Computational Analysis Revealed Five Novel Mutations in Human *IL2RG* gene Related to X-SCID

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ABSTRACT

Background: *X linked severe combined immunodeficiency (X-SCID) is a life-threatening disorder. It is due to mutation of the interleukin two receptor gamma-chain (IL2RG) gene. Nonsynonymous SNPs (nsSNPs) are the most common polymorphism, known to be deleterious or disease-causing variations because they alter protein sequence, structure, and function. Objective: is to reveal the effect of harmful SNPs in the function and structure of IL2RG protein.* ***Method:*** *Data on IL2RG was investigated from dbSNP/NCBI database. Prediction of damaging effect was done using sift, polyphen, provean and SNAP2.more software were used for more analysis: phd-snp, and and go, Pmut, Imutant.modeling was done using chimera and project hope. Gene interaction was done by gene mania.3UTR prediction was done using polymiRTS software.* ***Result:*** *The in-silico prediction identified 1479 SNPs within IL2RG gene out of which 253 were coding SNPs, 50 took place in the miRNA 3 UTR, 21 occurred in 5 UTR region and 921 occurred in intronic regions. a total of 12 missense nsSNPs were found to be damaging by both a sequence homology-based tool (SIFT) and a structural homology-based method (PolyPhen), Five of them were novel; rs1322436793(G305R), rs1064794027(C182Y), rs111033620(G114D), rs193922347 (Y105C) and rs1293196743(Y91C), Two SNPs(Rs144075871 and rs191726889) out of 50 in the 3UTR region were predicted to disrupt miRNAs binding sites and affect the gene expression.* ***Conclusions:*** *Computational analysis of SNPs has become a very valuable tool in order to discriminate neutral SNPs from damaging SNPs. This study revealed 5 novel nsSNPs in the IL2RG gene by using different software and 21 SNPs in 3UTR. These SNPs could be considered as important candidates in causing diseases related to IL2RG mutation and could be used as diagnostic markers.*

Keywords: X linked severe combined immunodeficiency (X-SCID), interleukin 2 receptor gamma-chain (*IL2RG), single* nucleotide polymorphism (SNP), nonsynonymous Single Nucleotide Polymorphisms (nsSNPs), bioinformatics.

1. Introduction:

Severe combined immunodeficiency (SCID) [OMIM: #300400] is a rare life-threatening condition characterized by impaired cellular and humoral immunity [(1)]. X linked severe combined immunodeficiency (X-SCID; 308380) is the commonest type of SCID (~50%) [(2-5)]. Interleukin 2 receptor gamma-chain gene (*IL2RG)* mutation is responsible of X-SCID [(6-9)]. Interleukin 2 receptor gamma-chain gene (*IL2RG)* [OMIM: *308380] is positioned on chromosome Xq13 and its product is common gamma protein [(10-12)]. The common gamma is an elemental structure of the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21[(13, 14)].These signaling cytokines are essential for lymphocytes development and function, they promotes regulation of T-cell growth , differentiation and peripheral tolerance, increasing of natural killer (NK) cytolytic activity and B cells differentiation[(6, 8, 15)] .T cell dysfunction effects immunoglobulin class switching of B cells that mean patients have compromised specific antibody responses , so they have defects in class-switched immunoglobulins (IgG, IgA, and IgE) with detectable IgM [(3)]. In classical X-SCID nonfunctional *IL2RG* results in deficiency of T and natural killer (NK) lymphocytes and malfunctioning B lymphocytes T (-), NK (-) B (+). non-classical X-SCID (312863) may have a missense mutation or other potentially non-loss of function result is low numbers of T cells while NK and B cells are normal in numbers T (low) NK (+) B (+) [(16-18)]. Male patients with classical X-SCID suffer from severe opportunistic infections and they usually die during infancy if not treated while non-classical X-SCID has less severe type of disease [(19-21)].

There are large numbers of information about *IL2RG* mutations associated with X-SCID found in the literatures [(4, 16, 22, 23)]. Moreover, there are many publications covering polymorphism of *IL2RG* single nucleotide polymorphism (SNPs) like: D39N, G114D, C115F, C115R, I153N, L162H, C182R, L183S, R222C, R224W, R226H, W240C, S241I, R285Q, L293Q [(12, 16, 17, 23-27)]. Also, a large numbers of missenses mutations or nonsynonymous (SNP) of *IL2RG* are collected in international databases. Despite this there are insufficient studies investigating all the harmful nsSNPs of *IL2RG*. The aim of this study to the possible effect of *IL2RG* SNPs on function and structure. Single Nucleotide Polymorphisms (SNPs) are variation of DNA sequence in which a single nucleotide is alter. It is the most common polymorphism [(28)]. It can occur in coding or non-coding DNA sequence, SNPs is detected in the coding region of people leading to genetic variation [(28,29)]. Non-synonymous SNPs (ns SNPs) are present in

coding region of genome which is frequently leads to alteration in amino acid residues of gene product. This single lost or additional nucleotide causes a frame shift mutation [30]. This effect changes the protein that is expressed, this possibly will cause a harmful mutation [31]. This is the first study investigate SNPs located in *IL2RG* gene using computational approach.

2. Materials and methods:

Data on *IL2RG* gene was obtained from national center for biological information (NCBI) web site (https://www.ncbi.nlm.nih.gov/) and the SNPs (single nucleotide polymorphisms) information was retrieved from NCBI SNPs database dbSNP (https://www.ncbi.nlm.nih.gov/snp/) (32). The gene ID and sequence was obtained from Uiprot (https://www.uniprot.org/). Analysis of the SNPs was done according to (figure 1).

2.1. SIFT: Sorting Intolerant from Tolerant software is available at (http://sift.bii.a-star.edu.sg/). SIFT is a sequence homology-based tool that sorts intolerant from tolerant amino acid substitutions and predicts whether an amino acid substitution in a protein will have a phenotypic outcome, considering the position at which the mutation occurred and the type of amino acid change. At the point when a protein sequence submitted, SIFT picks related proteins and acquired an alignment of these proteins with the query. According to the change on the type of amino acids appearing at each position in the alignment, SIFT calculates the probability that the substitution is tolerated or not. If this standardized esteem is less than a cutoff, the substitution is predicted to be deleterious. SIFT scores <0.05 are predicted to be intolerant or deleterious amino acid substitutions, whereas scores >0.05 are considered tolerant. [33]

2.2. PolyPhen-2: (http://genetics.bwh.harvard.edu/pph2/) **Poly**morphism **Phen**otyping **v2.** It is a tool that predicts conceviable impact of an amino acid ulteration on the structure and function of a human protein by using simple physical and comparative considerations. The submission of the sequence allows querying for a single individual amino acid substitution or a coding, non-synonymous SNP commented on in the SNP database. This software calculates position-specific independent count (PSIC) scores for each of the two variants and calculate the difference of the PSIC scores of the two variants. The higher a PSIC score difference, the higher functional effect to have.

PolyPhen scores were designated as probably damaging (0.95–1), possibly damaging (0.7–0.95), and benign (0.00–0.31). [34] [35]

2.3. Provean:**Pr**otein **Va**riation **E**ffect **An**alyzer software available at (http://provean.jcvi.org/index.php) it predict whether an amino acid substitution has an impact on the biological function of protein. Provean is useful in filtrating sequence variants to distinguish non-synonymous variants that are predicted to be practically imperative. [36]

2.4. SNAP2: is a trained classifier that is based on a machine learning gadget called "neural network". It distinguishes between effect and neutral variants/non-synonymous SNPs by taking differences of sequence and variant features in considerations. Most important input for the prediction is the evolutionary information taken from multiple sequence alignment. Also, structural features such as predicted secondary structure and solvent accessibility are considered. If available also annotation of the sequence or close homologs are used in. SNAP2 has persistent two-state accuracy (effect/neutral) of 82%. Software is available at (https://rostlab.org/owiki/index.php/Snap2).[37, 38]

2.5.PHD-SNP: prediction of human Deleterious Single Nucleotide Polymorphisms is accessible at (http://snps.biofold.org/phd-snp/phd-snp.html) .It is a Support Vector Machines (SVMs) based method trained to predict disease-associated nsSNPs using sequence information. The related transformaion is predicted as disease-related (Disease) or as neutral polymorphism (Neutral). [39]

2.6.SNP & GO: is a server for the prediction of single point protein mutations likely to be involved in the causing of diseases in humans. SNP&GO is accessible at (https://snps-and-go.biocomp.unibo.it/snps-and-go/). [40]

2.7. PMUT: available at (http://mmb.irbbarcelona.org/PMut/) is based on the use of different types of sequence information to detect mutations, and neural networks to analyse this information. It provides a very simple output: yes/no answer and a reliability index. [41]

2.8.I-MUTANT: available at (http://gpcr.biocomp.unibo.it/~emidio/I-Mutant3.0/old/IntroI-Mutant3.0_help.html) is a suit of support vector machine based predictors which integrated in a unique web server. It offers the chance to predict protein stability changes upon single site mutations starting from protein sequence

alone or protein structure if accessible. Also, it gives opportunity to predict human deleterious SNPs from the protein sequence alone. (42)

2.9. Project HOPE online software. It is a web service where the user can submit a sequence and mutation. The software gathers basic data from different sources, including calculations on the 3D protein structure, sequence commented in UniProt and prediction from other software. It gathers this information to give analysis for the effect of a certain mutation on the protein structure. HOPE will show the effect of that mutation in such a way that any one even those without a bioinformatics background can understand it. It allows the user to submit a protein sequence or an accession number of the protein of interest. In a next step the user can choose the mutated amino acid with a simple mouse click. In the final step the user can simply tap on one of the other 19 amino acid types that will become the mutant residue, and then full report well be available. HOPE is available at (http://www.cmbi.ru.nl/hope/method/). (43)

2.10.UCSF Chimera (university of California at san Francisco) (https://www.cgl.ucsf.edu/chimera/) is an exceedingly extensible program for interactive visualization and analysis of molecular structures and relative data, including density maps, supramolecular assemblies, sequence alignments, docking results, directions, and conformational troupes. High-quality images and animations can be generated. **Chimera** includes complete documentation and several tutorials. **Chimera** is developed by the Resource for Biocomputing , Visualization, and Informatics (RBVI), supported in part by the National Institutes of Health (P41-GM103311).(44)

2.11. PolymiRTS: used to predict 3UTR (un-translated region): polymorphism in microRNAs and their target sites available at (http://compbio.uthsc.edu/miRSNP/) it is a data base of naturally occurring DNA variations in microRNAs(miRNA) seed region and miRNA target sites. MicroRNAs combine to the transcript of protein coding genes and cause translational repression or mRNA destabilization. SNPs in microRNA and their target sites may influence miRNA-mRNA interaction, causing impact on miRNA-mediated gene repression. PolymiRTS database was made by examining 3UTRs of mRNAs in human and mouse for SNPs in miRNA target destinations. Then, the impact of polymorphism on gene expression and phenotypes are identified and then connected in the database. The PolymiRTS data base also includes polymorphism in

target sites that have been supported by variety of experimental methods and polymorphism in miRNA seed regions. [45, 46]

2.12. Gene interaction **GeneMAINIA** software that finds other genes that are related to a set of input genes, using a very large set of functional association data. Association data include protein and genetic interactions, pathways, co-expression, co-localization and protein domain similarity. GeneMANIA also used to find new members of a pathway or complex, find additional genes you may have missed in your screen or find new genes with a specific function, such as protein kinases. This software is available at (https://genemania.org/).[47]

