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Computational characterization of deleterious SNPs in Toll-like receptor gene that potentially cause mastitis in dairy cattle

Sundaresan Bhavaniramya[a,*], Ramar Vanajothi[b], Selvaraju Vishnupriya[a], Mohammad Saleh Al-Aboody[c], Rajendran Vijayakumar[c], Dharmar Baskaran[a,**]

[a] College of Food and Dairy Technology, TANUVAS, Chennai-600052, Tamil Nadu, India
[b] Department of Zoology, Fatima College, Madurai 625001, Tamil Nadu, India
[c] Department of Biology, College of Science in Zulfi, Majmaah University, Majmaah-11952, Saudi Arabia

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ABSTRACT

Mastitis is a highly prevalent disease in dairy cattle that cause high economic losses, because of the diminished production of milk and decreased milk quality. Toll-like receptors (TLRs) play a crucial role in the induction of innate immune responses and especially, in the mammary gland cells, it can pick out the invading pathogens. Gene expression shows considerable variation occurs in TLRs and several SNP's are linked with altered susceptibility to infectious diseases. Through this study, the deleterious SNPs in TLR2, TLR4, TLR6 and TLR10 are identified and reported along with available inhibitors interactions with native and mutant form of TLR. Initially, the experimentally reported non-synonymous SNPs of different types of TLRs is retrieved and analyzed by SFIT, PolyPhen, I- Mutant, Consurf and HOPE servers. The results obtained from various tools used in the study shows that the deleterious non-synonymous SNPs (nsSNPs) of TLR2 may responsible for the mutation of Arginine to Histidine at 563 and Threonine to Methionine at 605 and exhibit greatest impact on protein stability. Additionally, the 3D structure of both native and mutant forms of TLR2 is modeled and the interactions are analyzed with currently available inhibitors. Overall, the present study reveals the deleterious nsSNPs in TLR2 with mutation (T563H and T605M), which may affect the structure and function of TLR2.

1. Introduction

India is one of the well-known countries for its dairy products and one of the largest milk producers in the world with 165 million tons of milk during the year 2016–17. The dairy sector alone is contributing about 4% of the gross domestic product (GDP) and 26% of agricultural GDP (NAS, 2012). In considering the future demands, India has adopted the crossbreeding policy, in focus to enhance the milk production. However, dairy sector faces many challenges which affect the milk production and thereby leads to the greatest economic loss in dairy sectors. Among the various challenges, bovine mastitis is one of the adverse diseases in cattle associated with major economic importance and instigating the reduced milk quality and leading loss of milk production (Henna Hamadani et al., 2013; Yang et al., 2011; Das et al., 2016; Prathap Pragna et al., 2017). The clinical mastitis alone causes 2 billion dollars in the USA and 526 million-dollar losses in India, and it was estimated that approximately 70% of economic losses (Joshi and Gokhale, 2006; Vishnoi and Dang, 2007). Several bacterial species including *Escherichia coli, Streptococcus dysgalactiae, Streptococcus tubris and Streptococcus agalactiae* are important causative organisms of mastitis and played a major role in health of dairy cattle (Mason, 2005). Broadly speaking, the pathogen infection effectively activates the immune system in the udder of cattle animals at the same time the virulence of pathogens evokes the host immune system via various ways. The increased level of somatic cell in milk is one of the marked events which leads deterioraion in udder health and affects the quality of milk due to the presence of high amount of lipase and protease enzymes. These enzymes are extremely heat resistant and create difficulty during processing of milk and milk products (Kumar et al., 2013; Barbano et al., 2006; Belkaid and Hand, 2014).

Relevant to restrain the infection of pathogen activity, the mammalian innate immune system provides a host defense mechanism against a diversity of pathogens. In mammalian mammary gland, the immune system cells function with epithelial cells and are effectively recognize the invading pathogens via TLRs (Chen et al., 2013). The activation of TLRs significantly triggers the expression of inflammatory

* Corresponding author.
** Corresponding author. College of Food and Dairy Technology, TANVAS, Alamathi, Koduveli, Chennai, 600052, Tamil Nadu, India.
E-mail addresses: sbhavaniramya@gmail.com (S. Bhavaniramya), dharmabas@gmail.com (D. Baskaran).

