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IN SILICO ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN HUMAN NLRP7 GENE ASSOCIATION WITH RHMS

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Abstract-Single nucleotide polymorphisms (SNPs), present in the protein encoding regions of the genome can affect the structure and capacity of a protein. In this work, we have analyzed the mutations that can alter the function of the NLRP7 gene through computational strategies. NALP7 has recently been recognize as the adroit gene for familial repetitive Hydatidiform mole (FRHM), a attenuate autosomal (latent) recessive condition in which influences the people who have intermittent molar pregnancies of diploid bi-parental beginning. Here, we applied the distinctive computational tools like SIFT, polyphen, PROVEAN, I-Mutant-2.0, PANTHER, Phd-Snp, SNPs&GO, MutPred, NetsurfP. To understand the atomic arrangement in 3D space, the native and aberrant structures were modeled. Finally the structural analyses of native and mutant NLRP7 proteins were explored by utilizing Pymol and Hope project. Our Insilico analysis proposed that D657A variants of NLRP7 could directly or indirectly destabilize the amino acid interactions and hydrogen bond networks clarifies the functional deviations of protein to some extent. Screening of NLRP7, D657V variant might be valuable for disease molecular diagnosis and also to design the molecular inhibitors of NLRP7 pathways.
Keywords Single nucleotide polymorphisms, familial recurrent hydatidiform mole, computational tools.

1. INTRODUCTION

Hydatidiform mole (HM) is a distorted human pregnancy characterized by aberrant embryonic development and hyper expansion of the trophoblast. It happens early in pregnancy when an embryo does not completely develop. It occurs 1 of every 600 pregnancies in western countries but has higher frequencies in Southern and Asian countries. Recurrent hydatidiform moles (RHMs) are defined by the existence of two abnormal pregnancies and it influences 1.5–9.3% of women with a prior HM. In [1, 2], the authors exhibited that NLRP7 is a major gene for RHMs. In [3-7], the authors studies the NLRP7 codes for a nucleotide oligomerization domain-like receptor pyrine containing protein and plays a role in inflammatory response, trophoblastic tissue differentiation and proliferation and is part of the oocyte cortical cytoskeleton. Mutations in NLRP7 are responsible for recurrent RHMs [8-11]. More than 50 mutations in the NLRP7 gene have been found to cause recurrent Hydatidiform mole.

The type of genetic mutation that causes a single amino acid substitution (AAS) in a protein sequence is called nsSNPs. An nsSNPs could potentially impudence the function of the protein. The harmful nsSNPs for the NLRP7 gene have not been predicted to date in silico. Therefore we designed a strategy for analyzing the entire NLRP7 coding region. Different algorithms such as SIFT [12], polyphen [13-14], PROVEAN [15], I-Mutant-2.0 [16-17], PANTHER [18], Phd-Snp [19], SNPs&GO [20], MutPred [21], NetsurfP [22], Hope [23] were utilized to predict high-risk nonsynonymous single nucleotide polymorphisms (nsSNPs) in coding regions that are likely to have an effect on the function and structure of the protein.

2. MATERIAL AND METHOD

The data on human NLRP7 gene was collected from Online Mendelian Inheritance in Man (OMIM) and National Center for Biological Information (NCBI) web site. The SNPs information (Protein accession number and SNP ID) of the NLRP7 gene was retrieved from the NCBI dbSNP and SWISSProt databases. Each SNP has a unique reference ID (rs id) which gives information about the SNP including amino acid change and their respective positions along with corresponding accession ID

2.1 Validation of tolerated and deleterious snps (SIFT)

Sift predicts the tolerated and deleterious SNPs and identifies the impact of amino acid substitution on protein function and phenotype alterations. The concept for this technique is based on the evolutionary conservation of the amino acids within protein families. Highly conserved positions tend to be intolerant to substitution, whereas those with a low degree of conservation tolerate most substitutions. Therefore, changes at well-conserved positions tend to be predicted as deleterious or damaging [12]. The threshold intolerance score for SNPs is 0.05 or less

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2.2 Predicting the functional effect of amino acid substitutions

PolyPhen-2:

PolyPhen-2 (Polymorphism Phenotyping v2) is a tool which predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations. It calculates position-specific independent counts (PSIC) scores for each of the two variants, and then computes the PSIC score difference between them. Higher the PSIC score difference, higher the functional impact a particular amino acid is likely to have. A PSIC score difference = 1.5 is considered to be damaging [13-14].

