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Prediction of Deleterious Non-Synonymous Single Nucleotide Polymorphisms (Nssnps) of Human *TLR7* Gene

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Abstract

Toll-like receptors (TLRs) play a key role in innate immune response activation against viruses. *TLR7*, one of the TLRs family, is potentially important in controlling viral infection and the production of vaccines against the virus. The wide spectrum of discrepancy in response to antiviral drugs among different populations which is emerged by some pandemics like COVID-19 might be due to their different *TLR7* single nucleotide polymorphisms (SNPs). The present study aimed to investigate the consequences of 401 non-synonymous missense SNPs (nsSNPs) within *TLR7* on its protein structure, stability, and function by using specific bioinformatics tools. Seven bioinformatics tools were used to investigate 401 TLR7 nsSNPs from the dbSNP database. The results showed that the six variations, rs1171508003 (R262H), rs35160120 (F580S), rs968155471 (H587Q), rs202028806 (Y871D), rs1331496205 (W933S), and rs181600414 (R1004W), were found to be extremely deleterious by all of the employed bioinformatics tools. All six variations showed an impact on the protein's structure, function, and stability. Among them, Y871D (rs202028806) and R1004W (rs181600414) were revealed as the most damaging nsSNPs. This study suggested that the predicted six damaging variants of *TLR7* could indirectly or directly destabilize the structure of protein and deviate its function to some extent.

Keywords: prediction, nsSNPs, *TLR7*, human

التنبؤ بتعدد اشكال النيوكليوتيد المفردة الضارة لجين TLR7 في الانسان

جودت نوري

قسم التحليلات المرضية، كلية العلوم الطبية التطبيقية، جامعة كربلاء، كربلاء، العراق

الخلاصة

تلعب المستقبلات الشبيه بالصور دورا أساسيا في تنشيط الاستجابة المناعية الفطرية ضد الفيروسات. ويعد المستقبل الشبيه بالصور السابع احد افراد هذه العائلة وله فعالية مهمة في السيطرة على الإصابات الفيروسية وفي انتاج اللقاحات ضد الفيروسات. ان الطيف الواسع من الاختلافات في الاستجابة المناعية للأدوية المضادة للفيروسات بين مختلف المجتمعات والتي ظهرت نتيجة بعض الجائحات مثل جائحة كوفيد 19 قد تكون بسبب اختلاف تعدد اشكال النيوكليوتيد المفردة لجين TLR7. هدفت هذه الدراسة للتحري عن آثار 401 من تعدد اشكال النيوكليوتيد المفردة الضارة لجين TLR7 على تركيب ووظيفة وثباتية البروتين

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باستخدام سبعة من العدد المتخصصة للمعلوماتية الحيوية. أظهرت النتائج ان التغايرات الستة , rs1171508003 (R262H), rs35160120 (F580S), rs968155471 (H587Q), rs202028806 (Y871D), rs1331496205 (W933S), rs181600414 (R1004W) وجدت بانها ضارة الى درجة كبيرة من خلال جميع عدد المعلوماتية الحيوية المستخدمة. أظهرت جميع التغايرات الستة تأثيرا على تركيب و وظيفة و ثباتية البروتين. وظهرت التغايرات (rs202028806) Y871D و (rs181600414) R1004Wالأشد ضررا". اقترحت الدراسة ان التغايرات الستة لجين TLR7 والتي تم التنبؤ بضررها قد تزعزع استقرار تركيب ووظيفة البروتين بشكل مباشر او غير مباشر.

1. Introduction

Single nucleotide polymorphism (SNP) is a single nucleotide substitution (alteration) in deoxyribonucleic acid (DNA). In the human genome, SNPs are the most common cause of genetic variation. They make up nearly 90% of human genome polymorphism [1]. SNPs are different in their effects according to their location in DNA. Some are found in coding regions and some in the non-coding part of the DNA. Many SNPs have no effect on the cell function; however, some SNPs could be a predisposing factor to some diseases by influencing the response to a particular drug [2]. Nonsynonymous SNPs (nsSNPs), also called missense variations, resulted in a change in the amino acid sequence of the protein. particularly important as they result in nearly 50% of the known genetic variations related to human inherited diseases [3]. The nsSNPs are one of the major causes of the functional diversity of proteins in human populations. The recognition of the SNPs responsible for particular diseases or syndrome appears to be a difficult problem and Solving it requires investigation of a high number of SNPs in applicant genes [4]. The selection of the SNPs to be screened, on the other hand, is critical to the effectiveness of association studies. One option to address this issue is to use specific prediction bioinformatics algorithms to rank SNPs based on their functional relevance, which could help distinguish neutral SNPs from damaging ones. Otherwise, a huge number of individuals might be required to screen SNPs and their statistical significance [5], [6].