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Table 1
Shows of nonsynonymous SNPs predicted with SIFT and Polyphen chosen SNPs with PSIC SD equal 1 and TOLERANCE INDEX rang (0–0.001).

S.No	SNPs	Nucleotide change	Amino acid change	SIFT score	Type	PolyPhen (PICS Score)	Type
1	rs8193049	A/C	N151T	1	Tolerated	0.00	Benign
2	rs8193053	C/A	A347E	0.89	Tolerated	0.508	Possible damaging
3	rs8193055	A/G	K381R	0.009	Deleterious	0.423	Possible damaging
4	rs8193066	G/A	V501I	0.723	Deleterious	0.015	Benign
5	rs8193069	C/T	T674I	0.777	Deleterious	0.00	Benign
6	rs68268260 (TLR2)	C/T	R563H	0.001	Deleterious	0.906	Possible damaging
7	rs68343170 (TLR2)	G/A	T605M	0.027	Deleterious	0.215	Benign
8	rs68268270 (TLR6)	A/C	L43R	0.040	Deleterious	1.000	Possible damaging
9	rs55617286 (TLR10)	G/C	I/M	0.005	Deleterious	1.000	Possible damaging

Table 2
Shows of nonsynonymous SNPs predicted with PhD-SNP.

S.No	SNPs	Type	Ploymorphism
1	rs55617286 (TLR10)	Neutral (6)	Disease related polymorphism
2	rs68268270 (TLR6)	Neutral (3)	Disease related polymorphism
3	rs68343170 (TLR2)	Neutral (8)	Disease related polymorphism
4	rs68268260 (TLR2)	Neutral (5)	Disease related polymorphism
5	rs8193053 (TLR4)	Neutral (5)	Disease related polymorphism
6	rs8193055 (TLR4)	Neutral (2)	Disease related polymorphism

Table 3
Represents the stabilities of nsSNPs by I-Mutant 2.0 and results of MutPred.

S.No	SNPs	I-Mutant	MutPred	
		DGG Score	Amino acid Change	MutPred Score
1	rs8193055 (TLR4)	−0.05	A347E	0.308
2	rs8193053 (TLR4)	−0.05	K381R	0.348
3	rs68268260 (TLR2)	−0.64	R563H	0.437
4	rs68343170 (TLR2)	0.01	T605M	0.252
5	rs68268270 (TLR6)	−0.03	L43R	0.873
6	rs55617286 (TLR10)	−1.06	I134M	0.205

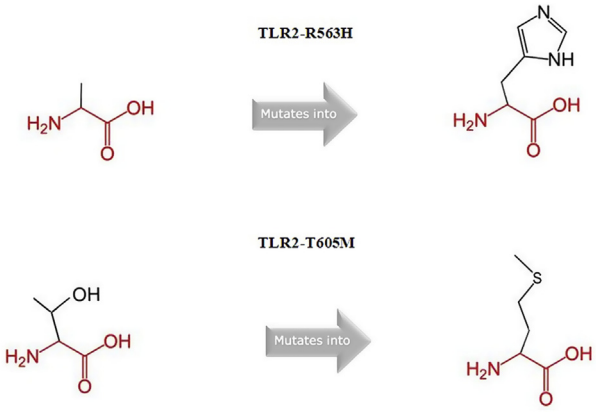

Fig. 1. Predicted mutation of LTR2 R563H and T605M by HOPE program.

cytokines subsequently regulate the immune reaction, cell differentiation and apoptosis (Ibeagha-Awemu et al., 2008; Yang et al., 2007; Cates et al., 2009; Diamond et al., 2009). TLRs are expressed in antigen-presenting cells and encodes type I transmembrane protein consists of a N-terminal leucine rich repeats (LLR), which play a crucial role in ligand recognition and transmembrane domain and C-terminal intracellular Toll/IL-1 receptor domain are involved in signal transduction (Vasselon and Detmers, 2002; Kaisho and Akira, 2006; West et al., 2006). The mammalian TLRs consist of six gene family members such as TLR1, TLR2, TLR4, TLR5, TLR6, TLR9 and TLR10 and are meritoriously involved in recognition of pathogen associated molecular patterns (PAMPs) and initiate the development of full immunological response (Lester and Li, 2013). TLR2 and TLR4 have been linked up with mastitis in cattle and play key role in host inflammatory mastitis. Various works have been taken away to discover the impact of genetic polymorphism in cattle animals which are associated with immune response. Opsal et al. (2008) reported that the dense linkage map comprising single nucleotide polymorphism in TLR2 and TLR4 of bovine chromosome.