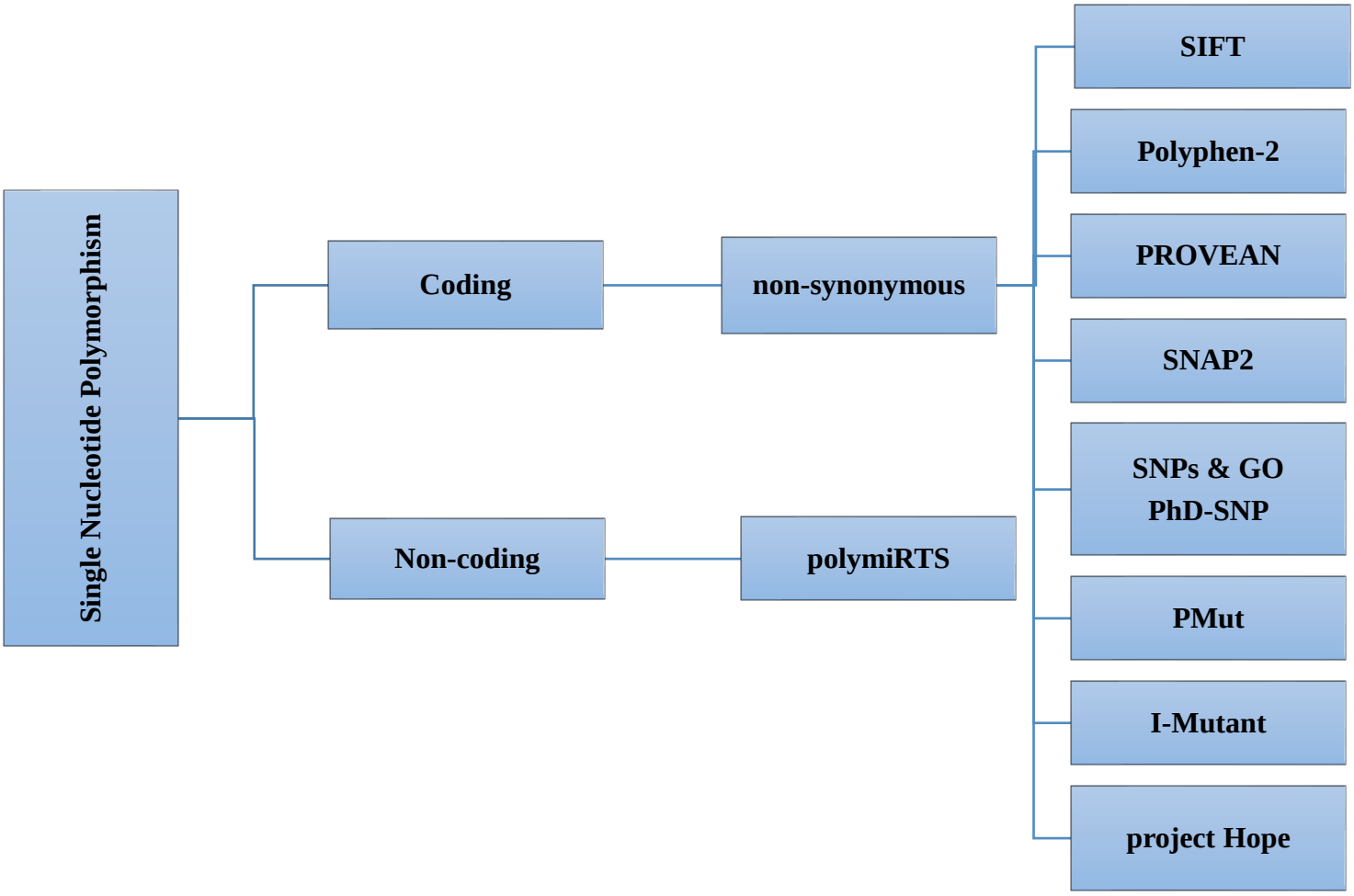


Figure (1): Flowchart of the software using in the analysis processes.

3. Results:

3.1. Retrieval of SNPs from the Database:

A total of 1479 SNPs within *IL2RG* gene -at the time of the study- were retrieved from dbSNP database, out of which 253 were coding SNPs, 50 took place in the miRNA 3′ UTR, 21 occurred in 5′ UTR region and 921 occurred in intronic regions.

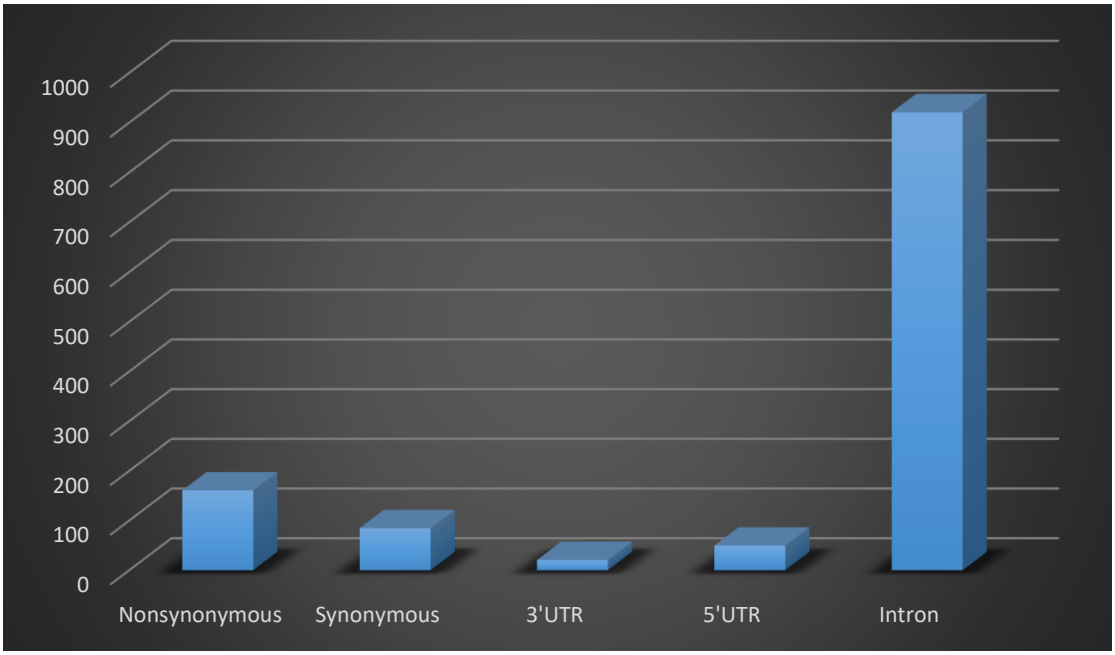


Figure (2): distribution of nonsynonymous, synonymous, 3′ UTR, 5′UTR and intronic SNPs for *IL2RG* retrieved from NCBI database.

3.2. Table (1) Prediction of functional effect of Deleterious and damaging nsSNPs by SIFT, Polyphen-2, PROVEAN and SNAP2:

Chr. Position*	dbSNP ID	Amino Acid Change	SIFT Prediction	SIFT score	Polyphen-2 Prediction	Polyphen-2 Score	PROVEAN Prediction	PROVEAN score	SNAP2 Prediction	SNAP2 Score
71108288	rs1322436793	G305R	Deleterious	0	probably damaging	1	Deleterious	-3.457	effect	76
71109267	rs1057517950	W240R	Deleterious	0	probably damaging	1	Deleterious	-13.448	effect	91
71109309	rs869320659	R226C	Deleterious	0.01	probably damaging	1	Deleterious	-6.126	effect	49
71109315	rs869320658	R224W	Deleterious	0	probably damaging	1	Deleterious	-7.601	effect	90
71109321	rs111033618	R222C	Deleterious	0.03	probably damaging	1	Deleterious	-4.007	effect	72
71110205	rs1064794027	C182Y	Deleterious	0.02	probably damaging	1	Deleterious	-10.504	effect	55
71110506	rs137852511	L151P	Deleterious	0.02	probably damaging	1	Deleterious	-3.865	effect	19
71110615	rs111033622	C115R	Deleterious	0	probably damaging	1	Deleterious	-11.55	effect	99
71110617	rs111033620	G114D	Deleterious	0.04	probably damaging	1	Deleterious	-6.538	effect	98
71110644	rs193922347	Y105C	Deleterious	0.05	probably damaging	1	Deleterious	-8.563	effect	43
71110686	rs1293196743	Y91C	Deleterious	0.01	probably damaging	1	Deleterious	-7.039	effect	47
71110906	rs1057520293	L87P	Deleterious	0.01	probably damaging	1	Deleterious	-5.396	effect	83

*Chr: chromosome

3.3. Table (2) prediction of Disease Related and pathological effect of nsSNPs by PhD-SNP, SNPs & GO and PMut:

Chr. Position*	dbSNP ID	Amino Acid Change	SNP & GO Prediction	RI*	Probability	PhD-SNP Prediction	RI*	Probability	PMUT Prediction	
71108288	rs1322436793	G305R	Disease	5	0.727	Disease	6	0.793	**0.76 (88%)**	Disease
71109267	rs1057517950	W240R	Disease	6	0.795	Disease	3	0.643	**0.81 (89%)**	Disease
71109309	rs869320659	R226C	Disease	1	0.528	Disease	4	0.7	**0.79 (89%)**	Disease
71109315	rs869320658	R224W	Disease	6	0.807	Disease	7	0.857	**0.87 (92%)**	Disease
71109321	rs111033618	R222C	Disease	0	0.509	Disease	4	0.709	**0.66 (85%)**	Disease
71110205	rs1064794027	C182Y	Disease	6	0.823	Disease	8	0.917	**0.73 (87%)**	Disease
71110506	rs137852511	L151P	Disease	1	0.546	Disease	4	0.707	**0.61 (83%)**	Disease
71110615	rs111033622	C115R	Disease	6	0.779	Disease	6	0.791	**0.90 (93%)**	Disease
71110617	rs111033620	G114D	Disease	3	0.661	Disease	5	0.758	**0.75 (87%)**	Disease
71110644	rs193922347	Y105C	Disease	3	0.627	Disease	4	0.697	**0.79 (89%)**	Disease
71110686	rs1293196743	Y91C	Disease	2	0.599	Disease	3	0.672	**0.76 (88%)**	Disease
71110906	rs1057520293	L87P	Disease	2	0.586	Disease	5	0.726	**0.87 (91%)**	Disease

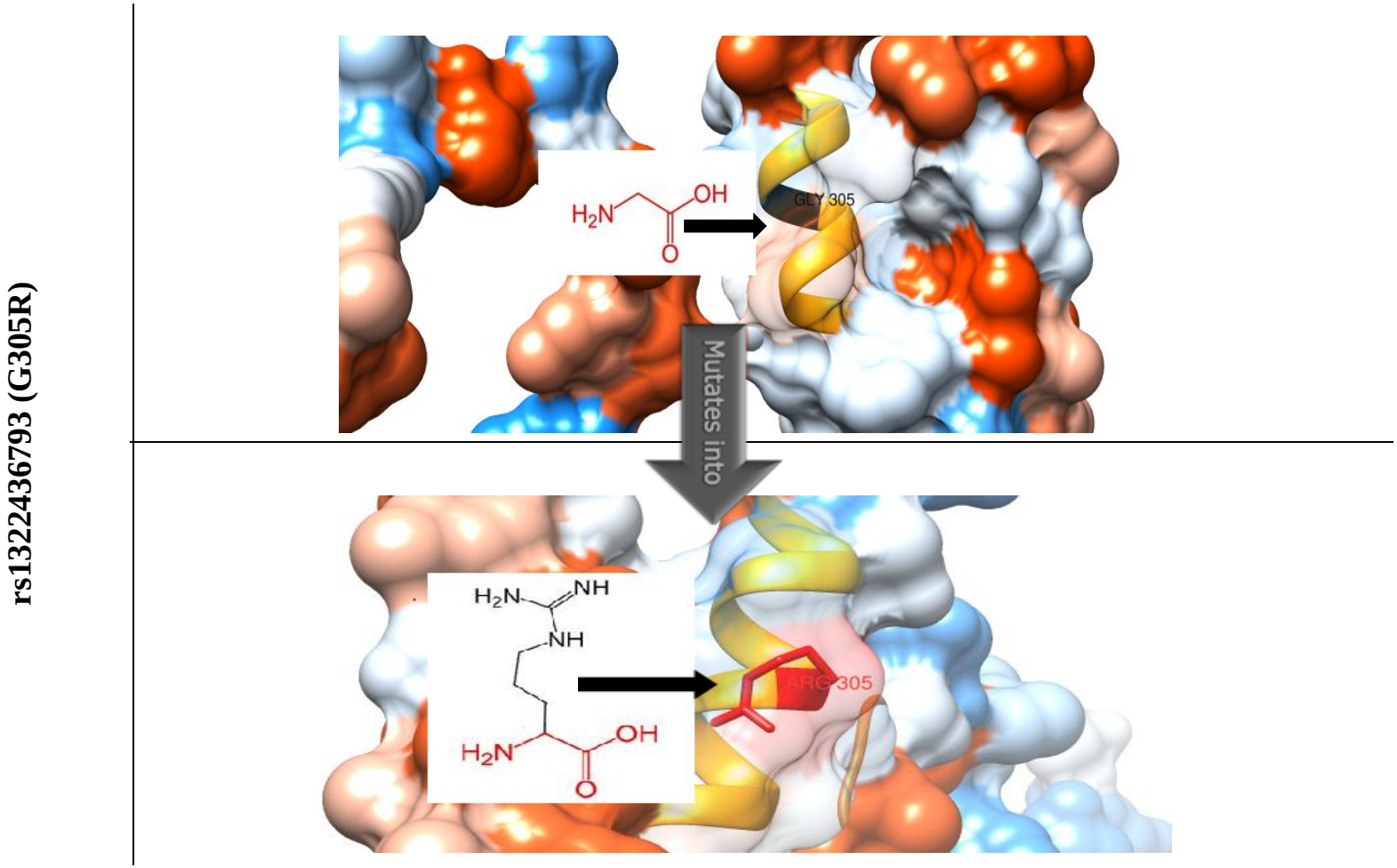
***** Chr: chromosome ***** RI: reliability index

