

Polymorphism of Bovine Forebrain Embryonic Zinc Finger Like (FEZL) Gene Associated with Resistance to Mastitis in Indian Cattle

Rajesh Kumar Somasundaram^{1*}, Iswar Dayal Gupta², K. N. Raja³, Kathiravan Periasamy⁴ and Saravanan Ramasamy⁵

¹Executive Veterinary Officer, RVC Branch, HQ 33 Corps, PIN 908533, 2CBPO, C/o 99APO

²Dairy Cattle Breeding Division, National Dairy Research Institute, Karnal, Haryana, INDIA

³National Bureau of Animal Genetic Resources, Karnal, Haryana, INDIA

⁴Animal Production and Health Laboratory, Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna, AUSTRIA

⁵Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, INDIA

*Corresponding Author: drsrajeshkumar@gmail.com

How to cite this paper:

Somasundaram, R., Gupta, I., Raja, K., Periasamy, K., & Ramasamy, S. (2020). Polymorphism of Bovine Forebrain Embryonic Zinc Finger Like (FEZL) Gene Associated with Resistance to Mastitis in Indian Cattle. *International Journal of Livestock Research*, 10(8), 144-149. doi:

<http://dx.doi.org/10.5455/ijlr.20200425120435>

Received : Apr 25, 2020
Accepted : Jul 05, 2020
Published : Aug 31, 2020

Copyright © Somasundaram *et al.*, 2020

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0). <http://creativecommons.org/licenses/by/4.0/>

Abstract

Bovine forebrain zinc finger like (FEZL) is one of the candidate genes associated with mastitis resistance in dairy cattle. The present study was undertaken to evaluate variation in exon 1 of FEZL gene in Bos indicus (Sahiwal) and crossbred cattle. A total of 114 Karan Fries cattle and 111 Sahiwal cattle were genotyped for 12G/13G polymorphism associated with mastitis resistance/susceptibility. The results revealed a predominant presence of 13G allelic variant in Indian cattle with an overall frequency of 98.2%. In Karan Fries cattle, 96.5% animals possessed 13G/13G homozygous genotype while 3.5% animals possessed 12G/13G heterozygous genotype. The allelic variant (12G) reportedly associated with mastitis resistance was completely absent in Bos indicus (Sahiwal) cattle. The observed inheritance of 12G resistant alleles in Karan Fries cattle might have been derived through introgression from exotic Friesian cattle. Screening FEZL locus in additional samples and breeds may help to establish association with mastitis in Indian cattle.

Keywords: FEZL gene, Cattle, Mastitis Resistance



Introduction

Mastitis is inflammation of mammary gland resulting from introduction and multiplication of pathogenic microorganisms such as *Staphylococcus aureus* and *Streptococcus agalactiae* in the udder. It causes huge economic losses in dairy cattle by adversely affecting animal health, quality of milk produced and the economics of milk production. Selective breeding of dairy cattle for reduced susceptibility/increased resistance to mastitis is a potential long-term solution to mitigate the disease. However, the genetic progress is hindered by low estimated heritability's that ranged from 0.048 to 0.134 (Chegini *et al.*, 2016). Indirect selection based on somatic cell score and candidate gene markers may help increasing the efficiency of such breeding programs. A fair amount of genetic research related to udder health has already been performed that resulted in the identification of several quantitative trait loci (QTLs) related to mastitis resistance in cattle (Ogorevc *et al.*, 2008; 2009). Beyond fine mapping, the ultimate target of QTL analysis is the identification of causal gene or QTN (quantitative trait nucleotide) itself which facilitate detection of resistance genes and alleles (Rainard and Riollet, 2005). Bovine forebrain embryonic zinc finger like (FEZL) is one of the candidate genes associated with resistance to mastitis in dairy cattle.