PROVEAN (Protein Variation Effect Analyzer) is a sequence based predictor that estimates the effect of protein sequence variation on protein function [15]. It is based on a clustering method where BLAST hits with more than 75% global sequence identity are clustered together and top 30 such clusters from a supporting sequence are averaged within and across clusters to generate the final PROVEAN score. A protein variant is predicted to be "deleterious" if the final score is below a certain threshold (default is -2.5), and is predicted to be "neutral" if the score is above the threshold.

2.3 Predicting the Stability Changes Caused by Single Amino Acid Polymorphisms Using Support Vector Machine

(I-Mutant 2.0):

I-Mutant2.0 is a support vector machine (SMV) based tool for the automatic prediction of protein stability changes caused by single point mutations. it calculate the unfolding Gibbs free energy value of the mutated protein minus the unfolding Gibbs free energy value of the native protein (kcal mol). Positive G values meant that the mutated protein has higher stability and negative values indicate lower stability [16-17].

2.4 Predicting Disease Associated Variations

PANTHER:

PANTHER program is a protein family and subfamily database which predicts the frequency of occurrence of amino acid at a particular position in evolutionary related protein sequences. The threshold subPSEC score of -3 has been assigned below which the predictions are considered as deleterious [18].

PHD-SNP:

PhD-SNP is SVM based classifier, trained over the million amino acid polymorphism datasets

Using supervised training algorithm. It predicts whether the given amino acid substitution leads to disease associated or neutral along with the reliability index score [19].

SNP&GO:

The SNPs&GO algorithms predict the impact of protein variations using functional information encoded by Gene Ontology (GO) terms of the three main roots: Molecular function, Biological process, and Cellular component [20]. SNPs&GO is a support vector machine (SVM) based web server to predict disease related mutations from the protein sequence, scoring with accuracy of 82% and Matthews correlation coefficient equal to 0.63. SNPs&GO collects, in a unique framework, information derived from protein sequence, protein sequence profile and protein functions. The prediction results based on the discrimination among disease related and neutral variations of protein sequence. The probability score higher than 0.5 reveals the disease-related effect of mutation on the parent protein function.

MUTPRED:

MutPred is a web based tool, used to predict the molecular changes associated with amino acid variants [21]. It uses SIFT, PSI-BLAST, and Pfam profiles along with some structural disorder prediction algorithms, including TMHMM, MARCOIL, I-Mutant 2.0, B-factor prediction, and DisProt. Functional analysis includes the prediction of DNA-binding site, catalytic domains, calmodulin binding targets, and posttranslational modification sites [21].

Combining the scores of all four servers, the accuracy of prediction rises to a greater extent and finally we filtered the most disease-associated mutation.

2.5 Biophysical validation of nsSNPs

NetSurfP predicts the surface and, solvent accessibility of amino acids, using the amino acid FASTA sequence. The solvent accessibility has been predicted in two classes as either buried or exposed, based on the accessibility of the amino acid residues to the solvent, respectively.

The reliability of this prediction method is in the form of Z-score. The Z-score highlights the surface prediction reliability, but is not associated with the secondary structure [22].

2.6 3D structure prediction

I-tasser:

Evaluation of the structural stability between the native and mutant proteins was performed based on the availability of a 3D structure of a protein in the PDB data base. In the case of NLRP7 a 3D crystallographic structure was not available in PDB. Hence to obtain a 3D structure of NLRP7, the amino acid sequence in FASTA format was submitted to the I-Tasser server [23]. It is based on multiple threading alignments and iterative structural assembly simulations. The prediction of the

accuracy of the model depends upon a confidence score (C-score) based on the quality of the threading alignments and structural assembly refinement simulations [24].
The C-score lies in the range of −5 to 2, with higher values depicting the high confidence for predicted model. The C-score has a correlation with the TM-score and RMSD. If the native structure is known, the TM-score and RMSD are used to measure the accuracy of predicted structure else, these are used to predict the quality of the modeling prediction by calculating the distance between two predicted models. The TM-score is a recently proposed scale to solve the local error problem of RMSD. A TM-score >0.5 high lights a model of correct topology and a TM-score < 0.17 indicates a random similarity. The 3D structure of NLRP7 was visualized by PyMOL.

