Bio-informatic Analysis of Missense Single Nucleotide Polymorphisms (SNPs) in Human CD38 Gene Associated with B-Chronic Lymphocytic Leukemia

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ABSTRACT

Background: CLL: Chronic lymphocytic leukemia is a chronic type of haematological malignancies that evoked from lymph proliferative origin of bone marrow and secondary lymphoid tissue, resultant in proliferation and progressive accumulation of distinct monoclonal CD5 /CD19 /CD23 B lymphocytes in the bone marrow, peripheral blood, and lymphatic organs. **CD38** is a multifunctional ecto-enzyme, known to be a direct contributor in pathogenesis of CLL by poorly understood mechanism. Even though , it highly expressed in CLL. At specific position of CD38 gene sequence, substitution of single nucleotide may result in change in amino acid that ends by consequent alteration of protein structure. **Aim:** To study CD38 polymorphism and to predict its effect on structure and subsequently function of CD38 molecule. **Methodology and Result**: The bioinformatic analysis of CD38 gene had been carried out by using several soft wares. Functional analysis by SIFT,Polyphen2, and PROVEAN reveled 12 deleterious SNPs. These SNPs were further analyzed by SNAP2, SNP@GO. PMut, STRING and other soft wars. Furthermore, Stability analysis was done using I-Mutant and MUpro software where seven SNPs were found to decrease the stability of the protein by I-Mutant ,while two SNPs increase it. At the same time, eight SNPs were found to decrease the stability by Mupro software while only one SNP is predicted to increase it. Finally, Physiochemical analysis was done using Project Hope. **Conclusion:** In summary, CD38 genotype seems to have twelve SNP that possibly will result in deleterious effect on Protein Structure. This genetic variation eventually will lead to alteration in potential molecule functions .Which effect the progression of CLL By the end.

Keywords: B-Chronic lymphocytic leukemia, missense single nucleotide polymorphism, and CD38 gene.

Introduction:

Chronic lymphocytic leukemia (CLL) is a chronic type of hematological malignancies that evoked from lymph proliferative origin and derived from bone marrow and secondary lymphoid tissue which accompanied by exaggerated monoclonal proliferation of mature B lymphocytes[(1)] **,** result in progressive accumulation of distinct monoclonal CD5+/CD19+/CD23+ B lymphocytes in the bone marrow, peripheral blood, and lymphatic organs[(2)]**.** On an other hand ,CLL is the most common type of leukemia

among adults[3]. furthermore, It is a Malignancy that mostly affects elders; 80% of those patients are aged more than 50 years [4,5] although ,B-CLL is molecularly heterogeneous disease[6] but the exact etiology of this leukemia is not well understood .[7] In fact, The clinical course for B-CLL is highly variable ,fortunately this matter recently were solved by the analysis of novel molecular prognostic factors including CD38 and ZAP-70 expression.[8]

CD38(Cluster Of Differentiation 38) also known as ADPRC1, it is a type II trans membrane glycoprotein in nature which is defined as a multifunctional ecto-enzyme that expressed normally at high levels on plasma and nuclear membranes of B cell precursors, germinal center B cells, and plasma cells, with low expression on circulating B cells.The gene that encodes human CD38 has been mapped in the short arm of chromosome 4 (4p15) with its coding sequence organized in eight exons and more than 98% of the gene is represented by introns [9].

CD 38 Structure:

As a Trans membrane protein, full-length human CD38 comprised from 300 amino acid residues connecting to three domains: a short intracellular domain, a single trans membrane helix domain, and a large extracellular domain. The distribution of amino acids among domains as following: Cytoplasmic domain from 1-21, Trans membrane domain 22-42 and extracellular domain 43-300 amino acid.

As known, intracellular domain act as enzyme and extra cellular domain as receptor. The extracellular domain is an ecto-enzyme (EC3.2.2.5) which produce cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) from its substrates NAD and NADP, in that order. [10]

The biological functions of CD38 molecule is varied depends on the cells type and CD38 maturation state.CD38 is known to be functionally active in signal transduction, cell adhesion and calcium signaling. The binding to the ligand CD31, initiates a signaling cascade that includes phosphorylation of sequential intracellular targets and increases cytoplasmic Ca^{+2} levels, mediating different biological CD38 (CD38 molecule**)**[11]. Additionally, another events of CD38 involvement in biological activities had been noted (e.g., activation, proliferation, apoptosis, cytokines secretion and homing),which depends on the cells type. CD38 accomplished its role As an enzyme, generate ng cADPR, ADP-ribose and NAADP CD38 as a products of AD+/NADP+ metabolism. These products bind different receptors and channels (IP3 receptors IP3R, Ryanodine receptor RyR and Transient receptor potential cation channel subfamily M member 2 TRPM2) and are involved in the regulation of intracellular Ca^{+2} and activation of critical signaling pathways connected to the control of cell metabolism, genomic stability, apoptosis, cell signaling, inflammatory response and stress tolerance.[12] Several studies reported over expression of CD38 in CLL.[13] Recent findings have suggested that CD38 is not only a prognostic marker, but also the key component of the pathogenic network underlying B CLL and T. [14]

Single nucleotide polymorphisms (SNPs) in CD38 gene were detected in numerous haematological disorders such as CLL, multiple myeloma, Correlectal cancer and SLE as well as other disorders like Diabetes and AutismREF. Most of the studies were used molecular tools, till now no studies talking a bioinformatics analysis of CD38. In the current study we performed a comprehensive bioinformatics analysis to predict the possible SNPs in CD38 gene and assessing its effects on the structure and function of **ADPRC1** protein.[15-19]

Method:

2.1. Retrieving nsSNPs:SNPs associated with CD38 gene were obtained from the Single Nucleotide Polymorphism database (dbSNP) in the National Center for Biotechnology Information (NCBI). (http://www.ncbi.nlm.nih.gov/snp/).