3.4. Table (3) Prediction of nsSNPs Impact on Protein structure Stability by I-Mutant:

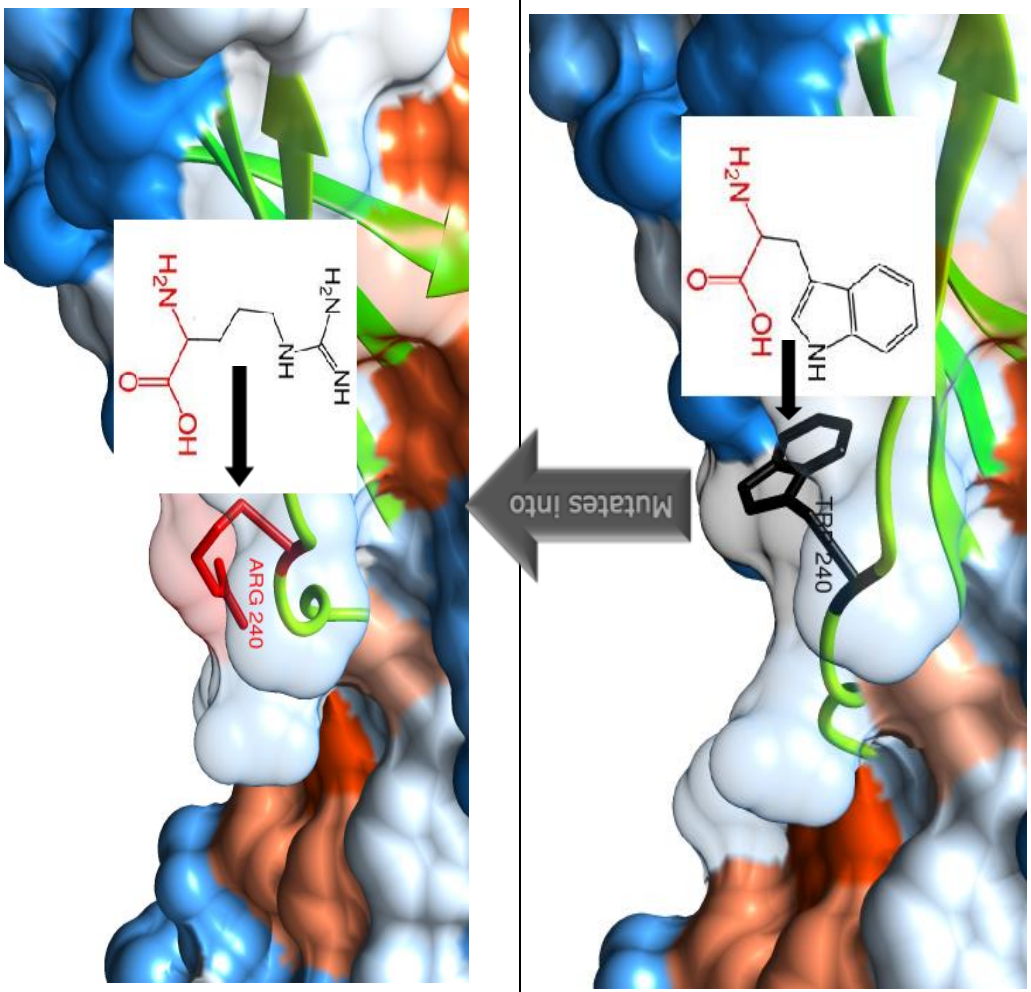
Chr. Position*	dbSNP ID*	Amino Acid Change	IMUTANT PREDICTION	SCORE
71108288	rs1322436793	G305R	DECREASE	-0.56
71109267	rs1057517950	W240R	DECREASE	-0.71
71109309	rs869320659	R226C	DECREASE	-1.27
71109315	rs869320658	R224W	DECREASE	-0.64
71109321	rs111033618	R222C	DECREASE	-1.4
71110205	rs1064794027	C182Y	DECREASE	-0.48
71110506	rs137852511	L151P	DECREASE	-1.49
71110615	rs111033622	C115R	INCREASE	0.03
71110617	rs111033620	G114D	DECREASE	-0.94
71110644	rs193922347	Y105C	DECREASE	-1.05
71110686	rs1293196743	Y91C	DECREASE	-1.23
71110906	rs1057520293	L87P	DECREASE	-1.65

* Chr: chromosome. *db: database.

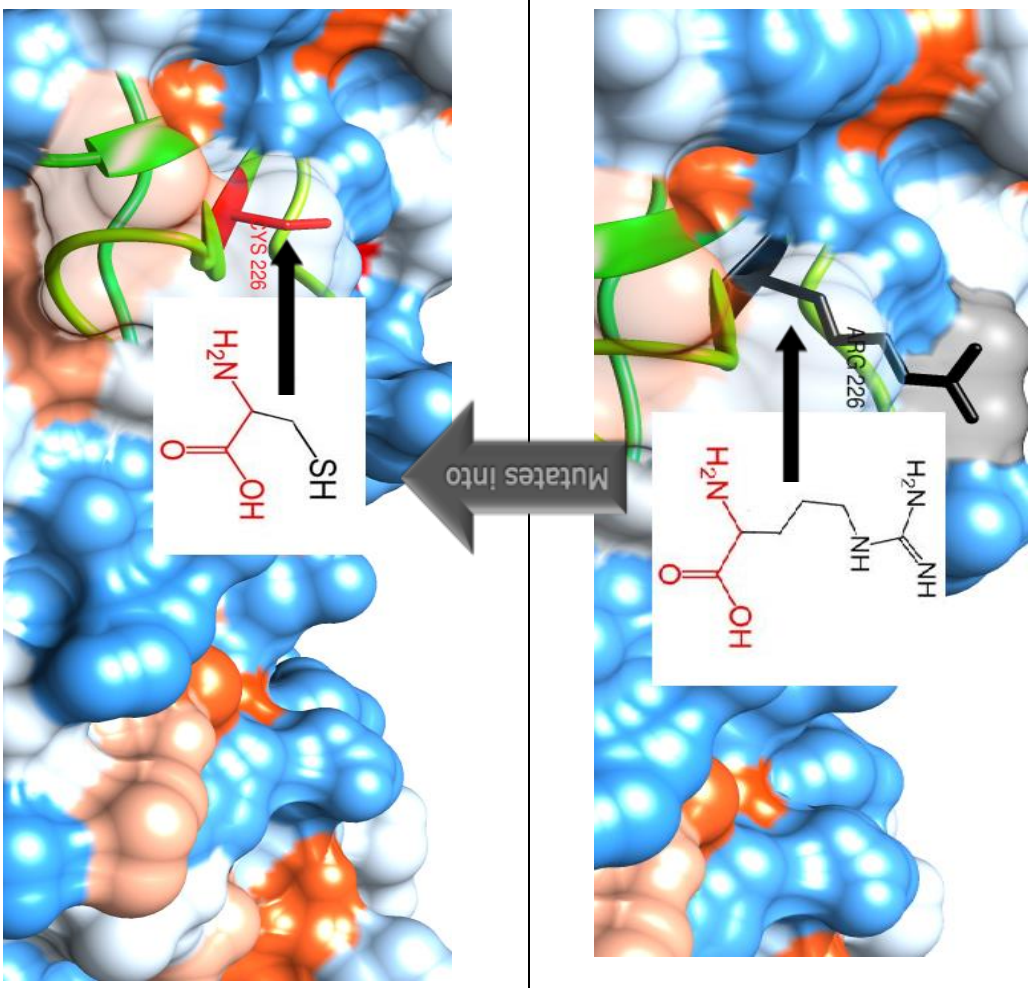
3.5. Figure (3) Modeling of wild and Mutant Structure by Project HOPE and Chimera:



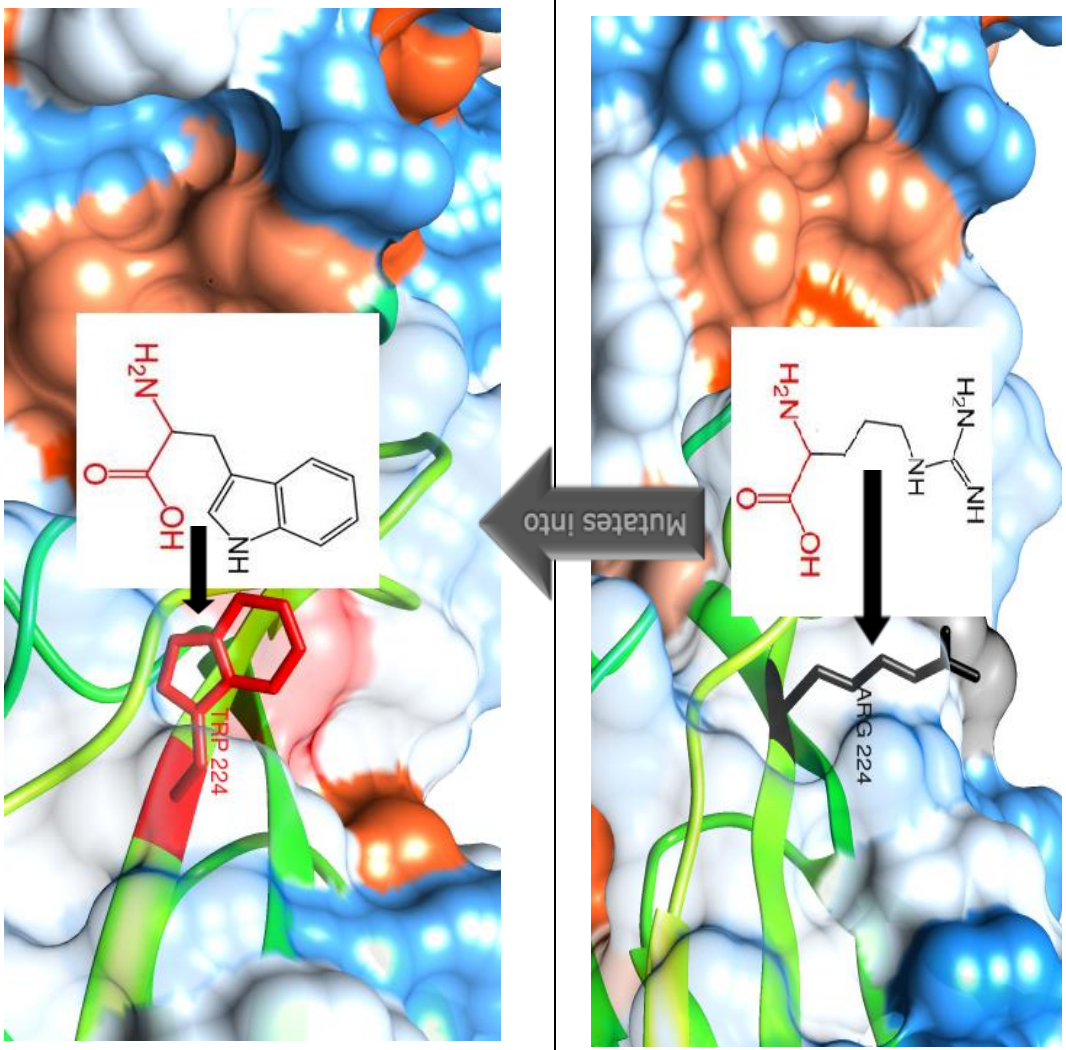
rs1057517950 (W240R)



