

Original Research Article

<https://doi.org/10.20546/ijcmas.2021.1001.334>

Molecular Characterization and Expression Profile of Partial *TLR2* Gene in Malnad Gidda Cattle

G. M. Satheesha^{1*}, R. Nagaraja², H. M. Yathish¹, A. Sahadev¹, R. Jayashree³,
A. M. Kotresh³ and S. Naveenkumar¹

¹Department of Animal Genetics and Breeding, Veterinary College, Bangalore, India

²Veterinary College, Gadag

³Veterinary College, Shivamogga, Karnataka, India

*Corresponding author

ABSTRACT

Keywords

Polymorphism,
Malnad gidda,
RFLP, Sequencing

Article Info

Accepted:
18 December 2020
Available Online:
10 January 2021

Toll like receptors (TLRs) are the cell surface receptors which mainly act by activating innate and adaptive immunity. These TLR's play very important role in disease resistance through the recognition of pathogen-associated molecular patterns (PAMPs). Among all the TLRs identified *TLR2* is mainly involved in the recognition of multiple pathogenic microorganisms. In the present study partial region exon-2 of *TLR2* gene covering 997bp region was characterized in Malnad gidda cattle which is one of the draft breed of cattle which is famous for its disease resistance property. The partial exon-2 of *TLR2* gene is amplified using self designed primers and subjected for RFLP analysis using *BsaA1* restriction enzyme which revealed AB genotypes with fragments of sizes 71, 926 and 997bp exhibiting monomorphic pattern. The allelic frequency of A and B allele are 0.5 and the genotypic frequency of the AB genotype is 1. Further, the observed heterozygosity, polymorphic information content (PIC) and allelic diversity are 1.0, 0.3750 and 0.5 respectively. Sequence analysis of Malnad gidda cattle revealed three SNPs when compared to *Bos Taurus* sequence.

Introduction

The indigenous cattle or Zebu cattle in India is scientifically known as *Bos indicus* which are very robust, resilient and adaptable to the harsh climate and environment of their respective breeding tracts. Among many indigenous breed, Malnad gidda is one of the unique cattle which belongs to Malnad and coastal region of Karnataka. They are very popular for their strength to adopt and

withstand a wide range of local climatic conditions of the hilly terrain of western ghat and they play a significant role in contributing to the local economy and livelihood security to its owners by way of providing milk, draught power and manure with minimum or zero inputs besides their inherent role in sustainable ecological balance. Malnad Gidda cows are small in size with compact body frames, weighing between 80 and 120 Kg. They are reared under a low input low output

system. The milk yield varies from 1.5 to 4 liters a day and the milk and milk products are known for a unique taste and creaminess.

Malnad gidda cattle are found resistant to many diseases when compared to crossbred cattle and have the ability to withstand stressful environmental conditions (Das *et al.*, 2009; Ramesha *et al.*, 2015). They are highly adaptable to harsh climatic conditions even during heavy rainfall (Ramesha *et al.*, 2007). Malnad gidda cattle are known to be better resistant against Foot and Mouth disease (Das *et al.*, 2009) or bacterial and parasitic diseases compared to other cattle breeds of the region. However, scientific data on the mechanisms of disease resistance in Malnad gidda cattle is clearly lacking.

The ability of a dairy cow to resist contagious disease is in part related to the activity of its immune system which consists of a variety of mechanisms and processes which are capable of both non-specifically and specifically targeting invading microorganisms (Ingvarsen and Moyes, 2013). Pattern recognition receptors (PRRs) are known to play an important role in the innate or non-adaptive immune response as they detect the presence of pathogens that breach an animal's surface defences (Janeway and Medzhitov, 2002). A major group of PRRs are the Toll-like receptors (TLRs) which play very important role in immunity of the individual. After pathogen-associated molecular patterns (PAMP) recognition, TLRs activate cellular signaling pathways to induce immune response genes, including inflammatory cytokines (Medzhitov and Janeway, 2000). Ten different Toll-like genes have been identified within the cattle genome. Among them *TLR2* has been first identified as antibacterial molecule that recognizes peptidoglycan of gram positive bacteria which is activated inside the phagosome by peptidoglycans (Campos *et al.*, 2001). It was

reported that the expression of *TLR2* gene strongly increased in different body tissue following infection in cattle (Goldammer *et al.*, 2004).