The study carried out by, Jann et al. (2008) reported that the TLR2 polymorphisms at 227, 305 and 326 position are functionally important and are studied as candidate of SNPs for immune related response in cattle. TLR2 is mainly recognizing the bacterial cell wall component such as polysaccharides and highly expressed during mastitis caused by pathogens. It clearly indicated that TLR genes play a key role in host response to inflammatory mastitis caused various bacterial species. Single nucleotide polymorphisms (SNPs) are single base variants present in the coding region and leads substitution of amino acid in the corresponding protein molecule. This mutation or replacement of single amino acid substitutions might alter the structure, function and stability of protein subsequently leads phenotypic effect (Yates and Sternberg, 2013; Ng and Henikoff, 2006; González-Castejón et al., 2011; Rodriguez-Casado, 2012). Several studies have been reported that the SNPs relationship with different diseases and related protein target (Barreiro et al., 2008; Wolf et al., 2012; Abecasis and Cookson, 2000). Prediction of SNPs can serve as a useful genetic marker and used to study the deleterious effect of SNPs on protein structure and function. Numbers of computational tools like SIFT, Polyphen and I-Mutant are often used for the analysis of SNPs and provides the basic information to understand the genetic basis of complex diseases. Based on the above information, present study was designed to identify the deleterious SNPs' mutation in the different TLR gene via *in silico* analysis to investigate the genetic variation and alteration in the function of TLR gene.

2. Methodology

The experimentally reported SNPs of four different TLR genes such as TLR2, TLR4, TLR6 and TLR10 is taken from literature (Fisher et al., 2011) for prediction of deleterious SNPs and damaging amino acid substitution. The protein sequences in the form of FASTA format were retrieved from UniProt at ExPASy database. The data were subjected various computational software as follows.

2.1. Analysis of functional effects of coding nsSNPs by SIFT method

Prediction of single amino acid substitutions and its deleterious effects on protein structure and function have the greatest attention to understand the mutation associated polymorphism in various diseases. Hence, in the present study, one of the Bioinformatics tools SIFT was used to identify the deleterious nsSNPs in four different TLR genes. SIFT is a multistep homology-based procedure which predicts the conservation level of amino acid position of protein. Initially, it searches the similar sequence and chooses closely related sequence which share similar function, then in the next step; it obtains the multiple alignment

Fig. 2. Analysis of evolutionary conserved amino acid residues of TLR2 (A) and TLR4 (B) by ConSurf. The color-coding bar shows conservation score. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

of the chosen sequence and calculates the normalized probabilities for all possible amino acid substitutions. Amino acid/Nucleotide substation at each position with normalized probabilities less than 0.005 are predicted as deleterious and those greater than or equal to 0.05 are predicted to be tolerated (Ng and Henikoff, 2011; Ramensky et al., 2002). (Available at http://sift.jcvi.org/).

2.2. Polyphen2: for simulation of functional changes on coding nsSNPs

Polyphen2 is a widely used online program to predict the possible effect of amino acid substitution on both structure and function level based on several criteria including phylogenetic, structural information, sequence of the protein. Basically, Polyphen server gets amino acid sequence or UniProt protein ID as input to search the 3D structure, multiple sequence alignments of similar sequence and amino acids count information together with sequence position and amino acid variants, then calculate position-specific independent counts (PSIC) scores for each of the two variants and then compute the difference between the PSIC scores. A PSIC score difference was assigned the SNPs using the following categories; probably damaging, possibly damaging and potentially damaging (González-Pérez and López-Bigas, 2011; Ramensky et al., 2002; Vanajothi et al., 2012). (Available at: http://genetics.bwh.harvard.edu/pph).