Toll-like receptors (TLRs) are innate immune receptors that play a key role in innate immune activation, cytokine generation, adaptive immune system indirect stimulation, and pathogen-associated molecular pattern recognition (PAMPs) [7], [8]. TLRs have ten members in the human. TLR1, TLR2, TLR4, TLR5, and TLR6 are located on the cell membrane, while TLR3, TLR7, TLR8, and TLR9 are located in endosomes (intracellular vesicles) [9]. Sentinel cells such as natural killer cells (NK), dendritic cells (DC), B-cells, T-cells, and macrophages express TLRs [10]. TLR7 is expressed on DC and monocyte-macrophages, and when activated, it produces IL-6, IL-1, monocyte chemoattractant protein-1, type 1 interferon, and TNF-alpha [11]. TLR7 is an X-chromosome gene that produces an endosome membrane protein that recognizes single-strand RNA (ssRNA) and synthesized oligonucleotides. Therefore, they could be involved in recognizing the viral RNA genome [12]. SNPs, which can affect a protein's normal function and denature its structure by affecting its stability, folding form, and ligand-binding site, have been linked to a variety of diseases in previous researches [13], [14]. The impact of nsSNPs on the function and structure of TLR7 protein is still unknown; therefore, in the present study, we investigate and analyze the negative effect of nsSNPs on TLR7 protein by using several bioinformatics tools

2. Materials and Methods

The data on 401 human *TLR7* gene missense SNPs (SNP ID, location, allele, residue change, protein accession number) was retrieved from the dbSNP database of the National Center for Biotechnology Information (NCBI) website (https://www.ncbi.nlm.nih.gov/snp/?cmd=search) and Ensemble genome browser (https://www.ensembl.org/).

Identification of damaging nsSNPs.

In this study, seven different bioinformatics tools were utilized to predict the structural and functional effect of nsSNPs recruited from Ensemble and dbSNP databases on protein. These tools include:

SIFT: Sorting Intolerant From Tolerant (SIFT) [https://sift.bii.a-star.edu.sg/], which is a web-based bioinformatics tool used to discriminate damaging nsSNP from tolerated once based on the homology of sequence [15]. Using SIFT, the prediction score was calculated using a range of values, with a score of ≥ 0.05 regarded as tolerated, and a score of ≤ 0.05 as deleterious [16].

PROVEAN: Protein Variation Effect Analyzer (PROVEAN) [http://provean.jcvi.org/] is a bioinformatics tool that predicts how amino acid substitutions affect a protein's biological activity. It is useful for identifying non-synonymous variants that are predicted to be functionally important [17]. This tool is based on a pairwise sequence alignment score obtained by fast computation approach that enables the creation of predictions of precomputed PROVEAN for twenty single amino acid alterations at every amino acid position [18]. A score of more than (2.5) was regarded neutral. And a score less than (-2.5) was considered damaging [19].

PolyPhen-2: Polymorphism Phenotyping v2 (PolyPhen-2) [http://genetics.bwh.harvard.edu/pph2/] is another bioinformatics tool used extensively to predict the impact of SNPs on the structure and function of the protein [20], [21]. FASTA format sequence of the protein, amino acid position, protein or SNP identifier, and variant details are the input options for the PolyPhen-2 [22]. The available prediction models are HumDiv and HumVar [23]. HumVar identifies the SNPs with a major phenotypic effect based on PSIC (Position-Specific Independent Counts) score, whereas HumDiv identifies the less damaging SNPs [24]. According to PolyPhen-2, the variation is ranged from benign (score = 0.00 – 0.14), possibly damaging (score = 0.15 – 0.84), and probably damaging (score = 0.85 – 1.00). The score ranged from zero (0.00) for neutral variation to one (1.00) for deleterious variation [25].

SNAP2: Screening for Non-acceptable Polymorphism v2 (SNAP2) [https://rostlab.org/services/snap2web/] is a bioinformatics tool that predicts the functional effects of sequence variants on the protein. SNAP2 is a trained classifier based on neural network, a machine learning device, which discriminates between effect and neutral nsSNPs by taking variants features into account. A score of +100 (dark red) or -100 (dark blue) on the heat map indicates whether an amino acid change is pathogenic or absolutely neutral [26].