Therefore, the present study was carried out with the objective to explore polymorphic patterns in the partial coding region of *TLR2* genes using PCR-RFLP technique and comparative bioinformatic analysis of resultant *TLR2* gene sequences of Malnad Gidda cattle with the available databases.

Materials and Methods

DNA extraction and PCR analysis

The random blood samples were collected from 110 unrelated Malnad gidda cattle from different parts of Western Ghats area where these animal breeding tracts are distributed. Genomic DNA was isolated from blood samples of Malnad gidda cattle by high salt DNA extraction method as described by Miller *et al.*, (1988) with some modifications. PCR was used to amplify partial coding regions of exon-2 of *TLR2* with self designed primers by using primer-3 software. The details were given in table-1.

The PCR conditions were standardized by adopting various primer concentrations and annealing temperatures to get an optimum yield of the desired fragment. The standardized concentration of the components used in the reaction mixture for amplification of *TLR2* gene in Malnad Gidda cattle have been given in Table 2.

Different thermal profiles/ PCR program were tested to optimize the program that gives the best amplification of partial coding regions of *TLR2* gene. The optimized thermal profile for amplification of partial coding regions of *TLR2* gene is initial denaturation at 95°C for 3 min followed by cyclic denaturation at 95°C

at 30 sec, followed by annealing at 56⁰C for 30sec and extension at 72⁰C for 1 min along with final extension at 72⁰C for 10min. Obtained amplicons were further subjected for PCR-RFLP analysis by using *BsaAI* for identification of single-nucleotide polymorphism (SNP) in fragments under study, these restriction enzymes were identified using NEB-cutter V2.0 online tool.

PCR-RFLP

After verifying the specific amplification of targeted regions of *TLR2* amplicons were digested with *BsaAI* restriction enzyme. To optimize the concentration of each component to be used for restriction digestion, various combinations of reaction mixture were tried. Restriction digestion of amplicons was carried out with incubation at 37⁰ C for 15 min followed by inactivation at 65⁰C for 20 minutes along with reaction components. The representative PCR products from *TLR2* gene of Malnad Gidda cattle with respect to different RFLP patterns were submitted for custom sequencing by using forward and reverse primers. The sequences obtained were analyzed, consensus was created, annotated and multiple sequence analysis was performed by using DNA star and Bio Edit software's.

Results and Discussion

The fragment-1, corresponding to exon-2, covering 997bp region of *TLR2* gene was amplified using TLR2_E2_F1-F and TLR2_E2_F1-R primers. All the DNA samples of Malnad gidda cattle produced satisfactory amplification. The amplification of fragment-1 of *TLR2* gene was confirmed by resolving the PCR products on 1.5% agarose gel (Fig.1). RFLP analysis using *BsaAI* enzyme revealed AB genotypes with fragments of sizes 71, 926 and 997bp exhibiting monomorphic pattern. The allelic

frequency of A and B allele are 0.5 and the genotypic frequency of the AB genotype is 1. Further, the observed heterozygosity, polymorphic information content (PIC) and allelic diversity are 1.0, 0.3750 and 0.5 respectively. Genotype AA and BB was totally absent in the studied populations. The representative gel pictures showing the band pattern in PCR - RFLP for fragment-1, corresponding to exon-2 of *TLR2* gene are presented in Fig.2. The gene and genotype frequency for these in Malnad gidda cattle is given in Table 3. Similarly, in Deoni cattle, Shreekant *et al.*, (2017) identified AA and AB genotype by using *PstI* and *TseI* enzyme. In Malnad gidda cattle population, the present investigation revealed that the significant departure of *TLR2_E2-F1_BsaAI*, marker from Hardy-Weinberg Equilibrium (HWE) was observed.

The nucleotide sequence Clustal W alignment report with respect to 997bp sized fragment-1, corresponding to exon-2 of *TLR2* gene, pertaining to *BsaAI* restriction enzymes, was carried out. Sequence analysis confirmed the presence of 997bp intron-1 (partial) and 5' UTR (partial) and partial coding sequence. Sequence analysis revealed no SNPs in the restriction sites of *BsaAI* enzyme in TLR2_E2-F1. However, three SNP's viz. T>C, G>A and G>A, when compared to *Bos Taurus* sequence, were found at 250th (Fig.3 a), 266th (Fig. 3 a) and 695th (Fig.3 b) position resulted from transition (Table 4).

