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COMPUTATIONAL ANALYSIS OF FUNCTIONAL SINGLE NUCLEOTIDE POLYMORPHISM OF HUMAN EUKARYOTICS TRANSLATION INITIATION FACTOR2 B1 *(EIF2B1)* GENE

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ABESTRACT

Introduction: Leukoencephalopathy with vanishing white matter (VWM) is a progressive disorder that mainly affects the brain and spinal cord. This disorder causes deterioration of the central nervous system's white matter, which consists of nerve fibers covered by myelin sheath; it is highly complex and not fully understood yet. This study aimed to perform a computational analysis of the nsSNPs in the *EIF2B1* gene, to identify the possible mutations and propose a modeled structure for the mutant protein that potentially affects its function. **Methods:** The nsSNPs were analyzed using 8 prediction software tools: SIFT, Polyphen-2, Provean, PhD-SNP, SNP&GO, I-Mutant 3.0, Mupro and Project Hope respectively. **Results:** Fifteen nsSNPs were found to be deleterious and damaging by SIFT, and 14 nsSNPs by PolyPhen-2, 12 nsSNPs by Provean, 5 nsSNPs were observed to be highly deleterious and damaging as in the 8 software. **Conclusion:** Five highly deleterious, damaging and disease related nsSNPs (rs113994007, rs150217005, rs201516905, rs375037006, and rs377538766) were detected at *EIF2B1* gene. These nsSNPs can be considered as candidate nsSNPs in people with VWM after further conformation using laboratory techniques.

KEYWORDS: Leukoencephalopathy (VWM), nsSNPs, *EIF2B1*, SIFT, Polyphen-2 and Project Hope.

INTRODUCTION

Leukoencephalopathy with vanishing white matter (VWM, OMIM306896) /childhood ataxia with central hypomyelination (CACH) are an autosomal recessive neurological disorder showing white matter rarefaction and cystic degeneration.[1,2,3,4,5] VWM is the first known hereditary human disease caused by direct defects in the initiation of the protein translation process, it is highly complex and is not fully understood yet.[6,7,8,9]

A single nucleotide polymorphism (SNP) is a variation in a single base DNA that occur normally throughout a person's genome, non-synonymous single nucleotide polymorphisms (nsSNPs) lead to an amino acid change in the protein product[10,11,12,13], fall into two classes :disease-associated (deleterious) and benign (no observable phenotypic effect).[11]

A previous study showed that mutations in the genes encoding the beta-subunit of the eukaryotic translation initiation factor eIF2B which is pivotal in translation of mRNAs into proteins can cause VWM[14,15,16], eIF2B is a ubiquitously expressed protein complex consisting of five subunits(eIF2Bα, β, γ, δ and ε)[17,18], that is regulating the rate of protein synthesis under different stress conditions such as fever.[19,20,21,22,23] eIF2B is a guanine nucleotide-exchange factor, that joint to its factor eIF2, which has a key role in mRNA translation initiation through its guanine nucleotide exchange (GEF) activity from guanine diphosphate (GDP) to guanine triphosphate (GTP), The eIF2•GTP product is not stable unless tRNAiMet joins to form the ternary complex.[24,25,3,26]

VWM syndrome is characterized by progressive cerebellar ataxia, spasticity, optic atrophy with loss of vision and epilepsy may occur. Febrile infections and minor head trauma leads to motor deterioration and may provoke rapid neurological deterioration following normal development.[27,28,29] Magnetic resonance imaging (MRI) shows a diffuse abnormality of the cerebral white matter increased with time, and replaced by cerebrospinal fluid, VWM patients died after a variable period of years, usually following an episode of fever and coma.[30,31,32,33,34]

In the present study we aimed to perform a computational analysis of the nsSNPs that have been

reported in *EIF2B1* gene, to identify the possible mutations and propose a modeled structure for the mutated protein that potentially affects the function.

MATERIAL AND METHODS

The critical step in this work was to select SNPs in the coding region, that are nsSNPs and analyzing them using 8 prediction software tools: (SIFT, Polyphen-2, Provean, SNP&GO, PhD-SNP, I-Mutant 3.0, Mupro, and Project Hope) to predict the mutation of *EIF2B1* gene that associated with VWM. nsSNPs information were obtained from the SNPs database (dbSNPs) (http://www.ncbi.nlm.nih.gov/snp). Gene's functions and other genes that related to *EIF2B1* gene were obtained from GeneMANIA (http://www.genemania.org/).

