In Silico Analysis and Modeling of Novel Pathogenic Single Nucleotide Polymorphisms (SNPs) in Human *CD40LG* Gene

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Abstract:

Background: The X-linked hyper-immunoglobulin M syndrome (XHIGM) is a rare, inherited immune deficiency disorder. It is more common in males. Characterized by elevated serum IgM levels and low to undetectable levels of serum IgG, IgA and IgE. Hyper-IgM syndrome is caused by mutations in the *CD40LG* gene. Located in human Xq26. CD40LG acts as an immune modulator in activated T cells. **Method**: we used different bioinformatics tools to predict the effect of each SNP on the structure and function of the protein. **Result**: 8 novel SNPs out of 233 were found to have most deleterious effect on the protein structure and function. *While modeling of* nsSNPs was studied by Project HOPE software. **Conclusion**: Better understanding of Hyper-IgM syndrome caused by mutations in CD40LG gene was achieved using in silico analysis. This is the first in silico functional analysis of CD40LG gene and 8 novel mutations were found using different bioinformatics tools, and they could be used as diagnostic markers for hyper-IgM syndrome. These 8 novel SNPs may be important candidates for the cause of different types of human diseases by *CD40LG* gene.

Keywords: hyper-IgM syndrome, CD40LG gene, SNPs, in silico analysis, diagnostic markers.

Introduction:

The X-linked hyper-immunoglobulin M syndrome (XHIGM) is a rare, inherited immune deficiency disorder.[1-6] autosomal recessive HIES (AR-HIES) is the most frequent form of the Hyper IgM syndrome which is characterized by elevated serum IgM levels and low to undetectable levels of serum IgG, IgA and IgE..[7-10] it is more common in males. [10-15]

Hyper-IgM syndrome is caused by mutations in the *CD40LG* gene.[16-19] located in human Xq26.[20] *CD40LG* acts as an immune modulator in activated T cells[21] Inactivation of *CD40LG* gene resulted from the insertion of an AluYb8 element in exon 1 responsible for a total deficiency of CD40 ligand expression by T lymphocytes[22] Interestingly, some study shows that, not all mutations occur in *CD40LG* gene may cause XHIGM [23] Different mutations have been reported. [23-32]

As far as we know there are two options for treatment, either hematopoietic stem cell transplantation or intravenous immunoglobulin replacement therapy. [33] but it is sometimes completely ignored because of low recognition and limited knowledge of this rare disease.[34] Mutations in *CD40LG* gene may also cause: Sickle cell anemia, systemic lupus erythematosus, malignancies, and discusses neuroendocrine tumors (NETs) arising in other immunocompromised states. Of all primary immune deficiency diseases, NETs appear to be distinctive to XHIGM patients. An outcome for XHIGM patients with NETs is poor, and the mechanism behind this association remains unknown.[35-37]

The aim of this study is to identify functional SNPs within dbSNP located in coding region of *CD40LG* gene using in silico analysis. The usage of in silico analysis has strong influence on the identification of candidate SNPs since they are easy and less costly, and can assist in pharmacogenomics by identifying high risk SNP variants contributing to drug response as well as developing novel therapeutic elements and diagnostic markers .[38-42]

Materials and Methods:

Data mining:

The data on human *CD40LG* gene was collected from National Center for Biological Information (NCBI) web site.(https://www.ncbi.nlm.nih.gov/) and the protein sequence was collected from Uniprot (https://www.uniprot.org/).

SIFT:

We used SIFT to observe the effect of A.A. substitution on protein function. SIFT predicts damaging SNPs on the basis of the degree of conserved amino A.A. residues in aligned sequences to the closely related sequences, gathered through PSI-BLAST.[43] It is available at (http://sift.jcvi.org/).

PolyPhen:

PolyPhen (version 2) We used PolyPhen to study probable impacts of A.A. substitution on structural and functional properties of the protein by considering physical and comparative approaches.[44] It is available at (http://genetics.bwh.harvard.edu/pph2/).

Provean:

Provean is a software tool which predicts whether an amino acid substitution or indel has an impact on the biological function of a protein. It is useful for filtering sequence variants to identify nonsynonymous or indel variants that are predicted to be functionally important.[45] It is available at (https://rostlab.org/services/snap2web/).

SNAP2:

SNAP2 is a trained classifier that is based on a machine learning device called "neural network". It distinguishes between effect and neutral variants/non-synonymous SNPs by taking a variety of sequence and variant features into account.[46] It is available at (https://rostlab.org/services/snap2web/).

SNPs&GO:

SNPs&GO is an accurate method that, starting from a protein sequence, can predict whether a variation is disease related or not by exploiting the corresponding protein functional annotation. SNPs&GO collects in unique framework information derived from protein sequence, evolutionary information, and function as encoded in the Gene Ontology terms, and outperforms other available predictive methods.[47] It is available at (http://snps.biofold.org/snps-and-go/snps-and-go.html).

PHD-SNP:

An online Support Vector Machine (SVM) based classifier, is optimized to predict if a given single point protein mutation can be classified as disease-related or as a neutral polymorphism, it is available at: (http://snps.biofold.org/phd-snp/phdsnp.html).

I-Mutant 3.0:

I-Mutant 3.0 Is a neural network based tool for the routine analysis of protein stability and alterations by taking into account the single-site mutations. The FASTA sequence of protein retrieved from UniProt is used as an input to predict the mutational effect on protein stability. [48] It is available at (http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi).

MUpro:

MUpro is a support vector machine-based tool for the prediction of protein stability changes upon nonsynonymous SNPs. The value of the energy change is predicted, and a confidence score between -1 and 1 for measuring the confidence of the prediction is calculated. A score <0 means the variant decreases the protein stability; conversely, a score >0 means the variant increases the protein stability.[49] It is available at (http://mupro.proteomics.ics.uci.edu/).

GeneMANIA:

We submitted genes and selected from a list of data sets that they wish to query. GeneMANIA approach to know protein function prediction integrate multiple genomics and proteomics data sources to make inferences about the function of unknown proteins.[50] It is available at (http://www.genemania.org/).

Structural Analysis:

Developing 3D structure of mutant STAT3 gene:

The 3D structure of human CD40LG protein is not available in the Protein Data Bank. Hence, we used RaptorX to generate a 3D structural model for wild-type CD40LG. RaptorX is a web server predicting structure property of a protein sequence without using any templates. It outperforms other servers, especially for proteins without close homologs in PDB or with very sparse sequence profile.[51] It is available at (http://raptorx.uchicago.edu/).

Modeling Amino Acid Substitution:

UCSF Chimera is a highly extensible program for interactive visualization and analysis of molecular structures and related data, including density maps, supramolecular assemblies, sequence alignments, docking results,conformational analysis Chimera (version 1.8).[52] It is available at (http://www.cgl.ucsf.edu/chimera/).

