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DYSFUNCTIONAL NLRP1 LEADS TO COMPROMISED IMMUNE RESPONSE AND INCREASED SUSCEPTIBILITY TOWARDS VARIOUS DISEASES

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Abstract - The study delineates effect of a polymorphism in NLR family pyrin domain containing 1 (NLRP1) protein which causes dysfunction, leading to a compromised immune response resulting in increased susceptibility of the host towards the foreign pathogenic infections. NLRP1 inflammasome promotes the activation of interleukin IL-1, IL-18, IL-33 which are effective mediators of inflammatory innate immune response to tissue damage and pathogen invasion. The retrieval of SNPs of the NLRP1 gene has been carried out through dbSNP. The deleterious SNPs were discerned using the computational tools SIFT, I-MUTANT, PhD-SNP and PROVEAN. The SNP V939M of NLRP1 was predicted to be the most deleterious on the basis of the outcomes from the computational tools. The protein structure of NLRP1 was retrieved from PDB (Protein Data Bank) and the structural mutations were carried out in Pymol. (patchdock)

keywords - NLRP1, Dysfunction, Innate Immunity, Inflammasome, Interleukin IL-1, Caspase-1, V939M

INTRODUCTION

The elimination of invading microorganisms is a prerogative for the survival of multicellular organisms, which is inclusive of humans. To ensure the removal of harmful pathogens, eukaryotic hosts have evolved an arsenal of defense mechanisms to sense and destroy invading microbes [1]. The first step in ensuring the safety of multicellular organisms is taken by innate immunity. This includes pathogen recognition and eradication. Divergent to adaptive immunity, which employs a distinctive and very specific repertory of genetically selected lymphocytes, innate immunity is able to dispatch cells with broad microbicidal activity immediately after the microbe is identified. A critical property of the innate immune system is its ability to discriminate microbes from 'self' through the recognition of conserved microbial structures called 'pathogen-associated molecular patterns' (PAMPs), such as lipopolysaccharide (LPS), peptidoglycan, flagellin and microbial nucleic acids [2] . NLRs are activated by bacterial, fungal, or viral molecules that contain pathogen-associated molecular patterns (PAMPs)

or by non-microbial danger signals (DAMPs) released by damaged cells [3] . Unlike membrane-bound TLRs, which sense PAMPs on the cell surface or in endosomes, NLRs recognize microbial molecules in the host cytosol. After microbial recognition, both TLRs and NLRs induce the activation of host signaling pathways, which lead to innate and adaptive immune responses [4].

The NLR (nucleotide-binding domain leucine-rich repeat containing) family is found in plants and animals, and serves as crucial regulators of inflammatory and innate immune response, though its functions are likely to extend greatly beyond innate immunity, and even beyond the immune system [5] . The family consists of 23 family members in humans, whereas the mouse genome contains at least 34 NLR-encoding genes[6] .

Most NLRs have a tripartite structure that consists of a variable amino terminal domain, a centrally located nucleotide-binding oligomerization domain (Nod) that mediates the formation of self oligomers, and a carboxy-terminal leucine-rich repeat that detects PAMPs [1] . Their oligomerization leads to the formation multiprotein inflammasome complexes that serve as platforms for the recruitment, cleavage, and activation of inflammatory caspases [2] .

NLRP1 was first described to form an inflammasome complex in 2002 [7], sparking intense interest in the involvement of this protein and its dysfunction in the formation of various diseases [8] . It is a 1473 amino acid long protein located at chromosome 17 at the cytogenic band location 17p13.2 which is the short arm of chromosome 17 (https://www.ncbi.nlm.nih.gov/genome/tools/gdp). NLRP1 is also known as CARD7, NLRP1, NALP1, NALP1 HUMAN, NLR family, pyrin domain containing 1.

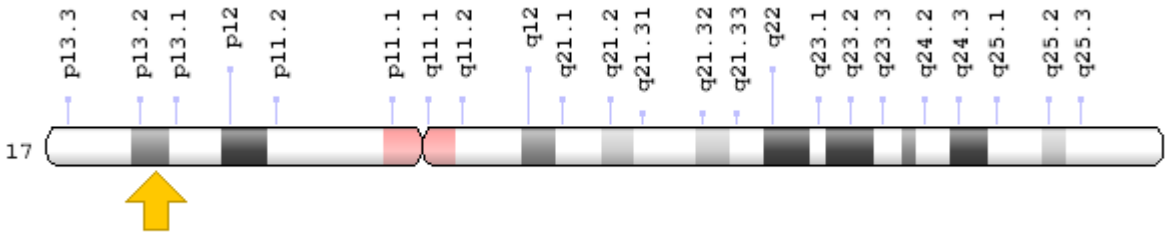


Fig.1. Structure of chromosome 17; yellow arrow depicts location of NLRP gene at position 17p13.2.