The sequence and natural variants of CD38 protein were obtained from the UniProt database as it considered as the most reliable and unambiguous database for protein sequences.[20] (https://www.uniprot.org/).

2.2. Identifying the most damaging nsSNPs and disease related mutations:

Five different bioinformatics tools were utilized to predict functional effects of nsSNPs obtained from dbSNP database on the protein. These algorithmic include: SIFT, PROVEAN, PolyPhen-2, SNAP2 and PhD-SNP.

The SNPs predicted deleterious by at least three softwares were considered high risk nsSNPs and investigated further.

2.2.1. SIFT Server: Phenotypic effects of amino acid substitution on protein function were predicted by using Sorting Intolerant From Tolerant (SIFT) server, which is a powerful tool used to fulfill this purpose. A list of nsSNPs (rsIDs) from NCBI's dbSNP database was submitted as a query (original) sequence to SIFT to predict tolerated and deleterious substitutions for every position of a protein sequence. . (Available at: http://sift.bii.a-star.edu.sg/). [21-24]

2.2.2. Provean Server:(Protein Variation Effect Analyzer) is the second software tool used. It also predicts the effect of an amino acid substitution on the biological function of a protein. It predicts the damaging effects of any type of protein sequence variations to not only single amino acid substitutions but also in-frame insertions, deletions, and multiple amino acid substitutions. (Available at: http://provean.jcvi.org/index.php). [25-26]

2.2.3. Polyphen-2 Server: Polymorphism Phenotyping v2.0 (PolyPhen-2) is another online tool that predicts the possible effects of an amino acid substitution on the structure and function of the protein. The results are classified into "PROBABLY DAMAGING" that is the most disease causing with a score near to 1 (0.7-1), "POSSIBLY DAMAGING" with a less disease causing ability with a score of 0.5-0.8 and "BENIGN" which does not alter protein functions with a score closer to zero; (0-0.4). (Available at: http://genetics.bwh.harvard.edu/pph2/). [27-29]

2.2.4. SNAP2

It is a trained functional analysis web-based tool that differentiates between effect and neutral SNPs by taking a variety of features into account. SNAP2 has an accuracy (effect/neutral) of 83%. It is considered an important and substantial enhancement over other methods.[30]

2.2.5. CONDEL:

Condel is a method to assess the outcome of non-synonymous single nucleotide variants SNVs using a consensus deleteriousness score that combines various tools (SIFT, Polyphen2, MutationAssessor, and FATHMM). The result of the prediction are either deleterious or neutral with a score ranging from zero to one.[31]

2.3 Prediction of Disease Associated SNPs

2.3.1. SNPs & Go server:

An online web server that used to ensure the disease relationship with the studied single nucleotide polymorphisms SNPs. It gives three different results based on three different analytical algorithms;

Panther result, PHD-SNP result, SNPs &GO result. (Available at: http://snps-and-go.biocomp.unibo.it/snps-and-go/) .[32]

2.3.2. PMUT Server:

PMUT is a powerful web-based tool used for the prediction of pathological variants on proteins. The prediction results are catogrized as "Neutral" or "Disease". It is available at (http://mmb.irbbarcelona.org/PMut).[33]

2.4 Prediction of nsSNPs Impact on the Protein Stability by I-Mutant2.0

2.4.1 I-Mutant:

A online web-tool that is used for the prediction of protein stability changes upon single point mutations, determining whether the mutation increases or decreases the protein stability. (Available at: http://gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant2.0/I-Mutant2.0.cgi). [34]

2.4.2 Mupro

. It is a support vector machine-based tool for the prediction of protein stability changes upon nonsynonymous SNPs. +e 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. [35]

2.5 Identification of Functional SNPs in Conserved Regions by ConSurf

2.5.1 ConSurf analysis:

It is a web server that offers evolutionary conservation summaries for proteins of known structure in the protein data bank. ConSurf spots the parallel amino acid sequences and runs multialignment methods.[36]

2.6 Analyzing the Effect of nsSNPs on Physiochemical Properties by Mutpred

2.6.1MutPred server:

MutPred2 is a standalone and web application developed to classify amino acid substitutions as pathogenic or benign in human. In addition, it predicts their impact on over 50 different protein properties and, thus, enables the inference of molecular mechanisms of pathogenicity. [37]

2.7 Prediction of structural effect of point mutation on the protein sequence using Project HOPE

Project HOPE: It is an online web-server used to analyze the structural and functional variations on protein sequence that have been resulted from single amino acid substitution. It searches protein 3D structures by collecting structural information from a series of sources, including calculations on the 3D coordinates of the protein and sequence annotations from the UniProt database. Protein sequences are submitted to project HOPE server then HOPE builds a complete report with text, figures, and animations. It is available at (http://www.cmbi.ru.nl/hope).[38]

2.8 Protein-protein interactions analysis:

The **STRING** database aims to collect, score and integrate all publicly available sources of protein–protein interaction information, and to complement these with computational predictions.