Identification of Functional SNPs in Conserved Regions by using ConSurf server:

ConSurf web server provides evolutionary conservation profiles for proteins of known structure in the PDB. Amino acid sequences similar to each sequence in the PDB were collected and multiply aligned using CSI-BLAST and MAFFT, respectively. The evolutionary conservation of each amino acid position in the alignment was calculated using the Rate 4Site algorithm, implemented in the ConSurf web server. The algorithm takes explicitly into account the phylogenetic relations between the aligned proteins and the stochastic nature of the evolutionary process. Rate 4 Site assigns a conservation level for each residue using an empirical Bayesian inference. Visual inspection of the conservation patterns on the 3-dimensional structure often enables the identification of key residues that comprise the functionally-important regions of the protein.[53, 54] It is available at (http://consurf.tau.ac.il/).

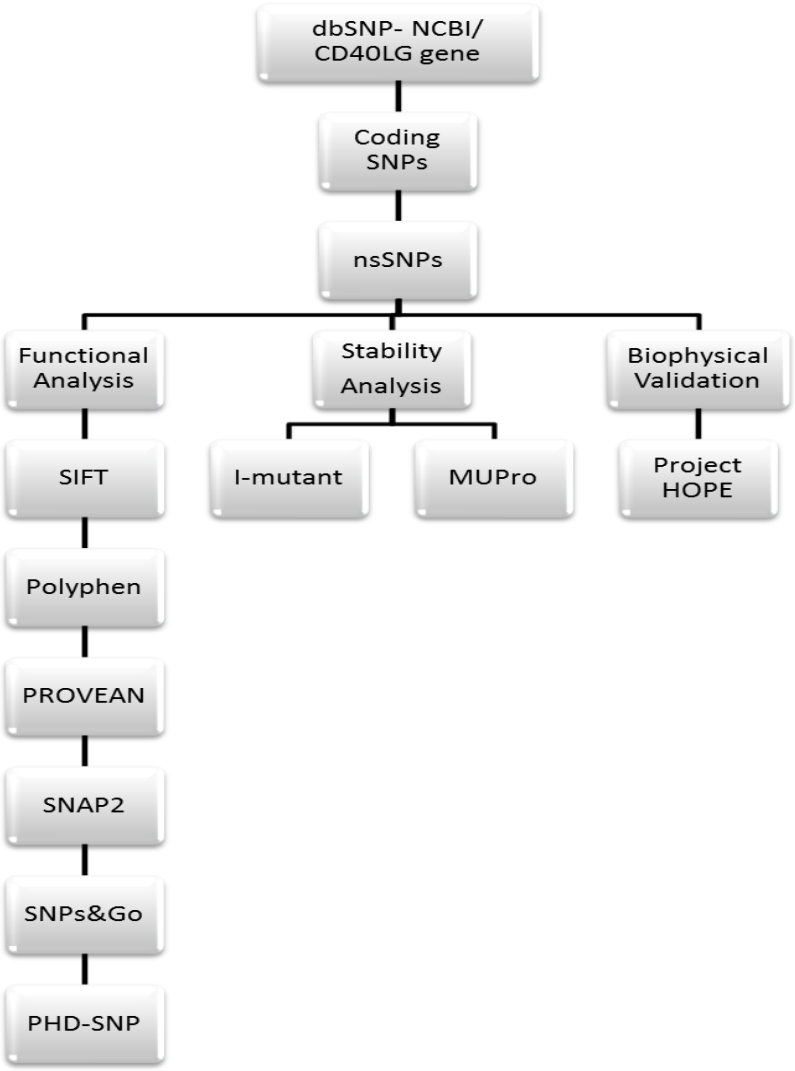


Figure 1: Workflow Software's used in SNPs analysis.

Results:

Table (1): Damaging or Deleterious nsSNPs associated variations predicted by various softwares:

dbSNP rs#	SUB	SIFT PREDICTION	SIFT SCORE	POLYPHEN PREDICTION	POLYPHEN SCORE	Prediction (cutoff= -2.5)	PROVEAN score	SNAP2 PREDICTION	SNAP2 SCORE
rs750090324	Y5C	Damaging	0	probably damaging	0.999	Deleterious	-3.085	effect	64
rs104894778	A123E	Damaging	0	probably damaging	1	Deleterious	-3.3	effect	97
-	A123E	Damaging	0	probably damaging	1	Deleterious	-3.3	effect	97
rs104894777	W140G	Damaging	0	probably damaging	1	Deleterious	-9.404	effect	96
rs886039326	G144E	Damaging	0.02	probably damaging	0.993	Deleterious	-3.333	effect	70
rs773698908	M148K	Damaging	0.02	possibly damaging	0.501	Deleterious	-3.107	effect	85
rs104894769	L155P	Damaging	0.01	probably damaging	1	Deleterious	-3.089	effect	92
-	L155P	Damaging	0.01	probably damaging	1	Deleterious	-3.089	effect	92
rs786205606	Y169C	Damaging	0	probably damaging	1	Deleterious	-8.868	effect	60
rs756468554	Y170C	Damaging	0	probably damaging	1	Deleterious	-7.592	effect	69
rs200840715	R181W	Damaging	0	probably damaging	0.999	Deleterious	-2.675	effect	65
rs993299705	I223F	Damaging	0.01	probably damaging	0.998	Deleterious	-2.768	effect	62
rs104894768	G227V	Damaging	0	probably damaging	1	Deleterious	-6.598	effect	95
rs1313764088	V237A	Damaging	0.01	probably damaging	0.968	Deleterious	-3.208	effect	40
rs193922136	T254M	Damaging	0.01	probably damaging	1	Deleterious	-4.261	effect	66
rs1057521128	F256S	Damaging	0.01	probably damaging	1	Deleterious	-7.425	effect	86
rs1477466218	G257D	Damaging	0	probably damaging	1	Deleterious	-6.453	effect	86

Table (2): Disease effect nsSNPs associated variations predicted by SNPs&GO and PhD-SNP:

dbSNP rs#	SNPs&GO prediction	RI	score	PhD-SNP predicton	RI	score
rs104894777	Desease	2	0.592	Desease	4	0.722
rs886039326	Desease	1	0.559	Desease	6	0.797
rs104894769	Desease	0	0.511	Desease	5	0.752
rs786205606	Desease	7	0.832	Desease	8	0.889
rs756468554	Desease	6	0.812	Desease	8	0.898
rs104894768	Desease	3	0.634	Desease	4	0.692
rs1057521128	Desease	1	0.542	Desease	2	0.612
rs1477466218	Desease	3	0.63	Desease	0	0.506