The NLRP1 inflammasome, plays a crucial role in innate immunity and inflammation. It initiates the formation of a inflammasome polymeric complex, made of NLRP1, CASP1, and possibly PYCARD. Activation of NLRP1 inflammasome also in turn, stimulates HMGB1 secretion, which is a type of chemokine. The active cytokines along with the chemokines stimulate inflammatory responses. Inflammasomes can also induce pyroptosis, which is an inflammatory form of programmed cell death [5, 9] .

MATERIALS AND METHODOLOGY

2.1 Data Mining

The datasets of SNPs were retrieved from dbSNP database of NCBI. The literature related to the SNPs was acquired from PubMed database. The protein structure of NLRP1 was obtained from PDB.

2.2 Prediction of effect of SNPs

There are a number of softwares and computational tools accessible to identify the SNPs as deleterious or neutral, based on the structural variations and constancy after a point mutation [10] . The SIFT and PhD-SNP software

were used to discover the outcomes to reveal any discrepancies in the protein sequence of NLRP1, signifying any possibly damaging effects on the sequence homology whereas I-Mutant and PROVEAN helped to identify any protein stability changes upon single point mutations and understand the biological utility of these single point mutations respectively.

2.2.1. SIFT

Sorting Intolerant from Tolerant (SIFT) is a software which foresees whether an amino acid change disturbs protein utility built on sequence homology and the physical properties of the amino acids. SIFT predicts whether the SNPs will be deleterious or tolerant. The SIFT system predicts the influence of coding variants on protein function [11]. (https://sift.bii.a-star.edu.sg/)

2.2.2. PhD-SNP

PhD-SNP is one of the computational tools which is used to predict the effect of both the coding and non-coding regions. A technique built on support vector machines (SVMs) that beginning from the protein order data can expect whether a different phenotype arose from a nsSNP can be linked to a genetic illness in humans. It categorizes the SNPs in disease and neutral based on the protein arrangement [12] . (https://snps.biofold.org/phd-snp/phd-snp.html)

2.2.3. I-Mutant

Estimation of the protein constancy deviates upon point mutation from the protein structure or sequence is regained from this tool. I-Mutant is a support vector machine (SVM)-based tool for the automatic prediction of protein stability changes upon single point mutations. I-Mutant predictions are performed starting either from the protein structure or, more importantly, from the protein sequence (https://omictools.com/protein-stability-change-prediction-category). I-Mutant can be expended equally as a classifier for guessing the signal of the protein steadiness vary upon mutation and by means of a regression estimator for guessing the related ΔG values. It shows the decrease or increase in stability[13] .(http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi)

2.2.4. PROVEAN

PROVEAN (Protein Variation Effect Analyzer) is a software device which expects if an amino acid exchange or indel has an influence on the biological utility of a protein[14]. PROVEAN is beneficial for sifting sequence variants to recognize nonsynonymous or indel variations that are expected to be functionally vital. It classifies the SNPs into deleterious and neutral (http://provean.jcvi.org/index.php).

2.3 Structure Alteration

The structural modifications were carried out using Pymol. The PDB structure of LRR5 domain of NLRP1 was mutated to create the structure with desired SNP. Pymol has function of mutating any amino acid in the protein makeup[15] . The amino acid was transformed to a new amino acid at the required place to get the mutant protein structure.(https://pymol.org/2/)

2.4 Swiss PDB Viewer

The energy minimization values were obtained using Swiss PDB Viewer after carrying out the structural modifications. This application is used to obtain amino acid mutations, H-bonds, angles and distances between atoms. (https://spdbv.vital-it.ch/)

2.5 PROCHECK

PROCHECK uses stereochemical considerations for overall assessment of stereochemistry of any given structure and highlights the regions for further investigations. (https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/)

2.6 STRING

The STRING database contains information from numerous sources, including experimental data, computational prediction methods and public text collections[16] .It was used to study the protein-protein interactions of Nlrp1 and analyse the effective binding proteins. (https://string-db.org)

2.7 Protein-Ligand Interaction

PatchDock was used to study the structural binding of protein-protein complexes and the interaction of the ligands, binding protein with the native and mutated protein structure of NLRP1. With the input of protein PDB codes and structures to the software server efficient docking of NLRP1 with CASP1 and APAF1 was performed. (https://bioinfo3d.cs.tau.ac.il/PatchDock/php.php)

2.8 ConSurf

The degree of evolutionary conservation of an amino acid Valine at position 939 in LRR5 domain of protein NLRP1 was studied using ConSurf software to infer balance between its natural tendency to mutate and the overall need to retain the structural integrity and function of the macromolecule. The server automatically collects homologues, infers their multiple sequence alignment and reconstructs a phylogenetic tree that reflects their evolutionary relations.[17]

RESULTS

1. Deleterious SNP Retrieval:

An overall of 19834 SNPs were obtained from dbSNP database for protein NLRP1. These SNPs were also put forward through SIFT. The software identified 5 SNPs that were found to have high frequency which proved to be deleterious. It showed a score ≥ 0.01. Thus, this gene was considered for further testing by various database screening softwares. Further screening of these SNPs by various database testing softwares (Ex. iMutant, SNAP, Provean and PhDSNP) revealed **rs61754791 (V939M)** with an amino acid change from valine to methionine at position 939 of the NLRP1 protein structure with maximum deleterious effect across all the database testing software.