GeneMANIA (http://www.genemania.org/)
It is a standalone tool for making fast and efficient gene function predictions and finds other genes that are related to an input gene, by using a very large association data including 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, missed genes in the screen or find new genes with a specific function.[35]

Sorting Intolerant from Tolerant (SIFT) (http://siftdna.org/www/SIFT_dbSNP.html)
It is an algorithm that predicts the potential impact of amino acid substitutions on protein function and phenotype alteration, SIFT has become one of the standard tools for characterizing missense variation. The main principle of this program is to generate alignments with a large number of homologous sequences, and assigns scores to each residue ranging from zero to one. The threshold intolerance score for nsSNPs is 0.05 or less.[36]

Polymorphism Phenotyping v2 (PolyPhen-2) http://genetics.bwh.harvard.edu/pph2/
This server has been used to predict the amino acid change on the structure and function of a protein. This prediction is based on phylogenetic and structural information characterizing the substitution, this program calculates position-specific independent count scores (PSIC) for each of the two variants, and then computes. The higher the PSIC score difference is the higher is the functional impact a particular amino acid substitution is likely to have. PolyPhen-2 scores were assigned benign (0.00–0.90) potentially damaging (1.0–1.50) possibly damaging (1.40–1.90), probably damaging (2.00 or more).[37]

Protein Variation Effect Analyzer (Provean) (http://provean.jcvi.org/about.php)
Prediction software of protein sequence variations involved in human diseases, including substitutions, insertions, deletions, frameshifts, and non-sense mutations. The program alignment-based score measures query sequence and a protein sequence homolog before and after the introduction of an amino acid variation to the query sequence. PROVEAN results showed that the scoring scheme performs well in separating disease-associated variants from common polymorphisms for human protein variations.[38]

I-Mutant version 3.0 (http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi)
Is a support vector machine (SVM)-based tool for the prediction direction of protein stability free-energy change (ΔΔG or DDG) on a specific nsSNP. I-Mutant 3.0 can be used as a classifier for predicting the protein stability change upon single point mutations. it is evaluate the stability change starting from the protein structure and/or sequence. The nsSNPs are then classified as disease-related or neutral polymorphisms.[39]

Mupro (http: //mupro.proteomics.ics. uci.edu) and server is available at http:// www.igb.uci.edu/servers/servers.html
This is a set of machine-learning programs use support vector machine and neural networks, It predict whether a single-site amino acid mutation increases or decreases the protein stability. The input provided to software is amino acid sequences followed by mutation positions, native and substituted amino acid.[40]

Single nucleotide polymorphism database and gene ontology (SNPs&GO) (http: //snps. biofold.org/phd-snp/phd-snp.html)
Support vector machine (SVM) based server which is freely available predictive software. It exploits protein functional annotation, which classifies single nucleotide variation as disease-related (desired output set to 0) and neutral polymorphism (desired output set to 1). Input query for SNPs&GO is an UNIPROT Accession Number, Mutation Position and wild-type as well as mutant-type residue. Its collects in unique information derived from protein sequence, function and evolutionary information that encoded in the Gene Ontology terms, and outperforms other available predictive methods.[41]

Predictor of Human Deleterious Single Nucleotide Polymorphisms (PhD-SNP)
(http://snps. biofold.org/phd-snp/phd-snp.html) Is a SVM based tools that uses to predict the new phenotype derived from a nsSNP is disease-related (disease) or neutral polymorphism based on a supervised training algorithm. Input query for PhD-SNP is Protein sequence from Uniprot, after providing position and the new amino acid residue.[42]

Project Hope (http: //www. cmbi.ru.nl/hope/home)
Hope is automatic program that analyzes the structural and functional effects of point mutations. It is provide the 3D structural visualization of mutated proteins. Project HOPE Input method is carries the protein sequence and selection of Mutant variants. It is output in the form of structural variation between mutant and wild type residues, it gives the results by using UniProt and

DAS servers. Homology models are built with yet another Scientific Artificial Reality Application (YASARA). Hope builds a report with text, figures, and animations that is easy to use for (bio) medical.[43]

RaptorX (http://raptorx.uchicago.edu/)
Is a protein structure prediction server developed by Xu group its use to predicting 3D structures for protein sequences without close homologs in the Protein Data Bank (PDB). RaptorX delivers high-quality structural models for many targets takes 35 min to processing a sequence of 200 amino acids.[44] EIF2B1 *Protein* sequences of the most deleterious nsSNPs were presented to RaptorX server to get the model sequence as PDB file that had been visualize by chimera program.