PANTHER: Protein Analysis Through Evolutionary Relationship (PANTHER) [http://www.pantherdb.org/] database provides tools for functional analysis of genes or proteins. PANTHER uses substitution Position-Specific Evolutionary Conservation (subPSEC) scores to estimate the changes in protein function caused by nsSNPs. A score equal to zero (0) is predicted benign, while a score less than (-3) is predicted to be deleterious [27].

GOR IV: GOR IV [https://npsa-prabi.ibcp.fr/NPSA/npsa_gor4.html] is another powerful tool to predict protein secondary structure upon SNPs. For each secondary structure at each amino acid location, this tool provides probability values. GOR IV, the most recent version, employs all potential pair frequencies across a range of amino acid residues, as stated by Garnier *et al.* (1996) [28]. GOR IV output shows a sequence and predicted secondary structure in rows (include E=extended, H=helix, beta-strand, and C=coil). It calculates the probability values for each secondary structure at every amino acid location.

MUpro: MUpro [http://mupro.proteomics.ics.uci.edu/] is a tool used to predict how single amino acid substitution affects the stability of protein [29]. By using a support vector machine (SVM) and neural networks, the MUpro predicts the sign of energy change, which measures the effects of amino acid substitution on the stability of the protein. A confidence score

ranged between (-1) and (1), a score of > 0 means the amino acid substitution increases protein stability, while a score of < 0 decreases the protein stability [29].

3. Results and Discussion

nsSNPs retrieved from dbSNP database

In this study, the dbSNP database provided by NCBI and Ensemble Genome Browser databases were exploited to achieve all types of SNPs of *TLR7* gene including inframe variants, intronic, synonymous, missense, etc. dbSNP results revealed that 6356 SNPs were found throughout the *TLR7* gene, of which 401 were nsSNPs, 267 were synonymous, 4574 were intronic, and the remaining SNPs were of various types (till 14 September 2021). We investigated only the 401 nsSNPs.

Predicting deleterious nsSNPs in *TLR7*

All the 401 nsSNPs in the *TLR7* gene were subjected to seven different prediction bioinformatics tools to investigate if these SNPs affect the function, structure, or stability of the TLR7 protein. The bioinformatics tools that used were: SIFT, SNAP2, PROVEAN, PANTHER, PolyPhen-2, GOR IV, and MUpro. Out of 401 nsSNPs subjected to tools analysis, only 6 nsSNPs were predicted to be deleterious in all of the bioinformatics tools and matched the requirements, putting them in the high-risk category (Table 1). Those six nsSNPs include rs1171508003 G/A, rs35160120 T/C, rs968155471 T/A, rs202028806 T/G, rs1331496205 G/C, and rs181600414 C/T which are protein substitution R262H, F580S, H587Q, Y871D, W933S, and R1004W respectively.

The SIFT tool analysis showed that the six nsSNPs were predicted to be deleterious and the amino acid alterations of these SNPs were given a score of zero (0) for all (Table 1). Prediction of nsSNPs with SNAP2 tool revealed two types of mutation, effect (pathogenic) or neutral. By using SNAP2 it was identified that the amino acid changes of all the six nsSNPs were pathogenic with a score ranging from 49 to 87 (Table 2). The analysis of the nsSNPs using PROVEAN tool showed that the six nsSNPs were predicted to be deleterious with a score ranging between (-12.694) and (-4.759) (Table 3). Prediction of nsSNPs using PANTHER tool revealed that the amino acid substitution of the six nsSNPs were probably damaging with a prediction score of 910 for all (Table 4). The prediction analysis of PolyPhen-2 tool showed that the amino acid substitution of the six nsSNPs were probably damaging with a score of 0.997 for R1004W and a score of 1 for the rest of the five amino acid substitution (Table 5).

Table 1- List of variants analyzed by SIFT tool.

NO.	Variant ID	Location	Allele	Amino acid substitution	SIFT score	SIFT prediction
1	rs1171508003	X:12886293	G/A	R262H	0	Deleterious
2	rs35160120	X:12887247	T/C	F580S	0	Deleterious
3	rs968155471	X:12887269	T/A	H587Q	0	Deleterious
4	rs202028806	X:12888119	T/G	Y871D	0	Deleterious
5	rs1331496205	X:12888306	G/C	W933S	0	Deleterious
6	rs181600414	X:12888518	C/T	R1004W	0	Deleterious

Table 2-Prediction of nsSNPs effect using SNAP2 tool.