Table 1: Combined biocomputational results of softwares for respective SNP's.

SNP	**AA CHANGE**	**SIFT**		**I-MUTANT**		**PhDSNP**		**PROVEAN**	
		Effect	Score	Effect	RI	Effect	RI	Score	Effect
rs146932154	M1154T	Deleterious	0.009	Decrease	9	Disease	5		
rs146932154	M1184T	Deleterious	0.011	Decrease	8	Neutral	6		
rs146932154	M1188T	Deleterious	0.012	Decrease	7	Neutral	6	-0.706	Neutral
rs61754791	**V939M**	**Deleterious**	**0.007**	**Decrease**	**3**	**Disease**	**8**	**-2.076**	**Neutral**
rs115872694	G354D	Deleterious	0.002	Decrease	9	Disease	6	-6.656	Deleterious

2.Protein Structure Building:

The protein structure of NLRP1 was retrieved from PDB database (PDB ID:4im6). The position 939 of the native NLRP1 protein structure was highlighted, while the mutated version of the NLRP1 protein showed the amino acid change (also highlighted) from valine to methionine at position 939.
Various tools of the Pymol were used to analyse and record changes within the native structure to obtain the mutated structure of the NLRP1 gene Fig 2: (B).

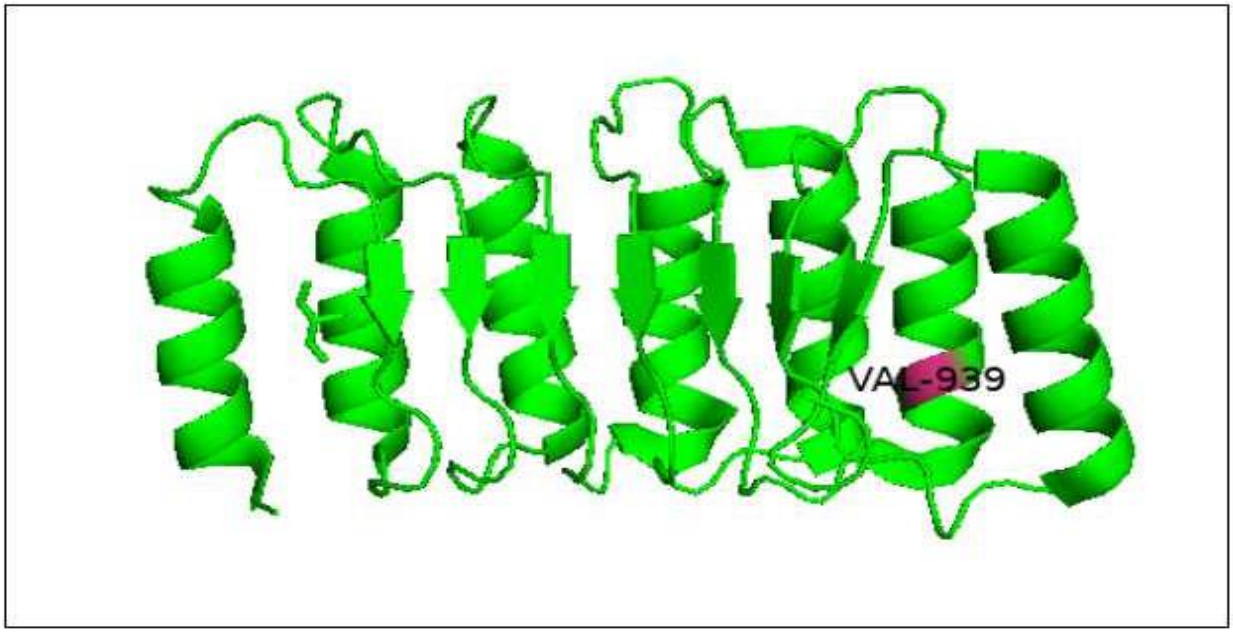


Figure 2:(A)