Table (3): stability analysis predicted by I-Mutant version 3.0 and MUPro (also Show the 8 novel mutations):

dbSNP rs#	SUB	SVM2 Prediction Effect	RI	DDG Value Prediction	Mupro Prediction
rs104894777	W140G	Decrease	9	-2.06	Decrease
rs886039326	G144E	Decrease	1	-0.68	Decrease
rs104894769	L155P	Decrease	6	-1.6	Decrease
rs786205606	Y169C	Decrease	2	-1.14	Decrease
rs756468554	Y170C	Decrease	3	-1.3	Decrease
rs104894768	G227V	Decrease	1	-0.14	Decrease
rs1057521128	F256S	Decrease	6	-1.5	Decrease
rs1477466218	G257D	Increase	0	-0.69	Decrease

Table (4): The *CD40LG* gene functions and its appearance in network and genome:

Function	FDR	Genes in network	Genes in genome
necrotic cell death	3.46E-05	4	22
necroptotic process	3.46E-05	4	18
programmed necrotic cell death	3.46E-05	4	19
positive regulation of I-kappaB kinase/NF-kappaB signaling	3.46E-05	6	138
regulation of I-kappaB kinase/NF-kappaB signaling	0.000103	6	185
regulation of immune effector process	0.000103	6	184
I-kappaB kinase/NF-kappaB signaling	0.000133	6	198
positive regulation of cytokine production	0.000151	6	207
toll-like receptor 4 signaling pathway	0.000183	5	107
positive regulation of defense response	0.000232	6	231
cellular response to interleukin-4	0.000285	3	11
CD40 receptor complex	0.000285	3	11
response to interleukin-4	0.00035	3	12
toll-like receptor signaling pathway	0.000405	5	137
pattern recognition receptor signaling pathway	0.000767	5	158
innate immune response-activating signal transduction	0.000767	5	160
production of molecular mediator of immune response	0.000879	4	69
activation of innate immune response	0.000879	5	168
TRIF-dependent toll-like receptor signaling pathway	0.00123	4	76
MyD88-independent toll-like receptor signaling pathway	0.001365	4	79
regulation of endothelial cell apoptotic process	0.001381	3	22
cell-type specific apoptotic process	0.001381	5	194
positive regulation of innate immune response	0.001381	5	191
toll-like receptor 3 signaling pathway	0.001387	4	83
endothelial cell apoptotic process	0.001887	3	25
regulation of innate immune response	0.003909	5	246
B cell activation	0.005198	4	119
cytokine production involved in immune response	0.006123	3	38
regulation of production of molecular mediator of immune response	0.009243	3	44
regulation of cysteine-type endopeptidase activity	0.015054	4	160
side of membrane	0.015581	4	163
B cell proliferation	0.015581	3	54
regulation of defense response to virus	0.02744	3	66
response to interleukin-15	0.02744	2	10
regulation of necrotic cell death	0.02744	2	10
response to virus	0.032144	4	205
lymphocyte activation involved in immune response	0.032144	3	72

cytoplasmic pattern recognition receptor signaling pathway in response to virus	0.035169	2	12
receptor complex	0.035169	4	213
cellular response to virus	0.035169	2	12
regulation of multi-organism process	0.035534	4	216
regulation of response to biotic stimulus	0.049587	3	87
positive regulation of apoptotic process	0.058691	4	249
regulation of endopeptidase activity	0.059151	4	251
leukocyte activation involved in immune response	0.059317	3	96
adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	0.059317	3	98
cell activation involved in immune response	0.059317	3	96
regulation of peptidase activity	0.059317	4	258
positive regulation of programmed cell death	0.059317	4	254
receptor signaling protein activity	0.059317	3	98
positive regulation of cell death	0.065646	4	268
T cell apoptotic process	0.072358	2	20
isotype switching	0.072358	2	20
somatic recombination of immunoglobulin genes involved in immune response	0.072358	2	20
somatic diversification of immunoglobulins involved in immune response	0.072358	2	20
inflammatory response	0.072358	4	283
regulation of leukocyte differentiation	0.072358	3	109
negative regulation of cytokine production	0.07391	3	111
tumor necrosis factor receptor superfamily binding	0.074167	2	21
positive regulation of NF-kappaB transcription factor activity	0.074167	3	112
defense response to virus	0.080025	3	116
NIK/NF-kappaB signaling	0.083661	2	23
B cell activation involved in immune response	0.083661	2	23
somatic recombination of immunoglobulin gene segments	0.083661	2	23
lymphocyte proliferation	0.089235	3	123
leukocyte mediated immunity	0.09	3	124
mononuclear cell proliferation	0.090774	3	125

***FDR:** false discovery rate is greater than or equal to the probability that this is a false positive.

Table (5) The gene co-expressed, share domain and Interaction with *CD40LG* gene network:

Gene 1	Gene 2	Weight	Network group
IL2RG	FASLG	0.014963	Co-expression
KCNA3	IL4	0.013943	Co-expression
NFKB2	BIRC3	0.011734	Co-expression
IL2RG	BIRC3	0.011704	Co-expression
IL4	CD40LG	0.009129	Co-expression

REL	IL4R	0.011926	Co-expression
KCNA3	CD40LG	0.010907	Co-expression
ICOS	CD40	0.012159	Co-expression
TRAF3	ICOS	0.008684	Co-expression
FASLG	ICOS	0.013282	Co-expression
BIRC3	ICOS	0.008743	Co-expression
BIRC3	TRAF3	0.011122	Co-expression
IL2RG	CD40	0.006743	Co-expression
IL2RG	ICOS	0.00355	Co-expression
IL2RG	IL4R	0.006168	Co-expression
IL2RG	BIRC3	0.003711	Co-expression
IL2RG	NFKB2	0.006361	Co-expression
KCNA3	IL2RG	0.005632	Co-expression
FASLG	ICOS	0.021807	Co-expression
TNF	JAK3	0.006956	Co-expression
BIRC3	CD40	0.017045	Co-expression
NFKB2	BIRC3	0.022512	Co-expression
IL2RG	IL4R	0.012735	Co-expression
IL2RG	BIRC3	0.011602	Co-expression
IL2RG	NFKB2	0.02143	Co-expression
KCNA3	CD40	0.017026	Co-expression
BIRC3	CD40	0.016166	Co-expression
NFKB2	CD40	0.019693	Co-expression
IL2RG	CD40	0.008382	Co-expression
IL2RG	ICOS	0.007516	Co-expression
IL2RG	BIRC3	0.005774	Co-expression
IL2RG	NFKB2	0.006811	Co-expression
KCNA3	ICOS	0.02028	Co-expression
KCNA3	IL2RG	0.006984	Co-expression
BIRC3	CD40LG	0.01894	Co-expression
IL2RG	JAK3	0.008937	Co-expression
BIRC3	CD40	0.00956	Co-expression
BIRC3	TNF	0.021513	Co-expression
NFKB2	CD40	0.008004	Co-expression
NFKB2	TNF	0.012938	Co-expression
NFKB2	BIRC3	0.015358	Co-expression
IL2RG	NFKB2	0.020683	Co-expression
KCNA3	MAP3K1	0.008914	Co-expression
BIRC2	TNF	0.01865	Co-expression
BIRC2	BIRC3	0.028052	Co-expression
IL4R	TRAF3	0.014878	Co-expression
TNF	FASLG	0.013372	Co-expression
REL	JUND	0.008833	Co-expression