2.7 Project HOPE:
Project Have yOur Protein Explained is an automatic mutant analysis server to study the insight structural features of native protein and the variant models. HOPE provides the 3D structural visualization of mutated proteins, and gives the results by using UniProt and DAS prediction servers. Input method of Project HOPE carries the protein sequence and selection of Mutant variants. HOPE server predicts the output in the form of structural variation between mutant and wild type residues [25].

3. RESULTS AND DISCUSSION

To determine the deleterious non-synonymous single nucleotide polymorphisms (nsSNPs), which might be involved in inducing disease associated phenomena, is now among the most important field of computational genomic research. The disease associated mutations can be identified with the help of genome sequencing and its analysis. The advanced method in computational biology has now enabled us to determine the deleterious nsSNPs in the target candidate genes. Computational methods were applied to study the protein structural and functional effect on point mutation at molecular level. In this investigation we implemented multiple computational methods to identify the most likely pathogenic mutations in NLRP7 gene. Our results also revealed that implementations of different algorithms often serve as powerful tools for prioritizing candidate functional nsSNPs. Here we used SIFT, PolyPhen, Provean, I-Mutant 2.0, PANTHER, PhD-SNP, SNP&GO and Mutpred tools to examine the most deleterious and disease associated nsSNPs from the SNP dataset. The combination of methods based on evolutionary information and protein structure and functional parameters were used in order to increase the prediction accuracy.

3.1 Screening of Deleterious nsSNPs
In SIFT, out of 35 mutations 13 mutations were predicted to be deleterious with the score ≤0.05 (table-1) remaining 22 were predicted as tolerated. A total of 18 nsSNPs were predicted to be damaging and the remaining 17 nsSNPs were categorized as benign with Polyphen 2.0 and the results were listed in Table 1. All the nsSNPs submitted to PolyPhen 2.0 and SIFT were also submitted as input to the I-Mutant-2.0server. 14 mutations were affecting the stability of the protein structure.

Table 1: list of nsSNPs analysis by sift, polyphen, provean and I-mutant respectively

Rs ID	Amino acid Change	Sift	score	Polyphen	score	provean	score	I-mutant3.0	score
Rs104895503	N913S	ıaging	0.04	Dam	0.97	·terious	-4.219	Decrease	-1.93
Rs61742869	R795C	rated	0.07	Dam	0.93	ıral	-1.485	Decrease Decrease	-0.41
Rs104895552	C761Y	ıaging	0.00	Dam	0.99	·terious	-9.667	Decrease	-1.46
Rs104895512	L750V	rated	0.32	Dam	0.94	ıral	-0.129	Decrease	0.04
Rs104895540	D722G	rated	0.43	Dam	0.91	·terious	-3.534	Decrease	-1.43
Rs104895525	R721W	ıaging	0.02	ıgn	0.241	·terious	-5.016	Decrease	-0.18
Rs104895526	A719V	ıaging	0.02	Dam	0.95	·terious	-2.608	Decrease	-1.34
Rs104895550	P716A	rated	0.71	Dam	0.94	·terious	-4.926	Decrease	-0.96
Rs104895535	R701C	ıaging	0.04	ıdam	0.99	ıral	-1.956	Decrease Decrease	-0.58
Rs77072552	V699I	rated	0.36	ıgn	0.00	ıral	0.744	Decrease	-0.29
Rs104895502	R693P	rated	0.81	Dam	0.99	·terious	-3.695	Decrease	-0.26
Rs104895502	R693W	ıaging	0.01	Dam	1.000	·terious	-5.019	Decrease	0.03
Rs104895508	D657V	ıaging	0.01	Dam	0.99	·terious	-4.284	Decrease	-1.22
Rs104895549	P651S	rated	0.66	ıgn	0.195	ıral	0.079	Decrease	-0.32
Rs56273180	A536T	rated	0.78	ıgn	0.003	ıral	1.307	Decrease	-2.35
Rs61743949	K511R	rated	0.53	ıgn	0.006	ıral	1.399	Decrease	-1.85
Rs61738423	E507V	rated	0.35	ıgn	0.124	·terious	-3.486	Decrease	-0.83
Rs61747407	A494T	rated	0.60	ıgn	0.105	ıral	-0.387	large Decrease	-0.68
Rs775881	G487E	rated	1.00	ıgn	0.00	ıral	1.135	Decrease	-1.75
Rs61747414	A481T	rated	0.22	ıgn	0.105	ıral	-0.468	Decrease	-1.33
Rs1654364	F430L	rated	0.14	ıgn	0.001	·terious	-3.198	Decrease	-1.09
Rs1654635	M427T	rated	0.59	ıgn	0.004	ıral	1.383	Decrease	-1.45
Rs104895510	C399Y	ıaging	0.02	Dam	1.00	·terious	-6.695	Decrease	0.04
Rs104895548	L398R	ıaging	0.00	Dam	1.00	·terious	-5.079	Decrease	-1.07
Rs104895557	G380R	ıaging	0.00	Dam	1.00	·terious	-6.282	Decrease	-1.11
Rs10418277	K379N	ıaging	0.03	ıgn	0.009	·terious	-3.405	Decrease	-1.07
Rs775882	V319I	rated	0.64	ıgn	0.004	ıral	0.045	Decrease	-1.05
Rs79513034	L311I	rated	0.41	Dam	0.92	ıral	-0.727	Decrease	-0.38
Rs77812009	Q310R	rated	0.57	ıgn	0.002	ıral	1.140	Decrease	-0.12
Rs78096121	F250C	ıaging	0.00	Dam	0.998	·terious	-7.097	Decrease	-0.89
Rs61732584	L234S	rated	0.75	Dam	0.868	ıral	-1.468	large Decrease	-0.08
Rs104895529	M192L	rated	0.41	ıgn	0.438	ıral	-2.313	Decrease Increase	-0.70
Rs61746625	R156Q	rated	0.60	ıgn	0.449	ıral	-0.276		-1.26
Rs104895509	C84Y	ıaging	0.04	Dam	0.594	·terious	-3.115		-0.82
Rs75678776	S24R	rated	0.75	ıgn	0.411	ıral	0.397		-0.54