Among the 3 SNPs identified 2 are non synonymous SNPs due to transition of bases namely at position 266 and 695 which lead to change in amino acid sequence from glycine (G) to serine (S) and valine (V) to isoleucine (I) at position 68 and 211 respectively(Fig.4).

The annotated sequence of TLR2_E2-F1 was used as query and subjected to nucleotide blast at NCBI (<https://blast.ncbi.nlm.nih.gov>)

/Blast.cgi), which revealed 99.80, 99.60, 99.79, 97.89, 96.69 and 96.25 per cent identity with *Bos indicus* (EU413951.1), *Bos taurus* (EU746465.1), Vechur cattle (KT862891.1), *Bubalus bubalis* (EU178742.1), *Capra hircus* (JQ911706.1) and *Ovis aries* (EU580543.1) respectively.

The phylogenetic tree was constructed using NCBI-BLAST by pairwise alignment

neighbor joining method utilizing the nucleotide sequence of TLR2_E2-F-1 of Malnad gidida by comparing with *TLR2* gene belonging to different breeds and species namely *Bos indicus* (EU413951.1), *Bos taurus* (EU746465.1), Vechur cattle (KT862891.1), *Bubalus bubalis* (EU178742.1), *Capra hircus* (JQ911706.1) and *Ovis aries* (EU580543.1) whose data is available at NCBI data base (Fig. 5.).

Table.1 Details of Primer used for amplification of *TLR2* gene in Malnad Gidda cattle

Gene	Primer's Name	F/R	5'-Primer sequence-3'	Length (bp)	Product Size (bp)	Region amplified
TLR2	TLR2_E2 – F1	F	GCCATGATGTCAAACA CAGTC	21	997	Partial 5'UTR,CDS1 partial & Partial intron1
		R	CGTCTCCACATTACCT AGGTGTC	23		

F: forward, R: reverse, bp: base pair, GC: guanine cytosine content, Tm°: melting temperature

Table.2 PCR reaction mix standardized for amplification of *TLR2* gene

Sl.No	PCR component	Volume(µl)
1	2x Red PCR Master Mix	12.5
2	Forward primer (varied concentrations)	1.0
3	Reverse primer (varied concentrations)	1.0
4	Genomic DNA	1.0
5	Nuclease free water	9.5
	Total	25

Table.3 Gene and genotype frequency for fragment-1, corresponding to exon-2 of *TLR2* gene in Malnad gidida cattle

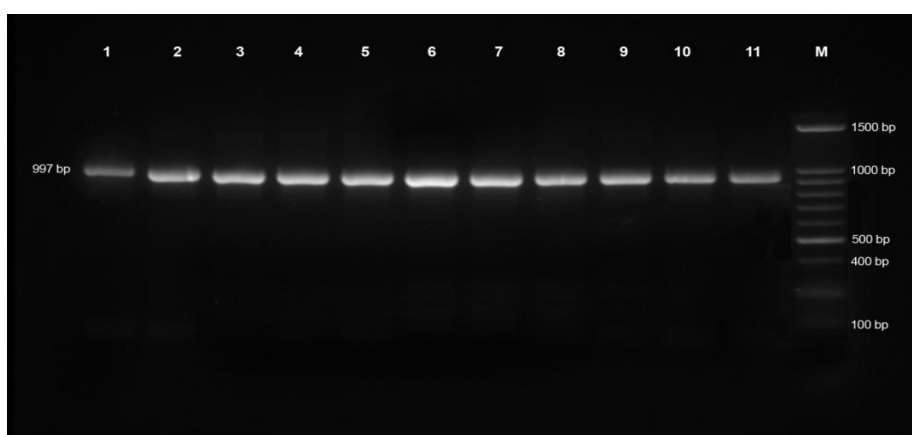
Gene_Frag.	No. of Indi.	RE	Gene frequency		Genotype frequency			PIC	Heter.	Alle. Divr.
			'A' allele	'B' allele	AA	AB	BB			
TLR2_E2-F1	102	BsaA1	0.5000	0.5000	-	1.0	-	0.3750	1.0000	0.5000