KEGG

(Kyoto Encyclopedia of Genes and Genomes: http://www.genome.jp/kegg/)[39] is a database resource which embraces genomic, chemical and systemic functional information, gene lists from fully sequenced genomes that interrelated to higher-level systemic functions of the cell, the organism and the ecosystem. KEGG is used broadly as a reference knowledge base designed for explanation of large-datasets that created by genome sequencing and other experimental technologies to support basic research, and practical applications of human diseases, drugs and other health-related matters.

3.Result:

3.1 SNP Dataset from dbSNP

The reference sequence of CD38 protein (ID: P28907) was retrieved from National center NCBI databases along with related single nucleotide polymorphisms SNPs.

3.2. Prediction of Functional Mutations

Furthermore these SNPs(51) were submitted to SIFT server for prediction of function effect where 12 SNP were found to have deleterious effect on Protein (table1). Further functional analysis was done using polyhen2 , PROVEAN and SNAP2, where 9 SNPs were found deleterious by the four previous software (table 2).

3.3 Prediction of Disease Associated SNPs

3. 4 Prediction of nsSNPs Impact on the Protein Stability

Stability analysis was done using Imutant and MUpro software where seven SNPs were found to decrease the stability of the protein by Imutant ,while two SNPs(T148M,T144N) increase it. At the same time, eight SNPs were found to decrease the stability by Mupro software while only one SNP(P98L) are predicted to increase it.(Table3).

3. 5 Analysis of Conservation profile

3.6 Analyzing the Effect of nsSNPs on Physiochemical Properties Mutpred

Physiochemical analysis was done using Project Hope . A summary of the predicted effect on the structure and physiochemical priorities were summarized in (Table 4).

Table 1 : functional analysis of SNPs in CD38 protein using SIFT,POLYPHEN2,PROVEAN, SNAP2, and CONDEL.

SNP	AMINO_ACID	SIFT_SCORE	SIFT_PREDICTION	POLY-2 SCRE	POLY-2 PREDICTION	PROVEAN score	PROVEANPrediction	SNAP2 SCORE	SNAP2	CONDEL	CONDEL_LABEL
rs1800561	R140W	0.012	DELETERIOUS	0.999	probably damaging	-4.403	Deleterious	93	effect	0.409454	Neutral
rs1388666 92	T148M	0.006	DELETERIOUS	1	probably damaging	-3.866	Deleterious	67	effect	0.55141	DELETERIOUS
rs1492990 47	K234N	0.012	DELETERIOUS	0.998	probably damaging	-3.692	Deleterious	71	effect	0.553697	DELETERIOUS
rs1501718 47	S193C	0	DELETERIOUS	1	probably damaging	-4.771	Deleterious	72	effect	0.516534	Neutral
rs1999987 92	D147G	0.018	DELETERIOUS	0.999	probably damaging	-5.34	Deleterious	87	effect	0.4864	Neutral
rs2016838 43	F284C	0.008	DELETERIOUS	1	probably damaging	-3.036	Deleterious	10	effect	0.419877	Neutral
rs2018169 57	D179G	0.035	DELETERIOUS	0.999	probably damaging	-3.79	Deleterious	38	effect	0.377277	Neutral
rs3692141 60	T144N	0.014	DELETERIOUS	1	probably damaging	-3.596	Deleterious	50	effect	0.581067	DELETERIOUS
rs3696742 33	P98L	0.007	DELETERIOUS	1	probably damaging	-9.568	Deleterious	48	effect	0.502413	Neutral

Table 2: functional analysis of SNPs in CD38 protein using : SNP@GO, PHD-SNP, PANTHER, and PMut.

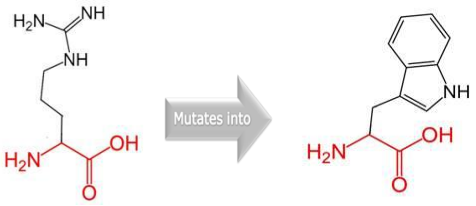
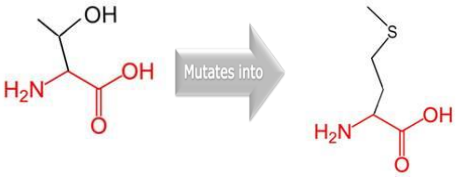
AMINO_ ACID	**Prediction PANTHER**	**RI**	**Probability**	**Prediction SNP & GO**	**RI**	**Probability**	**Prediction-PHD-SNP**	**RI**	**Probability**	Pmut prediction	**Pmut result**
R140W	**Disease**	8	0.925	**Neutral**	5	0.237	**Neutral**	4	0.288	Neutral	**0.08 (96 %)**
T148M	**Disease**	1	0.567	**Neutral**	6	0.224	**Disease**	0	0.504	Neutral	**0.13 (95 %)**
K234N	**Disease**	3	0.674	**Neutral**	1	0.461	**Disease**	1	0.544	Neutral	**0.22 (92 %)**
S193C	**Disease**	7	0.844	**Disease**	2	0.613	**Disease**	4	0.708	Disease	**0.85 (91 %)**
D147G	**Disease**	5	0.746	**Disease**	2	0.622	**Disease**	4	0.685	Disease	**0.53 (80 %)**
F284C	**Disease**	7	0.839	**Neutral**	2	0.409	**Disease**	1	0.557	Neutral	**0.16 (93 %)**
D179G	**Disease**	2	0.579	**Neutral**	2	0.375	**Disease**	0	0.51	Neutral	**0.23 (91 %)**
T144N	**Disease**	1	0.534	**Neutral**	2	0.38	**Disease**	2	0.586	Neutral	**0.31 (89 %)**
P98L	**Disease**	8	0.879	**Disease**	2	0.579	**Disease**	2	0.58	Disease	**0.63 (84 %)**