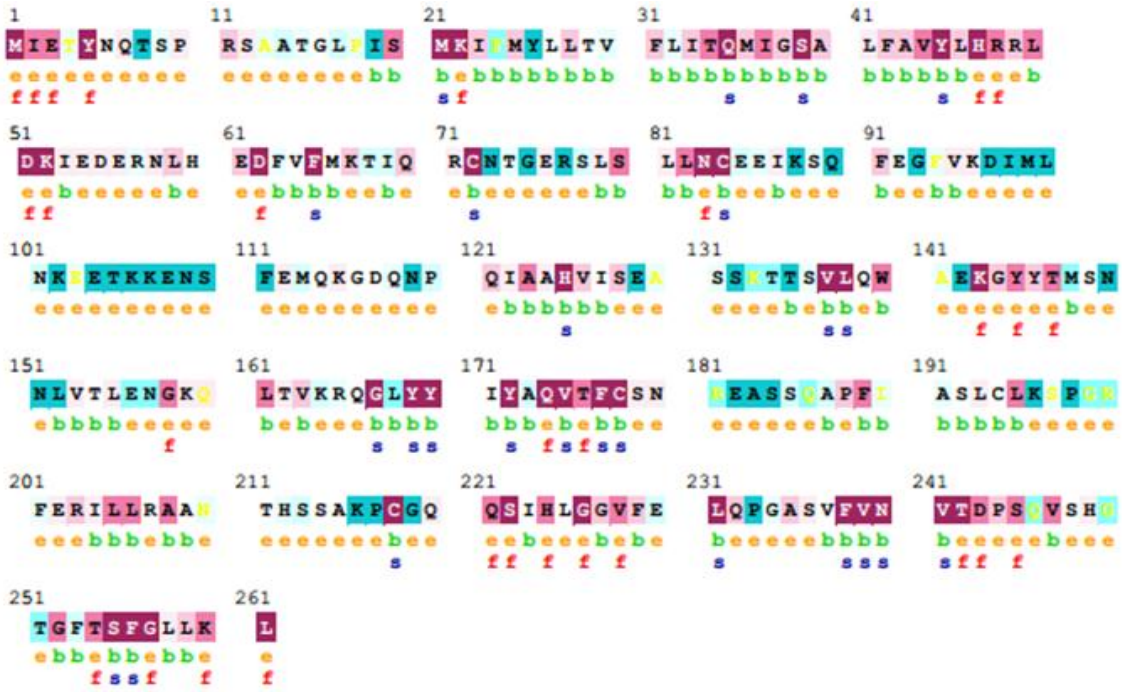
BIRC3	TRAF3	0.013216	Co-expression
NFKB2	TRAF3	0.022174	Co-expression
NFKB2	TNF	0.022091	Co-expression
NFKB2	BIRC3	0.013708	Co-expression
MAP3K1	REL	0.01427	Co-expression
IL4R	CD40	0.005425	Co-expression
REL	IL4R	0.011723	Co-expression
BIRC3	CD40	0.004387	Co-expression
NFKB2	CD40	0.011266	Co-expression
IL2RG	CD40	0.005195	Co-expression
IL2RG	IL4R	0.007494	Co-expression
BIRC2	BIRC3	0.006445	Co-expression
TNF	ICOS	0.014079	Co-expression
JUND	IL4R	0.009701	Co-expression
BIRC3	CD40	0.010649	Co-expression
RNF128	TDP2	0.006606	Co-expression
FASLG	CD40LG	0.017517	Co-expression
JAK3	CD40LG	0.020611	Co-expression
TNF	CD40LG	0.018848	Co-expression
BIRC3	CD40	0.019717	Co-expression
NFKB2	BIRC3	0.018493	Co-expression
IL2RG	CD40	0.007105	Co-expression
IL2RG	ICOS	0.004861	Co-expression
IL2RG	BIRC3	0.005204	Co-expression
IL2RG	NFKB2	0.007049	Co-expression
KCNA3	CD40LG	0.01689	Co-expression
KCNA3	ICOS	0.016696	Co-expression
KCNA3	IL2RG	0.006413	Co-expression
NFKB2	CD40	0.011105	Co-expression
NFKB2	IL4R	0.01556	Co-expression
NFKB2	TNF	0.011411	Co-expression
NFKB2	BIRC3	0.011462	Co-expression
KCNA3	BIRC3	0.006626	Co-expression
BIRC2	BIRC3	0.012962	Co-expression
BIRC2	NFKB2	0.00852	Co-expression
BIRC2	BIRC3	0.016683	Co-expression
REL	TNF	0.010127	Co-expression
BIRC3	CD40	0.00818	Co-expression
IL2RG	JUND	0.009352	Co-expression
IL2RG	NFKB2	0.009404	Co-expression
BIRC2	CD40	0.010793	Co-expression
BIRC2	IL4	0.008467	Co-expression
BIRC2	TNF	0.006199	Co-expression

BIRC2	BIRC3	0.007214	Co-expression
BIRC3	ICOS	0.020492	Co-expression
BIRC2	REL	0.010786	Co-expression
REL	JUND	0.023229	Co-expression
IL2RG	REL	0.012688	Co-expression
BIRC2	BIRC3	0.008191	Co-expression
TNF	JAK3	0.026589	Co-expression
BIRC3	REL	0.009161	Co-expression
IL2RG	JAK3	0.011127	Co-expression
IL2RG	IL4R	0.004268	Co-expression
IL2RG	TNF	0.010053	Co-expression
NFKB2	CD40	0.005998	Co-expression
NFKB2	BIRC3	0.014023	Co-expression
KCNA3	MAP3K1	0.003699	Co-expression
BIRC2	TNF	0.008398	Co-expression
BIRC2	BIRC3	0.015018	Co-expression
FASLG	CD40LG	0.015669	Co-localization
JAK3	CD40LG	0.014456	Co-localization
JAK3	FASLG	0.014457	Co-localization
BIRC3	CD40LG	0.014902	Co-localization
BIRC3	FASLG	0.020128	Co-localization
KCNA3	CD40LG	0.013906	Co-localization
KCNA3	JAK3	0.021973	Co-localization
JAK3	CD40LG	0.009828	Co-localization
TNF	IL4R	0.021817	Co-localization
IL2RG	CD40LG	0.004545	Co-localization
KCNA3	CD40LG	0.008507	Co-localization
KCNA3	IL2RG	0.005577	Co-localization
BIRC2	BIRC3	0.015573	Co-localization
CD40	CD40LG	0.192562	Pathway
ICOS	CD40LG	0.129156	Pathway
TRAF3	CD40LG	0.060989	Pathway
TRAF3	CD40	0.129558	Pathway
IL4	CD40LG	0.05932	Pathway
JAK3	CD40LG	0.023855	Pathway
JAK3	IL4	0.01561	Pathway
IL4R	CD40LG	0.049297	Pathway
IL4R	IL4	0.03226	Pathway
IL4R	JAK3	0.012973	Pathway
TNF	FASLG	0.01287	Pathway
JUND	CD40LG	0.055153	Pathway
REL	CD40LG	0.05313	Pathway
MAP4K4	CD40LG	0.048895	Pathway