Forebrain embryonic zinc finger-like (FEZL) factor is involved in transcriptional regulation of neuronal development (Hirataet *et al.*, 2004). The FEZL gene variations have been showed to have significant association with somatic cell count (Sugimoto *et al.*, 2006; 2011; 2013) and clinical/sub-clinical mastitis (Ali *et al.*, 2019) in cattle. A three-nucleotide insertion mutation within exon1 region of bovine FEZL gene resulted in an additional glycine residue within a stretch of 12G(glycine) region. This additional glycine residue i.e. 13G stretch has been reported to be associated with higher somatic cell counting Holstein-Friesian cattle (Sugimoto *et al.*, 2006). Mastitis induces FEZL expression in mammary gland, and induced FEZL promotes expression of the axon-attracting molecule semaphorin 5A (SEMA5A). Enhanced SEMA5A induces expression of at least nine genes related to host immune response, including TNF- α and IL-8. FEZL with 12G glycine stretch promotes higher semaphorin 5A expression as compared to FEZL with 13G glycine stretch (Sugimoto *et al.*, 2013). The relative lower expression of semaphorin 5A in cattle having FEZL 13G variant leads to impaired immune response and increased susceptibility to mastitis.

Considering the physiological basis for association of FEZL gene variation with mastitis resistance/susceptibility, it would be of significance to estimate the frequency distribution of 12G/13G allelic variant in the Indian cattle populations. Till date, very little information is available on FEZL allelic variants in *Bos indicus* cattle and their crossbreeds in India. Hence, the present study was undertaken with the objectives of estimating the frequency distribution of reportedly susceptible (13G) and resistant (12G) FEZL allelic variants in Sahiwal (*B. indicus*) and Karan Fries (*B. indicus* X *B. taurus*) cattle.

Materials and Methods

Sampling and DNA Extraction

Blood samples were collected from 111 Sahiwal and 114 Karan Fries cattle maintained at Cattle Farm, National Dairy Research Institute (NDRI), Karnal. About 10 ml of blood was collected in a sterile 0.5% EDTA (10 μ l/ml of blood) coated vacutainers by jugular venipuncture method. The collected blood was stored in the refrigerator at 4°C till further processing for extraction of DNA. DNA was isolated from blood using standard phenol-chloroform method as described in Sambrook and Russell (2001) with minor modifications. The quality of DNA was checked by comparing the bands with reference DNA in 0.8% agarose gel electrophoresis and the quantity was determined using Nanodrop.

Genotyping FEZL Allelic Variants

In order to genotype 12G/13G polymorphism within exon 1 of FEZL gene, methodology proposed by Sugimoto *et al.* (2011) was used. The PCR reaction was performed with the following primer pairs: Forward: 5'(FAM)-ACTCTGAGCTCTGGAAAAGCAG-3'; Reverse: 5'-CACACGCCACAAGTTGGTTT-3'. Polymerase chain reaction was carried out in a final reaction volume of 20 μ L, containing ~50ng of genomic DNA, 5 pmole of each primer, 200 μ M of each dNTP, 2.5 μ L of 10X buffer with 1.5 mM MgCl₂ and one unit of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). Amplification was performed using programmable thermal cyclers (PTC-200, MJ Research, USA) with an initial denaturation at 95°C for 2.5 min followed by 35 cycles of 94°C - 30s, 57°C- 30s and 72°C - 1min, with a final extension for 5 min at 72°C. Following PCR, alleles were resolved using

an ABI automated DNA analyzer (Applied Biosystems, USA) along with GS500LIZ as internal lane control. Genotype data were extracted using GENEMAPPER 4.0 (Applied Biosystems, USA). The methodology proposed by Sugimoto *et al.* (2011) to genotype 12G/13G variation within FEZL gene focused on estimating the size difference of amplicon covering the INDEL polymorphism. The forward primer conjugated with FAM dye was utilized to detect amplicons of ~130bp size (Figure 1) during the process of fragment length analysis in the automated DNA analyzer.

