Original Research

# SNP1 (G-1539A) of Toll- Like Receptor 4 Gene Polymorphism in Indigenous Cattle of North East India *vis-a-vis* Crossbred Cattle

**Chukham Gohain[1], Keyolenu Yore[1], Lalhruaitluangi[1], G. Poulinlu[2], P. Mayengbam[3], N. Shyamsana Singh[4] and T.C. Tolenkhomba[5]***

College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, INDIA

[1,4&5]Department of Animal Genetics and Breeding
[2]Department of Veterinary Microbiology
[3]Department of Veterinary Physiology and Biochemistry

***Corresponding author:** drkhomba10@gmail.com

| Rec. Date: | Jun 16, 2018 06:00 |
| --- | --- |
| Accept Date: | Aug 12, 2018 04:44 |
| DOI | 10.5455/ijlr.20180616060026 |

## Abstract

*The study was conducted to study the polymorphism in bovine Toll-like Receptor 4 gene in 160 indigenous cattle of North East India (viz. Manipur, Mizoram and Assam) and crossbred cattle. Two different variants of SNP1 (G-1539A) of TLR4 gene viz. A and G were detected by PCR-RFLP using BglI. The frequency of A allele was predominant (0.513) among Manipur indigenous cattle. On the contrary, the frequency of G allele was predominant in indigenous cattle of Mizoram and Assam and crossbred cattle. Among the genotypes, GG genotype was found in moderate to intermediate frequency among the local cattle of North East India. While the genotype GG was high in the crossbred population. The population conforming to equilibrium indicated lack of selection pressure in these cattle population.*

**Key words:** Indigenous Cattle, Gene Frequency, North East India, Toll- like Receptor 4 Gene

**How to cite:** Gohain, C., Yore, K., Renthlei, L., Mayengbam, P., Singh, N., & Tolenkhomba, T. (2018). SNP1 (G-1539A) of Toll- like Receptor 4 Gene polymorphism in Indigenous Cattle of North East India vis-a-vis Crossbred Cattle. International Journal of Livestock Research, 8(12), 58-63. doi: 10.5455/ijlr.20180616060026

## Introduction

Toll-like receptors 4 (TLR4) have been identified as crucial molecules for detection of invading pathogens and induction of host defense mechanism through recognition of pathogen-associated specific molecular patterns (Netra *et al.,* 2017). TLR4 gene have been found associated with mastitis in cattle and play a central role in innate immunity. Goldammer *et al.* (2004) suggested that TLR2 and TLR4 genes played a role in the host response to inflammatory mastitis. TLR-4 is able to recognize Gram-negative bacteria lipopolysaccharide (endotoxin) such as *Escherichia coli* and *Klebsiella*, cell wall components of other

important bacteria and fungi such as *Mycobacterium tuberculosis*, *Aspergillus fumigatus, Cryptococcus neoformans* and *Candida albicans*, as well as cellular stress components, such as heat shock proteins, fibrinogen, among others (Bannerman, 2009). TLR4 is critical in the immune response against Gram negative bacteria and virus (Mariotti *et al.,* 2009). Researchers have focused on identifying more informative genetic markers to allow faster and more accurate selection of cattle resistant to mastitis (Wang *et al*., 2007, Wiggans *et al*., 2011, Chauhan *et al.*, 2016) and milk production (Noori *et al*., 2013). The indigenous cattle of Northeast India are mainly of the non-descript type. They are known for their adaptability and resistance to diseases, which need to be conserved as conservation and improvement of animal genetic resources available in different regions have been globally accepted.

The present work was therefore undertaken to study the genetic polymorphisms in SNP1 (G-1539A) position of TLR4 gene in the indigenous cattle of the three North East state of India vis-à-vis crossbred cattle by PCR-RFLP method.

## Materials and Methods

### Experimental Animal and Blood Sampling

The study was conducted on a total of 160 unrelated indigenous cattle (*Bos indicus*) of North East India (*viz*., Manipur, Mizoram and Assam) and crossbred cattle. A total of 40 animals each were selected from the indigenous animals of each state and crossbred cattle. These animals were randomly selected from field(s), private farm(s), institute(s) and organized herd(s) maintained in these states of North East India. Blood samples were collected aseptically from the jugular vein of the selected animals in vacutainer tubes containing EDTA. Cold chain was maintained during the transit of the sample from farm to laboratory and stored in deep freezer at –20˚C till further use.