MAP4K4	CD40	0.103865	Pathway
MAP4K4	TRAF3	0.032897	Pathway
MAP4K4	TNF	0.012563	Pathway
BIRC3	FASLG	0.023192	Pathway
BIRC3	TNF	0.010785	Pathway
BIRC3	MAP4K4	0.022639	Pathway
NFKB2	CD40LG	0.042237	Pathway
NFKB2	REL	0.024756	Pathway
MAP3K1	CD40LG	0.028289	Pathway
MAP3K1	CD40	0.060093	Pathway
MAP3K1	TRAF3	0.019033	Pathway
MAP3K1	TNF	0.007269	Pathway
MAP3K1	MAP4K4	0.015259	Pathway
IL2RG	CD40LG	0.028033	Pathway
IL2RG	IL4	0.018345	Pathway
IL2RG	JAK3	0.007377	Pathway
IL2RG	IL4R	0.015245	Pathway
BIRC2	TNF	0.016131	Pathway
BIRC2	BIRC3	0.029068	Pathway
CD40	CD40LG	0.046437	Pathway
TRAF3	CD40LG	0.119048	Pathway
TRAF3	CD40	0.151661	Pathway
IL4	CD40LG	0.026394	Pathway
JAK3	CD40LG	0.024033	Pathway
JAK3	CD40	0.030616	Pathway
JAK3	IL4	0.017402	Pathway
IL4R	CD40LG	0.041845	Pathway
IL4R	IL4	0.0303	Pathway
IL4R	JAK3	0.027589	Pathway
TDP2	CD40LG	0.136808	Pathway
TDP2	CD40	0.174285	Pathway
MAP4K4	TNF	0.041482	Pathway
BIRC3	CD40LG	0.032145	Pathway
BIRC3	CD40	0.040951	Pathway
BIRC3	TRAF3	0.104984	Pathway
BIRC3	TNF	0.016886	Pathway
BIRC3	MAP4K4	0.069639	Pathway
MAP3K1	CD40LG	0.031848	Pathway
MAP3K1	CD40	0.040573	Pathway
IL2RG	CD40LG	0.019385	Pathway
IL2RG	IL4	0.014036	Pathway
IL2RG	JAK3	0.012781	Pathway
IL2RG	IL4R	0.022253	Pathway

RNF128	IL4	0.115976	Pathway
BIRC2	CD40LG	0.076342	Pathway
BIRC2	CD40	0.097255	Pathway
BIRC2	TRAF3	0.249331	Pathway
BIRC2	TNF	0.040103	Pathway
BIRC2	BIRC3	0.067323	Pathway
CD40	CD40LG	0.607524	Physical Interactions
TRAF3	CD40	0.095902	Physical Interactions
IL4R	IL4	0.796225	Physical Interactions
BIRC3	CD40	0.330114	Physical Interactions
NFKB2	REL	0.047741	Physical Interactions
IL2RG	JAK3	0.53926	Physical Interactions
CD40	CD40LG	0.08088	Physical Interactions
TRAF3	CD40	0.013307	Physical Interactions
HPR	CD40LG	0.523536	Physical Interactions
IL4R	CD40	0.054789	Physical Interactions
IL4R	IL4	0.343558	Physical Interactions
IL4R	JAK3	0.050891	Physical Interactions
TDP2	CD40	0.07344	Physical Interactions
TDP2	TRAF3	0.025738	Physical Interactions
BIRC3	CD40LG	0.039862	Physical Interactions
BIRC3	CD40	0.018714	Physical Interactions
BIRC3	TRAF3	0.006558	Physical Interactions
BIRC3	REL	0.020398	Physical Interactions
NFKB2	REL	0.048602	Physical Interactions
MAP3K1	MAP4K4	0.019982	Physical Interactions
IL2RG	JAK3	0.04765	Physical Interactions
IL2RG	IL4R	0.074022	Physical Interactions
RNF128	CD40LG	0.164195	Physical Interactions
BIRC2	CD40LG	0.032488	Physical Interactions
BIRC2	CD40	0.015252	Physical Interactions
BIRC2	TRAF3	0.005345	Physical Interactions
BIRC2	TNF	0.010899	Physical Interactions
BIRC2	BIRC3	0.007517	Physical Interactions
BIRC2	NFKB2	0.017911	Physical Interactions
TRAF3	CD40	0.475875	Physical Interactions
IL4R	IL4	0.372073	Physical Interactions
IL2RG	IL4	0.399409	Physical Interactions
IL4R	IL4	1	Physical Interactions
TNF	ICOS	0.102404	Physical Interactions
NFKB2	REL	0.173546	Physical Interactions
TRAF3	CD40	0.036334	Physical Interactions
IL4R	IL4	0.454577	Physical Interactions