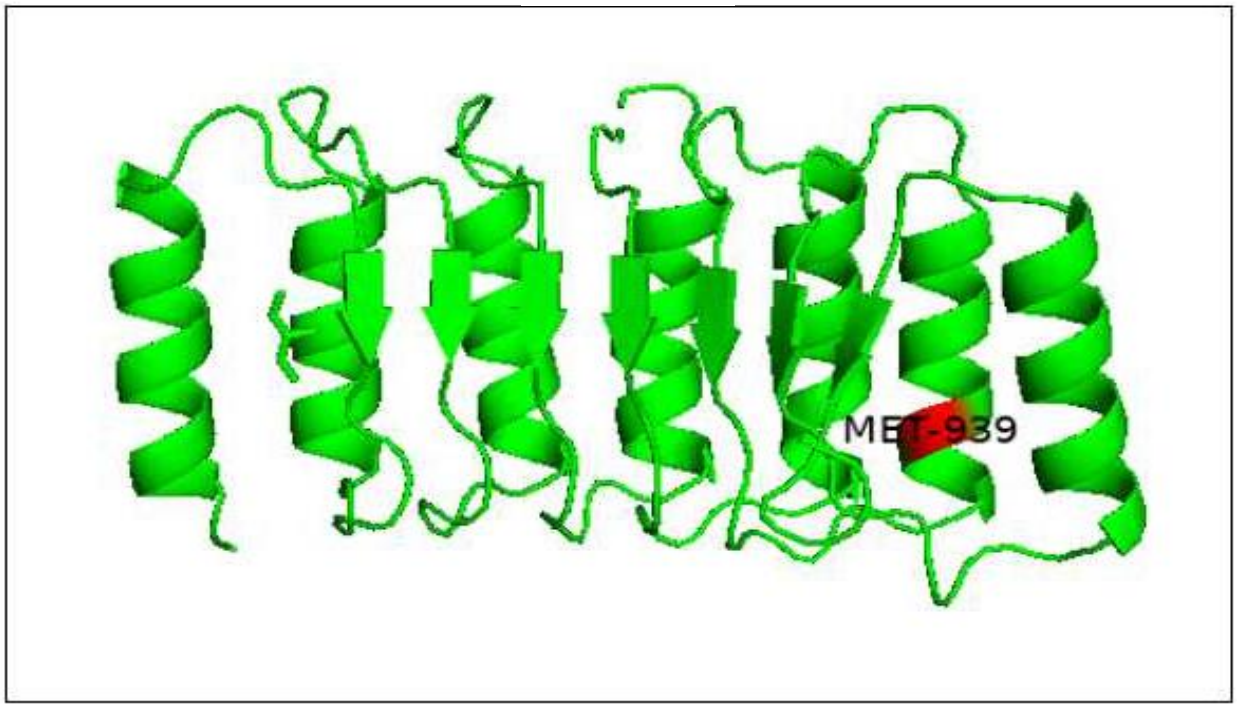


Figure 2: (B)

Figure 2: (A). NLRP1 Native Protein Structure; (B). NLRP1 Mutated Protein Structure

3. Structural Analysis:

3.1 Energy Minimization

The Energy Minimization values were obtained from Swiss PDB Viewer. The mutated protein structure of NLRP1 shows an increase in the total energy as compared to that of the native protein structure of NLRP1 as shown in Table 2.

Table 2: Energy Minimization values.

Protein	Bond	Angle	Torsion	Improper	Non bonded	Electrostatic	Total
Native	121.076	707.713	886.216	232.735	-6803.57	-7908.24	-12764.069
Mutated	221.870	711.209	920.426	243.588	-6510.31	-7649.40	-12062.615

3.2 Ramachandran Plot:

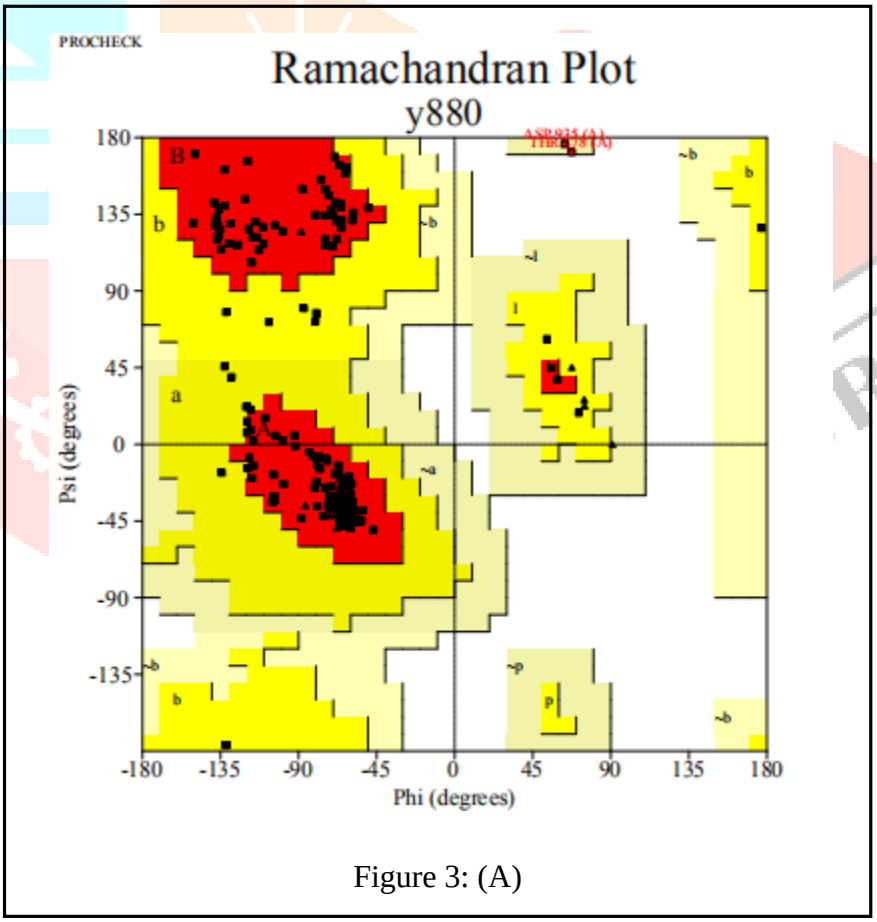


Figure 3: (A)