PIC: Polymorph Info Content, Heter.: Heterzygosity, Alle. Divr.: Allelic diversity

Table.4 Identified SNPs and type of mutation in Malnad gidda TLR2_E2-F1

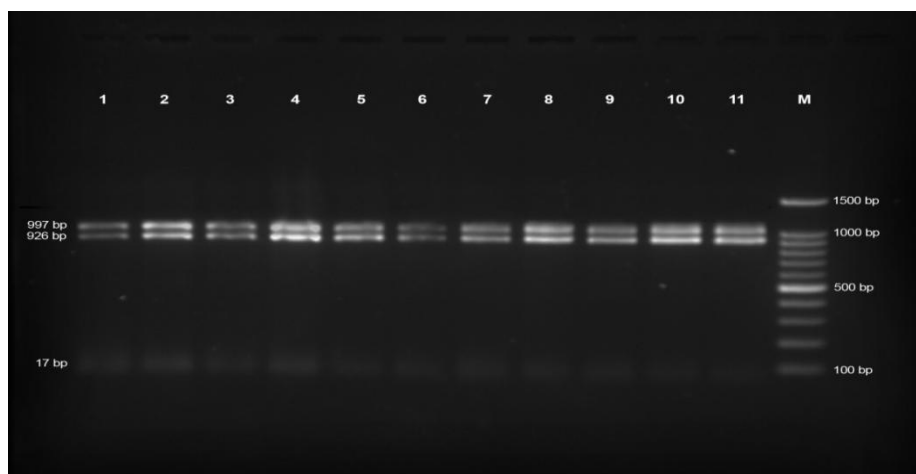
Sl. No.	SNP	Position	Nucleotide		Type of mutation	Amino acid substitution
			Bos taurus (From NCBI)	Malnad gidda cattle		
1	T>C	250	T	C	Transition	G to C
2	G>A	266	G	A	Transition	--
3	A>G	695	G	A	Transition	V to I

Fig.1 PCR amplified product of fragment 1 (997bp) corresponding to exon2 of TLR2 gene in Malnad gidda cattle



Lane 1 to 11 : 997bp amplicons
Lane M : 100bp Molecular Marker

Fig.2 PCR product of TLR2_E2-F1 after digestion with restriction enzyme *Bsa*AI for detection of gene polymorphism in Malnad gidda cattle



Lane 1-7 : AB genotype (71, 926 and 997bp)
Lane M : 100bp ladder

Fig.3a Chromatograph showing T>C and G>A transition for TLR2_E2-F1 gene at position 250 and 266 respectively