Table3. Analysis of ADPRC 1 using I-Mutant Suite

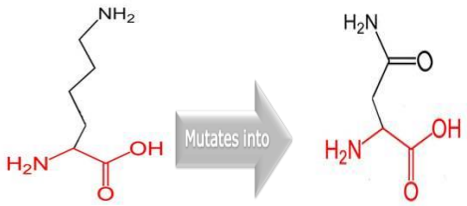
Residue	**Imutant Prediction Effect**	**Value**	Mupro Prediction Effect (Stability)	Value
R140W	Decrease	0.13	Decrease	-0.743
T148M	Increase	-0.07	Decrease	-0.471
K234N	Decrease	-0.23	Decrease	-0.666
S193C	Decrease	-0.60	Decrease	-0.168

D147G	Decrease	-0.92	Decrease	-1.514
F284C	Decrease	-1.90	Decrease	-1.285
D179G	Decrease	-1.39	Decrease	-1.131
T144N	Increase	-0.52	Decrease	-0.770
P98L	Decrease	-0.30	Increase	0.217

Table 4. the physiochemical proprieties induced by the SNPs in CD38 gene as predicted by Hope software.

SNPs	Secondary structure	Hope report summary
R140W		rs1800561(R140W) this mutation resulted in a change of Arginine amino acid R changed to Tryptophan amino acid W at position 140. The wild-type residue R is not conserved at this position. The mutation is associated with development of type II diabetes. The mutant amino acid has larger size, different charge and hydrophobicity in comparison to the wild type, this will have negative effect on external interactions.
T148M:		rs138866692(T148M) this mutation resulted in a change of Threonine amino acid T changed to Methionine M at position 148.Threoinie is very conserved at this position, The wild-type residue is involved in forming a cysteine bridge, furthermore it forms a hydrogen bond with Leucine at position 145. These bonds will be disrupted by this mutation. Methionine is also bigger in size than the wild-type residue. Along with that, it has different hydrophobicity, which affect the level of external interactions.

K234N:



rs149299047 (K234N) this mutation resulted in a change of Lysine amino acid K changes to Asparagine N at position 234. Lysine is very conserved at this position. The wild-type residue forms a hydrogen bond and salt bridge with Glutamic Acid at position 198, which will be negatively affected by this mutation.The difference in charge and the small size of Asparagine will lead to loss of external interactions.

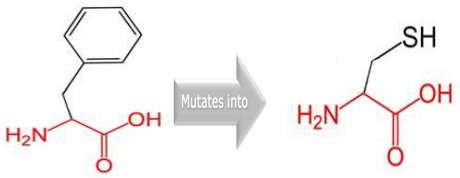
S193C

rs150171847 (S193C) this mutation resulted in a change of Serine amino acid S changed into a Cysteine C at position 193. Serine residue is very conserved at this position. Furthermore, this mutation will cause loss of hydrogen bonds in the core of the protein leading to distortions in the correct folding.

D147G

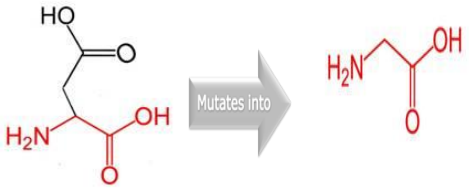
rs199998792 (D147G) this mutation resulted in a change of Aspartic Acid D changed into a Glycine G at position 147. Aspartic Acid is very conserved at this position, it is involved in forming a cysteine bridge. Furthermore, it forms a hydrogen bond with Threonine at position 136. Glycine is smaller and has different charge and hydrophobicity, which will damage the external interaction.

F284C



rs201683843 (F284C) this mutation resulted in a change of Phenylalanine F changed into a Cysteine C at position 284. Phenylalanine occurs often at this position. Phenylalanine and Cysteine differ in size. Cysteine is smaller, which will cause a possible loss of external interactions.

D179G



rs201816957 (D179G) this mutation resulted in a change of Aspartic Acid D changed into a Glycine G at position 179. Aspartic Acid occurs often at this position.it can forms a hydrogen bond with Arginine at position 47.It is also involved in forming a cysteine bridge; the mutation will cause disruption of these bonds. This mutation will lead to the loss of hydrophobicity and charges of the wild amino acid. Furthermore, the small size of Glycine will produce an empty space in the core of the protein.

T144N

rs369214160(T144N) this mutation resulted in a change of Threonine amino acid T changes into a Asparagine n at position 144. Threonine is 100% conserved at this position, therefore the mutation is probably damaging for the protein.

The wild-type residue involves in forming cysteine bridge and forming a hydrogen bond with Aspartic Acid at position 147. Asparagine is larger in size, furthermore, this mutation will cause loss of hydrophobicity interaction.

Analysis of Conservation profile ConSurf analysis predicted P98L, T144N and D147G to be exposed highly conserved and exposed, i.e., a functional residue, which suggests that these positions are important for the ADPRC 1 function. ConSurf analysis also predicted T148M and S193C to be buried and conserved residue, i.e., a structural residue (Fig.1 , Table5).