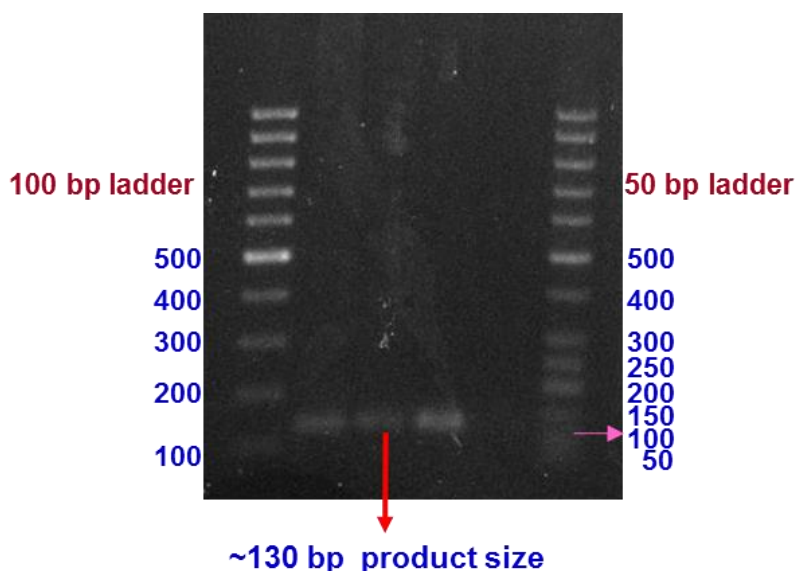
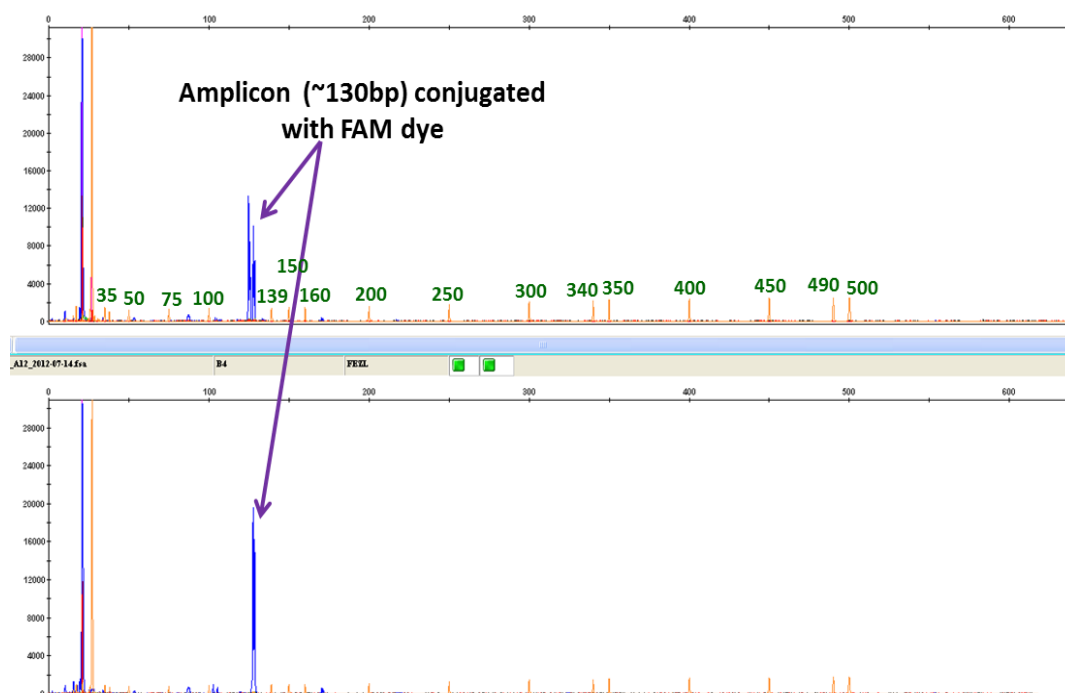


Figure 1: Amplified PCR product labeled with FAM dye and resolved in 2.5% agarose gel electrophoresis prior to genotyping

The genotypes were called based on specific peaks corresponding to different allele sizes. The expected amplicon size for 12G homozygous genotype was a single peak at 125 base pairs; single peak at 128 base pairs corresponded to 13G homozygous genotype while double peaks at 125 and 128 base pairs corresponded to 12G/13G heterozygous genotypes. The raw data of representative samples from ABI automated DNA analyzer using GENEMAPPER software is presented in Supplementary Figure 1.