NFKB2	REL	0.00236	Physical Interactions
CD40	CD40LG	0.105262	Physical Interactions
TRAF3	CD40	0.023258	Physical Interactions
JAK3	CD40	0.033453	Physical Interactions
IL4R	CD40	0.04218	Physical Interactions
IL4R	IL4	0.104439	Physical Interactions
IL4R	JAK3	0.035954	Physical Interactions
TDP2	CD40	0.077995	Physical Interactions
TDP2	TRAF3	0.046223	Physical Interactions
BIRC3	CD40	0.078458	Physical Interactions
NFKB2	REL	0.037047	Physical Interactions
IL2RG	IL4	0.095945	Physical Interactions
IL2RG	JAK3	0.03303	Physical Interactions
IL2RG	IL4R	0.041646	Physical Interactions
RNF128	CD40LG	0.306793	Physical Interactions
BIRC2	CD40	0.041099	Physical Interactions
TRAF3	CD40	0.101777	Predicted
NFKB2	REL	0.060585	Predicted
BIRC2	BIRC3	0.482262	Predicted
FASLG	CD40LG	0.245935	Predicted
FASLG	CD40	0.03527	Predicted
JAK3	CD40	0.046533	Predicted
IL4R	CD40	0.188654	Predicted
TNF	CD40LG	0.152204	Predicted
TNF	CD40	0.021828	Predicted
BIRC3	TRAF3	0.068934	Predicted
NFKB2	TRAF3	0.024427	Predicted
BIRC2	CD40	0.048271	Predicted
IL2RG	IL4	0.664124	Predicted
MAP3K1	MAP4K4	0.258003	Predicted
IL2RG	JAK3	1	Predicted
FASLG	CD40LG	0.041645	Shared protein domains
TNF	CD40LG	0.038418	Shared protein domains
TNF	FASLG	0.050344	Shared protein domains
NFKB2	REL	0.049596	Shared protein domains
IL2RG	IL4R	0.041143	Shared protein domains
BIRC2	BIRC3	0.047106	Shared protein domains
FASLG	CD40LG	0.064011	Shared protein domains
TNF	CD40LG	0.064011	Shared protein domains
TNF	FASLG	0.064011	Shared protein domains
NFKB2	REL	0.049118	Shared protein domains
MAP3K1	MAP4K4	0.003437	Shared protein domains
BIRC2	BIRC3	0.056869	Shared protein domains



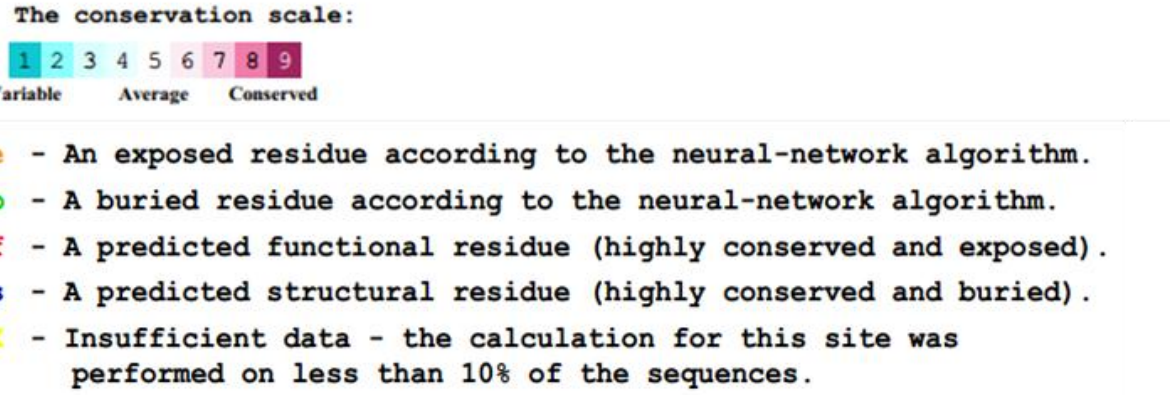


Figure 2: Unique and conserver regions in CD40LG protein were determined using Consurf.

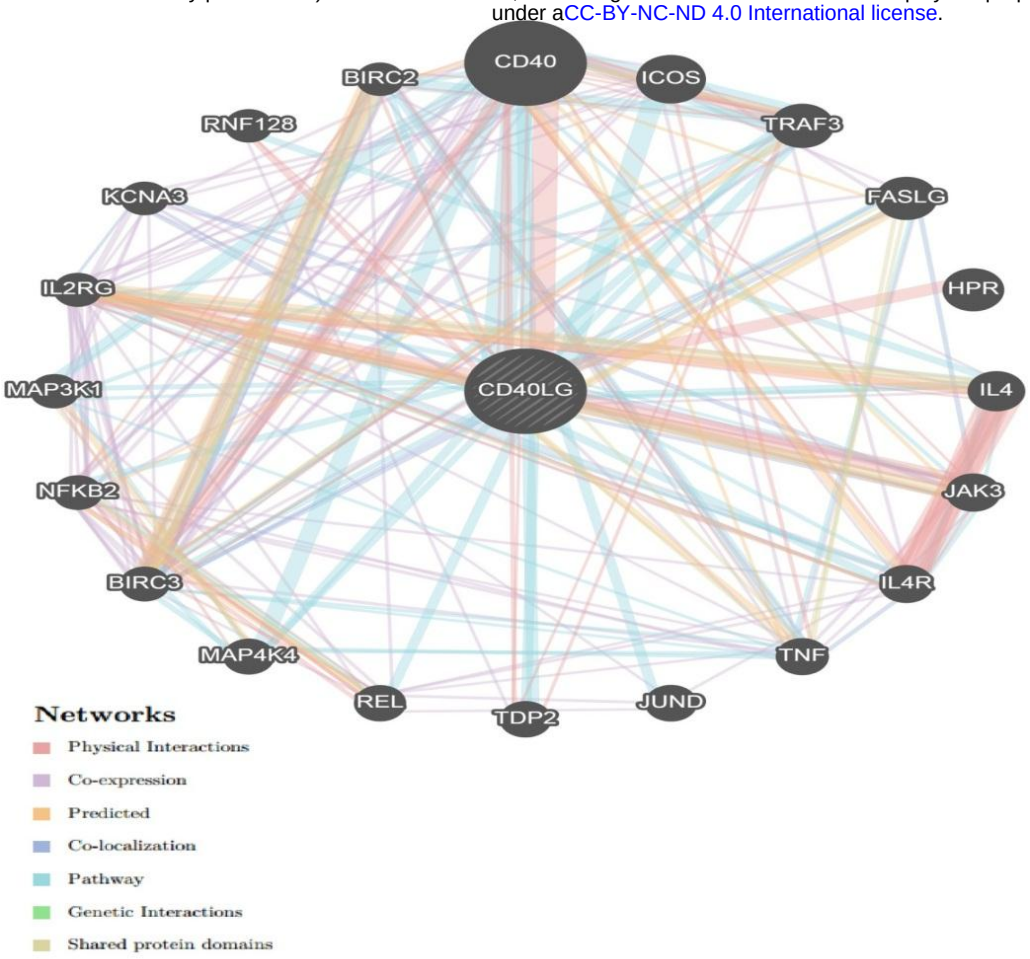


Figure 3: Interaction between *CD40LG* and its related genes.