2.3. Analyzing stability change in mutation by I-mutant 2.0

I-Mutant is a support vector machine and worked based on the Protherm database for the automatic prediction of protein stability changes upon a single amino acid mutation. I-Mutant estimates the value of free energy change of computing the unfolding Gibbs free energy value (DG) for the wild type protein and subtracting it from that of the mutant protein. The predicted results of all the mutations in TLR gene may change the protein stability with related free energy by $\Delta\Delta G$ value = ΔG (mutant protein) – ΔG (wild type) in kcal/mol at default pH

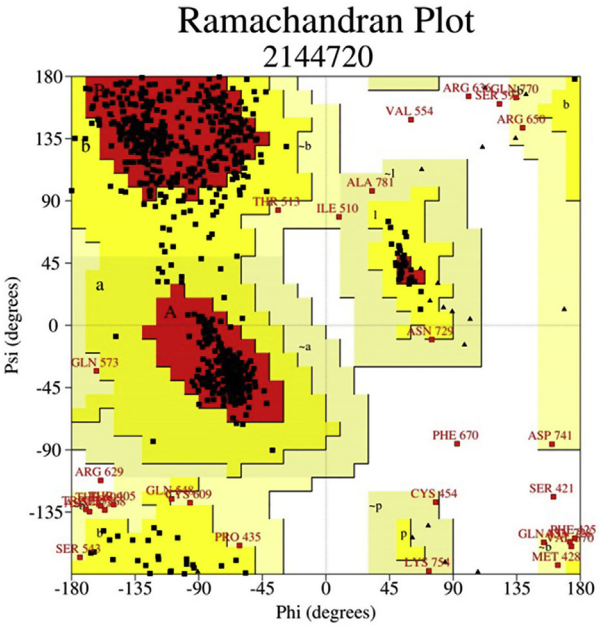

Fig. 3. Ramachandran map of LTR2 protein. The plot calculation on the three-dimensional (3D) model of LTR2 protein was calculated with the PROCHECK program.

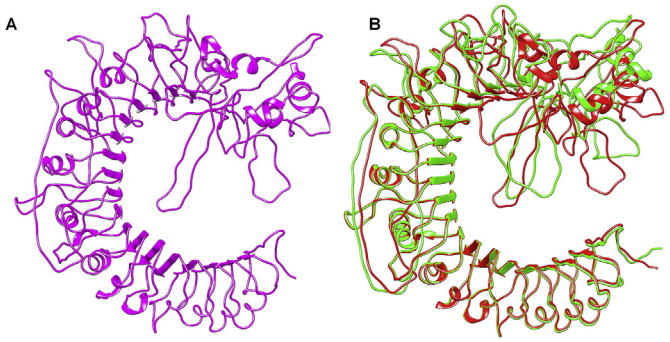

Fig. 4. (4a)Superimposed structure of native protein LTR2 (Pink) (4b); Native (red) and Mutant 1 (Green) and mutant 2 (Blue). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Shows the molecular interaction results of mutant and native protein with drug molecules.

Structure	Ligand	Docking Score	No. Hydrogen bond interaction	Interaction residues
Native	Oxytetracycline	−9.648	1	Glu430
	Sulphadimidine	−8.49	2	Pro429 (2), His289
	Sulphadiazine	−5.892	3	Pro429 (2)
Mutant 1 (R563H)	Oxytetracycline	−10.44	1	Glu430
	Sulphadimidine	−6.52	1	Ser330
	Sulphadiazine	−5.63	1	His289
Mutant 2 (T605M)	Oxytetracycline	−9.752	1	Glu430
	Sulphadimidine	−6.03	1	His289
	Sulphadiazine	−6.027	1	His289

7 and 25 °C. Four different outputs can be retrieved in this tool based on the selected mode. The positive DDG value indicates that the mutated proteins have high stability, whereas the negative value indicates lower stability (Capriotti et al., 2006; Capriotti et al., 2006). Available at http://gpcr2.biocomp.unibo.it/cgi/predictors/IMutant3.0/I-Mutant3.0.cgi.