### Genomic DNA Isolation

Genomic DNA was extracted using GeneJET Genomic DNA Purification Mini Kit (K0782, Thermo Fisher Scientific) according to the instruction manual. The quantity and quality of DNA were checked with a NanoDrop MultiscanGo Spectrophotometer (Thermo Scientific, USA). The primers and restriction enzyme used for PCR-RFLP analysis are given in Table 1.

**Table 1:** Gene location of locus, size of PCR product, primer sets, annealing temperature and restriction enzyme used for RFLF analysis

| Gene Position (primer) | | Primer Sequence (5´ - 3´) | RE | Product Size (bp) | TA (°C) | Reference |
|---|---|---|---|---|---|---|
| SNP1 | F | TTC TTC AAC CCA ACC CAC CT | *Bgl I* | 546 | 56 | Li *et al.*, 2014 |
| (G-1539A) | R | GCC CTG GCT CAC CAC AAC TA | | | | |

### PCR and RFLP



 CC BY NC SA DOI 10.5455/ijlr.20180616060026

The PCR amplification was carried in a 25 µl of 10X PCR buffer, 2mM of $MgCl_2$,200 µM of each dNTPs, 5 pM each of primers, 2 U Taq DNA polymerase and 60 ng genomic DNA. The following cycles were applied: at 95ºC for 5 min, followed by 35 cycles of – 95ºC for 30 sec, 56ºC for 45 sec, 72ºC for 30 sec and final synthesis at 72ºC for 10 min. The amplified DNA was digested with *Bgl*-I enzyme by incubating at 37ºC for 3 hours. The digested products were separated in 2.5% agarose gel in 0.5 X TAE containing 1.0 µM ethidium bromide and visualized under UV trans-illuminator and photograph were taken using Gel Doc system.

**Statistical Analysis**

The allele and genotype frequency calculation as well as the chi-square test were carried out by using the Popgene32 software (Yeh *et al.,* 1997).

**Results and Discussion**

**Genetic Polymorphism in SNP1 (G-1539A) of TLR4 Gene**

The digestion of 546 bp PCR amplified fragment with *Bgl*I yielded three types of restriction patterns and accordingly three genotypes were identified. The genotype AA showed a 546 bp fragment since there was no restriction site for the enzyme in A allele. The GG genotype produced a 423 bp and 123 bp fragments. The heterozygous GA genotype yielded a restriction pattern of 3 (546 bp, 423 bp and 123 bp) fragments (Fig. 1).

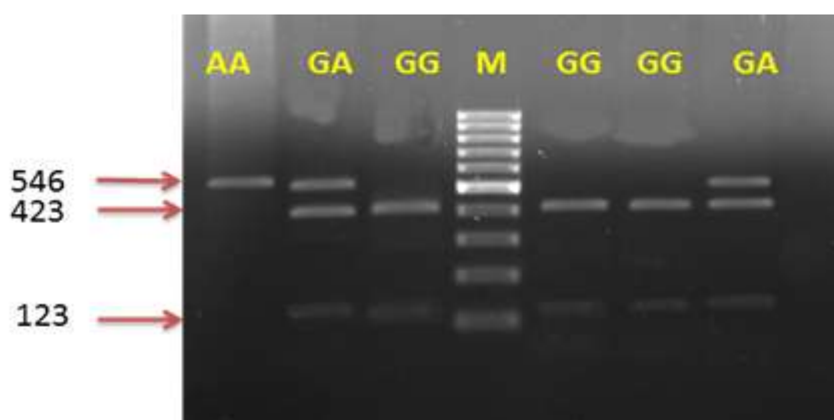


**Fig. 1:** Genotype of SNP1 (G-1539A) of TLR4 gene digested with *Bgl*I in 3 % agarose gel

The SNP1 (G-1539A) was found to be polymorphic with two alleles G and A in all the four cattle populations studied. Similar polymorphism of this locus / position was also reported by Li *et al*. (2014) in Chinese Holstein cattle.