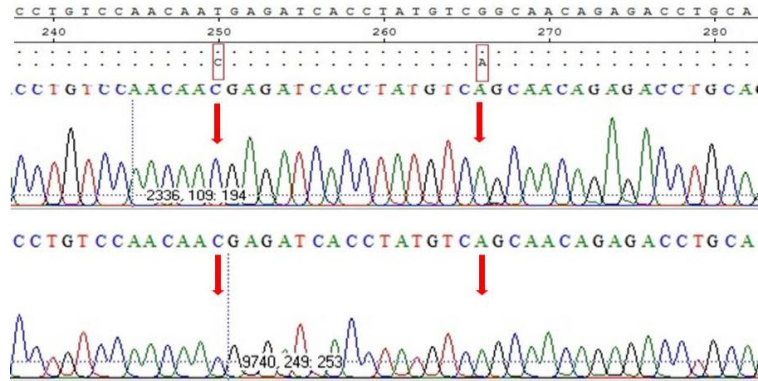


Fig.3b Chromatograph showing G>A transition for TLR2_E2-F1 gene at position 695

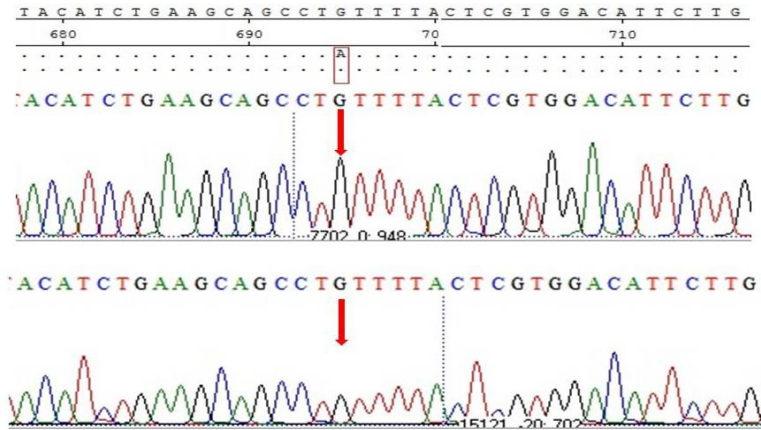


Fig.4 Amino acid sequence alignment report of fragment-1 corresponding to exon-2 of TLR2_E2-F1/*BsaAI* analysis in Malnad gidda cattle

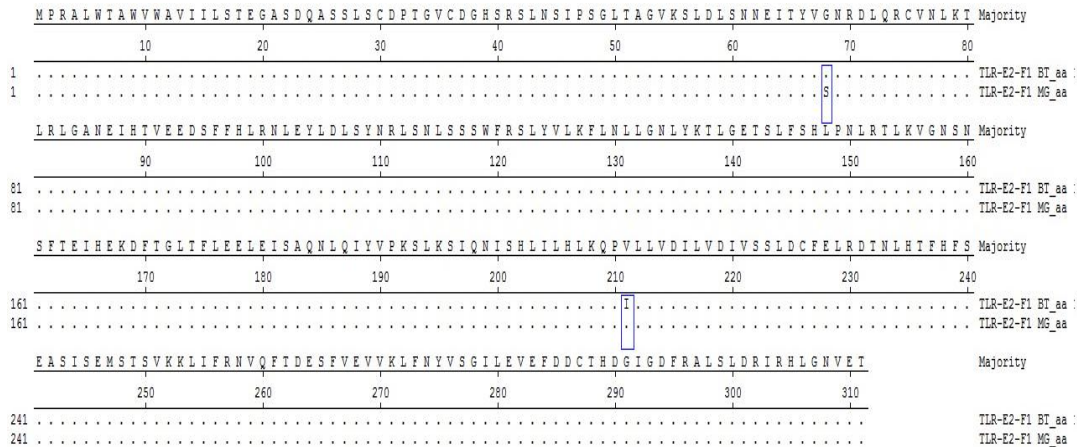
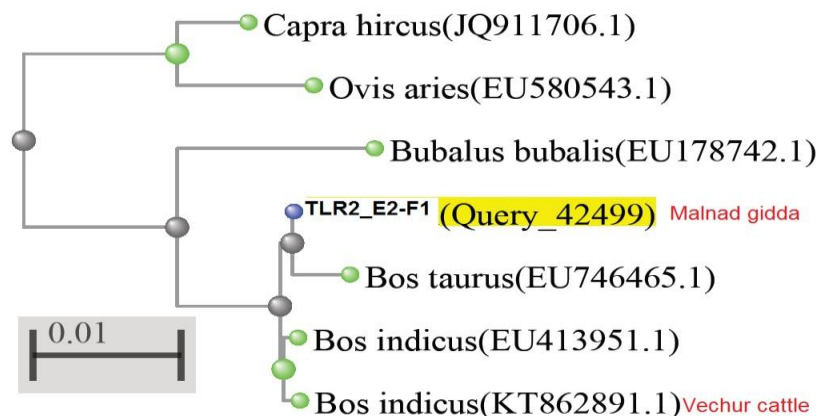


Fig.5 Phylogenetic tree utilizing the nucleotide sequence of TLR2 gene belonging to different Breed/species available at NCBI and comparing with TLR2_E2-F1 sequence of Malnad gidda using the neighbor joining procedure (Figure at nodes indicates percent bootstrap value out of 1000 replication)



Annotated sequence of Malnad gidda cattle was compared with sequence of Vechur cattle with genebank accession no. KT862891.1 (Shivakumara *et al.*, 2015) where it showed 99% identities with Vechur cattle and at position 44, T is replaced by G and at position 704, T is replaced by A. But when compared with the gene sequence of other *Bos indicus* breed (Tantia *et al.*, 2008) with gene accession no. EU413951.1, and cross breed cattle (Seabury and Womack, 2008, EU746457.1) showed 99% identity with Malnad gidda gene sequence.

