

# IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN *TOLL-LIKE RECEPTOR* GENES - *TLR2*, *TLR4* AND *TLR9* BY NEXT GENERATION SEQUENCING IN VECHUR CATTLE

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## ABSTRACT

Toll like receptors play a key role in the innate immune system. These receptors are expressed in membrane compartments and on the cell surface of both immune cells like leukocytes and non-immune cells like epithelial cells and endothelial cells. The *TLR* genes, *TLR2*, *TLR4* and *TLR9* were involved in the recognition of pathogens and activation of innate immunity. *TLR9* gene is particularly involved in the recognition of viral pathogens and thereby initiating antiviral responses. DNA isolated from blood sample of Vechur cattle well known for disease resistance was subjected to exome sequencing by next generation sequencing approach. Bioinformatics analysis of data by comparing with *Bos taurus* reference genome identified twenty SNPs (9 silent and 11 missense) in *TLR2* gene, thirteen SNPs (11 silent and 2 missense) in *TLR4* gene and 2 silent SNPs in *TLR9* gene.

**Keywords:** Vechur cattle, exome sequencing, *TLR* genes, single nucleotide polymorphism

## INTRODUCTION

As per National Bureau of Animal Genetic Resources (NBAGR), Vechur is an indigenous cattle breed of Kerala. The population of this cattle breed showed a drastic reduction during the last few decades due to the popularity of high producing crossbred animals throughout Kerala. These crossbred animals showed an increased susceptibility to various infectious diseases, at the same time Vechur cattle is believed to be resistant to most of these diseases. When an infectious agent enters a living organism, the challenging role of the host immune system is to recognize it as a foreign agent and to mount a rapid defense response. The first line of defense against an invading agent is the innate immune response. Members of toll like receptor family play an important role in pathogen recognition and activation of innate immunity. Takeda and Akira (2003) performed functional characterization of Toll like receptors and stated that these gene products control innate immune responses. They also opined that TLR mediated innate immunity activation

controls host's defense against pathogen and immune disorders. Bovine *TLR2* gene is located on chromosome 17, bovine *TLR4* is on chromosome 8 and bovine *TLR9* is on chromosome 22. *TLR2* gene has the ability to recognize widest range of pathogen associated molecular patterns and involved mainly in recognition of mycobacteria and gram positive bacteria (Borrello *et al.*, 2011). *TLR4* gene is involved in the recognition of bacterial lipopolysaccharides (Yan, 2006). Four members of *TLR* family (*TLRs* 3, 7, 8 and 9) were considered as the candidate genes for viral disease resistance or susceptibility studies in cattle (Cargill and Womack, 2007). *TLR9* gene is involving in the recognition of viral pathogen associated molecular patterns which are essential to initiate an antiviral response.

#### MATERIALS AND METHODS

Blood sample collected from the Vechur cattle maintained in Vechur Cattle Conservation Unit of Kerala Veterinary and Animal Sciences University was used for the present study.

DNA was isolated from whole blood collected from Vechur cattle using phenol chloroform method with modifications. The quality of isolated DNA was checked by agarose gel electrophoresis and the purity of DNA samples were checked using Nano drop spectrophotometry. DNA samples showing optical density ratio 260/230 between 1.7 and 1.8 indicating good quality DNA was used further.

The isolated genomic DNA was sheared and the coding regions of Vechur cattle genome was captured using All Exon Bovine Agilent Sure Select - 54Mb capture Kit and captured fragments were

sequenced using Illumina HiSeq 2500 platform. Sequenced fragments were then aligned to *Bos taurus* reference genome using Burrows Wheeler Aligner. A variant was the base pair unable to match with reference sequence (Nayarisseri *et al.*, 2013). The variants were identified using the Samtools Version-0.1.18.

#### RESULTS AND DISCUSSION

Mapping and analysis of the obtained sequence reads with *Bos taurus* reference genome revealed the presence of 20 SNPs in *TLR2* gene (11 missense and 9 silent), 13 SNPs in *TLR4* gene (2 missense and 11 silent) and 2 silent SNPs in *TLR9* gene. Details of the identified variants are given in Table 1.

Out of the total variants identified, 13 were missense which resulted in an amino acid change in corresponding proteins. The remaining 22 SNPs were silent which did not involve any amino acid changes. Degeneracy of genetic code (different codons specifying the same amino acids) implies that these mutations in coding sequences as silent, not affecting the protein sequence (Goymer, 2007).

Out of 13 missense mutations 11 were in *TLR2* gene. These missense mutations include one with the replacement of an amino acid threonine with methionine. Threonine is a small polar neutral amino acid and methionine is a sulphur containing amino acid usually packed in the protein interior thereby becoming less reactive (Betts and Russel, 2003). Second missense mutation in *TLR2* gene substituted the amino acid arginine with histidine. Arginine is a positively charged amphipathic molecule containing hydrophobic and polar areas whereas the substituted amino acid

histidine is polar and neutral. Fourth missense mutation in *TLR2* gene changed the amino acid serine with alanine. Serine is a polar neutral amino acid and alanine is a small hydrophobic amino acid. The remaining seven missense mutations in *TLR2* gene include the replacement of neutral polar amino acid asparagine with another neutral polar amino acid serine, neutral polar amino acid histidine with another neutral polar amino acid glutamine, hydrophobic isoleucine with hydrophobic valine, amphipathic arginine with another amphipathic amino acid lysine, hydrophobic phenyl alanine with another hydrophobic amino acid leucine.