Table 2: List of nsSNPs predicted as disease associated by Panther, PHD-SNP and SNP&GO server.

Rs ID	Amino acid change	Panther	PHD-SNP	SNP&GO
Rs104895503	N913S	Deleterious	Disease	Disease
Rs104895552	C761Y	Deleterious	Disease	Disease
Rs104895526	A719V	Tolerated	Neutral	Disease
Rs104895502	R693W	Tolerated	Neutral	Disease
Rs104895508	D657V	Tolerated	Neutral	Neutral
Rs104895510	C399Y	Deleterious	Disease	Disease
Rs104895548	L398R	Deleterious	Disease	Disease
Rs104895557	G380R	Deleterious	Disease	Disease
Rs78096121	F250C	Deleterious	Disease	Disease
Rs104895509	C84Y	Tolerated	Disease	Neutral

3.2 Prediction of Disease- Associated nsSNPs

PANTHER, PhD-SNP and SNPs&GO were performed to validate the results obtained from four tools. Out of 10 nsSNPs that predicted to be deleterious with SIFT, Polyphen, I-Mutant and PROVEAN; PANTHER predicted 6 nsSNP to be associated with disease (Table 2) PhD-SNP predicted 7 nsSNP and SNP&GO predicted 8 nsSNP,. Finally out of 35 nsSNP, we found 6 nsSNPs namely rs104895503 (N913S), rs104895552 (C761Y), rs104895510 (C399Y), rs104895557 (G380R) and rs78096121 (F250C) that are common in all (SIFT,Polyphen,I-Mutant,PROVEAN,PHD-SNP,SNP&GO,PANTHER) prediction.