 DOI 10.5455/ijlr.20180616060026

**Genotypic and Allelic Frequencies in SNP1 (G-1539A)/*Bgl* I**

In Manipur indigenous cattle, the frequency of the heterozygous GA genotype was slightly higher than the other two homozygous genotypes, which showed almost similar frequencies. The predominant genotypes in indigenous cattle of Mizoram and Assam were GG (0.50 and 0.55) and GA (0.42 and 0.45), but AA genotype was almost absent (0.08 and 0.00). However, GG genotype (0.70) was markedly higher than that of GA genotype (0.22) with a very low frequency of AA genotype (0.08) in crossbred cattle. In an earlier study (Li *et al*., 2014), higher frequency of GG genotype (52.12%) than those of GA (38.65%) and AA (9.23%) genotypes in Chinese Holstein had also been observed.

In the present study, the A and G allele frequency of SNP1 (G-1539A) were found to be almost similar (0.513 and 0.487) for indigenous cattle of Manipur. However, in indigenous cattle of Mizoram and Assam, the frequency of A allele (0.287 and 0.225) was lower than the G allele. Similar trend as in Mizoram and Assam indigenous cattle, was also observed in crossbred cattle (0.187 and 0.813). The present findings except in indigenous cattle of Manipur were in close agreement with the findings of Li *et al*. (2014), who reported that G allele was dominant among Chinese Holstein cattle with a frequency of 71.45% which was markedly greater than A (28.55%) allele. As the animals with GG genotype had much higher 305 days milk yield and SCS than those with the GA and AA genotypes (Li *et al*., 2014), the findings showed that no selection has been applied for genetic improvement of milk yield in the indigenous cattle of the three states studied. The results of higher frequencies of GG and GA genotypes with almost very low or nil AA genotype in Mizoram and Assam indigenous cattle might be due to sampling error. Whereas, in crossbred cattle, the findings of much higher frequency of GG genotype may be attributed to the genetic contribution of HF into the indigenous cattle of the North East region of India.

**Table 2:** Genotypic frequency of SNP1 (G-1539A) in indigenous cattle of Manipur, Mizoram and Assam and crossbred cattle

| Genotype | Types of Cattle | | | |
|---|---|---|---|---|
| | **Manipur** | **Mizoram** | **Assam** | **Crossbred** |
| AA | 0.30 (12) | 0.08 (3) | 0.00 (0) | 0.08 (3) |
| GA | 0.42 (17) | 0.42 (17) | 0.45 (18) | 0.22 (9) |
| GG | 0.28 (11) | 0.50 (20) | 0.55 (22) | 0.70 (28) |
| $\chi^2$ value | 0.89 $^{NS}$ | 0.06 $^{NS}$ | 3.37 $^{NS}$ | 2.74 $^{NS}$ |

*NS = Non significant at P<0.05; Values within the parentheses are the number of animals*





CC BY NC SA

DOI 10.5455/ijlr.20180616060026

**Table 3:** Allelic frequency of SNP1 (G-1539A) in indigenous cattle of Manipur, Mizoram and Assam and crossbred cattle

| Allele | Types of Cattle | Number | Frequency |
|---|---|---|---|
| A | Manipur | 42 | 0.513 |
| | Mizoram | 24 | 0.287 |
| | Assam | 18 | 0.225 |
| | Crossbred | 16 | 0.187 |
| G | Manipur | 38 | 0.487 |
| | Mizoram | 56 | O.713 |
| | Assam | 62 | O.775 |
| | Crossbred | 64 | 0.813 |
| Total | | 320 | |

**PIC and H values**

The present findings of PIC values (0.268 to 0.374) and H (0.320 to 0.498) indicated that the SNP1 (G-1539A) position showed moderate polymorphism and genetic variability in all the populations, which inferred the presence of genetic potential of this position of TLR4 gene for selection. The highest values of PIC and H for SNP1 (G-1539A) of TLR4 gene were observed in Manipur indigenous cattle compared to other populations implied that the polymorphism and the genetic variability in this position of TLR4 gene was higher than that of other populations.