Supplementary Figure 1: Unextracted raw data from ABI automated DNA analyzer showing the amplicon specific peak (Blue-Conjugated with FAM dye); Peaks in orange represent standard size DNA markers (GS500LIZ)

Results and Discussion

Frequency Distribution of FEZL Allelic Variants in Sahiwal and Karan Fries Cattle

The results of genotyping for the 12G/13G polymorphism within FEZL gene are presented in Table 1. The results revealed a predominant presence of 13G allelic variant in the studied Indian cattle with overall frequency of 98.2%.

Table 1: Results of genotyping 12G/13G polymorphism within exon 1 region of FEZL gene in Sahiwal and Karan Fries cattle

Genotype	Sahiwal		Karan Fries	
	No. Animals	Frequency	No. Animals	Frequency
13G/13G	111	1	110	0.965
12G/13G	0	0	4	0.035
12G/12G	0	0	0	0
Total	111	1	114	1

In Karan Fries cattle, 96.5% of the animals had 13G/13G genotype, while 3.5% of the animals possessed 12G/13G heterozygous genotype (Figure 2). In case of Sahiwal cattle, all the genotyped animals were found to be 13G/13G homozygotes with the absence of 12G alleles. Similar finding on the absence of 12G allele has been reported earlier in Jersey cattle. Sonstegard *et al.* (2005) reported the characterization of FEZL effects on somatic cell score in North American dairy cattle. They reported QTN genotypes for 2379 dairy bulls which included mostly Holstein and few Jersey sires. None of the Jersey sires (N=49) were found to possess 12G allelic variant (resistant variant) while less than 3% of the Holstein sires (N=2325) were found to possess the 12G allelic variant. Among these 2325 sires, only six (0.26%) were homozygous for 12G allelic variant. Later, in a study on Japanese Holstein cattle, Sugimoto *et al.* (2011) genotyped 12G/13G polymorphism in 917 Holstein sires and reported 97 sires with heterozygous genotypes (10.58%).