Chimera (http://www.cgl.ucsf.edu/chimera)
Software produced by University of California, San Francisco (UCSF) is a high-quality extensible program for interactive visualization and analysis of molecular structures and related data, including density maps, supramolecular assemblies, sequence alignments, docking results, and conformational ensembles.[45] Chimera (version 1.6.2) was used to generate the mutated 3D model models of each EIF2B1 protein.

RESULTS
The aim of this study was to investigate the effect nsSNPs in the *EIF2B1* gene on the protein function and structure. The nsSNPs were download from NCBI database (http://www.ncbi.nlm.nih.gov/projects/SNP); This downloaded nsSNPs were submitted to SIFT software and the results showed a total of 4030 SNPs at the time of the study, out of which 216 were *Homo sapiens*, 97 occurred in coding region (68 synonymous SNPs, 20 nsSNPs, 8 blank, 1 weak deleterious), 77 were in 3 U´TR, and 42 were in 5´ UTR.

To investigate the relation of *EIF2B1* gene with other genes, GeneMANIA results showed the interaction of the gene with 20 other genes. It is including functional and physical interaction, co-expression, co-location and pathway; it is also Share protein domains with 608,863 interactions from InterPro and with 457,054 interactions from Pfam (Figure 1).

The 20 nsSNPs were analyzed by SIFT software, after remove duplication 15 nsSNPs were predicted to be deleterious Table (1), These deleterious SNPs were analyzed using PolyPhen-2 and Provean software to predict the damaging SNPs Table (1). 14 nsSNPs were predicted as damaging in Poyphen-2, 12 nsSNPs damaging in Provean software.

The association of nsSNPs to disease using SNP&GO and PHD-SNP software revealed that, (5 in SNPs&Go,6 in PhD-SNP) were predicted to be disease related, (6 in SNPs&Go,5 in PhD-SNP) were predicted to be Neutral, and 4 nsSNPs Give no result in both software Table (2).

Prediction of the effect of the protein stability was done using I-Mutant3.0 and Mupro servers. I-Mutant3.0 results showed that the stability was decreased in 12 nsSNPs and increased in 3 nsSNP Table (2). Were all 15 nsSNP were decrease protein stability using Mupro software Table (2).

Five highly deleterious and damaging nsSNPs (rs113994007, rs150217005, rs201516905, rs375037006, rs377538766) predicted using previous software was submitted to project Hope software. It was describe the reaction, physiochemical properties of these Candidates, discus the conformational variations and interactions with the neighboring amino acids Table (3). The sequences of the eukaryotic translation initiation protein was obtained from ExPASy Database, illuminates these 5 highest nsSNPs. Chimera software was used to reveal the 3D structure for the truncated proteins with its new candidates Figure (2).

GeneMANIA RESULT

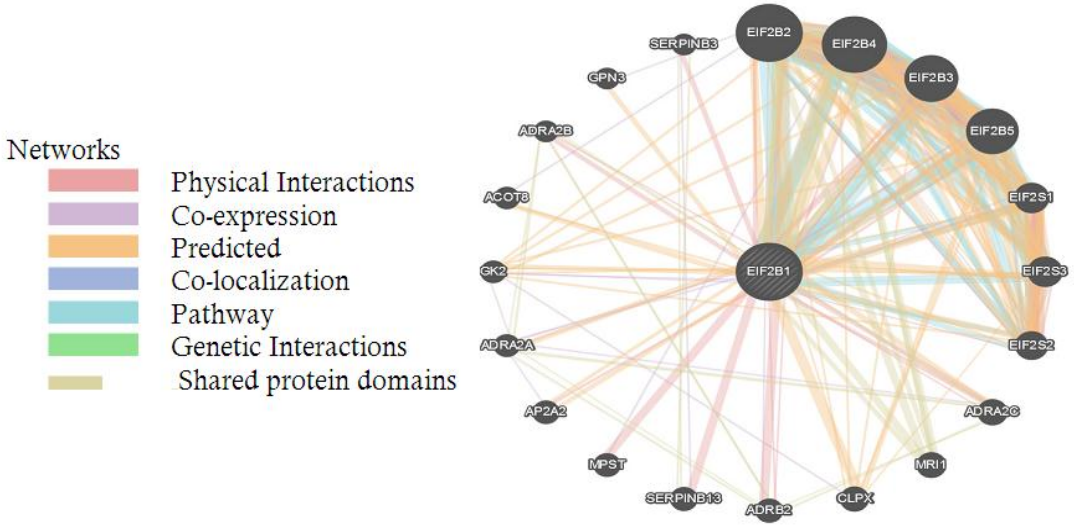


Table (1): List of nsSNPs with SIFT, POLYPHEN-2, PROVEAN results.