These 10 mutations were further analyzed by MutPred tool to predict the SNP disease association probability and probable change in the molecular mechanism in the mutant. We found "C761Y" to be highly deleterious with general probability (g) scores of 0.791 and were predicted to induce the loss of methylation at K760 residue with (p) score of 0.760, showing confident hypothesis. "A719V" was found to be highly deleterious with general probability (g) scores of 0.835 and was predicted to induce the loss of Phosphorylation residue at T718 with (p) score of 0.3045, showing confident hypothesis. "D657V" was found to be highly deleterious with general probability (g) scores of 0.841 and was predicted to induce the gain of moRF binding with (p) score of 0.0255, showing confident hypothesis. "L398R" was found to be highly deleterious with general probability (g) scores of 0.950 and was predicted to induce the loss of stability with (p) score of 0.1178, showing confident hypothesis. At the end of so many mutations considered, we screened C761Y, A719V, D657V and L398R as the most deleterious and disease associated mutation in NLRP7 gene (Table 3). This prediction could be endorsed with the observed experimental data

Table 3: The G score, P score, molecular variations and prediction reliability calculated from MUTPRED server. Here the most disease associated mutations are displayed in bold.

Amino acid change	G score	core	Mutpred molecular variation	Prediction reliability
N913S	0.933	045	Gain of loops	No reliable Inference
C761Y	0.561	60	Loss of methylation	Confident hypothesis
A719V	0.791	045	Loss phosphorylation	Confident hypothesis
R693W	0.776	324	Loss of disorder	No reliable Inference
D657V	0.833	255	Gain of moRF	Confident hypothesis
C399Y	0.845	299	Loss helix	No reliable Inference
L398R	0.835	178	Loss of stability	Confident hypothesis
G380R	0.799	483	Gain of methylation	Actionable hypothesis
F250C	0.817	265	Loss of catalytic residue	Actionable hypothesis
C84Y	0.605	635	Loss of disorder	No reliable Inference

Table 4: Surface accessibility of mutant variants in NLRP7

Amino acid change	Class assignment	ative surface accessibility	Absolute surface accessibility	Z-fit score for RSA prediction
N913S	Buried	88	42.105	-1.783
A719V	Buried	02	11.273	-0.580
D657V	Exposed	04	58.288	-2.065
L398R	Buried	41	7.562	0.295

3.3 Insilco biophysical validation of nsSNPs:

Based on the in silico analyses performed, 4 nsSNPs was selected for further analysis. The location and the type of a mutated residue affect the stability of the protein. In particular, as the solvent accessibility of a residue decreases, the stability of the protein due to mutation decreases.

NetSurfP Z-score allows the identification of the most reliable/unreliable predictions for both buried and exposed amino acids. A huge drift in the Z-score was observed for 1 nsSNPs as given in Table 4.

I-Tasser:

I-tasser tool created the 5 full- length models for NLRP7 protein. Among the 5 predicted models, model 2 carried the high-quality confidence in the form of C-score TM score and the RMSD. Mention the details in table-5. 3D structure of protein depicted in fig-1 &2 (wild and mutant proteins).

Table 5: I-tasser results carrying c-score.TM-score and RMSD regarding selected secondary structure (mutant protein)

Model	C-score	Exp.Tm-score	Exp.RMSD	No.of decoys	Cluster density
Model-1	-0.71	0.62±0.14Å	10.6±4.6Å	600	0.0890
Model-2	-0.53			549	0.1061
Model-3	-1.84			409	0.0286
Model-4	-2.62			180	0.0131
Model-5	-2.68			160	0.0124

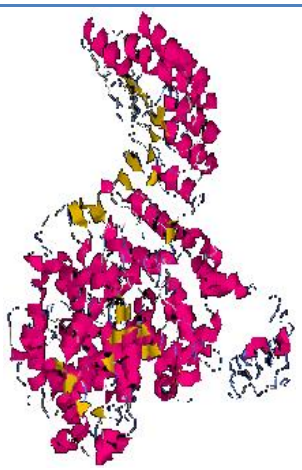


Fig: 1: 3D structure of NLRP7 predicted with I-Tasser (Wild)