NO.	Variant ID	Location	Allele	Amino acid substitution	SNAP2 score	SNAP2 prediction
1	rs1171508003	X:12886293	G/A	R262H	62	Effect
2	rs35160120	X:12887247	T/C	F580S	70	Effect
3	rs968155471	X:12887269	T/A	H587Q	49	Effect
4	rs202028806	X:12888119	T/G	Y871D	85	Effect
5	rs1331496205	X:12888306	G/C	W933S	51	Effect
6	rs181600414	X:12888518	C/T	R1004W	87	Effect

Table 3- Prediction of nsSNPs effect using PROVEAN tool.

NO.	Variant ID	Location	Allele	Amino acid substitution	PROVEAN score	PROVEAN prediction
1	rs1171508003	X:12886293	G/A	R262H	-4.759	Deleterious
2	rs35160120	X:12887247	T/C	F580S	-7.863	Deleterious
3	rs968155471	X:12887269	T/A	H587Q	-7.523	Deleterious
4	rs202028806	X:12888119	T/G	Y871D	-7.318	Deleterious
5	rs1331496205	X:12888306	G/C	W933S	-12.694	Deleterious
6	rs181600414	X:12888518	C/T	R1004W	-7.338	Deleterious

Table 4- List of variants analyzed by PANTHER tool.

NO.	Variant ID	Location	Allele	Amino acid substitution	PANTHER score	PANTHER prediction
1	rs1171508003	X:12886293	G/A	R262H	910	probably damaging
2	rs35160120	X:12887247	T/C	F580S	910	probably damaging
3	rs968155471	X:12887269	T/A	H587Q	910	probably damaging
4	rs202028806	X:12888119	T/G	Y871D	910	probably damaging
5	rs1331496205	X:12888306	G/C	W933S	910	probably damaging
6	rs181600414	X:12888518	C/T	R1004W	910	probably damaging

Table 5- variants evaluated using PolyPhen-2 tool.

NO.	Variant ID	Location	Allele	Amino acid substitution	PolyPhen-2 score	PolyPhen-2 prediction
1	rs1171508003	X:12886293	G/A	R262H	1	probably damaging
2	rs35160120	X:12887247	T/C	F580S	1	probably damaging
3	rs968155471	X:12887269	T/A	H587Q	1	probably damaging
4	rs202028806	X:12888119	T/G	Y871D	1	probably damaging
5	rs1331496205	X:12888306	G/C	W933S	1	probably damaging
6	rs181600414	X:12888518	C/T	R1004W	0.997	probably damaging

Predicting the effect of *TLR7* nsSNPs on protein secondary and tertiary structure.

In order to predict the effect of nsSNPs on protein secondary and tertiary structure, we used the GOR IV tool, which is a powerful prediction tool. The prediction analysis of GOR IV tool showed the contents of the normal secondary and tertiary structure of the TLR7 protein, which include 438 alpha helixes, 162 extended strands, and 449 random coils differed from the contents of structures with the six amino acids substitutions. The results showed that the amino acid substitution of the six nsSNPs affects on protein structure by altering the protein contents of alpha helixes, extended strands, and random coils. Table 6 summarizes the effect of amino acid substitution on protein secondary and tertiary structure.

Table 6-Prediction the effect of amino acid substitution on the secondary and tertiary structure of TLR7 protein using GOR IV.

Parameter	Normal protein No. (%)	R262H No. (%)	F580S No. (%)	H587Q No. (%)	Y871D No. (%)	W933S No. (%)	R1004W No. (%)
Alpha helix	438 (41.75)	438 (41.75)	439 (41.85)	439 (41.85)	438 (41.75)	443 (42.23)	436 (41.56)
310 helix	0	0	0	0	0	0	0
Pi helix	0	0	0	0	0	0	0
Beta bridge	0	0	0	0	0	0	0
Extended strand	162 (15.44)	161 (15.35)	162 (15.44)	162 (15.44)	160 (15.25)	158 (15.06)	161 (15.35)
Beta turn	0	0	0	0	0	0	0
Bend region	0	0	0	0	0	0	0
Random coil	449 (42.8)	450 (42.9)	448 (42.71)	448 (42.71)	451 (42.99)	448 (42.71)	452 (43.09)
Ambiguous states	0	0	0	0	0	0	0
Other states	0	0	0	0	0	0	0

Predicting the effect of nsSNPs on the stability of *TLR7* protein.