Table 5: Conservation profile of amino acids in ADPRC 1

Residue	Consurf	Class assignment	FUNCTION
R140	Variable	Exposed	-
T148	Highly Conserved	Buried	Structural
K234	Conserved	Exposed	-
S193	Highly Conserved	Buried	Structural
D147	Highly Conserved	Exposed	Functional
F284	Variable	Buried	-
D179	Conserved	Exposed	-
T144	Highly Conserved	Exposed	Functional
P98	Highly Conserved	Exposed	Functional

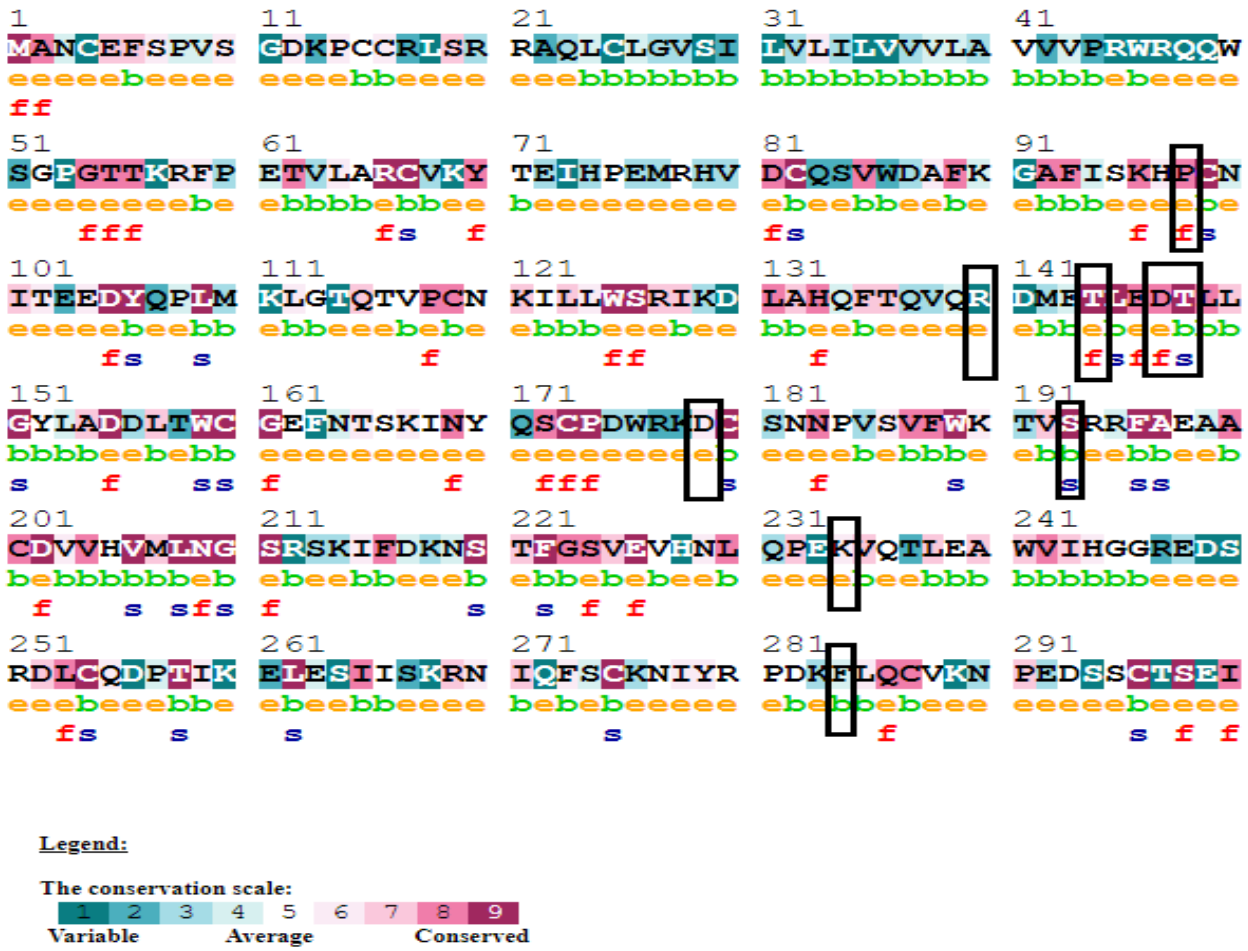


Fig1.. Analysis of evolutionary conserved amino acid residues of ADPRC 1 by ConSurf. The color coding bar shows the coloring scheme representation of conservation score

Protein-protein interactions analysis

From STRING protein-protein interaction analysis,CD38 was predicted with the strong interactions with BST1, ENPP1,ENPP3,NADK,NAMPT,NMNAT1,NMNAT2,NMNAT3,NNMT and PNP.(Fig 2) The STRING interaction result was further validated by using KEGG pathways for CALR. Interestingly, same set of proteins were involved in metabolic pathways , including Nicotinate and nicotinamide metabolism , Riboflavin metabolism , Pyrimidine metabolism , Pantothenate and CoA biosynthesis , Purine metabolism , Starch and sucrose metabolism, Calcium signaling pathway, Oxytocin signaling pathway in addition to Salivary secretion and Pancreatic secretion. Our literature search demonstrated that Pancreatic secretion pathway (hsa04972) is involved in pathways of Type 2 diabetes mellitus. (Fig 3)

