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In Silico Analysis of Single Nucleotide Polymorphisms (SNPs) in Human *MPL* Gene

Mohamed Mubarak Babeker[1, 2], Afra Mohamed Suliman Albakry[1], Mohammed Nagm Eldin Elsamani[3], Gihan Mossalami[2], Hind Abdelaziz Elnasri[1], Mona Abdelrahman Mohamed Khaier[1, *]

[1]Department of Molecular Biology and Bioinformatics, University of Bahri, Khartoum, Sudan

[2]Department of Haematology, Omdurman Ahlia University, Khartoum, Sudan

[3]Department of Clinical Biochemistry, Nile College, Khartoum, Sudan

Email address:

munakhaier@gmail.com (M. A. M. Khaier)

[*]Corresponding author

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Abstract: Thrombopoietin was shown to be the major regulator of megakaryocytopoiesis and platelet formation. The protein encoded by the c-mpl gene, CD110, is a 635 amino acid transmembrane domain, with two extracellular cytokine receptor domains and two intracellular cytokine receptor box motifs. Mutations to this gene are associated with myelofibrosis and essential Thrombocythemia. In essential Thrombocythemia, these mutations lead to the production of Thrombopoietin receptors that are constitutively activated, or constantly turned on, which results in the overproduction of abnormal megakaryocytes. MPL gene was investigated in NCBI database (http://www.ncbi.nlm.nih.gov/) and computational software's analyzed SNPs. SNPs in the coding region (exonal SNPs) that are non-synonymous (nsSNP) were analyzed by (sift, polyphen, Imutant and PHD-snp) softwares, and then SNPs at un-traslated region at 5' ends (5UTR) were analyzed too by SNPs Function prediction software. In this study, Bioinformatics' analysis of MPL gene initiated by SIFTand Polyphen-2server issued to review 197 SNPs and among this SNPs 23 pathological polymorphisms. Among these 23, 20 pathological polymorphisms were found to be very damaging, with higher Polyphen-2score, of the Polyphen-2 server (=1) and SIFT tolerance index of 0.000-0.005. Protein structural analysis was done by modeling of amino acid substitutions using Project Hope. AlsoI-Mutant software used to check their stability and the effect of the native and mutant residues protein and structure for all these pathological polymorphisms. We hope our results will provide useful information that is needed to help researchers to do further studies.

Keywords: *In silico* Analysis, MPL gene, SNPs, *SIFT*, *PolyPhen*-2, *I-Mutant 3.0* and *Project* Hope

1. Introduction

MPL is a protein in human encoded by (myeloproliferative leukemia protein) or CD110, also known as a Thrombopoietin receptor. [1] The Oncogene was identified in 1990 from the murine myeloproliferative leukemia virus and has the capability of immortalizing bone marrow hematopoietic cells from different lineages. In 1992, the human homologue, named, c-mpl, was cloned. The protein encoded by the c-mpl gene, CD110, is a 635 amino acid transmembrane domain, with two extracellular cytokine receptor domains and two intracellular cytokine receptor box motifs. Sequence data revealed that c-mpl encoded a protein that was homologous with members of the hematopoietic receptor superfamily. Presences of anti-sense oligodeoxynucleotides of c-mpl inhibited megakaryocyte colony formation. TPO-R deficient mice were severely thrombocytopenic, emphasizing the important role of CD110 and Thrombopoietin in megakaryocyte and platelet formation. Upon binding of Thrombopoietin, CD110 is

dimerized and the JAK family of non-receptor tyrosine kinases, as well as the STAT family, the MAPK family, the adaptor protein ShC and the receptors themselves become tyrosine phosphorylated [1]. MPL is expressed on early hematopoietic progenitors, megakaryocytes and platelets. Homozygous or compound heterozygous deleterious mutations in the MPL gene lead to congenital megakaryocytic thrombocytopenia (CAMT) [2]. The absence of MPL expression on platelets from CAMT patients has been described in a couple of case studies [3, 4]. However, reduced expression of *MPL* on platelets has also been demonstrated in patients with other forms of congenital thrombocytopenia, and in patients with inherited or acquired forms of thrombocytosis [5-7]. Mutations in MPL have been classified as being either type I or type II. The type I mutations cause, a complete loss of receptor activity and the type II mutations allow for the maintenance of some receptor function. Patients bearing the type I mutations exhibit bone marrow failure earlier than those with the type II mutations [3-8-9]. Mutations in this gene have been shown to cause familial aplastic anemia. [10] Mutations to this gene are also associated with myelofibrosis and essential thrombocythemia. [11]. In essential thrombocythemia, mutations occur at position 505 or 515. In myelofibrosis, a mutation occurs at position 515 (W515 mutation). These mutations lead to the production of thrombopoietin receptors that are constitutively activated, or constantly turned on, which results in the overproduction of abnormal megakaryocytes [12].

In the present study, we aimed to determine the influence of various polymorphisms in *MPL* gene on its protein structure that may have an important role in disease susceptibility. The harmful SNPs for the *MPL* gene have not been predictable to date *in silico*.

2. Material and Method

2.1. Sequence Datasets and Polymorphism Identification

*MPL*gene was investigated in dbSNP/NCBI database NCBI (http://www.ncbi.nlm.nih.gov/snp).197rsSNPs in the coding region were detected. Predictions of deleterious rsSNPs was performed by SIFT and Polyphen-2 software's. Prediction of change in stability due to mutation was performed by I-Mutant 3.0 While prediction of disease-related (disease) or neutral polymorphism was performed by PHD-SNP software. The functional impact of the deleterious SNPS was analyzed by project hope. Project hope software was used to highlight the changes occurred because of the deleterious SNPs at the molecular level of the protein 3D structure.