The impact of the six nsSNPs of the *TLR7* gene on the stability of the protein was predicted by MUpro tool by comparing changes of free energies (ΔΔG). The results showed that the amino acid substitutions of the six nsSNPs were identified to have a negative impact on protein stability. The six variants (R262H, F580S, H587Q, Y871D, W933S, and R1004W) showed ΔΔG values fewer than 0 kcal/mol (Table – 7), which would affect the function and structure of the protein.

Table 7-Prediction of protein structure stability using MUpro tool.

NO.	Amino acid substitution	WT	New	ΔΔG (kcal/mol)	Prediction confidence score	Protein stability
1	R262H	R	H	-0.065758698	-0.10873486	Decrease
2	F580S	F	S	-1.5330122	-0.84580361	Decrease
3	H587Q	H	Q	-0.90065946	-0.33974696	Decrease
4	Y871D	Y	D	-1.3860953	-0.93811023	Decrease
5	W933S	W	S	-1.2453685	-1	Decrease
6	R1004W	R	W	-1.1088291	-0.82867613	Decrease

Where, "WT" is the wild type amino acid, "New" is a mutant amino acid, and ΔΔG is the stability (ΔΔG > 0: increase stability, ΔΔG < 0: decrease stability).

TLR7 is a protein encoded by the *TLR7* gene found in humans on chromosome Xp22.2. It recognizes viruses with single-strand RNA such as HCV and HIV [30] and SARS-Co-V-2 [31]. TLR7 is involved in the pathophysiology of autoimmune disorders as well as antiviral immunity control. TLR7 expression varies from person to person and it is genetically determined. The existence of loss of function variations in the X-linked recessive *TLR7* gene, notably missense deleterious variations, may be the source of illness vulnerability to viruses like COVID-19 [32].

Many human single nucleotide polymorphisms are recognized in the human genome, providing an opportunity for future research for much understanding of the phenotype – genotype association. At the molecular level, bioinformatics algorithms are increasingly being utilized to predict disease-associated SNPs. Recently, recognition of SNPs by the computational method is favored in identifying the variations that alter the function and structure of proteins [33]-[35]. The nonsynonymous SNPs of the TLR7 gene have not been studied at the atomic level to see how they affect the structure and functional implications. Therefore, an effort was made to recognize SNPs that can affect and alter the function, structure, and stability of the *TLR7* gene. In the present study, the nsSNPs in the *TLR7* gene, have been linked to various human viral and autoimmune diseases. The 401 nsSNPs, which were retrieved from the dbSNP database, were submitted to the five bioinformatics tools (SIFT, SNAP2, PROVEAN, PANTHER, and PolyPhen-2), out of these 401 nsSNPs, by using all these tools, 6 nsSNPs were found to be deleterious which may significantly affect TLR7 protein function and/or structure. The critical point for protein function, activity, and regulation is protein stability. Decreased stability and misfolding are the major consequences of pathogenic nsSNPs [36]. The thermodynamic stability of a protein is measured by ΔG (folding free energy). The mutant and wild types of protein have their specific ΔG values. The ΔΔG, the free energy change between the wild and mutant types, is observed by the equation (ΔΔG = ΔG mutant – ΔG wild) [37]. This study showed that the six amino acid substitutions (R262H, F580S, H587Q, Y871D, W933S, and R1004W) were decreased protein stability by using MUpro tool. A combination of multiple prediction methods revealed better prediction results in many recent studies on deleterious nsSNPs classification, in MACC1 [38], NY-BR-1 [39], MBL2 [40], HBA1 [41], AHSP [42], MKRN3 [43], MTHFR [44], and BCL11A [45]-

[47]. The findings of the present study were not reported in any previous study. As a result, we need to validate the six variants to support our findings.

4. Conclusions

The analysis of our results concludes that the six amino acid substitutions (R262H, F580S, H587Q, Y871D, W933S, and R1004W) might directly or indirectly destabilize the structure of the TLR7 protein and deviate its function to some extent. Therefore, future studies can be designed by considering the present study results to analyze its biological context.

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