2.4. PHD-SNP

PHD-SNP is another type of online support vector machine-based program used to predict the single point amino acid mutation. It provides the information about whether the amino acid substitution leads to disease or natural along with the reliability index score. Available athttp://snps.biofold.org/phd-snp/phdsnp.html.

2.5. Examination of amino acid change by MutPred

MutPred tool was used to predict the molecular basis of the disease linked pathogenic amino acid substitution. Basically, it uses several characteristic features associated with protein structure, function, and evolution. It effectively predicts the missense amino acids by combining three servers such as SIFT, PSI-BLAST and Pfam. The score shows greater than 0.50 is considered as pathogenic. (Available at: http://mutpred2.mutdb.org/index.html).

2.6. Project HOPE

Project HOPE is an easy-to-use online program to analyses the structural modification and its intended mutation. It provides the 3D structural information of both wild and mutant type residues and 3D visualization of mutated proteins by using Uniprot and DAS prediction servers. (Available at: http://www.cmbi.ru.nl/hope/home).

2.7. Analysis of amino acid evolutionary conservation

The Consurf web server is used to predict the amino acid evolutionary conservation in TLR2 and TLR4. It uses an empirical Bayesian method to determine the conserved amino acids and used to identify the putative structural and functional residues. The results show conservation score with a range of 1–10. The range between 1-4 is considered as variable, 5–6 as intermediate and 7–9 as conserved amino acid position (Landau et al., 2005; Ashkenazy et al., 2016). Available at Consurf (http://consurf.tau.ac.il/).

2.8. Protein modeling and structural analysis

Based on the SIFT, PolyPhen2 and I-mutant results, it was confirmed that the nsSNPs of TLR2 is more deleterious and leads the amino acid substitution subsequently leads the structural and functional implications of the protein. Hence the TLR2 alone was selected for the molecular modeling studies. Due to the unavailability of 3D structure of TLR2, homology modeling was performed using modeler software. The BLASTP results showed that the crystal structure of *Mus muscus* (PDB ID: 5D3I) was identified as the closest match of TLR2 (*Bos taurus*) with 72% sequence similarity recommended for homology modeling (Selvaraj et al., 2016). Based on the DOPE score value, the best model was selected for further analysis (Selvaraj et al., 2015a,b). The structural refinement of the predicted model was evaluated by set of analysis, such ERROT, PROSA, PROCHEK and WHATCHECK. Mutation and energy minimization of both native and mutant proteins were performed by the SWISS PDB viewer. The divergence of mutant structure from native is due to mutation, deletion and insertion amino acids are evaluated by RMSD values and visualization of 3D structure and superimposition of native and mutant was assessed by Pymol software.

2.9. Molecular interaction analysis

Auto Dock software was used to evaluate the binding energy and interactions between native and mutant proteins with currently reported inhibitors which widely used to treat mastitis. Both the modeled