**Table 4:** PIC values and Heterozygosities (H) in TLR4 gene positions viz. SNP1 (G-1539A), in three native cattle of Manipur, Mizoram and Assam and Crossbred cattle

| Gene Position | Types of Cattle | No. of Animals | No. of Alleles | PIC | H |
|---|---|---|---|---|---|
| SNP1 (G-1539A) | Manipur | 40 | 2 | 0.374 | 0.498 |
| | Mizoram | 40 | 2 | 0.331 | 0.42 |
| | Assam | 40 | 2 | 0.287 | 0.348 |
| | Crossbred | 40 | 2 | 0.268 | 0.32 |

**Conclusion**

It can be concluded from above findings that PCR-RFLP is an efficient molecular technique for identification of the variants in SNP1 (G-1539A) position. The finding reveals that no selection has been applied for genetic improvement of milk yield in the indigenous cattle (Zebu) of the three states studied. Lower PIC values and heterozygosity suggested the scope for formulation of suitable breeding strategies to perpetuate the distribution of desirable alleles in these native cattle.

**Acknowledgment**

The authors are thankful to Central Agricultural University, Imphal, Manipur and all the staff of Department of Animal Genetics and Breeding, C.V. Sc. & A.H., Selesih, Aizawl, Mizoram for providing financial support and technical assistance respectively during the course of study





CC BY NC SA

DOI 10.5455/ijlr.20180616060026

**References**


1. Yeh FC, Yang RC, Boyle TBJ, Ye ZH and Mao JX. 1997. POPGENE the user friendly shareware for population genetic analysis. University of Alberta, Canada.
2. Goldammer T, Zerbe H, Molenaar A, Schuberth HJ, Brunner RM, Kata SR and Seyfert H. M. 2004.. Mastitis increases mammary mRNA abundance of beta defensin 5, toll-like-receptor 2 (TLR2) and TLR4 but not TLR9 in cattle. *Clin. Diagn. Lab. Immunol.* 11: 174–185.
3. Li Z, Zhang H, Wang H, Chen L, Wang L, Liu X, Song A and Ru C. 2014. Polymorphism in the promoter of TLR4 gene by PCR-RFLP and its association with somatic cell score in Chinese Holstein. *Arch. Tierz.* 57: 1-6.
4. Bannerman DD. 2009. Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows. *J. Anim. Sci.* 87(13): 10-25.
5. Mariotti M, Williams JL, Dunner S, Valentini A and Pariset L. 2009. Polymorphisms within the toll-like receptor (TLR)-2, -4, and -6 genes in cattle. *Diversity.* 1(1): 7–18.
6. Netra MK, Gahlot G, Ashraf M, Agrawal VK and Dhakad GS. 2017. Genotypic Variations of Toll-Like Receptors 4 Gene and its Association with Intra-Mammary Infections in Rathi Cattles. *Intl J Livestock Res.* 7(8), 274-280.
7. Wang X, Xu S, Gao X, Ren H and Chen J. 2007. Genetic polymorphism of TLR4 gene and correlation with mastitis in cattle. *Journal of Genetics and Genomics*. 34(5), 406-412.
8. Wiggans G R, Van Raden PM and Cooper TA. 2011. The genomic evaluation system in the United States: Past, present, future. *Journal of dairy science*. 94(6): 3202-3211.
9. Chauhan S, Das D, Mundhee U and Soumya NP. 2016. Association of polymorphism in exon 3.3 toll like receptor 4 gene with milk somatic cell count in Deoni and Holstein Friesian crossbred cattle. *J. Cell Tissue Res.* 16(2): 5655-5659.
10. Noori R, Mahdavi AH, Edriss MA, Rahmani HR, Talebi M and Soltani-Ghombavani M. 2013. Association of polymorphism in Exon 3 of toll-like receptor 4 gene with somatic cell score and milk production traits in Holstein dairy cows of Iran. *S. Afr. j. anim. sci.* 43(4), 493-498.




CC BY NC SA



DOI 10.5455/ijlr.20180616060026