The neutral and polar threonine is exchanged with a hydrophobic amino acid methionine which is mostly non-reactive. The neutral polar amino acid histidine is replaced with amphipathic glutamine and the small amino acid glycine is replaced with serine. The amino acid glycine has an unique functional role in binding with phosphates (Schulze-Gahmen *et al.*, 1996). So the replacement of this amino acid with any other amino acid is having a drastic impact on function (Betts and Russel, 2003).

Missense mutations identified in *TLR4*

genes included the change of a neutral polar amino acid asparagine with another neutral polar amino acid threonine and replacement of hydrophobic small amino acid alanine with amphipathic glutamic acid. The two SNPs identified in *TLR9* gene were silent without any effect on corresponding amino acids.

## SUMMARY

The single nucleotide polymorphisms identified in the genes can contribute as markers relating the predisposition or resistance to diseases. These variations in amino acids in the selected three genes could produce changes in the corresponding protein and thereby changes in protein function which might be a reason for increased resistance to several infectious diseases in Vechur cattle compared to other exotic breeds.

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**Table 1.** List of SNPs of *TLR2*, *TLR4* and *TLR9*

| Sl. No. | Gene        | Chromosome No. | Position | Reference base | Alternate base | Reference codon | Changed codon | Reference amino acid | Changed amino acid |
|---------|-------------|----------------|----------|----------------|----------------|-----------------|---------------|----------------------|--------------------|
| 1       | <i>TLR2</i> | 17             | 3950892  | G              | A              | CCC             | CCT           | P                    | P                  |
| 2       | <i>TLR2</i> | 17             | 3950973  | C              | T              | GAG             | GAA           | E                    | E                  |
| 3       | <i>TLR2</i> | 17             | 3951132  | A              | G              | ATT             | ATC           | I                    | I                  |
| 4       | <i>TLR2</i> | 17             | 3951162  | A              | G              | CAT             | CAC           | H                    | H                  |
| 5       | <i>TLR2</i> | 17             | 3951192  | G              | C              | CAC             | CAG           | H                    | Q                  |
| 6       | <i>TLR2</i> | 17             | 3951373  | G              | A              | ACG             | ATG           | T                    | M                  |

|    |      |    |           |   |   |     |     |   |   |
|----|------|----|-----------|---|---|-----|-----|---|---|
| 7  | TLR2 | 17 | 3951405   | C | A | GCG | GCT | A | A |
| 8  | TLR2 | 17 | 3951408   | T | G | GCA | GCC | A | A |
| 9  | TLR2 | 17 | 3951499   | C | T | CGC | CAC | R | H |
| 10 | TLR2 | 17 | 3951555   | A | G | TTT | TTC | F | F |
| 11 | TLR2 | 17 | 3951683   | A | C | TCA | GCA | S | A |
| 12 | TLR2 | 17 | 3951879   | T | G | GGA | GGC | G | G |
| 13 | TLR2 | 17 | 3951937   | T | C | AAC | AGC | N | S |
| 14 | TLR2 | 17 | 3951973   | G | A | ACG | ATG | T | M |
| 15 | TLR2 | 17 | 3952177   | C | T | AGA | AAA | R | K |
| 16 | TLR2 | 17 | 3952209   | A | T | CAT | CAA | H | Q |
| 17 | TLR2 | 17 | 3952506   | A | T | TTT | TTA | F | L |
| 18 | TLR2 | 17 | 3952556   | T | C | ATT | GTT | I | V |
| 19 | TLR2 | 17 | 3952985   | C | T | GGC | AGC | G | S |
| 20 | TLR2 | 17 | 3953001   | A | G | AAT | AAC | N | N |
| 21 | TLR4 | 8  | 108829437 | C | A | ACC | ACA | T | T |
| 22 | TLR4 | 8  | 108834032 | G | A | CAG | CAA | Q | Q |
| 23 | TLR4 | 8  | 108837116 | A | C | AAC | ACC | N | T |
| 24 | TLR4 | 8  | 108837704 | C | A | GCA | GAA | A | E |
| 25 | TLR4 | 8  | 108837783 | A | G | CCA | CCG | P | P |
| 26 | TLR4 | 8  | 108837831 | T | G | GGT | GGG | G | G |
| 27 | TLR4 | 8  | 108838185 | A | G | CAA | CAG | Q | Q |
| 28 | TLR4 | 8  | 108838320 | C | T | AGC | AGT | S | S |
| 29 | TLR4 | 8  | 108838431 | T | C | AGT | AGC | S | S |
| 30 | TLR4 | 8  | 108838491 | T | C | TGT | TGC | C | C |
| 31 | TLR4 | 8  | 108838539 | C | T | AAC | AAT | N | N |
| 32 | TLR4 | 8  | 108838656 | C | A | GGC | GGA | G | G |
| 33 | TLR4 | 8  | 108838692 | T | C | GAT | GAC | D | D |
| 34 | TLR9 | 22 | 49231107  | C | T | GCC | GCT | A | A |
| 35 | TLR9 | 22 | 49232646  | G | C | GCG | GCC | A | A |

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