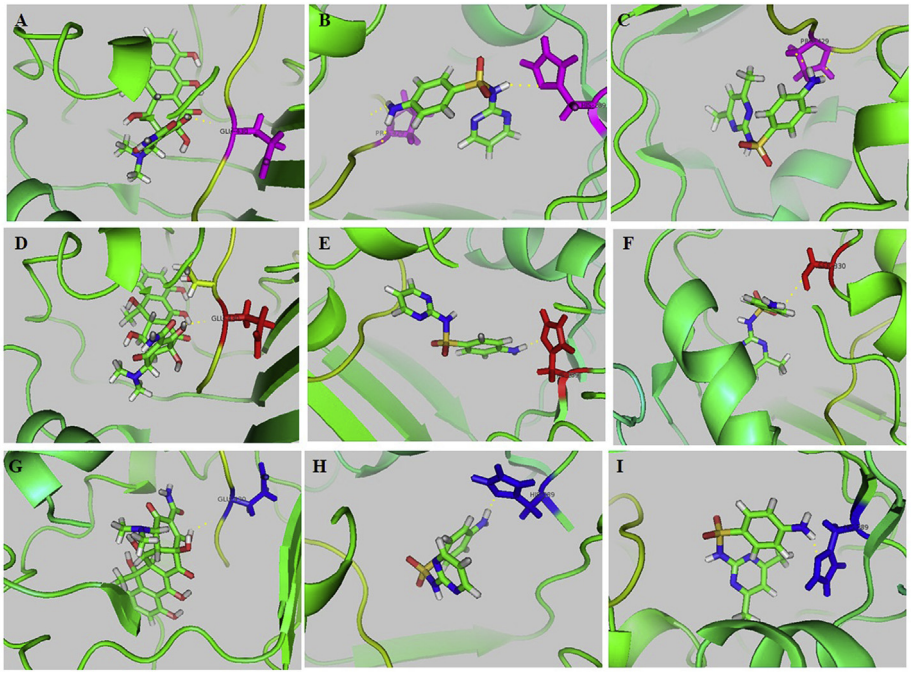

Fig. 5. Docked pose of Oxytetracycline, sulphadiazine and Sulphadimidine with native (A, B and C respectively), with mutant1 (D, E and F respectively) and mutant 2 (G, H and I).

native and mutant structures were prepared and saved as PDBQT format and the Auto Grid was generated with size set as 60 × 60 × 60 xyz points, grid space about 0.375 Å and grid center was designated at dimensions (x; −1.095, y; −1.554, z; −3.894). Auto Dock employs both iterated local and global optimizer (Blum et al., 2008). During the docking procedure, native and mutant protein and currently reported inhibitors are considered as rigid. Less than 1.0 Å RMSD were clustered together and the results were represented with the most favorable free energy binding. Each pose with lowest energy of binding or binding affinity was calculated and aligned with protein molecules for further analysis. The docked results were visualized using Schrödinger suite.

3. Results and discussion

Single nucleotide polymorphism is an important marker of choice for several genes associated diseases because SNPs is abundance, stable and is interspersed in the genome of every organism (Tchin et al., 2011). In the present study, experimentally predicted TLR gene single nucleotide polymorphisms were taken for computational analysis. About 45 non-synonymous in TLR2, 28 non-synonymous in TLR4, 15, 35 non-synonymous SNPs in TLR6 and TLR10 respectively were selected from the literature and are subjected to further analysis.

3.1. Analysis of functional consequences of coding nsSNPs by SIFT method and Polyphen2

Among the 28 nsSNPs in TLR4, rs8193055 was found to be deleterious with a tolerance index score 0.009 and 2 nsSNPs in TLR2, one nsSNP in TLR6 and one in TRL10 were found to be deleterious (Table 1). Of four TLR gene, 5 nsSNPs such as rs8193055, rs68268260, rs68343170 (TLR2), rs68268270 (TLR6) and rs55617286 (TLR10) showed a deleterious index score less than 0.05. This low tolerance index may lead the functional impact of amino acids and it also indicated that these predicted nsSNPs may influence the protein stability and its functionality (Ng and Henikoff, 2006). Further, the predicted deleterious nsSNPs were submitted to PolyPhen for analyzing the structural information in query sequences. Among the 9 predicted deleterious nsSNPs, 5 SNPs were predicted to be probably damaging and remaining were benign with Position-specific independent count (PSIC) score range from 0.00 to 1.00 (Table 1). The results obtained from both SIFT and PolyPhen servers indicated that the predicted deleterious nsSNPs shows significant correlations which could be précised important for the recognition of various disorders including mastitis.

3.2. Stability analysis of I-Mutant

The DDG score predicted by I-Mutant showed that the deleterious SNPs, such as rs8193055 (TLR4), rs68268260 (TLR2) and rs55617286 (TLR10) have the DDGI score value < −1.0 (Table 3). While other nsSNPs such as rs8193053 (TLR4), rs68343170 (TLR2), rs68268270 (TLR6) have a very low DDG score compared other SNPs. The high negative score of DDG values indicates that less stability, which may due to mutation (Capriotti et al., 2006). Based on the results predicted from above three programs, it was noticed that TLR2 and TLR4 were seen to be less stable, more deleterious and found to be possibly damaging. Hence, these two genes were taken for further analysis.

