International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2023; SP-8(2): 06-09

www.veterinarypaper.com
Received: 09-12-2022
Accepted: 12-01-2023

Komal
Ph.D. Scholar, Department of Animal Genetics and Breeding, LUVAS, Hisar, Haryana, India

Amandeep
Ph.D. Scholar, Department of Livestock Production Management, LUVAS, Hisar, Haryana, India

Ritu
Ph.D. Scholar, Department of Animal Nutrition, LUVAS, Hisar, Haryana, India

Corresponding Author:
Amandeep
Ph.D. Scholar, Department of Livestock Production Management, LUVAS, Hisar, Haryana, India

A review on candidate gene approach for bovine mastitis

Komal, Amandeep and Ritu

Abstract
Mastitis, being a multi-etiological disease, therefore it cannot be cured, however it can be controlled to a minor degree. Highly producing bovines suffer from mastitis widely but buffalo is comparatively less susceptible to mastitis because of its longer teats as well as thicker teat canal epithelium than cattle. This can be accomplished by breeding techniques, a decrease in pathogen exposure, and an increase in intramammary infection resistance. A generally advocated strategy, however, is marker assisted selection (MAS) using a candidate gene approach, which is focused on enhancing the host genetics. These genes are related to immune system including Toll-like receptor (TLR) genes which somehow are responsible for mastitis resistance, glycoprotein receptors, lactoferrin gene, neuropeptide receptors, etc., which are responsible for mastitis susceptibility and resistance. This review illustrates briefly a number of candidate gene identified for mastitis in bovines and also the methods to identify such candidate genes.

Keywords: Mastitis, teat, intramammary, candidate gene, breeding

Introduction
Mastitis is a complicated problem for lactating animals, as indicated by the fact that an estimated 30% of cases are ascribed to farming and improper management practices, 20% to the milking machine, and 20% to the genetics of the cow itself (Mein *et al.*, 2004) [1]. Dairy animals frequently contract the infectious illness clinical mastitis. According to Tiezzi *et al.* (2015) [2], it is typically described as an inflammation of the mammary gland that develops as a result of the admission and subsequent multiplication of pathogenic bacteria. This ultimately has a significant impact on the financial situation of farmers. According to Lush (1950) [3], "all quarters that are abnormal or that are producing abnormal milk" were considered to have clinical mastitis. This applies to any quarter with milk that is cloudy, clotted, sedimentary, or watery as well as any quarter with hardness, pain, or another abnormal condition of a same nature. Various microorganisms, including bacteria, fungus, yeasts, and algae, can cause mastitis. The agents that cause inflammation have an impact on how severe it is (Lavon *et al.*, 2011) [14]. For instance, *Staphylococcus aureus* induces subclinical infections while *E. coli* infections cause acute reactions. Pathogen-specific characteristics (the genes governing immune response), which have been linked to the occurrence of clinical mastitis, can be one of the clear signs of mastitis infection in cows.
Despite the fact that buffalo appear to be less susceptible to mastitis than cattle, maternal mastitis causes a significant mortality rate in the first three months of life in the calves, which further reduces buffalo output (Akhtar and Ali, 1994) [5]. During the first three months following calving, buffalo exhibit a 79% quarter-wise prevalence of intra-mammary infection. According to Joshi and Gokhale (2006) [6], mastitis is common in cows at 10–50% and in buffaloes at 5-20%. Due to decreased milk production, early culling, and treatment costs, mastitis has a severe influence on the water buffalo's economy. The teats of buffalo are longer and thicker than those of dairy cattle. Mastitis is less common in buffaloes than in dairy cattle because of thicker teat canal epithelium. In order to lower the frequency of mastitis in dairy animals, breeding techniques should be developed in conjunction with milk production. This is because clinical mastitis is heritable and has a negative link with milk yield. Mastitis is a low heritability trait, and Gomez-Raya *et al.* (1998) [7] found that low heritability traits have a higher power of detecting a particular effect's QTL. In addition, the SNPs discovered in the coding areas can be proven to be associated with the trait of interest.