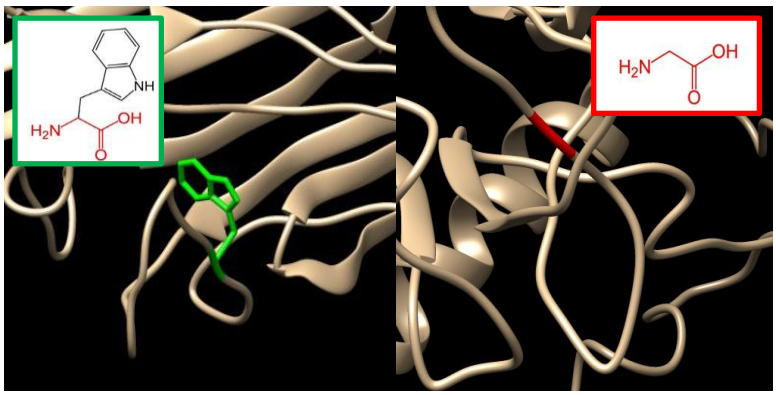


Figure 4: (W140G): The amino acid Tryptophan change to Glycine at position 140.

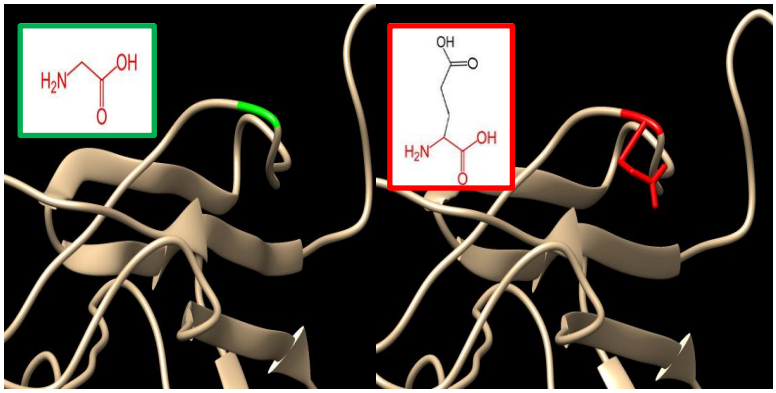


Figure 5:(G144E) The amino acid Glycine change to Glutamic Acid at position 144.

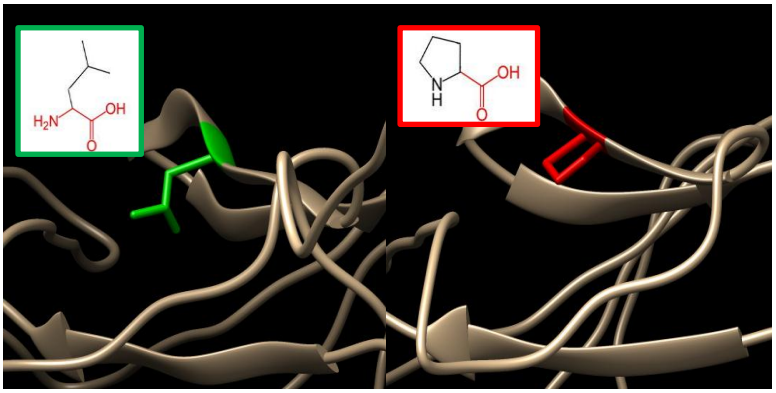


Figure 6:(L155P) The amino acid Leucine change to Proline at position 155.

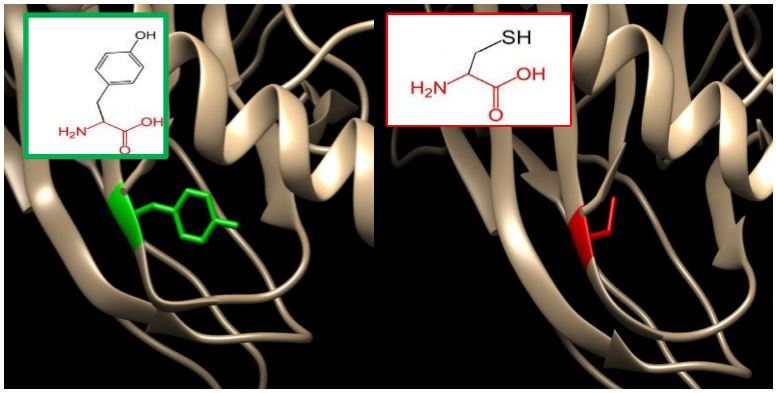


Figure 7 :(Y169C) The amino acid Tyrosine change to Cysteine at 169.

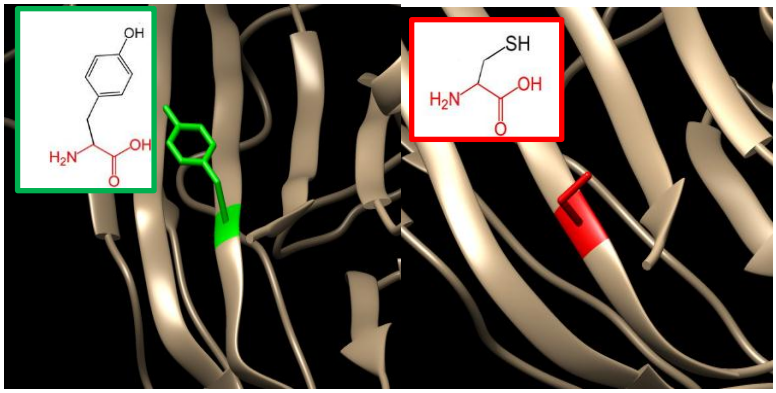


Figure 8: (Y170C) The amino acid Tyrosine change to Cysteine at 170.

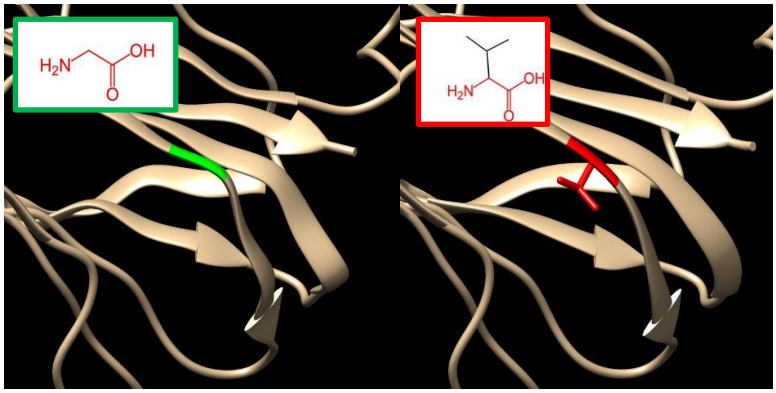


Figure 9: (G227V) The amino acid Glycine change to Valine at 227.