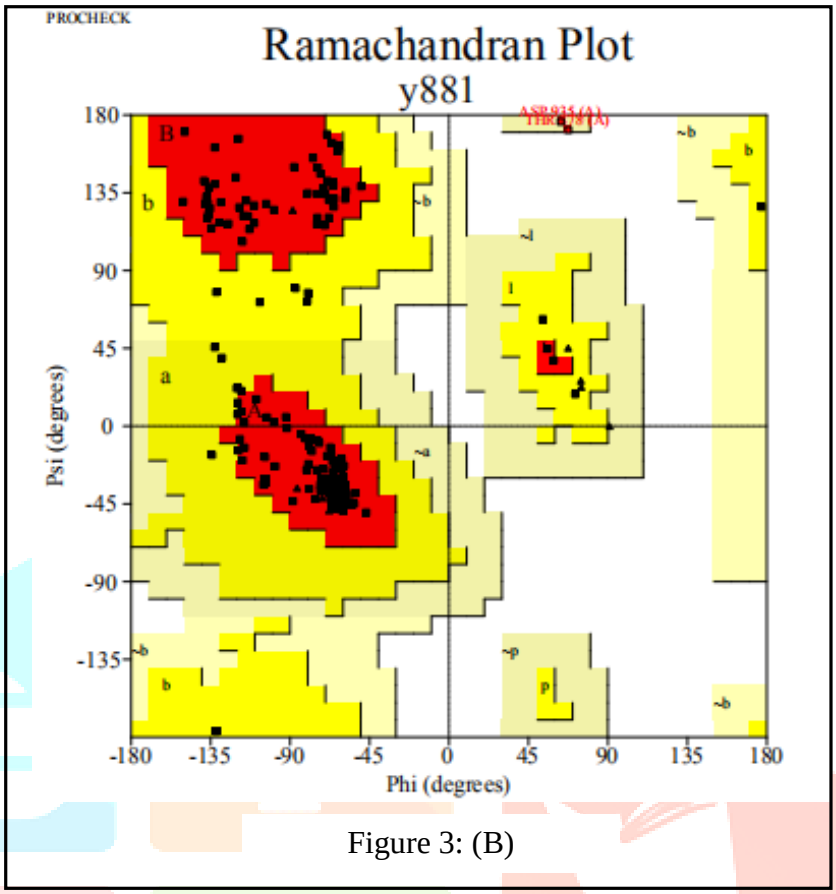


Figure 3: (B)

Figure 3: (A). Ramachandran Plot of Native NLRP1 Protein Structure; (B). Ramachandran Plot of Mutated NLRP1 Protein Structure

Table 3: Ramachandran Plot Statistics

Plot Statistics	Residues in favoured regions	Residues in additional allowed regions	Residues in generously allowed regions	Residues in disallowed regions	Number of non-glycine and non-proline residues	Number of end residues	Number of glycine residues (shown as triangles)	Number of proline residues	Total number of residues
Native	165 90.7%	15 8.2%	2 1.1%	0 0.0%	182 100%	1	9	6	198
Mutated	165 90.7%	15 8.2%	2 1.1%	0 0.0%	182 100%	2	9	6	199

The mutated protein structure shows 90.7% of residues in the most favored regions signifying that the given structure is a good quality model.

4. Protein-Protein Interaction

4.1 STRING

The interaction between NLRP1 and CASP1 was determined and is shown below -

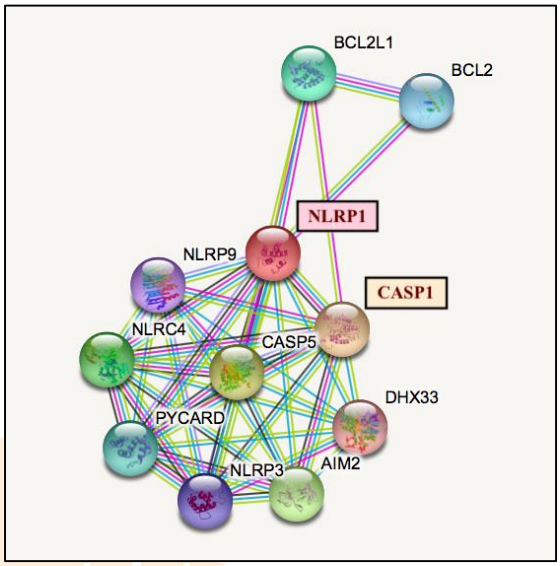


Figure 4: Interaction of NLRP1 with CASP1 and several other proteins.