3.3. PHD-SNP and project HOPE

PHD-SNP is significantly analyze the disease associated nsSNPs, the result obtained SIFT and PolyPhen software, it was noticed that about six nsSNPs such as rs8193055, rs8193053 in TLR4, rs68268260, rs68343170 in TLR2, rs6826870 in TLR6 and rs5561786 in TLR10 were predicted to be a neutral polymorphism (Table .2). Based on the results, the most deleterious and damaging nsSNPs of TLR2 rs68268260 and rs68343170 were subjected to HOPE software. The results obtained from HOPE indicated that the mutant residues histidine (rs68268260) and methionine (rs68343170) was bigger than wild-type residues at position 563 and 605 and located in the more hydrophobic than the wild-type residue (Fig. 1).

3.4. MutPred analysis

Diseases associated amino acids and the substitution profile was analyzed by MutPred software and the results were summarized in Table 3. It also identifies the molecular mechanism of the amino acid

replacement instigated by deleterious SNPs. The resulted p-values scores of the amino acid replacement greater than 0.5 indicate that those are considered as actionable hypothesis, whereas p-value score less than 0.05 are considered as confident hypothesis. Deleterious nsSNPs mutations in TLR2, TLR4 and TLR6 were subjected to MutPred analysis and the results shows that the probability of a deleterious mutation score for TLR4 nsSNPs mutations A347E (0.308), K381R (0.348), TLR2 mutation R563H (0.437), T605M (0.252) and TLR10 mutation L43R (0.873), I134M (0.205).

3.5. Structural conformation and conservation analysis

The evolutionary rate of amino acid sequence was calculated depending on the similarity among the proteins and homologous structure for predicting the amino acid substitution that may have a deleterious effect (Ashkenazy et al., 2010; Ramensky et al., 2002). Fig. 2A and B shows the results predicted by Consurf of TLR2 and TLR4 respectively, which contains about 9 color variations based on the conserved amino acids and the conservation scale of each residue indicated the evolutionary relationships of TLR2 and TLR4 with a homologous sequence (Celniker et al., 2013). The conservation scale e represents the exposed residues, b; is a buried residue, f; indicated the highly conserved functional residues, s; is a predicted structural and highly conserved residues in the protein. From the result, it was noticed that TLR2 variants such as R563and T605 have the conserved scale 3 and the predicted mutation of TLR4 such as A347 and T K381 have the conservation scale 5. The experimentally predicted results also revealed that theTLR2 SNPs (rs68268260 and rs68343170) were responsible for amino acid substitution present on the LRR-functional domain of the TLR2 gene (Prebavathy et al., 2015). The result revealed that the residue of TLR2 (R563 and T605) is present in the conserved region and buried, which makes it an important functional residue in TLR2.

3.6. Homology modeling and structure validation

The tertiary structure of protein molecules plays a key role to understand the ability of protein interaction with other molecules and to study its functions (Hasan et al., 2011; Alshatwi et al., 2011; Schneider et al., 2018). Therefore, prediction of the damaged coding nsSNPs at the structural level of protein is crucial to understand its activity. Based on the computational results TLR2 protein was selected for further structural related work. Homology modeling combined with molecular dynamics simulation will be a frat tool for understanding the structural features of proteins which lacks experimental structures (Selvaraj et al., 2018). There is no crystal structure of TLR2 of *Bos taurus* in protein data bank. Hence the 3D structure of the TLR2 was constructed through homology modeling approach with the template of TLR2 of *Mus muscus* (PDB ID: 5D3I) with 72% sequence identity. Then the structural geometry of the final model of TLR2 was evaluated with Ramachandran's plot calculation using PROCHECK program, it revealed the backbone and dihedral angles of the modeled structure contains 80.1% residues in the most favorable region, 15.1% and 3.2% in additionally allowed and generously allowed regions respectively (Fig. 3). In order to compare the stability of native proteins and the mutant structure at respective position, the mutation resides such as rs68268260 and rs68343170 of TLR2 were subjected to various *in silico* nsSNPs analysis tools. The position of the mutation and the associated amino acid of nsSNPs such as R/H at the position of 563 and T/M at the position of 605 were mapped on modeled structure. The mutation and energy minimization for the modeled structure was performed by using the SWISS-PDB viewer. The native and mutant structures (R563H and T605) can be seen that the total energy is found to be −27891.74, −24830.48 and −23388.04 respectively. The super impositions model of native and two mutant structures is performed using Chimera suite. Fig. 4A and B were a cartoon representation of the modeled protein of the native form (4A) and superimposed structure with both mutant forms (4B). The mutation and deleterious effects such as substitution, deletion and insertion are the characteristic event which leads the divergence of structure from native structure and subsequently alter the functional activity and the deviation between the mutant and native structures is evaluated with RMSD (Varfolomeev et al., 2002; Han et al., 2006; George Priya Doss et al., 2008; Selvaraj and Singh, 2018). Since, lower values compared to the native structure, it was suggested that the mutations have not caused any significant change in the mutant structures.