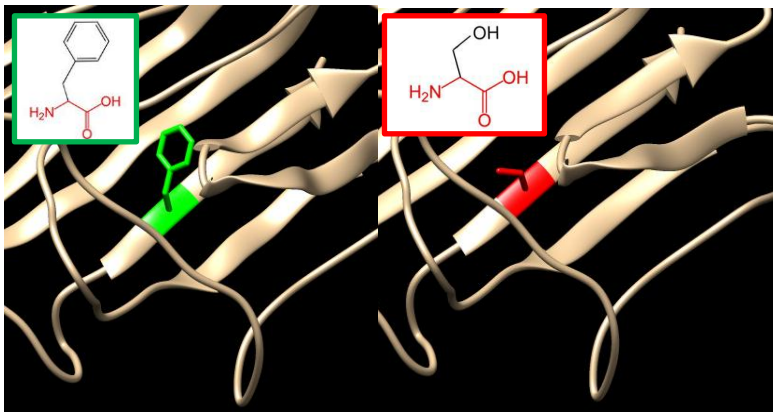


Figure 10:(F256S) The amino acid Phenylalanine change to Serine at 256.

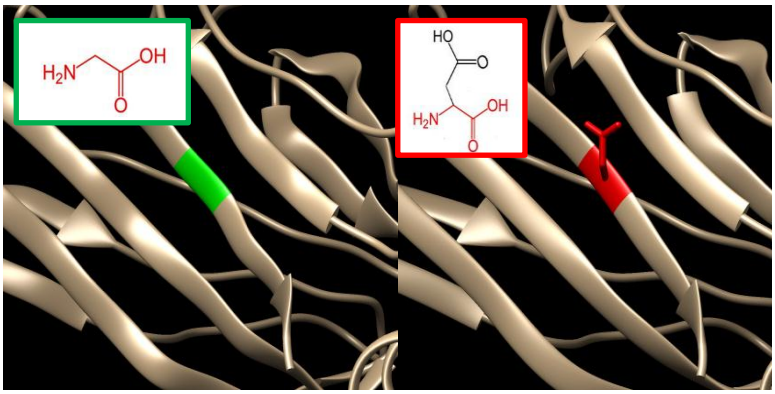


Figure 11:(G257D) The amino acid Glycine change to Aspartic Acid at 256.

Discussion:

8 novel mutations were found to have a damaging effect on the stability and function of the *CD40LG* gene using bioinformatics tools. The human immune system, especially the adaptive branch, significantly declines with ageing. Several distinct immunosenescent measures have already been described, yet data regarding to age-associated baseline alterations in immune cell function is limited.[55] Therefore, We investigated the effect of each SNP on the function and stability of the protein and gene expression of immune-related genes using different softwares with different parameters and aspects, in order to confirm the results we found and to minimize the error to the least percentage possible. The software used were SIFT, Polyphen-2, PROVEAN, SNAP2, PhD-SNP, SNP&GO, I-Mutant 3.0, MUPro, and Project HOPE. (figure 1)

We retrieved 322 SNPs from the dbSNP/NCBI Database, which was the total number of nsSNPs in the coding region of the *CD40LG* gene in *Homo sapiens*. There were 105 nsSNPs (missense mutations) then submitted them to functional analysis by SIFT, PolyPhen-2, PROVEAN and SNAP2. SIFT server predicted 38 deleterious SNPs, polyphen-2 predicted 56 damaging SNPs (19 possibly damaging and 37 probably damaging), PROVEAN predicted 23 deleterious SNPs and SNAP2 predicted 53 deleterious SNPs. After filtering the Quadra-positive deleterious SNPs we ended up with 15 SNPs (Table 1) and we submitted them to PhD-SNP and SNP&GO to further investigate their effect on the function. PhD-SNP predicted 15 disease-associated SNPs while SNP&GO predicted 8, so we filtered the double positive 8 SNPs (Table 2) and submitted them to I-Mutant 3.0 and MUPro to investigate their effect on the stability. All the SNPs were found to cause a decrease in the stability of the protein except for one SNP (G257D) predicted by I-Mutant 3.0 to increase the stability. (Table 3)

We submitted the most deleterious 8 SNPs to project HOPE which revealed that all the SNPs are located in a domain in the protein and thus might have a vital change in the structure and function of the protein and it may affect its ability to bind with its targets. And we used Chimera software to visualize the amino acids change (figures 4-11)

GeneMANIA revealed that *CD40LG* has many vital functions: adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains, B cell activation, B cell activation involved in immune response, B cell proliferation, cell activation involved in immune response, cell-type specific apoptotic process, endothelial cell apoptotic process, inflammatory response, isotype switching, leukocyte activation involved in immune response, leukocyte mediated immunity, lymphocyte activation involved in immune response, lymphocyte proliferation, mononuclear cell proliferation, positive regulation of apoptotic process, positive regulation of cell death, positive regulation of cytokine production, positive regulation of programmed cell death, production of molecular mediator of immune response, regulation of endothelial cell apoptotic process, somatic diversification of immunoglobulins involved in immune response, somatic recombination of immunoglobulin gene segments, somatic recombination of immunoglobulin genes involved in immune response, tumor necrosis factor receptor superfamily binding. We also analysed the correlations between significantly regulated genes, share similar protein domain, or participate to achieve similar function were illustrated by GeneMANIA and shown in figure 3, Tables (4&5).

We also used ConSurf web server; the nsSNPs that are located at highly conserved amino acid positions tend to be more deleterious than nsSNPs that are located at non-conserved sites. (Figure2).

Some study revealed that, patients with Hyper IgM syndrome are susceptible to malignancies of neuroendocrine origin know as neuroendocrine tumors (NETs), of all primary immune deficiency diseases, NETs appeared to be unique to XHIGM patients[35] The mechanism behind this association remains unclear, so we hope that this study will help in understanding and diagnosis of this disorder. We also believe that, any mutations in *CD40LG* may be a part of molecular events involved in the severity of Sickle cell disease and Down syndrome. [36, 56]

This is the first in silico analysis of *CD40LG* gene that used functional analysis to determine the deleterious SNPs. Our study matches the result from the NCBI that 6 SNPs are pathogenic (W140G and G227V are pathogenic while G144E, L155P, Y169C and F256S likely pathogenic) yet the remaining 2 SNPs (Y170C and G257D) were untested in the NCBI but found deleterious in our study. These SNPs may now be used as diagnostic markers for hyper IgM syndrome and may help in the understanding of the associated diseases. Finally some gratitude of wet lab techniques is suggested to support our findings.

Conclusion:

In this study the influence of functional SNPs in the *CD40LG* gene was thoroughly investigated through different bioinformatics prediction softwares. A total of 8 novel mutations (W140G, G144E, L155P, Y169C, Y170C, G227V, F256S, and G257D) were predicted to be responsible for the structural and functional modifications of *CD40LG* protein. It is evident from multiple in silico analysis tools that; these 8 nsSNPs might serve as, a novel diagnostic markers of Hyper-IgM syndrome. And can assist in pharmacogenomics by identifying high risk SNP variants contributing to drug response as well as developing novel therapeutic elements. And could help in the overall understanding of this disease.

Conflict of interest:

The authors have declared that no competing interest exists.

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- Sudan.

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