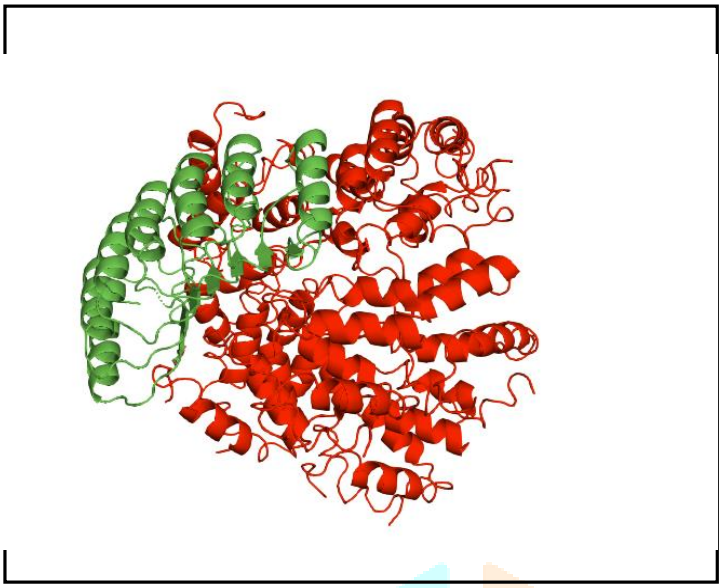
4.2 PatchDock Analysis:

4.2.1 CASP1

The protein-ligand docking of the gene NLRP1(green) and its binding protein CASP1(red) was undertaken in both the native and the mutated structure of the gene by the software PatchDock. Information of the PatchDock structures are tabulated below (Table No:4). Protein-ligand docking of the native NLRRP1 and CASP1 is shown in the Figure 4(A).similarly the docking between mutated NLRP1 and CASP1 is shown in the Figure 4(B).

Table 4 : PatchDock – NLRP1 and CASP1

Structure	Position	Score	Area	ACE	Transformation
Native	939	14760	2813.70	458.00	-2.82 -0.15 0.84 4.09 -12.97 -22.11
Mutated	939	14760	2813.70	458.00	-2.82 -0.15 0.84 4.09 -12.97 -22.11



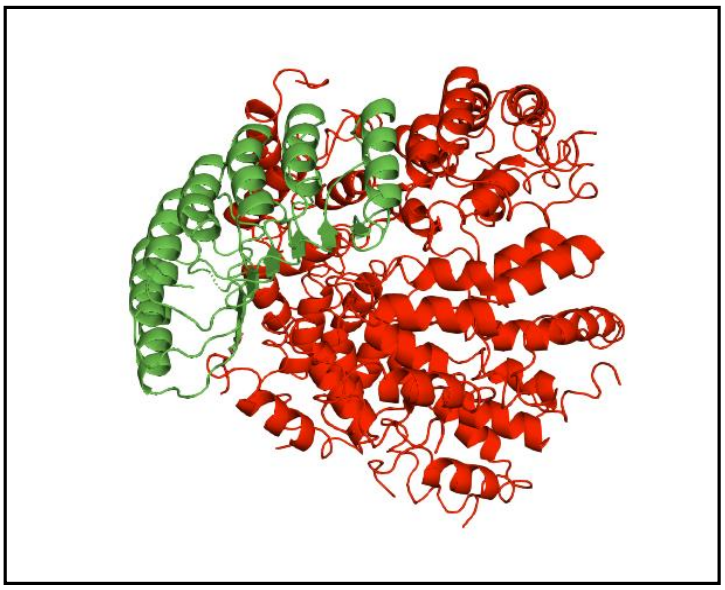


Figure 5: (A) Patch Dock structure of native NLRP1 (green) and CASP1 (red)

(B) Patch Dock structure of mutated NLRP1 and CASP1

4.2.2. APAF1

The protein-ligand docking of the gene NLRP1(green) and its binding protein APAF1(blue) was undertaken in both the native and the mutated structure of the gene by the software Patch Dock.Information of the patch dock structures are tabulated below (Table 5). Protein-ligand docking of the native NLRRP1 and APAF1 is shown in the Figure 5(A).similarly the docking between mutated NLRP1 and APAF1 is shown in the Figure 5(B).

Table 5 : PatchDock – NLRP1 and APAF1

Structure	Position	Score	Area	ACE	Transformation
Native	939	17878	2695.00	417.76	-2.17 -0.05 2.81 -16.04 -35.49 -19.21
Mutated	939	17878	2695.00	417.76	-2.17 -0.05 2.81 -16.04 -35.49 -19.21

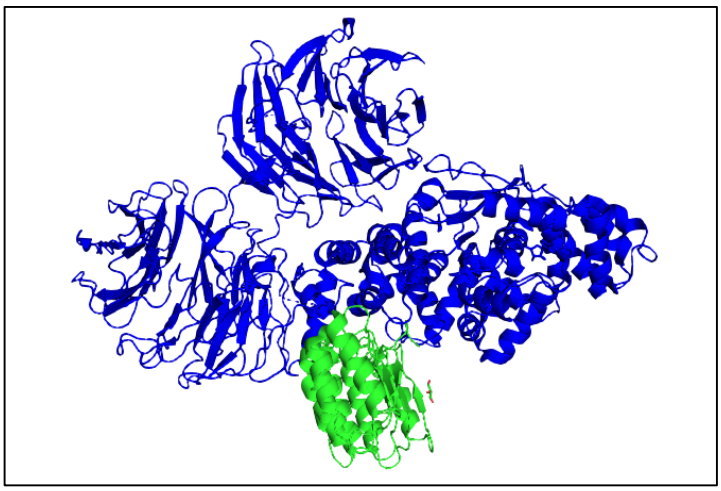


Figure 6 (A)