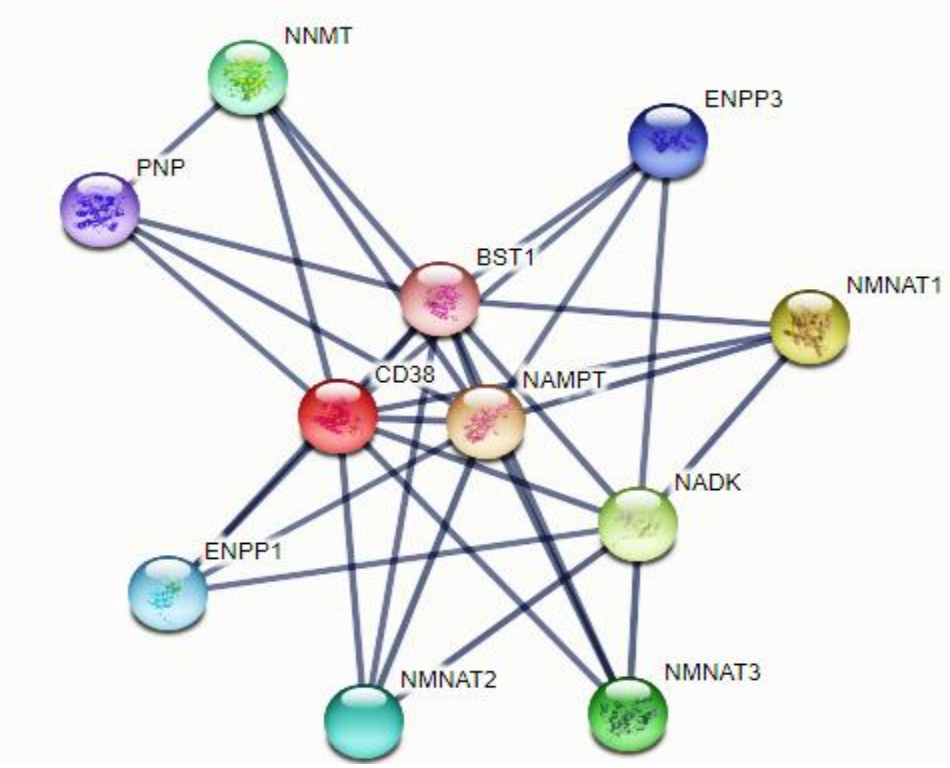


Figure 2 : Interaction between CD38 and its related protein

Predicted Functional Partners:

		Score
NAMPT	*Nicotinamide phosphoribosyltransferase; Catalyzes the condensation of nicotinamide with 5- phosphoribosyl-1-pyrophosphate to yield nicotinamide mo...*	0.941
NMNAT1	*Nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1; Catalyzes the formation of NAD(+) from nicotinamide mononucleotide (NMN) and A...*	0.933
NADK	*NAD kinase; Belongs to the NAD kinase family (591 aa)*	0.932
NMNAT3	*Nicotinamide/nicotinic acid mononucleotide adenylyltransferase 3; Catalyzes the formation of NAD(+) from nicotinamide mononucleotide (NMN) and A...*	0.926
NNMT	*Nicotinamide N-methyltransferase; Catalyzes the N-methylation of nicotinamide and other pyridines to form pyridinium ions. This activity is important fo...*	0.923
NMNAT2	*Nicotinamide/nicotinic acid mononucleotide adenylyltransferase 2; Catalyzes the formation of NAD(+) from nicotinamide mononucleotide (NMN) and A...*	0.922
ENPP1	*Ectonucleotide pyrophosphatase/phosphodiesterase family member 1; By generating PPi, plays a role in regulating pyrophosphate levels, and functions ...*	0.918
ENPP3	*Ectonucleotide pyrophosphatase/phosphodiesterase family member 3; Cleaves a variety of phosphodiester and phosphosulfate bonds including deoxyn...*	0.918
PNP	*Purine nucleoside phosphorylase; The purine nucleoside phosphorylases catalyze the phosphorolytic breakdown of the N-glycosidic bond in the beta- (d...*	0.914
BST1	*ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 2; Synthesizes the second messagers cyclic ADP-ribose and nicotinate-adenine dinucleotide phosphat...*	0.913