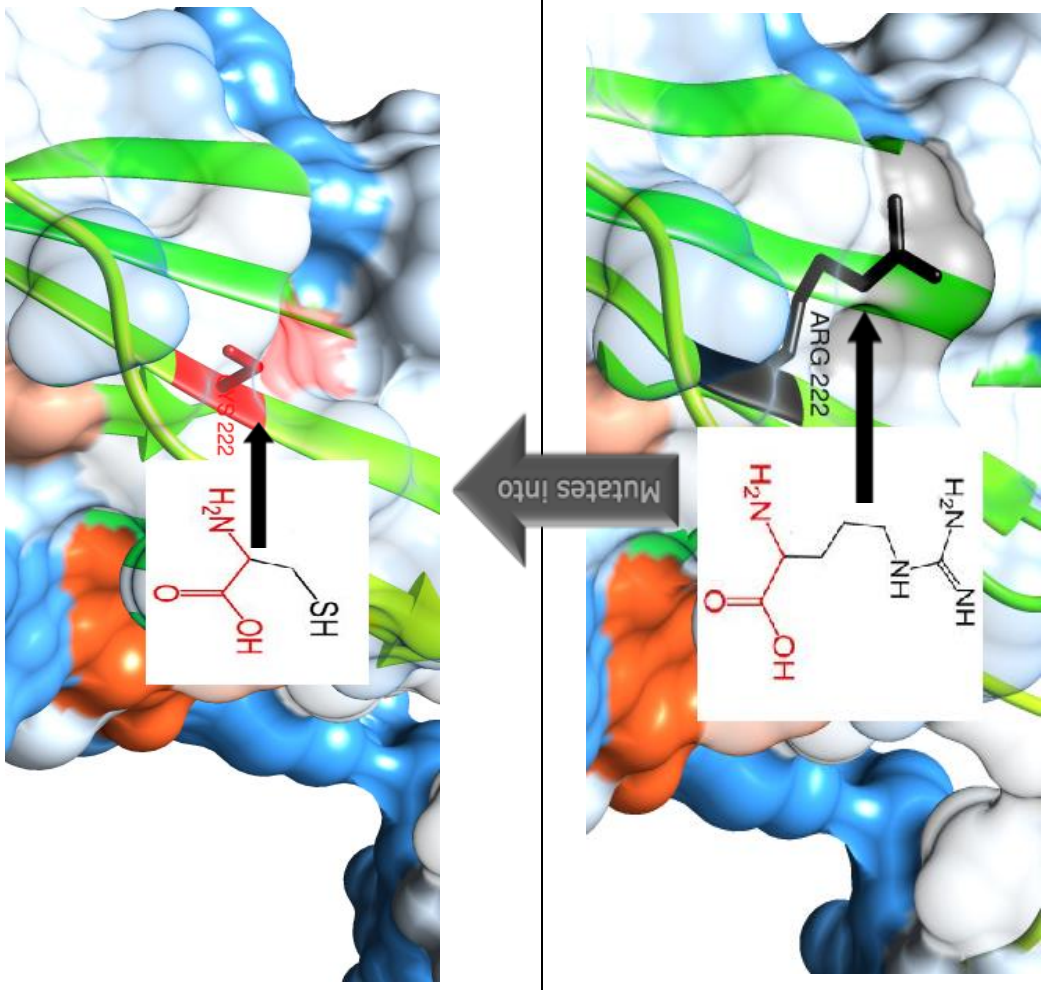
rs869320659 (R226C)



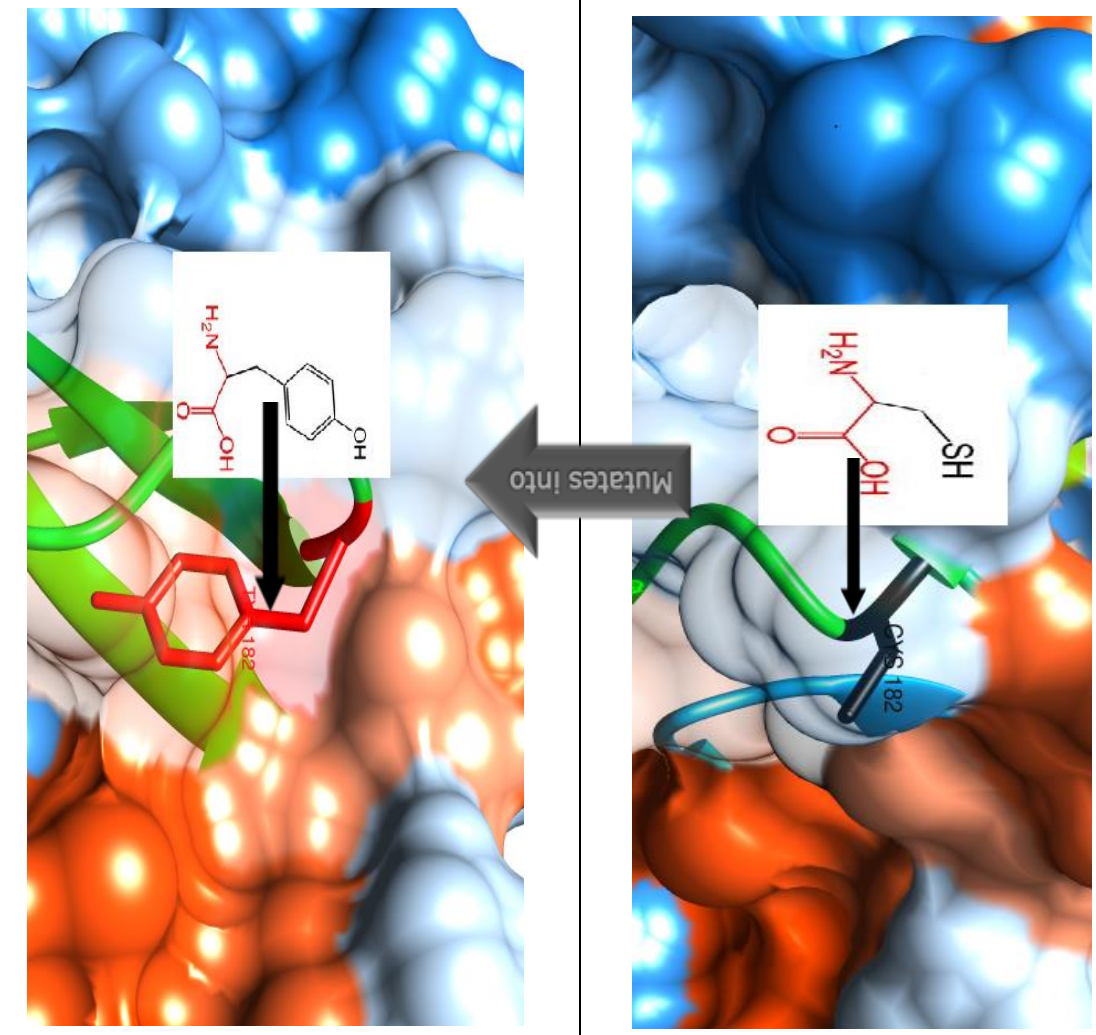
rs869320658 (R224W)



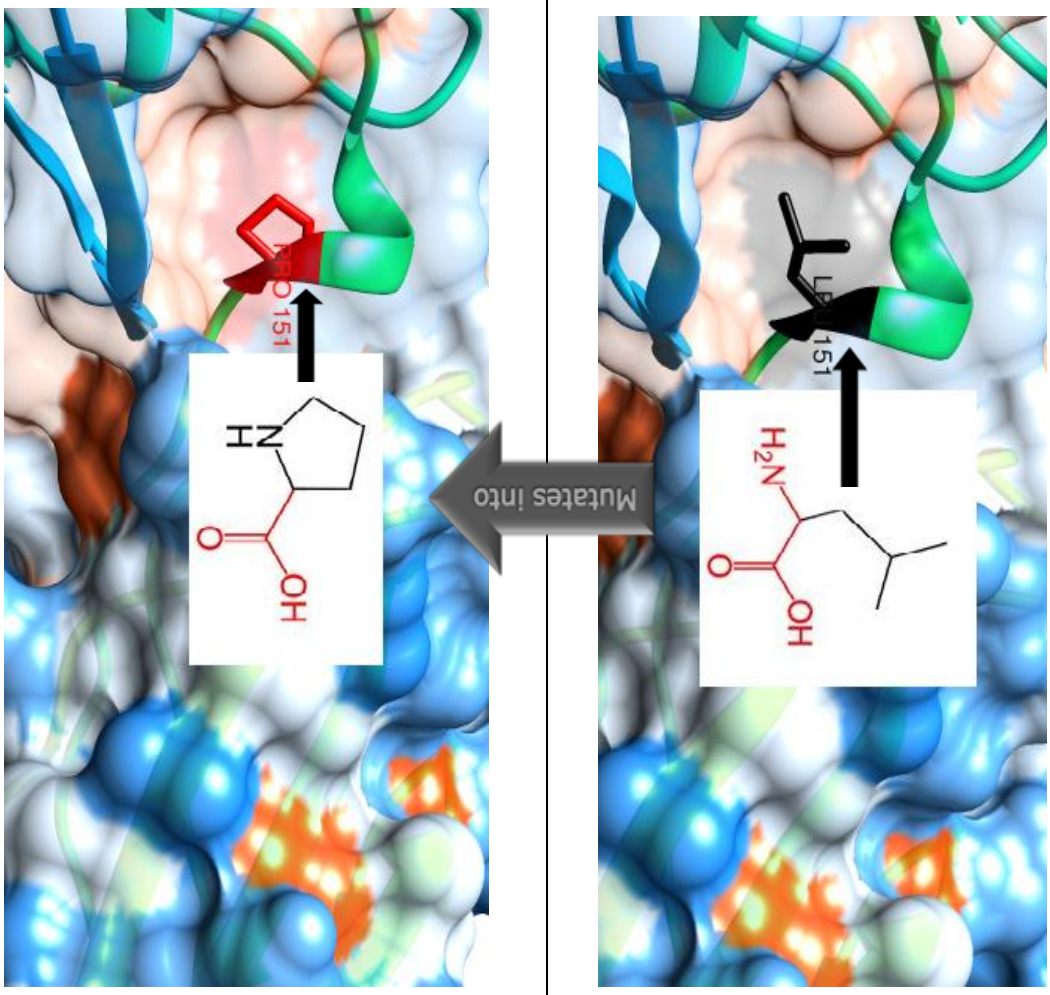
rs111033618 (R222C)



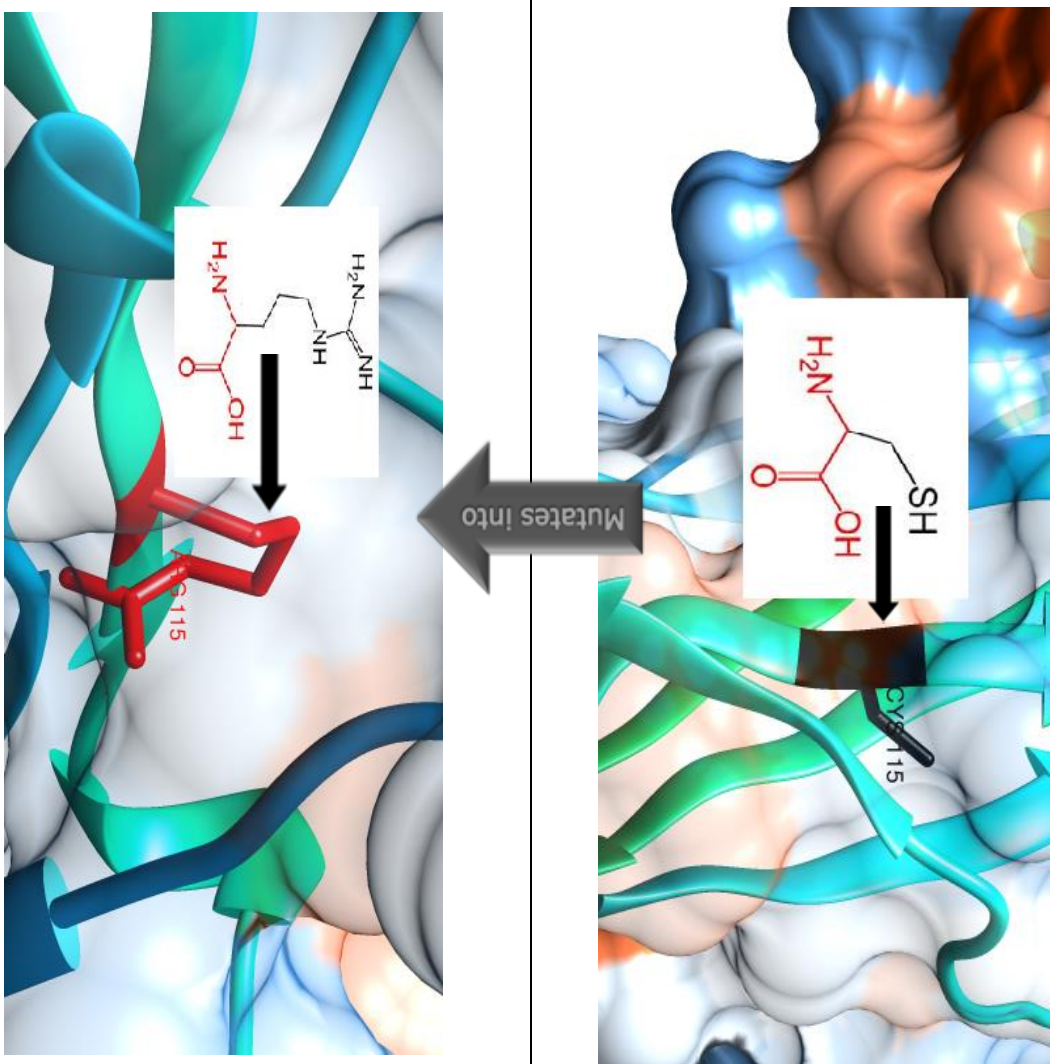
rs1064794027 (C182Y)