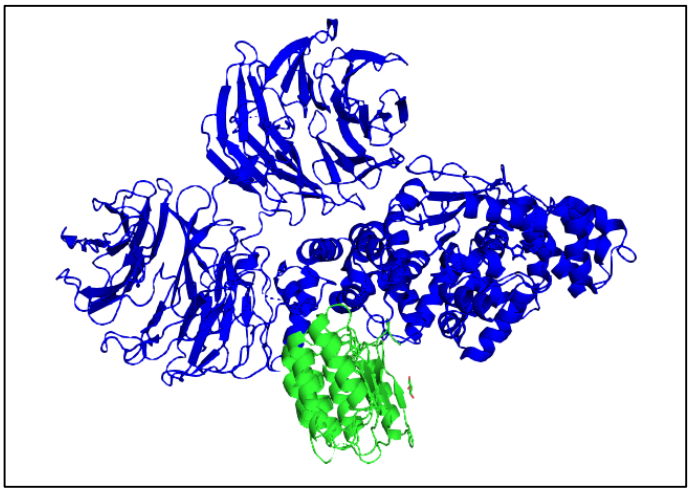


Figure 6 (B)

Figure 6: (A) Patch Dock structure of native NLRP1(green) and APAF1(blue)

(B)Patch Dock structure of mutated NLRP1 and APAF1

5. Conservation of amino acid

The reference sequence (SLKELDLQQNNLDDVGVRLLCE) for LRR5 domain of NLRP1 was used to obtain the Multiple Sequence Alignment (MSA) result using Consurf software (https://consurf.tau.ac.il). The MSA result in figure 6. depicts the conserved amino acid valine at position 939 and any change in it may be detrimental. Table 6 denotes the respective taxa for reference codes depicted in figure 6.

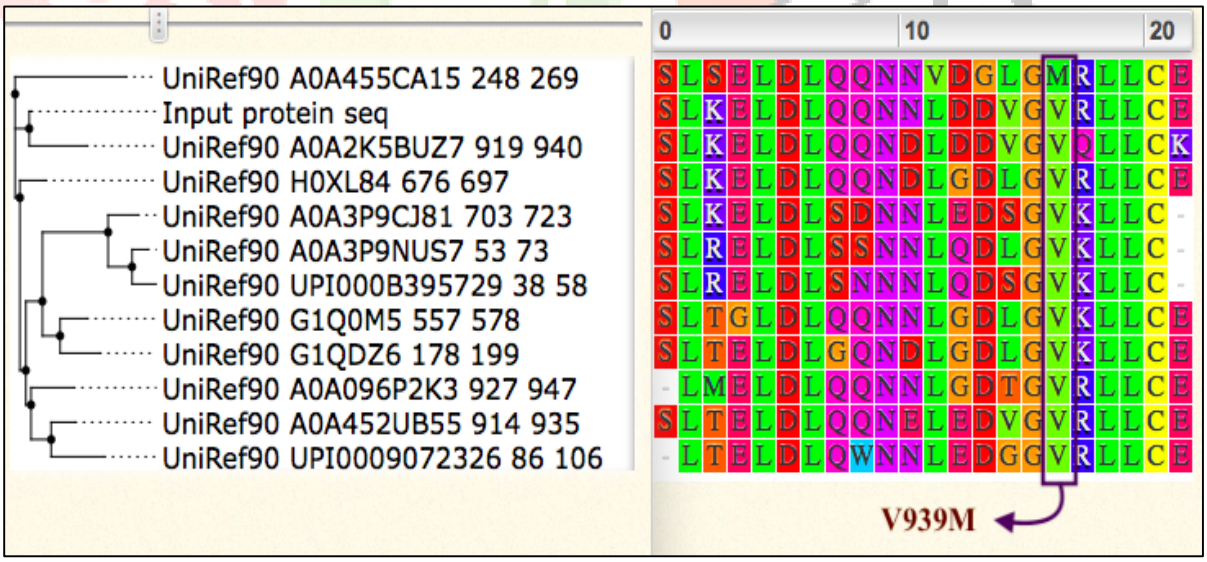


Figure 6: Conserved Multiple Sequence Alignment result of domain LRR5 of NLRP1 for SNP V939M.

Table 6: Taxonomiacal names for the conserved references.