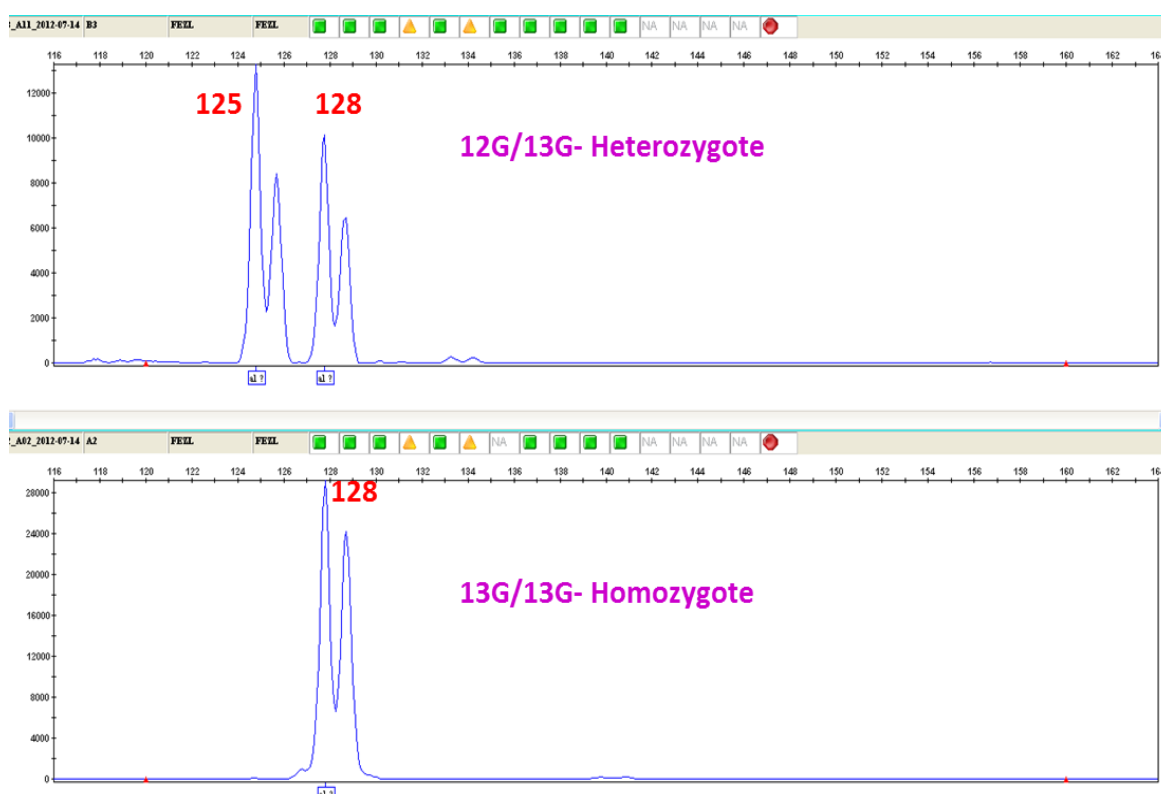


Figure 2: Extracted FEZL genotypes using GENEMAPPER with peaks at 125bp and 128bp for 12G/13G heterozygote (above) and 128 bp peak for 13G/13G homozygote (below)

The frequency of 12G/13G heterozygotes (3.5%) observed in Karan Fries cattle is lesser than those reported for

Holstein cattle. However, Sahiwal, the indigenous *Bos indicus* cattle was found to be almost fixed with 13G allele reportedly associated with higher somatic cell score. This finding seems to be interesting when considered with the consistent reports of higher mastitis incidence in Sahiwal cattle as compared to other breeds. Singh (1979) reported a comparison of mastitis incidence in indigenous and crossbred cattle with 31.1%, 20.9%, 16.3% and 15.3% in Sahiwal, Red Sindhi, Tharparkar and crossbred cattle. Satyapal (2003) reported an incidence of 20.66% incidence of mastitis in Sahiwal cattle and 14.12% in Karan Fries cattle. Similarly, Khate and Yadav (2010) reported mastitis incidence in 26.43% Sahiwal cattle and 18.91% Murrah buffaloes. Higher mastitis incidence in Sahiwal cattle as compared to Karan Swiss and Karan Fries cattle was also reported by Jingar *et al.* (2014) and Danish *et al.* (2018). Higher incidence of mastitis in Sahiwal cattle implies that they are relatively more susceptible to mammary gland infections as compared to other breeds. Further, complete fixation of susceptible alleles at FEZL-12G/13G locus along with genes at other loci could possibly be one of the potential reasons for the higher susceptibility of Sahiwal cattle to mastitis. Large scale association studies in Sahiwal cattle are needed to further validate the role of bovine FEZL allelic variants in the susceptibility/resistance to mastitis in *Bos indicus* cattle.

Another interesting feature of the present study is the presence of “12G-FEZL” allele in Karan Fries cattle, that has been reported to confer relatively more resistance to mastitis in Japanese Holstein-Friesians (Sugimoto *et al.*, 2006, 2011; 2013). The inheritance of this allele in Karan Fries cattle albeit at low frequency might have been through the Friesian genes which had been used as source of exotic germplasm for the development of this synthetic breed. It seems that the occurrence of 12G variant is found comparatively higher in *Bos taurus* cattle than *Bos indicus* cattle. However, such a proposition needs to be verified by genotyping this locus in a wider population of cattle including diverse indigenous cattle breeds.

Conclusion

In conclusion, the present study is the first report on the distribution of bovine FEZL allelic variants in Indian cattle. The results revealed a predominant presence of 13G allelic variant in the studied Indian cattle populations with an overall frequency of 98.2%. The allelic variant reportedly associated with mastitis resistance (12G) was completely absent in *Bos indicus* cattle while its frequency was about 3.5% in crossbred cattle. The observed inheritance of 12G resistant alleles in Karan Fries cattle might have been derived through introgression from exotic Friesian cattle. Screening FEZL locus in additional samples and breeds may help to establish association with mastitis in Indian cattle.

Acknowledgements

The authors are thankful to the Director, National Bureau of Animal Genetic Resources and Director, National Dairy Research Institute, Karnal, Haryana, India for providing necessary facilities to conduct the study.