3.7. Molecular interaction study

Recently, Oxytetracycline, Sulphadimidine and Sulphadiazine were recently shown promising results in clinical models against mastitis. Hence the 2D structure of currently reported molecules is retrieved from PubChem for molecular docking studies and the protein ligand docked complexes were visualized by using PyMol. (MacDiarmid, 1978; Königsson et al., 2001; Chandrasekaran, 2013). Molecular docking is one of the well-established and often used computational methods to predict the binding affinity of two molecules (Selvaraj et al., 2018; Ramar and Pappu, 2016). The binding affinity and interaction results of currently reported molecules are reported in Table 4. The methods of evaluating the accuracy of docking results of three reported inhibitors were determining how closely the lowest energy poses (binding conformation) predicted by the object docking score. It was noticed that there are three hydrogen bond interactions that exit between native form and sulphadiazine, while it shows one hydrogen bond interaction with Mutant 1 (R563H) and Mutant 2 (T605M). The amino acid residue His289 is involved in bond formation in both types. The hydrogen bond interactions of other inhibitors such as oxytetracycline and sulphadimidine also studied comparatively with native and mutant forms. Oxytetracycline forms one hydrogen bond interaction with both mutant form and native form with Glu430 of receptor molecules. On the other hand, sulphadimidine forms two hydrogen bond interactions with Pro429 (2) with native protein and form one hydrogen bond interaction Mutant 1 via Ser330, and Mutant 2 viz His289. Oxytetracycline possess the maximum docking score with native (-9.648 kcal mol^{-1}) and mutant 1 (-10.441 kcal mol^{-1}) and mutant 2 (-9.753 kcal mol^{-1}). Other two inhibitors were shows similar docking score with both mutant and native types (Table 4). The amino acid residues Pro429, His289 and Glu430 were common in bond formation with native and mutant form and this result indicates that those amino acid residues were playing a crucial role in both structure and function of TLR2. Among three inhibitors Oxytetracycline forms low docking energy with native than mutant structure. The docked complex Oxytetracycline with native and mutant types is shown in Fig. 5A, B and C. The docked pose of sulphadimidine and sulphadiazine with native and mutant types were shown in Fig. 5D, E and F and Fig. 5G, H and I respectively.

4. Conclusion

The genetic alterations, like deletion, replacement and insertion cause functional changes in a gene, that may lead pathological condition of an organism. Prediction of mechanisms involved in alterations in genes will endow with a clue for preventing several genes associated diseases. Several computational works are performed to elucidate those deleterious effects, possible mutations in TLRs genes its crucial role in mastitis and inflammatory disease especially in dairy cattle. Four TLRs genes such as LTR2, TLR4, LTR6 and LTR10 are taken for the analysis and results with LTR2 gene showing the significant deleterious effects which may affect the protein structure and its function. The 9 deleterious nsSNPs are identified as deleterious nsSNP from four different TLRs genes and shows the major mutations at the position R563H and T605M. Additionally, the interactions of currently available inhibitors are also analyzed with both native and mutant forms of TLR2 3D models shows that the drug molecules are exhibiting less binding

affinity with mutant type compared to native type. The overall findings of this research indicated that R563H and T605M mutations in LTR2 may be higher risk nsSNPs which may affect both structure and functions of TLR2.

Declaration of interest

All the authors confidently declare that there is nothing to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101151.

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