Reference Codes	Taxa
UniRef90_A0A2K5BUZ7_919_940	Platyrrhini
UniRef90_H0XL84_676_697	Otolemur garnettii
UniRef90_A0A452UB55_914_935	Ursus
UniRef90_A0A455CA15_248_269	Physeter macrocephalus
UniRef90_A0A096P2K3_927_947	Cercopithecidae
UniRef90_UPI0009072326_86_106	Alligator mississippiensis
UniRef90_A0A3P9NUS7_53_73	Poecilia reticulata
UniRef90_UPI000B395729_38_58	Fundulus heteroclitus
UniRef90_G1Q0M5_557_578	Myotis lucifugus
UniRef90_A0A3P9CJ81_703_723	Haplochromini
UniRef90_G1QDZ6_178_199	Myotis lucifugus

DISCUSSION

The NLRP1 proteins are pattern recognition receptors (PRRs) that act as cytoplasmic sensors of danger signals ranging from dividing bacteria to crystalline materials [7, 18, 19] . The human NLRP1 inflammasome was the first caspase-1-activating platform to be identified. [7]

Nuclear localization leucine-rich-repeat protein 1 (NLRP1) is a key regulator of the innate immune system, particularly in the skin where, in response to molecular triggers such as pathogen associated or damage-associated molecular patterns, the activated sensors oligomerize and recruit caspase-1 to a multiprotein complex (the inflammasome). Inflammasomes are composed of three proteins: (1) an NLR family member, (2) the adaptor protein apoptosis speck-like protein with a caspase recruitment domain (ASC), and (3) caspase-1. The inflammasome regulates caspase-1 processing and activity and, consequently, the levels of the active cytokines IL-1b and IL-18.

The NLRP1 inflammasome promotes caspase-1–dependent processing of bioactive interleukin-1β (IL-1β), resulting in IL-1β secretion and downstream inflammatory responses [20] . In the innate immune response, activation and processing of proinflammatory cytokines IL-1b, IL-18, and IL-33 are controlled by inflammatory caspase-1 and caspase-5 in cytoplasmic multiprotein complexes known as inflammasomes [7, 18, 19] . Inflammasome-mediated activation of caspase-1 allows it to cleave the precursors of the inflammatory cytokines IL-1b and IL-18, as well as currently unidentified substrates that cause the rapid lysis of macrophages and dendritic cells (pyroptosis).IL-1b and IL-18 are potent mediators of inflammation and initiate and/or amplify a wide variety of effects associated with innate immunity, host responses to tissue injury, and microbial invasion [21-24] . Increased mature IL-1β may contribute to the pathogenesis of autoimmunity, perhaps by functioning as an “adjuvant,” facilitating presentation of autoantigens that may trigger or provide specificity for the autoimmune response [20]. Interleukin-18 (IL-18) is activated by Caspase-1 in inflammasome complexes [25]. Caspases also have a role in inflammation, whereby it directly processes pro-inflammatory cytokines such as pro-IL1β. These are signalling molecules that allow recruitment of immune cells to an infected cell or tissue [26] .

The CARD of NLRP1 interacts selectively with the CARD domain of Apaf1, a caspase-activating protein that couples mitochondria-released cytochrome c (cyt-c) to activation of cytosolic caspases. APAF1 and procaspase9 interact and form a complex. NLRP1 increases the coimmunoprecipitation of the APAF1/procaspase9 complex, suggesting that NLRP1 enhances rather than inhibits interactions of these proteins. Cyt-c induces formation of a large holoenzyme complex (apoptosome) containing multiple Apaf1 and Casp9 molecules [27]. As the cyt-c is released it induces association of nlrp1 with the active capases. This causes mitochondrial release of cytochrome

c, which binds to cytosolic Apaf-1, promoting activation of pro-caspase-9 and subsequently pro-caspase-3 [28]. Caspase-3 is a key mediator of apoptosis and accelerates a cascade of caspases, leading to degradation of the cell [29].

The mutation in the amino acid sequence of the NLRP1 protein is proven to be deleterious as per the in-silico analysis. This polymorphism could impede the function of the NLRP1 protein and its binding efficiency. NLRP1 is a key regulator of the innate immune system and autoimmune system. It is involved in the detection of pathogens and activation of interleukins and caspases. Interleukins are an essential part of inflammatory response and caspases that help in the degradation of damaged cells affected by pathogens. This polymorphism leads to dysfunction of NLRP1 which leads to the compromised immune system of the host and a higher susceptibility to various diseases. NLRP1 is genetically associated with risk of several autoimmune diseases including generalized vitiligo, Addison disease, type 1 diabetes, rheumatoid arthritis, and others [7, 18, 19]. IL-1b has been implicated in the pathogenesis of several neurologic diseases including TBI, Alzheimer's disease, epilepsy, Parkinson's disease, and stroke [30].

CONCLUSION

After meticulous in-silico studies, we have found the SNP with rsID **rs61754791** to be most deleterious which exhibits the mutation with an amino acid change from Valine to Methionine at position 939 which is part of the LRR5 domain.

The study of the NLRP1 protein shows its importance in the innate immune system and autoimmune system and hence a deleterious mutation in the amino acid sequence causing a dysfunction of NLRP1 protein which leads to increased susceptibility of the host towards the pathogens due to weakened immune response.

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