It was concluded that, Malnad gidda *TLR2* gene is highly polymorphic. Three novel SNPs were identified in the partial coding region exon-2 of *TLR2* region out of which two were non synonymous that lead to change in amino acid sequence. The effect of non-synonymous SNPs on the structure and function of *TLR2* revealed that some of the SNPs possibly play important role in disease resistance or disease susceptibility, which can be further authenticated by association study with some of the important bovine infectious diseases.

References

- Das, D. N., Rao, M. K., Shrihari, V. G., Reddy, A.O. and Murthy, L. K. 2009. Characterization of natural resistance associated macrophage protein (NRAMP1) partial gene in Malnad Gidda cattle. *Indian Journal of Animal Sciences*. 79 (7):720-721.
- Ramesha, K.P., 2007. 'Role of indigenous cattle genetic resources in the modern IPR regime' invited lead paper presented at the international conference "Vishwa Gou Sammelan" at Hosanagar, Shimoga, Karnataka during 21-29 April 2007.
- Ramesha, K.P., Jeyakumar, S., Kataktalware, M.A., Bandla Srinivas, Das, D.N., Varalakshmi, S. and Nagaraja, 2015. In Book: Malnad Gidda: Unique Indigenous Cattle of Western Ghats, Published by the Head, Southern Regional Station, ICAR-National Dairy Research Institute, Bengaluru-560030, India. Pp : 45-55.
- Ingvarstsen K.L and Moyes K., 2013. Nutrition, immune function and health of dairy cattle. *Animal Genetics*, 7:

- 112–122.
- Janeway CA, JR and Medzhitov, R., 2002. Innate immune recognition. *Annual Review of Immunology*, 20: 197–216.
- Medzhitov, R., Janeway, C., 2000. Innate immunity: impact on the adaptive immune response. *N. Engl. J. Med.* 343:338-44.
- Campos, M.A., Almeida, I.C., Tacheuchi, O., Akira, S., Valenta, E.P., Procopio, D.O., Travossos, L.R., Golenbock, D.T. and R.T.Gazzinelli, 2001. Activation of Toll like receptor-2 by glycosyl phosphatidy linositol anchors from a protozoan parasite. *Journal of Immunology*, 167: 416-423.
- Goldammer, T., Zerbe, H., Molenaar, A., Schuberth, H. J., Brunner, R. M., Kata, S. R., and Seyfert, H.M., 2004. Mastitis Increases Mammary mRNA Abundance of Defensin 5, Toll-Like-Receptor 2 (*TLR2*), and *TLR4* but Not *TLR9* in Cattle, *Clinical and diagnostic laboratory immunology*, 11:174–185.
- Miller, S. A., Dykes, D. D. and Polesky, H. F., 1988. A sample salting out procedure for extraction of DNA from human nucleated cells. *Nucleic Acids Res.*, 16: 1215.
- Shreekant, S., 2017. Identification of Variants in Bovine *TLR2* Gene and its association with somatic cell count, Masters Thesis, submitted to Veterinary College, Bangalore, KVAFSU.
- Shivakumara, P.N., Aravindakshan,T.V.and Naicy,T., 2015. Molecular characterization of Toll-like receptor 2 in Vechur cattle, NCBI Gene bank accession No.KT862891.1.
- Tantia,M.S., Mishra,B., Bharani Kumar,S.T. and Vijn,R.K.,2008. *Bos indicus* Toll-like receptor 2, NCBI Gene bank accession No. EU413951.1.

How to cite this article:

Satheesha, G. M., R. Nagaraja, H. M. Yathish, A. Sahadev, R. Jayashree, A. M. Kotresh and Naveenkumar, S. 2021. Molecular Characterization and Expression Profile of Partial *TLR2* Gene in Malnad Gidda Cattle. *Int.J.Curr.Microbiol.App.Sci.* 10(01): 2879-2886.
doi: <https://doi.org/10.20546/ijcmas.2021.1001.334>