SNP	AMIN ACIDCHANGE	PROTEIN ID	SIFT SCORE	SIFT PREDICTION	Polyphen-2 prediction	PSIC	Provean prediction	PSIC
rs11572913	M1I	ENSP00000446429	0.03	DELETERIOUS	Benign	0	Neutral	0.197
rs113994007	N208Y	ENSP00000416250	0.001	DELETERIOUS	probably damaging	1	Deleterious	-7.644
rs139913280	V142M	ENSP00000416250	0.039	DELETERIOUS	possibly damaging	0.826	Neutral	-0.822
rs150217005	R43W	ENSP00000443172	0.003	DELETERIOUS	probably damaging	1	Deleterious	-6.236
rs150217005	R28W	ENSP00000444183	0.004	DELETERIOUS	probably damaging	0.99	Deleterious	-3.942
rs150217005	R15W	ENSP00000438060	0.004	DELETERIOUS	probably damaging	0.999	Deleterious	-4.183
rs184545759	D75H	ENSP00000443172	0.003	DELETERIOUS	possibly damaging	0.589	Neutral	-2.189
rs184545759	D60H	ENSP00000444183	0.005	DELETERIOUS	probably damaging	0.999	Deleterious	-3.737
rs184545759	D47H	ENSP00000438060	0.006	DELETERIOUS	probably damaging	1	Deleterious	-3.47
rs185814675	D18Y	ENSP00000443172	0.024	DELETERIOUS	possibly damaging	0.897	Deleterious	-2.806
rs201516905	F25S	ENSP00000443172	0	DELETERIOUS	possibly damaging	0.631	Deleterious	-2.842
rs201516905	F10S	ENSP00000444183	0.001	DELETERIOUS	probably damaging	1	Deleterious	-5.686
rs368027476	D191N	ENSP00000416250	0.04	DELETERIOUS	probably damaging	0.994	Deleterious	-4.094
rs375037006	G204R	ENSP00000416250	0	DELETERIOUS	probably damaging	1	Deleterious	-7.578
rs377538766	A219D	ENSP00000416250	0.001	DELETERIOUS	probably damaging	1	Deleterious	-5.162

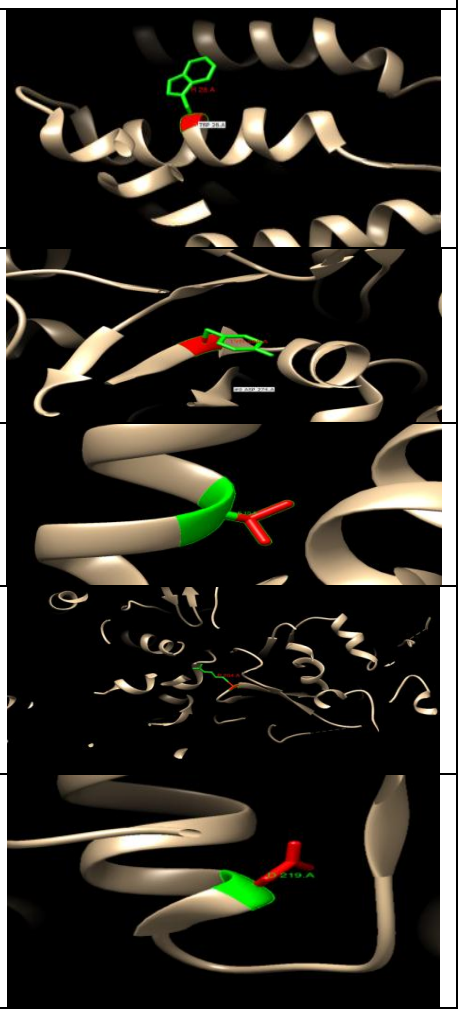
PolyPhen-2 result: POSSIBLY DAMAGING (less confident prediction) POROBABLY DAMAGING (more confident prediction) /, PSIC SD: Position-Specific Independent Counts software. Tolerance Index: Ranges from 0 to 1. The amino acid substitution is predicted damaging if the score is ≤ 0.05, and tolerated if the score is > 0.05.

Table (2): List of nsSNPs with SNP&GO, PhD SNP, I MUTANT 3.0, MUPRO results.