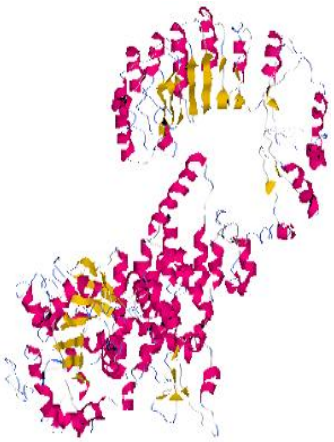
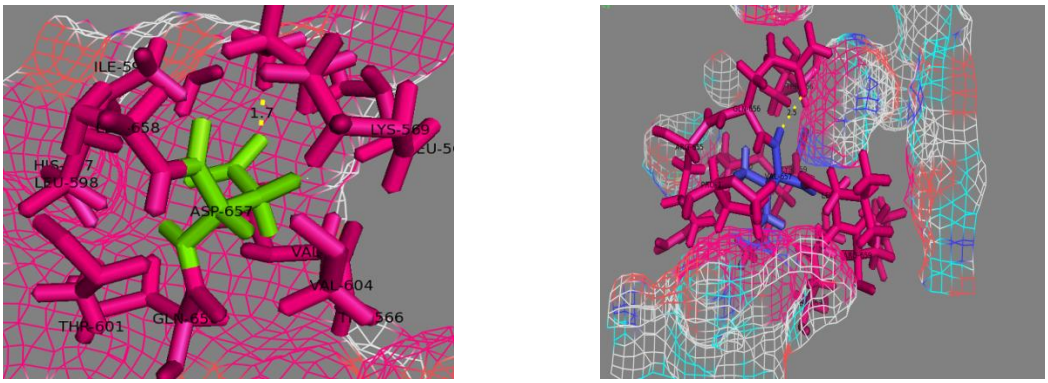
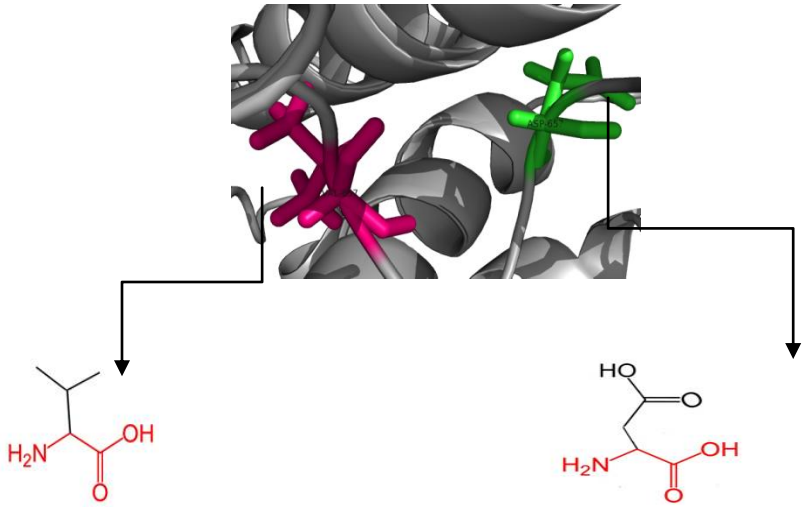


Figure 2: 3D structure of NLRP7 predicted with I-Tasser (Mutant)

Stuctural variation between wild and mutatnt protein structure were visualized by PYMOL, green color indicates wild amino acid (aspertic acid) while blue color indicates mutant protein (Valine) at position 657. (D657V)



The 3D analysis of wild type and mutant protein structures was performed by using project HOPE. Each amino acid in wild type and mutant structures carries specific properties like solvent accessibility, charge density, hydrophobicity, rigidity, molecular surfaces and electrostatic potential values. Native and mutant residues sometimes differ due to carrying such specific properties and can disrupt the structural and functional features of the original protein. Here we present the results upon each mutation and discuss the conformational variations and interactions with the neighboring amino acids. A/T mutation (rs104895508) resulted in a change of the aspartic acid to valine at position 657 (D657V). The mutant residue is smaller and less hydrophobic than the wild type (Fig. 2). The mutation will cause loss of hydrophobic interactions in the core of the protein. The mutation introduces a more hydrophobic residue at this position. This can result in loss of hydrogen bonds and/or disturb correct folding.



Mutated amino acid Wild amino acid

4. CONCLUSION

In modern genetic analysis, the characterization of disease-associated single nucleotide polymorphism is very encouraging. Bioinformatics has reduced the cost of genotyping and helped to increase genetic association studies. Hence we conducted a bioinformatics approach to analyze the disease associated nsSNPs of NLRP7. Out of 35 non-synonymous mutations, just one was predicted as deleterious by all software's. Structural analysis study suggested that structure and function of NLRP7 can be disturbed by single gene mutation (D657V). Therefore, this SNP can be strongly considered as key candidates in causing disease related to NLRP7 malfunction; hence it will help in effective drug discovery and developing precision medicines.

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