Candidate genes associated with mastitis

Mastitis cannot be cured, however it can be controlled to a minor degree. This can be accomplished by breeding techniques, a decrease in pathogen exposure, and an increase in intramammary infection resistance. The mastitis is managed and reduced using a variety of therapeutic, preventative, and management strategies. A generally advocated strategy, however, is marker assisted selection using a candidate gene approach, which is focused on enhancing the host genetics (Rasheed *et al.*, 2020) [8]. A number of candidate gene have been identified for mastitis in bovines (Table 1). BoLA-DRB3, IL8RA, TLR4, and LTF are highly associated with clinical mastitis and can be considered as important candidate genes for clinical mastitis studies. BoLA-DRB3 (Bos taurus major histocompatibility complex, class II, DRB3) is present on chromosome no. 23 of cattle which is responsible for somatic cell count and altered milk production traits. Also, BoLA-DRB3 and LTF genes show an association with both mastitis and production traits. Lactoferrin possesses strong iron binding properties, and plays an important role in host defense against microbial infection and anti-inflammatory activity, and therefore, strong functional candidate for mastitis resistance or susceptibility.

MUC1 is a glycoprotein mucin that is expressed in the apical cells of luminal tissues, including the luminal cells of the mammary gland. Its primary job is to defend the cell surface from outside microbes. The MUC1 gene showed a strong correlation with characteristics linked to mastitis resistance. It is polymorphic in the Murrah breed and might be a good marker for MAS for intra-mammary infection (da Rosa *et al.*, 2020) [9]. Another gene, osteopontin, was described by Alain *et al.* (2009) [10] as being expressed in a variety of immune cells and playing a function in cell attachment and wound healing by mediating cell activation and cytokine production. In order to create effective control measures against coliform mastitis, bulls with extreme estimated breeding values can be chosen for osteopontin (SPP1) polymorphism. In Chinese Holstein cows, Wang *et al.* (2015) [11] discovered 48 SNPs, mostly on the BTA 14, that were strongly related with SCS EBVs for mastitis resistance characteristics. With mixed model based single marker regression analysis (MMRA), their study discovered two genes (TRAPPC9 and ARHGAP39) as novel candidate genes of mastitis susceptibility in Holsteins.

In a GWAS utilising a high density SNP array, Kurz *et al.* (2019) [12] found 27 QTLs for mastitis resistance based on 117 single nucleotide polymorphisms (SNPs) in Holstein dairy cattle. One of the detected QTLs for the RAS guanyl-releasing protein 1 gene (RASGRP1), a potential gene for mastitis resistance, was one of the four QTLs that were connected to teat length. In their genome-wide association research, Miles *et al.* (2021) [13] identified 28 genomic regions that were significantly related (Bonferroni-corrected $p<0.05$). When these genomic regions were probed, they revealed five biologically plausible genes: ZNF683, DHX9, CUX1, TNNT1, and SPRY1. These genes' activities ranged from controlling cell proliferation to signalling the immune system, and they may be causing the development of mastitis. A novel locus and candidate genes with potential pleiotropic effects connected to mastitis were discovered by genetic analysis of the risk composite trait. In the Dutch HF population, Lee *et al.* (2021) [14] provided confirmation of the presence of a clinical mastitis resistance QTL that had previously been discovered on BTA6 in several cattle populations (Freebern *et al.*, 2020; Cai *et al.*, 2018; Abdel-Shafy *et al.*, 2014) [15, 16, 17] 4142 progeny tested bulls were used in GWAS to map the QTL. Due to the presence of the lead SNP in a non-coding region, the GWAS results of their study suggested that transcriptional regulation might be the underlying mechanism of the CM resistance QTL. The vitamin D binding protein (DBP)-encoding gene GC was shown to be the gene with the greatest functional relevance behind the CM resistance QTL.