SNP	probability	Score	PhD SNP prediction	Probability	score	SNP&GO prediction	Mupro	I mutant DDG	RI	SVM
rs11572913	0.035	9	Neutral	0.024	10	Neutral	decrease stability	-0.51	5	Decrease
rs113994007	0.955	9	Disease	0.898	8	Disease	decrease stability	-1.56	8	Decrease
rs139913280	0.377	2	Neutral	0.155	7	Neutral	decrease stability	0.3	4	Increase
rs150217005	-	-	No result	-	-	No result	decrease stability	-0.31	1	Decrease
rs150217005	0.758	5	Disease	0.591	2	Disease	decrease stability	-0.32	7	Decrease
rs150217005	0.402	2	Neutral	0.264	5	Neutral	decrease stability	0.28	1	Increase
rs184545759	-	-	No result	-	-	No result	decrease stability	-2	9	Decrease
rs184545759	0.45	1	Neutral	0.161	7	Neutral	decrease stability	-0.46	7	Decrease
rs184545759	0.332	3	Neutral	0.088	8	Neutral	decrease stability	-0.98	8	Decrease
rs185814675	-	-	No result	-	-	No result	decrease stability	0.21	6	Increase
rs201516905	-	-	No result	-	-	No result	decrease stability	-0.75	2	Decrease
rs201516905	0.834	7	Disease	0.763	5	Disease	decrease stability	-1.8	9	Decrease
rs368027476	0.647	3	Disease	0.367	3	Neutral	decrease stability	-0.72	4	Decrease
rs375037006	0.961	9	Disease	0.9	8	Disease	decrease stability	-0.27	3	Decrease
rs377538766	0.96	9	Disease	0.831	7	Disease	decrease stability	-0.56	3	Decrease

RI: Reliability Index DDG: $\Delta\Delta G$ sign SVM: support vector machine, DDG < 0: decrease stability, DDG >0 increase stability.

Table (3): HOPE describes the Amino acid change, reaction and physiochemical properties of these 5 Candidates.

SNPs	Amino acid change	size	Charge	Hydrophobicity	Chimera 3D structure
rs150217005	Arginine into a Tryptophan at position 28	bigger	Changed from positive charge into NEUTRAL	More hydrophobic	
rs113994007	Asparagine into a Tyrosine at Position 208	bigger	Both amino acid are NEUTRAL	More hydrophobic	
rs201516905	Phenylalanine into a Serine at position 10	smaller	Both amino acid are NEUTRAL	More hydrophobic	
rs375037006	Glycine into a Arginine at position 204.	bigger	Changed from NEUTRAL to positive charge	More hydrophobic	
rs377538766	Alanine into a Aspartic Acid at position 219.	bigger	Changed from NEUTRAL to negative charge	More hydrophobic	

DISCUSSION

This study aimed to analyze the nsSNPs identified in the *EIF2B1* gene. We found a total of 97 SNPs, 5 of which were predicted to adversely affect the function of the resulting protein. The residue affected by rs201516905 mutation leads to translation of a Serine instead of a Phenylalanine at position 10, and the mutant residue is located near a highly conserved region. The mutation result in a non-functional protein and this leads to mutation introduces a more hydrophobic residue at this position. This can result in loss of hydrogen bonds and/or disturb correct folding. The rs113994007caused conversion of amino acid Asparagine with a bigger and more hydrophobic Tyrosine at position (208) leading to bumps, loss of hydrophobic interactions and may disturb correct folding. This nsSNP was predicted to be disease related by PhD-SNP software. The rs150217005 result in replacement of Arginine with a Tryptophan at position (28) leads to loss of hydrophobic interactions and disturbance the site of modification is bigger, more hydrophobic according to Project hope software. The rs377538766 result in substitution of Alanine to Aspartic Acid at position 219, that is change of secondary structure and disturbs correct folding and phosphorylation modification sit according to project hope software. The rs375037006 result in substitution of a Glycine into an Arginine at position 204 means that a nonpolar amino acid will be replaced with a polar one. The original wild-type residue and newly introduced mutant residue differ in their electrochemical properties. The mutation results in incorporating an amino acid with a different level of hydrophobicity, this will affect hydrogen bond formation, and the mutant residue is located near a highly-conserved region.

CONCLUSIONS

In conclusion, the results suggest that the application of computational tools like SIFT, Polyphen-2, Provean, I-Mutant 3.0, Mupro, SNP&GO, PhD-SNP, and Project Hope may provide an alternative approach for selecting target nsSNPs for diagnostic purposes. The results of this study showed five damaging nsSNPs rs113994007, rs150217005, rs201516905, rs375037006, rs377538766 in the *EIF2B1* gene that are responsible for causing VWM disease.

Computational biology tools are very powerful, especially when provided with good data and used by experts. However, bioinformatics tools have their limitations; its results are predictions, which require confirmation using various methods such as functional studies. Hence, this results may provide useful information needed to help researchers to do further study in leukoencephalopathy with vanishing white matter especially in our country.

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