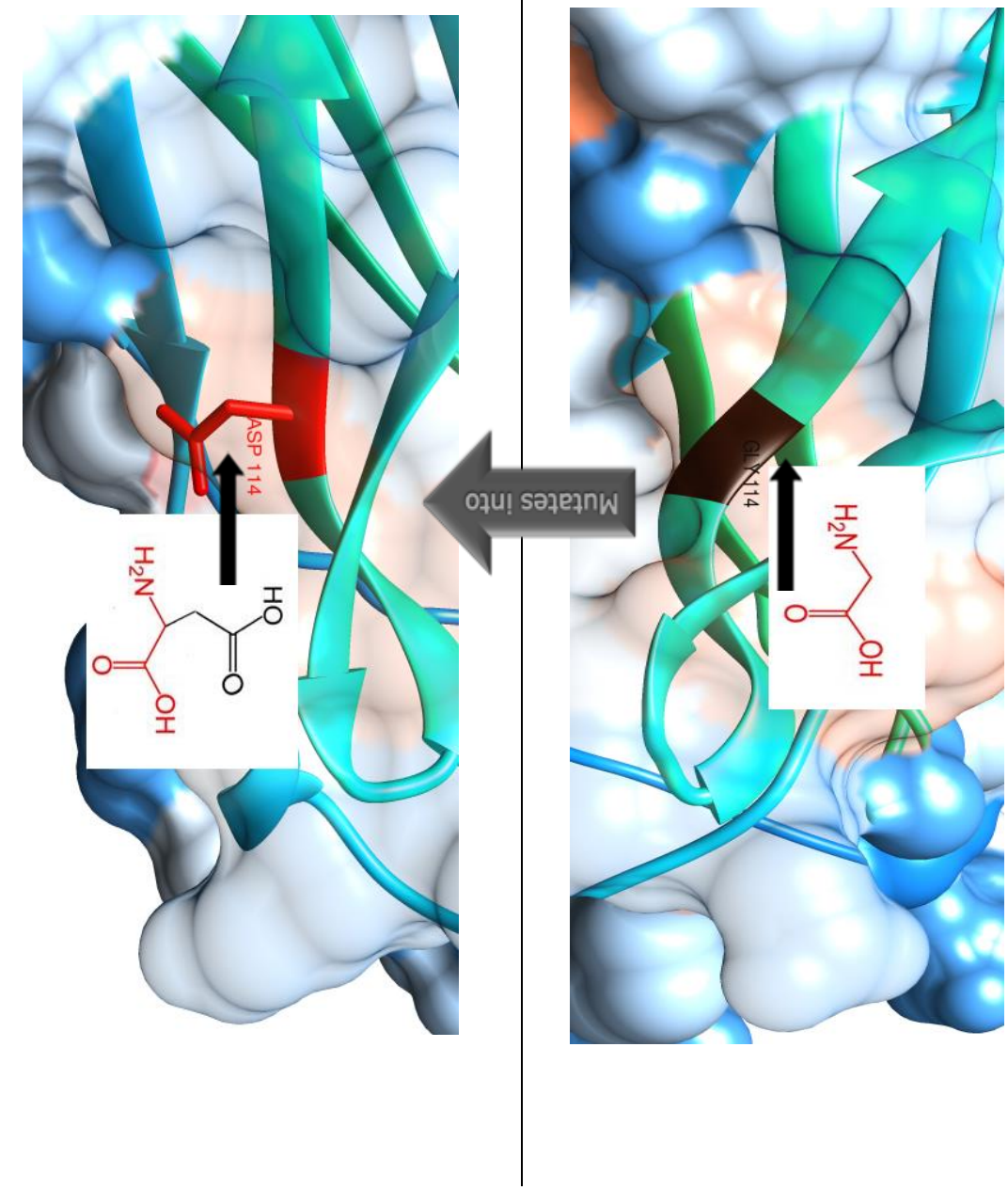
rs137852511 (L151P)



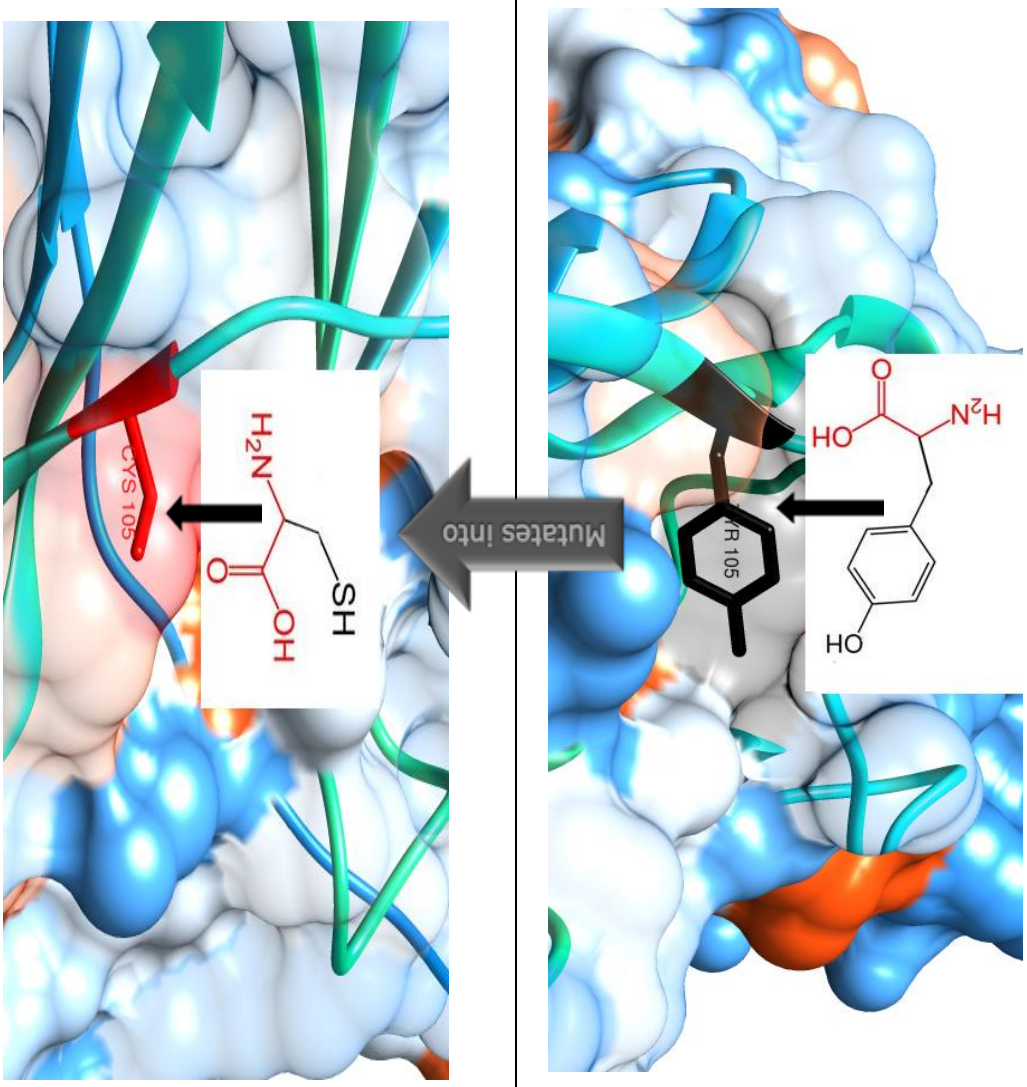
rs111033622 (C115R)



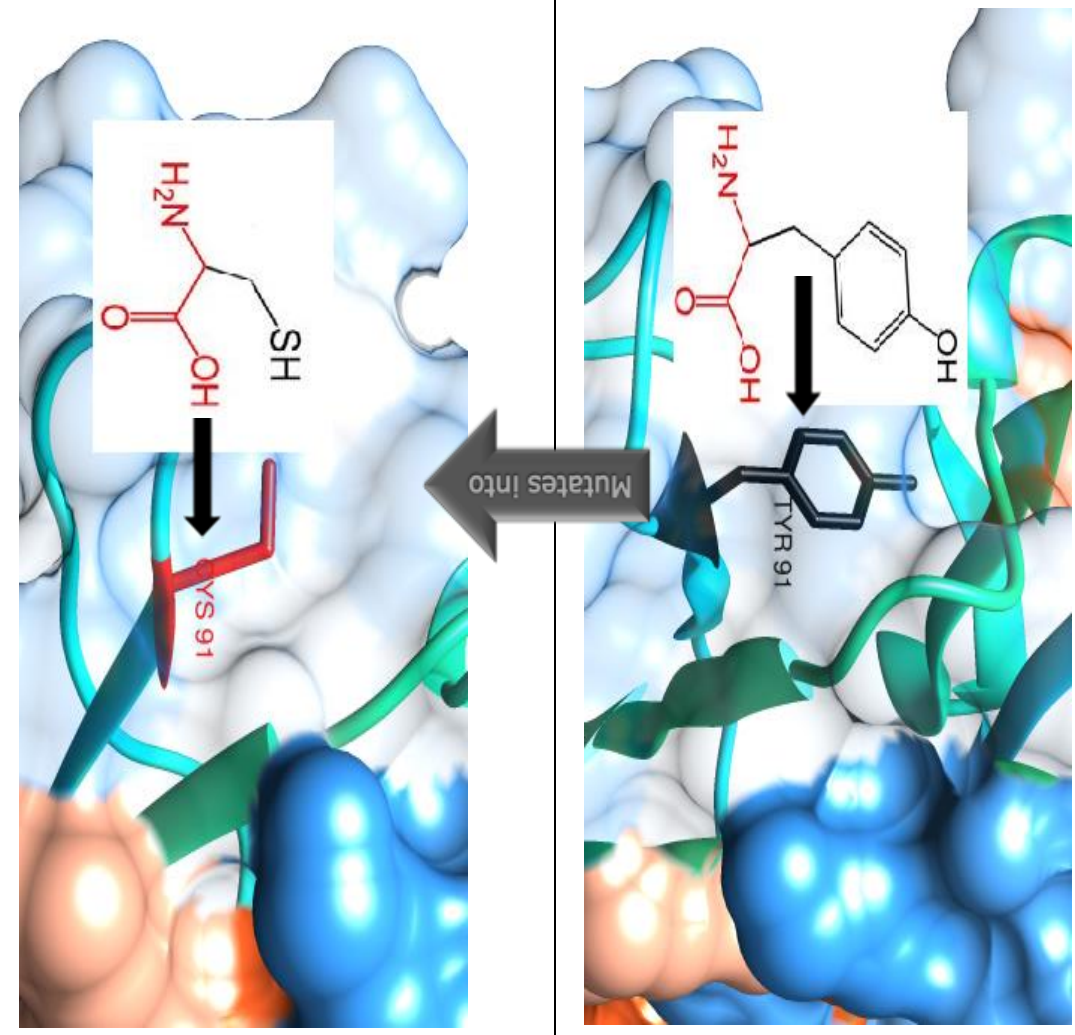
rs111033620 (G114D)