Table 1: Candidate genes identified for mastitis in bovines

S. No.	Candidate gene for mastitis	References
1.	BoLA-DRB3	(Nascimento *et al.*, 2006) [18]
2.	CXCR2	(Youngerman *et al.*, 2006) [19]
3.	Bovine lactoferrin gene (LTF)	(Wojdak *et al.*, 2006) [20]
4.	Caspase recruitment domain-containing protein 15 and TLR4 gene	(Wang *et al.*, 2007) [21]
5.	CXCR1	(Levya Baca *et al.*, 2007) [22]
6.	Osteopontin	(Alain *et al.*, 2009) [10]
7.	BRCA 1	(Deb *et al.*, 2014; Magotra *et al.*, 2015) [23, 24]
8.	CACNA2D1	(Magotra *et al.*, 2019) [25]
9.	Vit D-binding protein precursor, GC	(Sahana *et al.*, 2014) [26]
10.	TRAPPC9, ARHGAP39 (Mastitis susceptibility gene)	(Wang *et al.*, 2015) [11]
11.	LYK6, LYKD, LYNX1, LYPD2, SLURP1, PSCA (lymphocyte antigen-6 complex genes)	(Tiezzi *et al.*, 2015) [2]
12.	S100A8 (Calgranulin A) – Disease resistance	(Sulabh *et al.*, 2016) [27]
13.	Neuropeptide FF receptor 2, NPFFR2	(Zhang *et al.*, 2016) [28]
14.	COL4A1 gene (inflammatory associated fibroblasts in bovine mammary gland)	(Hu G *et al.*, 2017) [29]
15.	TLR-2, TLR-6 polymorphism	(Elmaghraby *et al.*, 2018) [30]
16.	PTK2B, SYK and TNFRSF21	(Yang *et al.*, 2018) [31]
17.	Bovine MBL 1 (Mannose-binding Lectin)	(Dhundwal *et al.*, 2019) [32]
18.	MUC1 gene (Mucin 1 glycoprotein)	(da Rosa *et al.*, 2020) [9]
19.	Mammary Serum amyloid A3.2 (M-SAA3.2), immunoglobulin J (joining) chain gene (JCHAIN), Nucleotide binding oligomerization domain containing 2 (NOD2)	(Moretti *et al.*, 2021) [33]
20.	NCBP1, FOXN3, HERC1	(Jaglan *et al.*, 2023) [34]

Genetic markers associated with mastitis incidence

The majority of association and quantitative trait locus (QTL) studies in large farm animals are conducted in outbred populations, which makes it challenging and less reliable to identify robust QTL and candidate genes because of the variability of genetic background and population-specific interactions between loci. In contrast to the scenario in model and laboratory animal species, where highly inbred lines and targeted gene knockouts are available, this situation is fundamentally different. Genetic markers are DNA sequences that have been linked to specific chromosomal regions and are indicative of particular traits. They exhibit polymorphism, which is the difference in markers between several members of the same species. The presence of polymorphisms is

directly correlated with the availability of genetic markers. According to Silveri *et al.* (2006) [35], the expression of micro RNAs (miRNAs) in the cow mammary gland may also have a significant impact on the regulatory pathways that control milk production, mastitis resistance, and susceptibility. The potential to use heterologous animal models for comparison research has been made possible by recent advances in molecular biology. Targeted gene disruption in mice revealed a number of abnormalities associated with the mammary gland. The publication of the cow genome sequence has made it possible to find additional markers and build synteny maps that incorporate information from other species. It is typically expensive and very time-consuming to screen the genome for QTL. Sharma *et al.* (2006) [36] looked for genome-wide QTL linked AFLP markers for mastitis resistance in Canadian Holsteins. Using AFLP and selective DNA pooling, cows were tested.

