens. Of great interest are IL-17 and IL-8 signaling pathways (Lewandowska-Sabat *et al.*, 2012). Study on SNPs in the chemokine genes *CCL2* and *IL8* and the chemokine receptor genes *IL8RA* and *CCR2* were assessed association with for somatic cell score (SCS). Two unreported SNPs were found in the *CCL2* gene and one SNP was found in the *CCR2* gene. Three in the *IL8* gene and five in

the IL8RA chemokine receptor were reported to have significant association with mastitis (Leyva-Baca *et al.* 2007). SNP which is located in the 5' upstream region of the IL8RA gene has significantly associated with neutrophil migration in response to mastitis (Leyva-Baca *et al.*, 2008). One potential marker is CXCR2, a chemokine receptor required for neutrophil migration to infection sites, which contains SNP within the gene. This approach of genetically identifying mastitis resistant cows may represent an effective means of marker-assisted selection for mastitis (Youngerman *et al.*, 2004). Compton *et al.*, (2008) suggested that gene polymorphisms of Beta defensin (Bdef) and Molecule possessing ankyrin-repeats induced by lipopolysaccharides (MAIL) as potential markers of mastitis resistance in dairy heifers. Sodeland *et al.*, (2011) found that on basis of genome-wide association study SNPs highly associated with clinical mastitis (CM) lie near both the gene encoding interleukin 8 on BTA6 and the genes encoding the two interleukin 8 receptors on BTA2. Among genes associated with reduced mastitis incidence particular attention is being paid to BoLA-DRB3 gene because of role of this gene plays in immune system (Ramirez, *et al.*, 2014). The *BoLA-DRB3* gene belongs to major histocompatibility complex (MHC) genes. The exon 2 of *BoLA-DRB3* locus is especially highly polymorphic. This region encodes the antigen adhesion site of MHC molecules and plays an essential role in regulation of the immune response against pathogens. Several studies have demonstrated that *BoLA-DRB3.2\*24* allele tended to be associated with mastitis susceptibility and *BoLA-DRB3.2\*3* tended to be associated with mastitis resistance (Rupp and Boichard, 2003). Analysis of *secreted phosphoprotein 1 (SPP1)* revealed four SNPs associated with SCS EBV in the third lactation. One SNP located in the promoter region of the SPP1 gene was significantly associated with somatic cell score across all three lactations in daughters of Canadian Holstein sires (Alain *et al.*, 2009). Chemotactic neutrophil migration from blood could be impaired due to decreased proportion of cells expressing the adhesion receptor CD62L (L-selectin) which is necessary for penetration through endothelium to sites of infection (Diez-Fraile *et al.*, 2004).  $\beta$ -defensin antimicrobial peptides a major component of oxygen-independent microbicidal system of polymorphonuclear (PMN) cells. It shown to have significant difference in somatic cell count between different genotypes of  *$\beta$ -defensin* suggesting use as genetic marker in breeding programmes aiming at selecting highly productive dairy cattle with increased resistance to udder infections (Das *et al.*, 2008). Bactericidal/permeability-increasing protein (BPI) is an inflammatory response molecule expressed primarily by neutrophils. It contributes to host innate immunity by directly killing and enhancing phagocytosis of Gram-negative bacteria and

neutralizing bacterial endotoxins. Thus, variation in the bovine BPI gene and associations somatic cell score (SCS), a measure highly correlated (0.80–0.90) to clinical mastitis in Holstein cattle (Connor *et al.*, 2008). Cathelicidins are peptide components of innate immune system of mammals. Apart from exerting a direct antibiotic activity, they can also trigger specific defence responses in the host. Their roles in various pathophysiological conditions have been studied in relation to mastitis. Indicated multiple roles for bovine cathelicidins in mastitis, with antimicrobial activities against causative pathogens and capacity to activate host cells (Tomasinsig *et al.*, 2010). Three SNPs of mannan-binding lectin (MBL) linked to greater risk of *S. aureus* infections were associated with SCS in Chinese Holstein and Luxi Yellow cattle (Wang *et al.*, 2011). Alpha-2-macroglobulin (A2M) has the function of binding to foreign peptides and particles, thereby serves as a defence barrier against pathogens in plasma and tissues of animals. Four novel SNPs in the promoter region were completely linked. Thus, the mutant type can be used as a potential functional marker for a mastitis resistance breeding program in dairy cows (Wang *et al.*, 2013). Also, in Chinese Holstein dairy cows it was found that polymorphism of *ATP1A1*, which encodes bovine  $Na^+$ ,  $K^+$  *ATPase*  $\alpha 1$  subunit is associated with mastitis (Liu *et al.*, 2012). *Calcium channel, voltage-dependent, alpha-2/delta subunit 1 (CACNA2D1)* is considered to be an important non-cytokine candidate gene influencing mastitis. *CACNA2D1* gene has been mapped to BTA18 and located within the genomic region of QTL for SCS and nearby SCC (Deb *et al.*, 2014). The single SNP in *bovine breast cancer 1 (BRCA1)* and their genetic effects on SCS were evaluated and a significant association with SCS was found in c.46126 G>T. Results from association analysis provided preliminary evidence that bovine *BRCA1* could be used as a candidate gene or molecular marker for improvement of bovine mastitis resistance traits in Chinese commercial cattle (Yuan *et al.*, 2012). Yang *et al.*, (2012) reported that polymorphism of bovine *complement component 4 (C4A)* was also related to mastitis resistance. A three-base insertion in a glycine-coding stretch of the gene for *forebrain embryonic zinc finger-like (FEZL)*, a transcription factor with a role in neuronal development also plays role in mastitis. Mastitis induced *FEZL* promotes expression of the axon-attracting molecule semaphorin 5A (*SEMA5A*) also induces *SEMA5A* expression in susceptible cattle but at a lower level than in resistant cattle. Enhanced *SEMA5A* induces expression of at least nine genes related to the host's immune response, including *TNF- $\alpha$*  and *IL-8*. Results shows animal susceptibility to mastitis results from an impaired immune response due to the lower transcription activity of susceptible *FEZL* (Sugimoto *et al.*, 2013).

## Conclusion

Understanding the genetic basis of mastitis resistant in cattle, there exist scopes for producing mastitis resistant animals for better economical production including animal welfare concern. Identification of various SNP associated with mastitis is very promising in devising a suitable control strategy in combating mastitis. One of the limiting factors would be that, though SNP found to be associated with mastitis in a particular population would not guarantee that such an association exists in another population. Hence it is necessary to ascertain the association of SNPs with mastitis in various populations. This enforce to study the mastitis resistant trait existing in indigenous breeds and to utilise those valuable genetic resources available. SNP studies in relation to mastitis would enhance the niche area of mastitis research and thereby help in devising a suitable control strategy against this economically important disease. There exist a negative correlation between milk production and mastitis resistant. When breeding strategies focus only on milk production, it may lead to a population of cattle highly susceptible to mastitis which is not of our desire. To avoid such conditions, mastitis resistant trait should be included in breeding strategies to produce genetically mastitis resistant animals using the wisdom gained on genetic basis of mastitis resistant

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