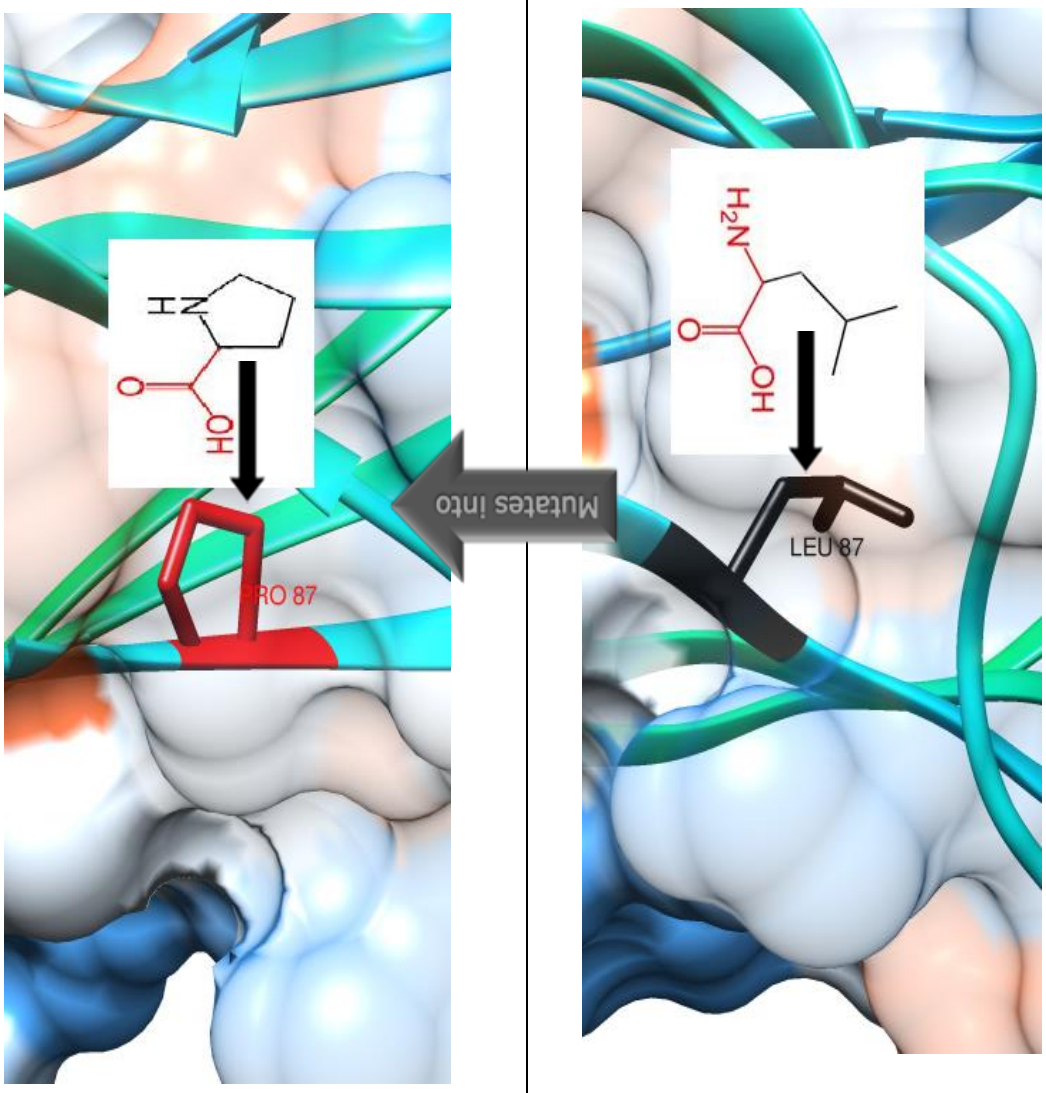
rs193922347 (Y105C)



rs1293196743 (Y91C)



rs1057520293 (L87P)



3.6. Table (4) prediction of SNPs at the 3'UTR Region using PolymiRTS:

Location	dbSNP ID	Variant type	Wobble base pair	Ancestral Allele	Allele	miR ID	Conservation	miRSite	Function Class	Exp Support	context+ score change
70327328	rs144075871	SNP	N	C	C	hsa-miR-1321	2	cCCTCCCTtcctc	D	N	-0.172
						hsa-miR-149-3p	2	CCCTCCCttcctc	D	N	-0.279
						hsa-miR-3202	7	ccctCCCTTCCtc	D	N	-0.227
						hsa-miR-4476	7	ccctcCCTTCCTc	D	N	-0.166
						hsa-miR-4728-5p	2	CCCTCCCttcctc	D	N	-0.288
						hsa-miR-4739	2	cCCTCCCTtcctc	D	N	-0.219
						hsa-miR-4756-5p	2	cCCTCCCTtcctc	D	N	-0.144
						hsa-miR-6785-5p	2	CCCTCCCttcctc	D	N	-0.26
						hsa-miR-6876-5p	7	ccctcCCTTCCTc	D	N	-0.138
						hsa-miR-6883-5p	2	CCCTCCCttcctc	D	N	-0.251

						hsa-miR-7515	3	cccTCCCTTCctc	D	N	-0.115
					A	hsa-miR-4802-5p	2	cCCTCCATtcctc	C	N	-0.192
						hsa-miR-6515-5p	2	CCCTCCAttcctc	C	N	-0.278
						hsa-miR-6797-5p	2	CCCTCCAttcctc	C	N	-0.215
70327365	rs191726889	SNP	N	T	T	hsa-miR-4476	6	CCTTCCTtttcgc	D	N	-0.141
						hsa-miR-6876-5p	6	CCTTCCTtttcgc	D	N	-0.132
					C	hsa-miR-4747-5p	6	CCTTCCCtttcgc	C	N	-0.151
						hsa-miR-5196-5p	6	CCTTCCCtttcgc	C	N	-0.179

3.7. IL2RG Gene Interactions and network predicted by Genemania:

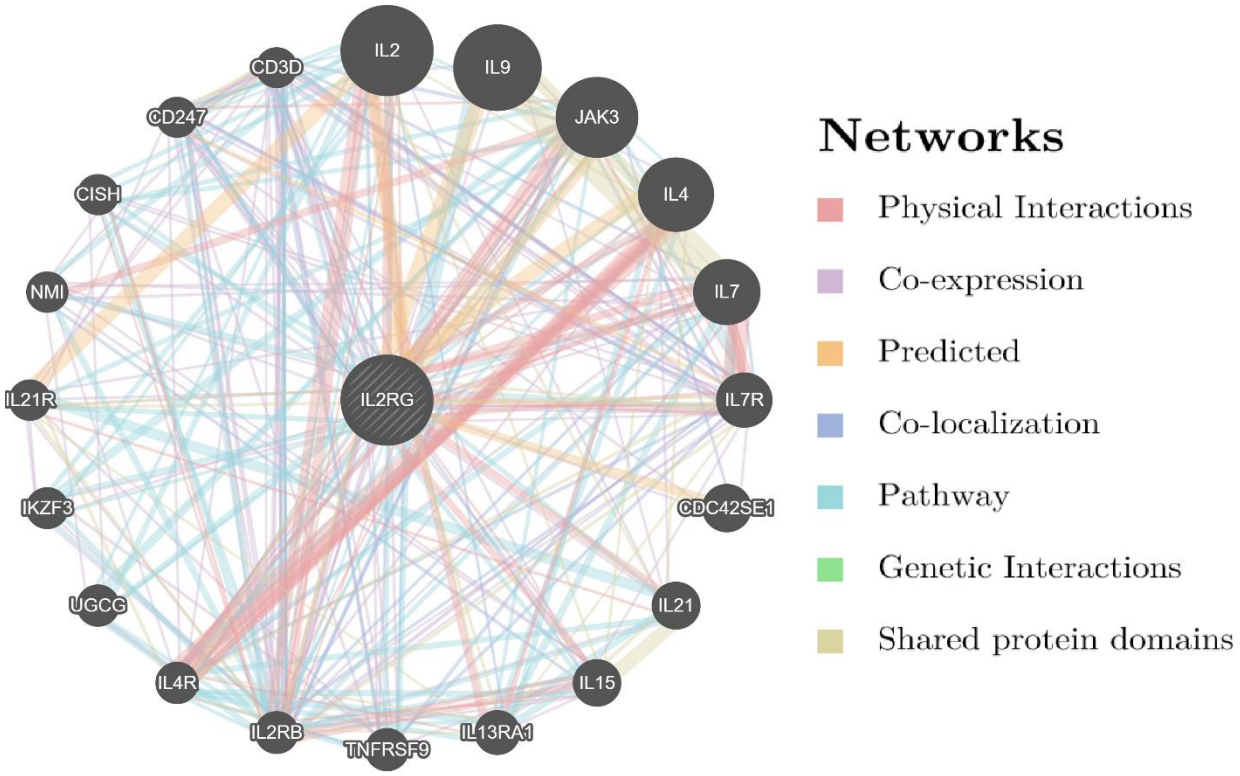


Figure (4): interactions between IL2RG and its related genes with paths colored according to their functions.

3.7.1 Table (7): The IL2RG gene functions and its appearance in network and genome:

Function	FDR	Genes in network	Genes in genome
response to interleukin-15	4.18E-09	5	10
regulation of lymphocyte activation	2.51E-07	8	208
regulation of leukocyte activation	4.01E-07	8	232
regulation of B cell proliferation	5.55E-07	5	31
regulation of cell activation	5.55E-07	8	254
regulation of T cell activation	1.08003E-06	7	169
regulation of B cell activation	4.88027E-06	5	50
B cell activation	5.47236E-06	6	119
B cell proliferation	5.65021E-06	5	54
T cell activation	6.68554E-06	7	242
cytokine receptor activity	6.68554E-06	5	59
positive regulation of T cell activation	6.68554E-06	6	131
positive regulation of B cell proliferation	7.05598E-06	4	20
lymphocyte differentiation	9.87751E-06	6	144

positive regulation of lymphocyte activation	1.43429E-05	6	155
positive regulation of leukocyte activation	2.02752E-05	6	166
positive regulation of cell activation	2.35959E-05	6	172
positive regulation of B cell activation	2.48256E-05	4	29
T cell differentiation	3.62913E-05	5	90
JAK-STAT cascade	3.64563E-05	5	91
regulation of lymphocyte proliferation	3.66906E-05	5	92
regulation of mononuclear cell proliferation	3.69875E-05	5	93
regulation of leukocyte proliferation	4.60727E-05	5	98
leukocyte differentiation	8.47764E-05	6	226
cellular response to interleukin-4	0.000126535	3	11
positive regulation of interleukin-17 production	0.000126535	3	11
lymphocyte proliferation	0.000126535	5	123
mononuclear cell proliferation	0.000128311	5	125
response to interleukin-4	0.00015697	3	12
leukocyte proliferation	0.00016322	5	133
positive regulation of tissue remodeling	0.000190764	3	13
regulation of lymphocyte differentiation	0.000290979	4	61
positive regulation of lymphocyte proliferation	0.000290979	4	61
positive regulation of mononuclear cell proliferation	0.000301688	4	62
positive regulation of leukocyte proliferation	0.000324077	4	64
tissue remodeling	0.000324077	4	64
interleukin-17 production	0.000368994	3	17
regulation of interleukin-17 production	0.000368994	3	17
regulation of tissue remodeling	0.001916034	3	29
regulation of leukocyte differentiation	0.002475089	4	109
inflammatory response	0.004895314	5	283
positive regulation of T cell proliferation	0.005951229	3	43
tyrosine phosphorylation of STAT protein	0.00793416	3	48
positive regulation of inflammatory response	0.00793416	3	48
regulation of T cell differentiation	0.010471188	3	53
growth factor activity	0.010838637	3	54
B cell differentiation	0.016878279	3	63
regulation of T cell proliferation	0.017327481	3	64
cytokine biosynthetic process	0.02036095	3	68
cytokine metabolic process	0.020846338	3	69
T cell proliferation	0.022264005	3	71

positive regulation of cytokine production	0.023751435	4	207
growth factor receptor binding	0.024248269	3	74
receptor complex	0.025555197	4	213
negative regulation of lymphocyte apoptotic process	0.029668545	2	13
T cell receptor complex	0.029668545	2	13
hemopoiesis	0.033704918	4	232
hematopoietic or lymphoid organ development	0.044189123	4	250
immune system development	0.055936889	4	267
negative regulation of leukocyte apoptotic process	0.095692758	2	24
JAK-STAT cascade involved in growth hormone signaling pathway	0.095692758	2	24