Identification methods for genes linked to mastitis

Microarray analysis allows researchers to simultaneously examine changes in the expression profiles of thousands of genes in response to a disease infection. Despite the fact that microarray analysis has grown to be a crucial tool in animal genomics, the main issue still remains the lack of a clear consensus on the microarray data processing techniques for the identification of differentially expressed genes. To date, 12 publications have described 107 genes with expression patterns linked to mastitis cases in cattle using microarrays, real-time PCR (Schwerin *et al.* 2003; Goldammer *et al.* 2004) [37, 38], and ELISA (Lee *et al.* 2006) [39]. *Staphylococcus. aureus*, *Streptococcus. uberis*, *Streptococcus. agalactiae*, coliforms, *Cornybacterium* spp., and yeast were used in the experiments on cattle and mice. In more than one (two to four) expression experiment, the differential expression of 11 genes (IL6, IL8, CD14, TLR4, IL1B, LBP, TLR2, C5AR1, TNF, IFNG and SAA3) during mastitis was validated. In addition, six genes (IL6, CD14, TLR4, IL1B, TLR2 and SAA3) were discovered to differ between mice and cattle.

Conclusion

There has been extensive research into the genetics of mastitis resistance in dairy cattle. Breeding techniques that only prioritise milk production could result in an unfavourable population of cattle that are very vulnerable to mastitis. Mastitis resistance features should be incorporated into breeding techniques to create animals who are genetically resistant to mastitis in order to prevent such diseases. By reducing the occurrence of economically significant diseases like mastitis, several molecular markers in cattle have proven to be helpful for dairy farmers and breeders in boosting milk output and various other performance aspects. It is effective to employ these molecular markers when making breeding and management choices.

References

1. Mein G, Reinemann D, Schuring N, Ohnstad I. R-MM-1: milking machines and mastitis risk–a storm in a teatcup. InProceedings of the National Mastitis Council Annual Meeting. 2004;176-188.
2. Tiezzi F, Parker-Gaddis KL, Cole JB, Clay JS, Maltecca C. A genome-wide association study for clinical mastitis in first parity US Holstein cows using single-step approach and genomic matrix re-weighting procedure. PLoS One. 2015;10(2):e0114919.
3. Lush JL. Inheritance of susceptibility to mastitis. Journal of Dairy Science. 1950;33:121-125.
4. Lavon Y, Leitner G, Moallem U, Klipper E, Voet H, Jacoby S, *et al.* Immediate and carryover effects of Gram-negative and Gram-positive toxin-induced mastitis on follicular function in dairy cows. Theriogenology. 2011;76(5):942-53.
5. Akhtar S, Ali S. Monitoring of bovine health problems of small dairy herds in Islamabad capital territory: design, data and disease frequencies. Tropical animal health and production. 1994;26(4):193-198.
6. Joshi S, Gokhale S. Status of mastitis as an emerging disease in improved and periurban dairy farms in India. Annals of the New York Academy of Sciences. 2006;1081(1):74-83.
7. Gomez-Raya L, Klungland H, Vage DI, Olsaker I, Fimland E, Klemetsdal G, *et al*. Mapping QTL for milk production traits in Norwegian cattle. InProc. 6th World Congr. Genet. Appl. Livest. Prod. 1998;26:429-432.
8. Rasheed A, Usman T, Niaz K. A Review on Bovine Mastitis with Special Focus on as a Potential Candidate Gene for Mastitis Resistance–A Review. Annals of Animal Science. 2020;20(3),735-755.
9. da Rosa FT, Moreira CG, Barbero MM, Hurtado Lugo NA, de Camargo GM, Aspicueta Borquis RR, *et al*. Associations between MUC1 gene polymorphism and resistance to mastitis, milk production and fertility traits in Murrah water buffaloes. Journal of Applied Animal Research. 2020;48(1):151-155.
10. Alain K, Karrow NA, Thibault C, St-Pierre J, Lessard M, Bissonnette N. Osteopontin: an early innate immune marker of Escherichia coli mastitis harbors genetic polymorphisms with possible links with resistance to mastitis. BMC genomics. 2009;10:1-7.
11. Wang X, Ma P, Liu J, Zhang Q, Zhang Y, Ding X, *et al*. Genome-wide association study in Chinese Holstein cows reveal two candidate genes for somatic cell score as an indicator for mastitis susceptibility. BMC genetics. 2015;16(1):1-9.
12. Kurz JP, Yang Z, Weiss RB, Wilson DJ, Rood KA, Liu GE, *et al*. A genome-wide association study for mastitis resistance in phenotypically well-characterized Holstein dairy cattle using a selective genotyping approach. Immunogenetics. 2019;71:35-47.
13. Miles AM, Posbergh CJ, Huson HJ. Direct phenotyping and principal component analysis of type traits implicate novel QTL in bovine mastitis through genome-wide association. Animals. 2021;11(4):1147.
14. Lee YL, Takeda H, Costa Monteiro Moreira G, Karim L, Mullaart E, Coppieters W, *et al*. A 12 kb multi-allelic copy number variation encompassing a GC gene enhancer is associated with mastitis resistance in dairy cattle. PLoS Genetics. 2021;17(7):e1009331.
15. Freebern E, Santos DJ, Fang L, Jiang J, Parker Gaddis KL, Liu GE, *et al*. GWAS and fine-mapping of livability and six disease traits in Holstein cattle. BMC genomics. 2020;21:1-1.
16. Cai Z, Guldbrandtsen B, Lund MS, Sahana G. Prioritizing candidate genes post-GWAS using multiple sources of data for mastitis resistance in dairy cattle. BMC genomics. 2018;19(1):1-1.
17. Abdel-Shafy H, Bortfeldt RH, Reissmann M, Brockmann GA. Validation of somatic cell score–associated loci identified in a genome-wide association study in German Holstein cattle. Journal of Dairy Science. 2014;97(4):2481-2486.