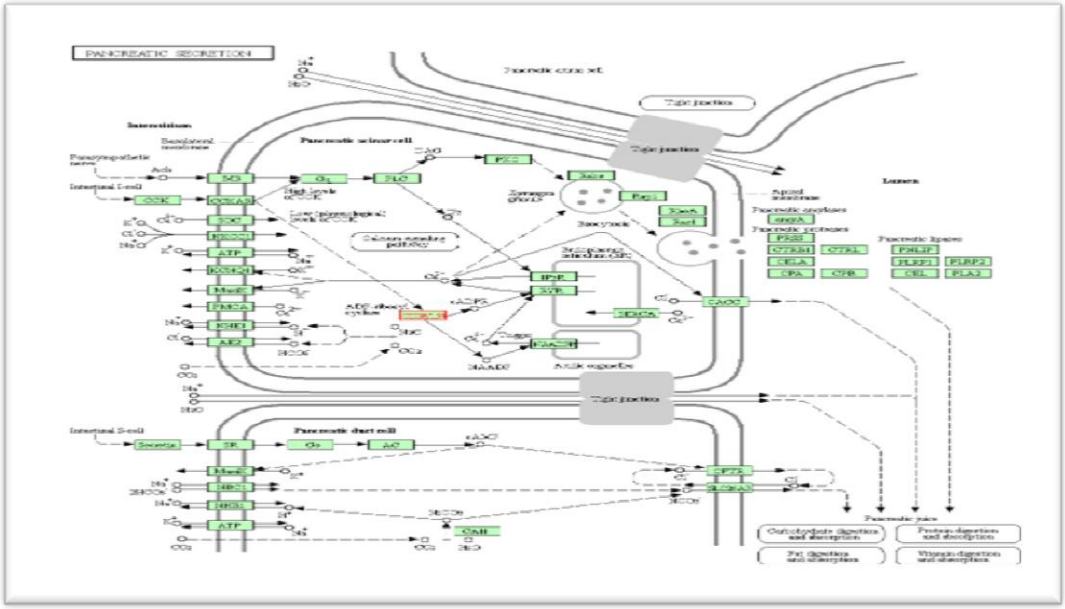


Figure3: Pancreatic secretion pathway in Type 2 diabetes mellitus

Analysis of the effect of nsSNPs in ADPRC by MutPred server:

The effect on the polymorphism in CD38 gene on ADPRC 1 protein was predicted using mutpred software which shows the following six SNPs (T148M, K234M, S193C, D147G, F284C, D179G,T144N, P998L) to be harmful in term of disturbing the molecular mechanisms, while the SNP R140W was predicted to be non-harmful with score of .275.(Table 6).

Table 6: Analysis of the effect of nsSNPs in ADPRC 1 structure, function, and evolution by MutPred server.

Amino acid Change	**Score (g)**	**Prediction**	**Top 5 Molecular Molecular Mechanism Disrupted (P)**
R140W	0.275	Non harmful	Loss of solvent accessibility (P = 0.086) Loss of helix (P = 0.1706) Gain of sheet (P = 0.1945) Loss of catalytic residue at R140 (P = 0.2226) Loss of disorder (P = 0.2286)
T148M	0.778	Highly harmful	Gain of catalytic residue at T148 (P = 0.0143) Loss of sheet (P = 0.1158) Gain of helix (P = 0.132) Loss of phosphorylation at T144 (P = 0.614) Loss of disorder (P = 0.6792)

K234N	0.701	Harmful	Loss of ubiquitination at K234 (P = 0.0116) Loss of methylation at K234 (P = 0.0138) Gain of loop (P = 0.0851) Loss of disorder (P = 0.2145) Loss of helix (P = 0.2662)
S193C	0.900	Highly harmful	Loss of MoRF binding (P = 0.0729) Gain of methylation at K190 (P = 0.078) Loss of ubiquitination at K190 (P = 0.1498) Loss of phosphorylation at S193 (P = 0.219) Loss of catalytic residue at S193 (P = 0.2472)
D147G	0.863	Highly harmful	Loss of stability (P = 0.1122) Gain of catalytic residue at D147 (P = 0.1166) Gain of sheet (P = 0.1945) Loss of helix (P = 0.3949) Loss of disorder (P = 0.6107)
F284C	0.854	Highly harmful	Gain of catalytic residue at L285 (P = 0.0249) Gain of disorder (P = 0.0444) Gain of ubiquitination at K289 (P = 0.0594) Loss of MoRF binding (P = 0.1028) Gain of sheet (P = 0.1208)
D179G	0.687	Harmful	Gain of MoRF binding (P = 0.065) Gain of methylation at K178 (P = 0.0896) Gain of relative solvent accessibility (P = 0.09) Loss of disorder (P = 0.1701) Loss of helix (P = 0.1706)
T144N	0.785	Highly harmful	Gain of relative solvent accessibility (P = 0.09) Loss of sheet (P = 0.1158) Gain of solvent accessibility (P = 0.1422) Loss of catalytic residue at E146 (P = 0.2117) Loss of loop (P = 0.2237)
P98L	0.895	Highly harmful	Gain of relative solvent accessibility (P = 0.0479) Gain of solvent accessibility (P = 0.0704) Loss of disorder (P = 0.0862) Loss of methylation at K96 (P = 0.0868) Gain of ubiquitination at K96 (P = 0.102)

Discussion:

CD38 is known to be a direct contributor in pathogenesis of CLL but still the exact mechanism is poorly understood. CD38 expression was observed to increase in CLL. More ever CD38 consider as a dual function molecule, it can plays that role by acting as a receptor/adhesion molecule as well as an ecto-enzyme. Depending on the cell type as well as CD38 maturation state, the downstream signaling and biological consequences of CD38 activation will take place[40-42]. This can be attributed to the fact that CD38 often associates and acts in synergy with other cell type specific surface receptors. In CLL cells, CD38 collaborates with the BCR/CD19 complex for signal transduction .[43-44]