3.7.2 Table (8). The genes co-expressed, co localized, Physical Interacted and share a domain with IL2RG:

Gene 1	Gene 2	Weight	Network group
NMI	IL2RG	0.007142981	Co-expression
CD247	IL2RG	0.011854455	Co-expression
CD3D	IL2RG	0.010006283	Co-expression
IL7R	IL2RG	0.015151113	Co-expression
TNFRSF9	IL2RG	0.01611196	Co-expression
CD247	IL2RG	0.012152404	Co-expression
CD3D	IL2RG	0.012304108	Co-expression
TNFRSF9	IL2RG	0.014275057	Co-expression
IL21R	IL2RG	0.011859321	Co-expression
CD247	IL2RG	0.01117192	Co-expression
CD3D	IL2RG	0.014610344	Co-expression
IL7R	IL2RG	0.003189589	Co-expression
TNFRSF9	IL2RG	0.006582764	Co-expression
IL2RB	IL2RG	0.007769996	Co-expression
IL4R	IL2RG	0.00616813	Co-expression
CD247	IL2RG	0.003073129	Co-expression
CD3D	IL2RG	0.002789903	Co-expression
IL4R	IL2RG	0.012734761	Co-expression
IL7	IL2RG	0.007766109	Co-expression
IL2RB	IL2RG	0.006711766	Co-expression
IKZF3	IL2RG	0.007105181	Co-expression
IL21R	IL2RG	0.003766455	Co-expression
CD247	IL2RG	0.005185788	Co-expression
CD3D	IL2RG	0.00601814	Co-expression

JAK3	IL2RG	0.008937181	Co-expression
CDC42SE1	IL2RG	0.011040032	Co-expression
IKZF3	IL2RG	0.009303174	Co-expression
CD3D	IL2RG	0.007054924	Co-expression
CD247	IL2RG	0.008991863	Co-expression
IL15	IL2RG	0.008256315	Co-expression
IL4R	IL2RG	0.007494276	Co-expression
IL7	IL2RG	0.004436255	Co-expression
IL7R	IL2RG	0.004471675	Co-expression
CD247	IL2RG	0.006826574	Co-expression
CD3D	IL2RG	0.003450207	Co-expression
NMI	IL2RG	0.010466014	Co-expression
CD3D	IL2RG	0.012631002	Co-expression
IL7R	IL2RG	0.010826766	Co-expression
JAK3	IL2RG	0.011127411	Co-expression
IL4R	IL2RG	0.004267869	Co-expression
IL15	IL2RG	0.009034835	Co-localization
TNFRSF9	IL2RG	0.008663382	Co-localization
CD247	IL2RG	0.010449201	Co-localization
CD3D	IL2RG	0.005962262	Co-localization
IL2	IL2RG	0.007554005	Pathway
IL9	IL2RG	0.1314403	Pathway
JAK3	IL2RG	0.007377151	Pathway
IL4	IL2RG	0.018344974	Pathway
IL7	IL2RG	0.024201902	Pathway
IL7R	IL2RG	0.027118763	Pathway
IL21	IL2RG	0.024172688	Pathway
IL15	IL2RG	0.024201902	Pathway
IL2RB	IL2RG	0.008450603	Pathway
IL4R	IL2RG	0.015245179	Pathway
UGCG	IL2RG	0.044504892	Pathway
IKZF3	IL2RG	0.03478117	Pathway
IL21R	IL2RG	0.03414414	Pathway
NMI	IL2RG	0.048489608	Pathway
CISH	IL2RG	0.029071633	Pathway
CD247	IL2RG	0.008765063	Pathway
CD3D	IL2RG	0.01024757	Pathway
IL2	IL2RG	0.008473313	Pathway
JAK3	IL2RG	0.012780515	Pathway
IL4	IL2RG	0.014036303	Pathway
TNFRSF9	IL2RG	0.09801694	Pathway
IL2RB	IL2RG	0.012710958	Pathway
IL4R	IL2RG	0.02225277	Pathway

UGCG	IL2RG	0.041583646	Pathway
IKZF3	IL2RG	0.035283282	Pathway
CISH	IL2RG	0.06464156	Pathway
CD247	IL2RG	0.013865737	Pathway
CD3D	IL2RG	0.013662125	Pathway
IL2	IL2RG	0.32064575	Physical Interactions
JAK3	IL2RG	0.5392597	Physical Interactions
IL7	IL2RG	0.2520761	Physical Interactions
IL15	IL2RG	0.19908392	Physical Interactions
IL2RB	IL2RG	0.13526194	Physical Interactions
IL2	IL2RG	0.48999068	Physical Interactions
JAK3	IL2RG	0.047650374	Physical Interactions
IL21	IL2RG	0.35071465	Physical Interactions
IL13RA1	IL2RG	0.32248512	Physical Interactions
IL4R	IL2RG	0.0740218	Physical Interactions
IL4	IL2RG	0.39940926	Physical Interactions
IL7	IL2RG	0.4357728	Physical Interactions
IL7R	IL2RG	0.33060825	Physical Interactions
IL7	IL2RG	0.25101426	Physical Interactions
IL7R	IL2RG	0.34089583	Physical Interactions
IL2	IL2RG	0.066997014	Physical Interactions
JAK3	IL2RG	0.03302985	Physical Interactions
IL4	IL2RG	0.09594477	Physical Interactions
IL7	IL2RG	0.22980283	Physical Interactions
IL7R	IL2RG	0.0603281	Physical Interactions
IL15	IL2RG	0.16295333	Physical Interactions
IL2RB	IL2RG	0.03907459	Physical Interactions
IL4R	IL2RG	0.041646395	Physical Interactions
IL9	IL2RG	0.5554309	Predicted
CDC42SE1	IL2RG	0.37048033	Predicted
IL2	IL2RG	0.6641241	Predicted
IL4	IL2RG	0.6641241	Predicted
JAK3	IL2RG	1	Predicted
IL7R	IL2RG	0.040768523	Shared protein domains
IL13RA1	IL2RG	0.034895726	Shared protein domains
IL2RB	IL2RG	0.040768523	Shared protein domains
IL4R	IL2RG	0.04114318	Shared protein domains
IL21R	IL2RG	0.04076852	Shared protein domains
IL7R	IL2RG	0.016019475	Shared protein domains
IL13RA1	IL2RG	0.06732253	Shared protein domains

4. Discussion:

IL2RG gene was investigated in dbSNP National Center of Biotechnology Information (NCBI public database). This gene containing a total of 253 SNPs in coding region, of which 153 were missense, 85 synonymous, eight nonsense, seven frame shift and 71 were in the non-coding region, of which 50 in 3'un-translated region (3' UTR) and 21 in 5' un-translated region (5'UTR). We selected the missense coding SNPs and 3′UTR SNPs for our investigation.

Our study revealed that 12 nsSNPs in the coding region were predicted to be damaging by various software. Five of them were novel; rs1322436793(G305R), rs1064794027(C182Y), rs111033620(G114D), rs193922347(Y105C) and rs1293196743(Y91C). The remaining seven nsSNPs were described by previous study to be damaging and disease related; rs1057517950 (W240R), rs869320659 (R226C), rs869320658 (R224W), rs111033618 (R222C), rs137852511 (L151P), rs111033622 (C115R) and rs1057520293 (L87P). Our study was in agreement with Weiping Tan et al who detected the rs1057517950 (W240R) mutation in a Chinese family with X-SCID. Their 4-month-old boy with X-SCID has a single point homozygous missense mutation within *IL2RG* exon 5: c. 718 T > C, p. W240R. The results of *IL2RG* sequencing showed that his mother and two sisters were heterozygous for the mutation and thus were asymptomatic carriers, while his father's *IL2RG* gene was normal [(48)]. In 2013 a study conducted by Alsina L et al described the vertical transmission of the somatic rs869320659 (R226C) *IL2RG* mosaicism from the mother to the child as the genetic mechanism underlying the patient with X-SCID [(49)]. In 1997 Sharfe N et al revealed that the rs111033618 (R222C) mutation leads to an atypical phenotype presentation of SCID with a normal thymus gland, mitogen response and normal numbers of T and B cells. However, despite the normal number of T cells, the T cells demonstrate a reduced ability to bind IL-2 and thus incomplete participation in antigenic responses [(50)]. Another study reported that in the rs111033618 (R222C) mutation signaling of γc cytokines for developmental processes was sufficient but the peripheral differentiation and function of T lymphocytes, B lymphocytes and NK cells was significantly reduced. Consequently, this resulted in more severe clinical phenotype than expected from T cell counts [(18)]. Several studies investigated the frequency and variety of *IL2RG* mutations that cause SCID detected the mutations that had been predicted by our study to be damaging; rs1057517950 (W240R) [(51)], rs869320659 (R226C) [(7, 51-53)] and rs869320658 (R224W) [(7, 51, 53)]. rs137852511 (L151P) mutation was detected in a 5-year-old boy by Speckmann C et al. This mutation was found in B, NK, and epithelial cells, whereas the gene

sequence T cells were normal, indicating reversion of the mutation in a common T-cell precursor. This genetic correction in T cells resulted in restoration of immune function and an a typical phenotype with mild immunodeficiency [(54)]. rs111033622 (C115R) was described in a one-year old boy with atypical X-SCID. The results of genetic analysis revealed normal expression of the γc chain and an absence of the γc gene mutation in the T cells. Reversion of the mutation in early T-cell precursors in this patient led to partially functional lymphocyte clones [(24)]. rs1057520293 (L87P) mutation had been described in X-SCID patient with mild phenotype and delayed diagnosis due to the reversion in a common lymphoid progenitor [(55)].

Two SNPs out of 50 at the 3'UTR were predicted to disrupt miRNAs binding sites and hence affect the gene expression. rs144075871 SNP and rs191726889 SNP contained (D) functional classes that disrupted a conserved miRNA site and (C) functional class that created a new site of miRNA.

In rs1322436793 (G305R) mutation the mutant residue is bigger than the wild-type residue, this might lead to bumps. The wild-type residue charge is neutral while the mutant residue charge is positive, this can cause repulsion of ligands or other residues with the same charge. The wild-type residue is more hydrophobic than the mutant residue. Moreover in both rs1322436793 (G305R) and rs111033620 (G114D) mutations the torsion angles for this residue are unusual. Only glycine is flexible enough to make these torsion angles, mutation into another residue will force the local backbone into an incorrect conformation and will disturb the local structure. The wild-type residue in rs1064794027 (C182Y) and rs111033620 (G114D) mutations was buried in the core of the protein, the mutant residue is bigger probably will not fit. The wild-type residue is more hydrophobic than the mutant residue. The mutation will cause loss of hydrophobic interactions in the core of the protein. The mutant residue in rs193922347 (Y105C) and rs1293196743 (Y91C) mutations is smaller than the wild-type residue. This will cause a possible loss of external interactions in (Y105C) and will cause an empty space in the core of the protein in (Y91C). The mutant residue is more hydrophobic than the wild-type residue. The mutated residue is located in a domain that is important for binding of other molecules and in contact with residues in a domain that is also important for binding. The mutation might disturb the interaction between these two domains and as such affect the function of the protein.

All eleven mutations (G → R, W → R, R → C, R → W, R → C, C → Y, L → P ,G → D, Y → C, Y → C) predicted a decrease of the protein stability, while only mutation (C → R) predicted an

increase of stability of protein. *IL2RG* gene function and activities illustrated by GENEMAN which showed that *IL2RG* gene has many functions such as cellular response to interleukin-4, cytokine receptor activity, response to interleukin-15, response to interleukin-4. *IL2RG* gene was predicted to be related to 20 genes (*CDC42SE1, IL9, TNFRSF9, IL21, IL4, IL13RA1, IL15, IKZF3, IL2, NMI, JAK3, UGCG, IL7, CISH, IL21R, ILR7, CD247, CD3D, IL2RB,* and *IL4R*).

This study is computational and has limitations; in vivo genetic analysis is recommended. This will improve our understanding of how complex human phenotype is inherited. SNPs revealed by this study could be considered as important candidates in causing diseases related to *IL2RG* mutation and could be used as diagnostic markers. We hope our results will provide useful information that needed to help researchers to do further studies and also may help in diagnosis and genetic screening of SCID

5. Conclusions:

Computational analysis of SNPs has become a very valuable tool in order to discriminate neutral SNPs from SNPs of likely functional importance and associated with disease. In this study we used different software to predict the damaging mutations of *IL2RG* gene; 12 nsSNPs were predicted by different software to be the most damaging mutations for *IL2RG* protein altering its size, charge, hydrophobicity, stability and physiochemical properties and thus leading to loss of the protein's function. Out of the 12 nsSNPs, 5 nsSNPs were novel and the rest were described by other studies to be disease related. Two SNPs out of 50 at the 3'UTR were predicted to disrupt miRNAs binding sites and hence affect the gene expression. This might result in alteration of the gene function.