18. Nascimento CS, Machado MA, Martinez ML, Silva MV, Guimarães MF, Campos AL, *et al*. Association of the bovine major histocompatibility complex (BoLA) BoLA-DRB3 gene with fat and protein production and somatic cell score in Brazilian Gyr dairy cattle (Bos indicus). Genetics and Molecular Biology. 2006;29:641-647.
19. Youngerman SM, Saxton AM, Oliver SP, Pighetti GM. Association of CXCR2 polymorphisms with subclinical and clinical mastitis in dairy cattle. Journal of Dairy Science. 2004;87(8):2442-2448.
20. Wojdak-Maksymiec K, Kmiec M, Ziemak J. Associations between bovine lactoferrin gene polymorphism and somatic cell count in milk. Veterinarni Medicina-Praha. 2006;51(1):14.
21. Wang Y, Chen T, Han C, He D, Liu H, An H, *et al*. Lysosome-associated small Rab GTPase Rab7b negatively regulates TLR4 signaling in macrophages by promoting lysosomal degradation of TLR4. Blood, The Journal of the American Society of Hematology. 2007;110(3):962-971.
22. Leyva-Baca I, Schenkel F, Sharma BS, Jansen GB, Karrow NA. Identification of single nucleotide polymorphisms in the bovine CCL2, IL8, CCR2 and IL8RA genes and their association with health and production in Canadian Holsteins. Animal genetics. 2007;38(3):198-202.
23. Deb R, Singh U, Kumar S, Singh R, Sengar G, Sharma A. Profiling of bovine breast cancer 1, early onset (BRCA1) gene among Frieswal (HF× Sahiwal) cows and their association with mastitis. National Academy Science Letters. 2014;37(6):579-583.
24. Magotra A, Gupta ID, Verma A, Alex R, Kumar V, Vineeth MR, *et al*. Characterization and validation of point mutation in breast cancer 1 (BRCA1) and its relationship with mastitis traits in Sahiwal. Int. J. of Recent Res. in Interdisciplinary Sci. 2015;3:22-26.
25. Magotra A, Gupta ID, Verma A, Alex R, Mr V, Ahmad T. Candidate SNP of CACNA2D1 gene associated with clinical mastitis and production traits in Sahiwal (Bos taurus indicus) and Karan Fries (Bos taurus taurus× Bos taurus indicus). Animal biotechnology. 2019;30(1):75-81.
26. Sahana G, Guldbrandtsen B, Thomsen B, Holm LE, Panitz F, Brøndum RF, *et al*. Genome-wide association study using high-density single nucleotide polymorphism arrays and whole-genome sequences for clinical mastitis traits in dairy cattle. Journal of dairy science. 2014;97(11):7258-7275.
27. Sulabh S, Verma A, Gupta ID, Kumar V. Genotyping of calgranulin A gene in Murrah buffaloes by PCR-RFLP. Indian Journal of Dairy Science. 2016;69:2.
28. Zhang Q, Guldbrandtsen B, Thomasen JR, Lund MS, Sahana G. Genome-wide association study for longevity with whole-genome sequencing in 3 cattle breeds. Journal of Dairy Science. 2016;99(9):7289-7298.