Conflict of Interests

There is no conflict of interest.

Publisher Disclaimer

IJLR remains neutral concerning jurisdictional claims in published institutional affiliation.

References

1. Ali, G.E., Ibrahim, M.A. and Zaki, S.M. (2019). Association assessment of single nucleotide polymorphism in Forebrain embryonic zinc finger-like (FEZL) gene with mastitis susceptibility in Holstein cattle (*Bos taurus*). *Large Animal Review*, 25, 163-171.
2. Chegini, A., Hossein-Zadeh, N.G., Hosseini-Moghadam, H. and Shadparvar, A.A. (2016). Estimation of genetic and environmental relationships between milk yield and different measures of mastitis and hyperkeratosis in Holstein cows. *Acta Scientiarum*, 38, 191-196.
3. Danish, Z., Bhakat, M., Paray, A.R., Lone, S.A., Rahim, A., Mohanty, T.K. and Sinha, R. (2018). Udder and teat morphology and their relation with incidence of sub-clinical and clinical mastitis in Sahiwal, Karan Fries cows and Murrah buffaloes. *Journal of Entomology and Zoology Studies*, 6(5), 2138-2141.

4. Hirata, T., Suda, Y., Nakao, K., Narimatsu, M., Hirano, T. and Hibi, M. (2004). Zinc finger gene fez-like functions in the formation of subplate neurons and thalamocortical axons. *Developmental Dynamics*, 230, 546–556.
5. Sambrook, J. and Russell, D.W. (2001). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
5. Jingar, S.C., Mehla, R.K., Singh, M. and Singh, P.K. (2014). Effect of stages and level of milk production on mastitis incidence in cows and Murrah buffaloes. *Journal of Bio Innovation*, 3(3), 117-123.
6. Khate, K. and Yadav, B.R. (2010). Incidence of mastitis in Sahiwal cattle and Murrah buffaloes of a closed organized herd. *Indian Journal of Animal Sciences*, 80, 467-469.
7. Ogorevc, J., Kunej, T and Dovč, P. (2008). An integrated map of cattle candidate genes for mastitis: a step forward to new genetic markers. *Acta agriculturae Slovenica*, 2, 85–91.
8. Ogorevc, J., Kunej, T., Razpet, A. and Dovč, P. (2009). Database of cattle candidate genes and genetic markers for milk production and mastitis. *Animal Genetics*, 40, 832–851.
9. Rainard, P. and Riollet, C. (2005). Innate immunity of the bovine mammary gland. *Veterinary Research*, 37, 369–400.
10. Sambrook, J. and Russell, D.W. (2001). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
11. Satyapal (2003). Investigation on health disorders in dairy cattle and buffaloes during pre and post-partum period. Ph.D Thesis submitted to NDRI, Karnal.
12. Singh, R. N. (1979). Influence of breed groups (Zebu X Exotic) on health, production and breeding efficiency. Ph.D Thesis. NDRI, Karnal.
13. Sonstegard, T.S., Van Tassell, C.P. and Li, R. (2005). Characterization of FEZL effects on SCS in a sample of North American Holsteins. *Journal of Animal Science*, 83 (Supplement), 201.
14. Sugimoto, M., Fujikawa, A., Womack, J.E and Sugimoto, Y. (2006). Evidence that bovine forebrain embryonic zinc finger-like gene influences immune response associated with mastitis resistance. *Proceedings of National Academy of Sciences*, 103 (17), 6454–6459
15. Sugimoto, M., Itoh, T., Gotoh, Y., Kawahara, T., Moriya, H., Uchimura, Y. and Sugimoto, Y. (2011). Enhanced clinical mastitis resistance in Holsteins with a FEZL p.Gly105(12_13) polymorphism. *Journal of Dairy Science*, 94, 2103-2107.
16. Sugimoto, M., Uchiza, M. and Kuniyuki, M. (2013). Effects of a Forebrain embryonic zinc finger-like p.Gly105(12_13) polymorphism on mastitis resistance: an embryo-transfer study. *Molecular Biology and Genetic Engineering*, <http://www.hoajonline.com/journals/pdf/2053-5767-1-1.pdf>