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7. References:

1. Chinn IK, Shearer WT. Severe Combined Immunodeficiency Disorders. Immunology and Allergy Clinics. 2015;35(4):671-94.
2. Michos A, Tzanoudaki M, Villa A, Giliani S, Chrousos G, Kanariou M. Severe combined immunodeficiency in Greek children over a 20-year period: rarity of gammac-chain deficiency (X-linked) type. J Clin Immunol. 2011;31(5):778-83.
3. Buckley RH, Schiff RI, Schiff SE, Markert ML, Williams LW, Harville TO, et al. Human severe combined immunodeficiency: genetic, phenotypic, and functional diversity in one hundred eight infants. The Journal of pediatrics. 1997;130(3):378-87.
4. Noguchi M, Yi H, Rosenblatt HM, Filipovich AH, Adelstein S, Modi WS, et al. Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. Cell 73: 147-157. 1993. J Immunol. 2008;181(9):5817-27.
5. Buckley RH. Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. Annu Rev Immunol. 2004;22:625-55.
6. Allenspach E, Rawlings DJ, Scharenberg AM. X-linked severe combined immunodeficiency. 2016.
7. Kong X, Liu N, Xu X, Wu Q, Zhao Z, Bai Q, et al. [Mutation analyses and prenatal diagnosis in two families of X linked severe combined immunodeficiency caused by *IL2RG* gene novel mutation]. Zhonghua Yi Xue Za Zhi. 2014;94(16):1227-31.
8. Bai QL, Liu N, Kong XD, Xu XJ, Zhao ZH. Mutation analyses and prenatal diagnosis in families of X-linked severe combined immunodeficiency caused by *IL2Rgamma* gene novel mutation. Genet Mol Res. 2015;14(2):6164-72.
9. DiSanto JP, Müller W, Guy-Grand D, Fischer A, Rajewsky K. Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor gamma chain. Proceedings of the National Academy of Sciences. 1995;92(2):377-81.
10. Poletti V, Charrier S, Corre G, Gjata B, Vignaud A, Zhang F, et al. Preclinical Development of a Lentiviral Vector for Gene Therapy of X-Linked Severe Combined Immunodeficiency. Mol Ther Methods Clin Dev. 2018;9:257-69.
11. Zhang C, Zhang ZY, Wu JF, Tang XM, Yang XQ, Jiang LP, et al. Clinical characteristics and mutation analysis of X-linked severe combined immunodeficiency in China. World J Pediatr. 2013;9(1):42-7.

12. Fanos JH, Davis J, Puck JM. Sib understanding of genetics and attitudes toward carrier testing for X-linked severe combined immunodeficiency. Am J Med Genet. 2001;98(1):46-56.
13. Asao H, Okuyama C, Kumaki S, Ishii N, Tsuchiya S, Foster D, et al. Cutting edge: the common γ-chain is an indispensable subunit of the IL-21 receptor complex. The Journal of Immunology. 2001;167(1):1-5.
14. Kovanen PE, Leonard WJ. Cytokines and immunodeficiency diseases: critical roles of the γc-dependent cytokines interleukins 2, 4, 7, 9, 15, and 21, and their signaling pathways. Immunological reviews. 2004;202(1):67-83.
15. Okano T, Nishikawa T, Watanabe E, Watanabe T, Takashima T, Yeh TW, et al. Maternal T and B cell engraftment in two cases of X-linked severe combined immunodeficiency with IgG1 gammopathy. Clin Immunol. 2017;183:112-20.
16. DiSanto JP, Dautry-Varsat A, Certain S, Fischer A, de Saint Basile G. Interleukin-2 (IL-2) receptor gamma chain mutations in X-linked severe combined immunodeficiency disease result in the loss of high-affinity IL-2 receptor binding. Eur J Immunol. 1994;24(2):475-9.
17. Schmalstieg FC, Leonard WJ, Noguchi M, Berg M, Rudloff HE, Denney RM, et al. Missense mutation in exon 7 of the common gamma chain gene causes a moderate form of X-linked combined immunodeficiency. The Journal of clinical investigation. 1995;95(3):1169-73.
18. Fuchs S, Rensing-Ehl A, Erlacher M, Vraetz T, Hartjes L, Janda A, et al. Patients with T+/low NK+ IL-2 receptor γ chain deficiency have differentially-impaired cytokine signaling resulting in severe combined immunodeficiency. European journal of immunology. 2014;44(10):3129-40.
19. Gathmann B, Grimbacher B, Beauté J, Dudoit Y, Mahlaoui N, Fischer A, et al. The European internet-based patient and research database for primary immunodeficiencies: results 2006–2008. Clinical & Experimental Immunology. 2009;157:3-11.
20. Mou W, He J, Chen X, Zhang H, Ren X, Wu X, et al. A novel deletion mutation in *IL2RG* gene results in X-linked severe combined immunodeficiency with an atypical phenotype. Immunogenetics. 2017;69(1):29-38.
21. Allenspach E, Rawlings DJ, Scharenberg AM. X-Linked Severe Combined Immunodeficiency. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al., editors. GeneReviews((R)). Seattle (WA)1993.

22. Tassara C, Pepper AE, Puck JM. Intronic point mutation in the *IL2RG* gene causing X-linked severe combined immunodeficiency. Hum Mol Genet. 1995;4(9):1693-5.

23. Pepper AE, Buckley RH, Small TN, Puck JM. Two mutational hotspots in the interleukin-2 receptor gamma chain gene causing human X-linked severe combined immunodeficiency. Am J Hum Genet. 1995;57(3):564-71.

24. Stephan V, Wahn V, Le Deist F, Dirksen U, Broker B, Muller-Fleckenstein I, et al. Atypical X-linked severe combined immunodeficiency due to possible spontaneous reversion of the genetic defect in T cells. N Engl J Med. 1996;335(21):1563-7.

25. Ishii N, Asao H, Kimura Y, Takeshita T, Nakamura M, Tsuchiya S, et al. Impairment of ligand binding and growth signaling of mutant IL-2 receptor gamma-chains in patients with X-linked severe combined immunodeficiency. J Immunol. 1994;153(3):1310-7.

26. Clark PA, Lester T, Genet S, Jones AM, Hendriks R, Levinsky RJ, et al. Screening for mutations causing X-linked severe combined immunodeficiency in the IL-2R gamma chain gene by single-strand conformation polymorphism analysis. Hum Genet. 1995;96(4):427-32.

27. O'Marcaigh AS, Puck JM, Pepper AE, De Santes K, Cowan MJ. Maternal mosaicism for a novel interleukin-2 receptor gamma-chain mutation causing X-linked severe combined immunodeficiency in a Navajo kindred. J Clin Immunol. 1997;17(1):29-33.

28. Frazer KA, Murray SS, Schork NJ, Topol EJ. Human genetic variation and its contribution to complex traits. Nature Reviews Genetics. 2009;10(4):241.

29. Collins FS, Brooks LD, Chakravarti A. A DNA polymorphism discovery resource for research on human genetic variation. Genome research. 1998;8(12):1229-31.

30. Medicine UNLo. What are single nucleotide polymorphisms (SNPs)? 2018 [Available from: https://ghr.nlm.nih.gov/primer/genomicresearch/snp.

31. Shastry BS. SNP alleles in human disease and evolution. Journal of human genetics. 2002;47(11):561.

32. Sherry ST, Ward M-H, Kholodov M, Baker J, Phan L, Smigielski EM, et al. dbSNP: the NCBI database of genetic variation. Nucleic acids research. 2001;29(1):308-11.

33. Hale LP, Buckley RH, Puck JM, Patel DD. Abnormal development of thymic dendritic and epithelial cells in human X-linked severe combined immunodeficiency. Clin Immunol. 2004;110(1):63-70.

34. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Current protocols in human genetics. 2013;76(1):7.20. 1-7.. 41.

35. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nature methods. 2010;7(4):248.

36. Choi Y, editor A fast computation of pairwise sequence alignment scores between a protein and a set of single-locus variants of another protein. Proceedings of the ACM Conference on Bioinformatics, Computational Biology and Biomedicine; 2012: ACM.

37. Bromberg Y, Rost B. SNAP: predict effect of non-synonymous polymorphisms on function. Nucleic acids research. 2007;35(11):3823-35.

38. Hecht M, Bromberg Y, Rost B. Better prediction of functional effects for sequence variants. BMC genomics. 2015;16(8):S1.

39. Capriotti E, Calabrese R, Casadio R. Predicting the insurgence of human genetic diseases associated to single point protein mutations with support vector machines and evolutionary information. Bioinformatics. 2006;22(22):2729-34.

40. Calabrese R, Capriotti E, Fariselli P, Martelli PL, Casadio R. Functional annotations improve the predictive score of human disease-related mutations in proteins. Human mutation. 2009;30(8):1237-44.

41. Ferrer-Costa C, Orozco M, de la Cruz X. Use of bioinformatics tools for the annotation of disease-associated mutations in animal models. Proteins: Structure, Function, and Bioinformatics. 2005;61(4):878-87.

42. Capriotti E, Fariselli P, Casadio R. I-Mutant2. 0: predicting stability changes upon mutation from the protein sequence or structure. Nucleic acids research. 2005;33(suppl_2):W306-W10.

43. Venselaar H, te Beek TA, Kuipers RK, Hekkelman ML, Vriend G. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. BMC bioinformatics. 2010;11(1):548.

44. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera—a visualization system for exploratory research and analysis. Journal of computational chemistry. 2004;25(13):1605-12.

45. Bhattacharya A, Ziebarth JD, Cui Y. PolymiRTS Database 3.0: linking polymorphisms in microRNAs and their target sites with human diseases and biological pathways. Nucleic acids research. 2013;42(D1):D86-D91.

46. Ziebarth JD, Bhattacharya A, Chen A, Cui Y. PolymiRTS Database 2.0: linking polymorphisms in microRNA target sites with human diseases and complex traits. Nucleic acids research. 2011;40(D1):D216-D21.

47. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic acids research. 2010;38(suppl_2):W214-W20.

48. Cavazzana M, Six E, Lagresle-Peyrou C, Andre-Schmutz I, Hacein-Bey-Abina S. Gene Therapy for X-Linked Severe Combined Immunodeficiency: Where Do We Stand? Hum Gene Ther. 2016;27(2):108-16.

49. Alsina L, Gonzalez-Roca E, Giner MT, Piquer M, Puga I, Pascal M, et al. Massively parallel sequencing reveals maternal somatic *IL2RG* mosaicism in an X-linked severe combined immunodeficiency family. J Allergy Clin Immunol. 2013;132(3):741-3 e2.

50. Sharfe N, Shahar M, Roifman CM. An interleukin-2 receptor gamma chain mutation with normal thymus morphology. The Journal of clinical investigation. 1997;100(12):3036-43.

51. Matsubara Y, Chiba T, Kashimada K, Morio T, Takada S, Mizutani S, et al. Transcription activator-like effector nuclease-mediated transduction of exogenous gene into *IL2RG* locus. Sci Rep. 2014;4:5043.

52. Yao C-M, Han X-H, Zhang Y-D, Zhang H, Jin Y-Y, Cao R-M, et al. Clinical characteristics and genetic profiles of 44 patients with severe combined immunodeficiency (SCID): report from Shanghai, China (2004–2011). Journal of clinical immunology. 2013;33(3):526-39.

53. Lebet T, Chiles R, Hsu AP, Mansfield ES, Warrington JA, Puck JM. Mutations causing severe combined immunodeficiency: detection with a custom resequencing microarray. Genetics in Medicine. 2008;10(8):575.

54. Speckmann C, Pannicke U, Wiech E, Schwarz K, Fisch P, Friedrich W, et al. Clinical and immunologic consequences of a somatic reversion in a patient with X-linked severe combined immunodeficiency. Blood. 2008;112(10):4090-7.

55. Hsu AP, Pittaluga S, Martinez B, Rump AP, Raffeld M, Uzel G, et al. IL2RG reversion event in a common lymphoid progenitor leads to delayed diagnosis and milder phenotype. J Clin Immunol. 2015;35(5):449-53.