29. Hu G, Li L, Xu W. Extracellular matrix in mammary gland development and breast cancer progression. Frontiers in Laboratory Medicine. 2017;1(1):36-39.
30. Elmaghraby MM, El-Nahas AF, Fathala MM, Sahwan FM, El-Dien MT. Association of toll-like receptors 2 and 6 polymorphism with clinical mastitis and production traits in Holstein cattle. Iranian Journal of Veterinary Research. 2018;19(3):202.
31. Yang F, Chen F, Li L, Yan L, Badri T, Lv C, *et al*. GWAS using 2b-RAD sequencing identified three mastitis important SNPs via two-stage association analysis in Chinese Holstein cows. bioRxiv. 2018, 434340.
32. Dhundwal K, Pander BL, Dalal DS, Magotra A, Mittal D, Singh M, *et al*. Characterization and validation of point mutation in MBL1 gene and its relationship with mastitis in Murrah buffalo (Bubalus bubalis). Buffalo Bulletin. 2019;38(3):451-457.
33. Moretti R, Soglia D, Chessa S, Sartore S, Finocchiaro R, Rasero R, *et al*. Identification of SNPs associated with somatic cell score in candidate genes in Italian Holstein Friesian bulls. Animals. 2021;11(2):366.
34. Jaglan K, Ravikumar D, Sukhija N, George L, Alex R, Vohra V, *et al*. Genomic clues of association between clinical mastitis and SNPs identified by ddRAD sequencing in Murrah buffaloes. Animal Biotechnology; c2023. p. 1-9.
35. Silveri L, Tilly G, Vilotte JL, Le Provost F. MicroRNA involvement in mammary gland development and breast cancer. Reproduction Nutrition Development. 2006;46(5):549-556.
36. Sharma BS, Jansen GB, Karrow NA, Kelton D, Jiang Z. Detection and characterization of amplified fragment length polymorphism markers for clinical mastitis in Canadian Holsteins. Journal of Dairy Science. 2006;89(9):3653-3663.
37. Schwerin M, Czernek-Schäfer D, Goldammer T, Kata SR, Womack JE, Pareek R, *et al*. Application of disease-associated differentially expressed genes–Mining for functional candidate genes for mastitis resistance in cattle. Genetics Selection Evolution. 2003;35:1-6.
38. Goldammer T, Zerbe H, Molenaar A, Schuberth HJ, Brunner RM, Kata SR *et al*. Mastitis increases mammary mRNA abundance of β-defensin 5, toll-like-receptor 2 (TLR2), and TLR4 but not TLR9 in cattle. Clinical and Vaccine Immunology. 2004;11(1):174-185.
39. Lee JW, Bannerman D, Paape M, Huang MK, Zhao X. Characterization of cytokine expression in milk somatic cells during intramammary infections with Escherichia coli or Staphylococcus aureus by real-time PCR. Veterinary Research. 2006;37(2):219-229.