The present study revealed that CD38 gene polymorphisms (CD38Missense SNPs) may result in, harmful alteration on entire protein structure, stability and physiochemical properties. Subsequently, this change will initiate modification on multiple functions of molecule, in addition to interaction between CD38 and other molecules in wide variety pathways [45]**.** One Study, was performed by Fadl, et al [46] in Sudan and concluded that Missense CD38 SNP (rs1800561) (418C>T, Arg140Trp) has higher genotypic frequency than wild type allele (CC), among CLL Sudanese patients. On another hand , a significant correlation between CD38 SNP and age of patients was found in her work, with a higher frequent among patients whose their ages (≤60) years , Concerning gender of patients , they found that {SNP were higher in males than in females} even though, they were stated The following: CD38 gene polymorphism caused significant increase in some hematological parameters {TWBCS count(C/L), Absolute Lymphocytes and PLTS count(C/L) } but no significant correlation between the SNP and Hb conc(g/dl).TWBCS count(C/L) and Absolute Lymphocytes count(C/L) were strongly correlated with CD38 gene SNP, although it seems to be weakly correlated with PLTS count(C/L). Further study done by Jamroziak et al in Poland [47]. They described that frequency of wild type allele (CC) allele was higher than CD38 gene polymorphism (CT) with (P-value=0.97) in CLL patients, against low (rs1800561 CT allele) frequency, they did not detect any individuals homozygous for this allele, which is consistent with Fadl's study. And it did not associate with age of patients, which is disagreed with Fadl's findings .variation in sample size and study population might explain the gap of results between these studies.Other study in Poland, that was published by Szemraj-Rogucka et al [48]**,**stated that, The patients who were diagnosed with MM ,Their median age never been differ significantly from wild-type(rs1800561) CC homozygotes(61 years) and others who have heterozygous (rs1800561) CT genotype (68 years)with P-value= 0.23.whereas ,they were reported that no Significant association between CD38 genotype and age nor gender of MM patients. According to P-value = 0.74 for rs1800561 CT analysis. Additional study that investigated CD38 polymorphism as risk of diseases is a study which conducted in Spain by González-Escribano et al [49]**.**Their results did not support the hypothesis that the CD38 gene polymorphism is related to the risk of SLE, that suggests no individual who have (CT genotype) were detected among patients with SLE, nor the healthy individuals. These results, support the absence (or the extremely low frequency) of this polymorphism in Spanish population. Former study which carried out by Yagui et al [50] in Japan. Noticed that, there is an significant association between CD38 gene polymorphism and type II Diabetes mellitus. By way of initiating Considerable declining in both ADP-ribosyl cyclase and cADPR hydrolase activities by nearby 50%, that could contribute to the diminishing of (in vivo) insulin secretion in patients with Type II diabetes mellitus. It had been suggested that (CT) allele frequency were significantly different between the type II diabetes mellitus patients, as (P-value= 0.004) .CD38 gene SNP may contribute to the development of type II diabetes mellitus in collaboration with other genetic defects in beta cell function and insulin action, this finding along support our Hypothesis, that CD38 gene polymorphisms could contributed to some diseases despite the fact that González-Escribano's study was disagreed with it.(Figure3).

Twelve SNPs were found in this work to be functional deleterious by three soft wares ,further analysis by the remnant software reveals nine of them were deleterious. Of these SNPs eleven, were novel(not reported before in the literature and one was reported in many studies as predisposing factor to some diseases. Likewise, stability analysis reported that Seven SNPs were decreased stability while two were increased stability. Even though, eight SNPs were decreased against one was increased by other software. Furthermore (rs 180056 1) SNP was reported before in the work of Fadl et al. where they found it higher in frequency than wild type allele that is suggested as risk factor for CLL. Project hope was used to

predict the effect of these SNPs on the physiochemical prorates of the protein where it were found to effect the size, charge and hydrophobic state of the protein s=confirming the deleterious effect of these SNPs. Moreover the effect on molecular mechanism was predicted using MutPred server. Software which shows that all the SNPs were harmful on the molecular level except R140W which was un anticipated result as this SNPs was reported in CLL patients , which raise the need for more focus on this SNP on further research.Our findings are agree with Fadl's while disagree with Jamroziak's findings. Also, Consistent with Yagui's and Inconsistent with Szemraj-Rogucka, González-Escribano regarding other diseases.It was reported in the literature that CD38 gene has complex relation with other genes. This relation was studied in this work by using STRING software, where it shows a complex interactions with other genes like PNP, BST1,NNMT, and many others.

Conclusion: The bioinformatics analysis has got highest concern to screen diseases associated nsSNP at molecular level. In this study a comprehensive bioinformatics analysis has been performed to investigate the effect of nsSNPs on structure and function of CD38. As CD38 involved in many pathways, hence any changes on it structure and function would have an impact on many pathways, involved in wide verity diseases. Nine nsSNPs were predicted to be the most damaging mutations for CD38 leading to loss or disturbance of the protein internal and external interactions and eventually loss of the protein's function as well as disease association. This is computational approach, thus wet-lab based studies with clinical evidences are necessary to accompaniment the findings of this study.

Recommendations: Additional wet-lab based researches are recommended to study outcomes that may occur as consequence of CD38 SNPs, which might amend other functions of CD38 molecule. As it was involved in many pathways that could end in wide verity diseases. Have a preference to use more soft wares, and to state advance study on a specific population, in purpose of taking genetic background of patients in consideration. As well as focusing on CD38 as flow marker and its impression on prognosis. Also, the effect of the CD38 genotype on the hematological findings has to be studied further, for the reason that those patients could be at risk of other haematopathological complications.

Declaration of interests:

The authors declare to have no conflict of interest regarding the making of this paper.

Funding source:

There is no funding source for this study.

Ethical Consideration:

This study is a bioinformatic approach and doesnt required ethical approval.

Author's Contribution:

This Study was fulfilled in collaboration among all authors. Author **H.A.O.F** designed the study, contributed in bioinformatic analysis, prepared and wrote the manuscript. Author **A.H.A.H** participated in the bioinformatic analysis, preparation and revision of manuscript. Author **S.G.E** established the protocol of the Study, accomplished the bioinformatic analysis and final revision of the study. All authors critically reviewed the manuscript and approved the final draft

Acknowledgment:

We would like to thank our families,frindes and teachers who always provid us a highly support.

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