2.2. Analysis of Functional Consequences of Coding nsSNPs by Sequence Homology Based Method

2.2.1. SIFT (Sorting Intolerant from Tolerant)

Single nucleotide polymorphism (SNP) studies and random mutagenesis projects identify amino acid substitutions in protein-coding regions. Each substitution has the potential to affect protein function. SIFT is a program that predicts by uses sequence homology whether an amino acid substitution affects protein function and hence, potentially alter phenotype. SIFT can distinguish between functionally neutral and deleterious amino acid changes in mutagenesis studies and on human polymorphisms by calculates the probability that an amino acid at a position is tolerated conditional on the most frequent amino acid being tolerated. The cutoff value in the SIFT program is a tolerance index of ≥ 0.05. If this normalized value is less than a cutoff, the substitution is predicted to be deleterious [13].

2.2.2. Simulation for Functional Change in Coding rsSNPs by Structure Homology Based Method (PolyPhen-2) (Polymorphism Phenotyping)

Is an automatic tool for prediction of the possible impact of an amino acid substitution on the structure and function of a human protein. The impact of amino acid allelic variants on protein structure and function can be predicted via analysis of multiple sequence alignments and protein 3D Structures. It Calculates Position-Specific Independent Counts (PSIC) scores for each of the two variants and then computes the PSIC score difference between them. The higher a PSIC score difference the higher functional impact a particular amino acid substitution is likely to have. PolyPhen-2 scores were designated as "probably damaging" (0.95–1), "possibly damaging" (0.7–0.95), and "benign" (0.00–0.31). "Error" (Not enough data to make a prediction [14].

2.3. Calculation of Stability of Predicted Mutations by Free Energy

I-Mutant 3.0 suite: Is a suit of Support Vector Machine (SVM) based predictors integrated in an unique web server. It offer the opportunity to predict automatically protein stability change upon single-site mutation starting from protein sequence alone from protein structure when available that calculates protein stability related to a single mutation in units of free energy and also predicts the deleterious SNPs from the human protein sequence [15].

2.4. (Predictor of Human Deleterious Single Nucleotide Polymorphisms)

This serveries available at (http://snps.biofold.org/phd-snp/phd-snp.html)

PHD_SNP is SVM-based classifier that is optimized to predict if a given single point protein mutation can be classified as disease-related or as neutral polymorphism. The input FASTA sequence of protein along with the residues change was submitted to PhD-SNP server for the analysis [16].

2.5. Modeling rsSNP Locations on Protein Structure

Modeling by *Project hope* CMBI (Centre for Molecular and BiomolecularInformatics) is a website where the user can upload the sequence and mutation of interest, HOPE collects

structural information from a series of sources, including calculations on the 3D protein structure, sequence annotations in UniProt (The mission of the Universal Protein Resource) (Consortium, 2014) and prediction from the Report of software. HOPE combines this information to give analyses the effect of a certain mutation on the protein structure. HOPE is an online web service where the user can submit a sequence and mutation (Venselaar and CMBI, 2012). or Chimera software (version 1.8) which is a highly extensible program for interactive visualization and analysis of molecular structures and related data [17].

2.6. Prediction for Gene Prioritization and Predicting Gene Function by GeneMANIA

GeneMANIA finds other genes that are related to a set of input genes, using a very large set of functional association data which include protein and genetic interactions, pathways, co-expression, co-localization and protein domain similarity. It can use GeneMANIA to find new members of a pathway or complex, find additional genes may have missed in screen or find new genes with a specific function, such as protein kinases [18].

3. Results

The SIFT software indicated 23 nsSNPs out of 197 as deleterious i.e. amino acid substitution affects protein function and hence, potentially alter phenotype. The results were shown in Table 1.

Table 1. *Results of SIFT score and prediction for MPL gene.*

SNP	Amino Acid Change	Protein ID	SIFT Score	SIFT Prediction
rs6088	E168K	ENSP00000361548	0.012	DELETERIOUS
rs28928907	R102P	ENSP00000361548	0	DELETERIOUS
rs28928908	P275T	ENSP00000361548	0.047	DELETERIOUS
rs113696793	D295G	ENSP00000361548	0.018	DELETERIOUS
rs117656396	L265F	ENSP00000361548	0.013	DELETERIOUS
rs121913611	R257C	ENSP00000361548	0	DELETERIOUS
rs137952228	T183M	ENSP00000361548	0.014	DELETERIOUS
rs139770726	E335K	ENSP00000361548	0	DELETERIOUS
rs141311765	Y252H	ENSP00000361548	0	DELETERIOUS
rs145714475	F245S	ENSP00000361548	0	DELETERIOUS
rs147608148	S402P	ENSP00000361548	0.024	DELETERIOUS
rs148276667	R75H	ENSP00000361548	0.018	DELETERIOUS
rs149810307	V368L	ENSP00000361548	0.038	DELETERIOUS
rs200454070	R426Q	ENSP00000361548	0	DELETERIOUS
rs201101813	D128Y	ENSP00000361548	0.002	DELETERIOUS
rs202137992	P173A	ENSP00000361548	0.031	DELETERIOUS
rs202206935	L109F	ENSP00000361548	0.014	DELETERIOUS
rs267598614	E99K	ENSP00000361548	0.032	DELETERIOUS
rs267598615	E149K	ENSP00000361548	0.012	DELETERIOUS
rs369486165	E332K	ENSP00000361548	0.011	DELETERIOUS
rs371232858	A418T	ENSP00000361548	0.026	DELETERIOUS
rs372164360	F315I	ENSP00000361548	0	DELETERIOUS
rs373621350	R390C	ENSP00000361548	0.018	DELETERIOUS

For Polyphen-2 there where, 20 nsSNPs predicted as damaging while three were benign, the damaging SNPs has impact on amino acid allelic variants, protein structure and function. The results were shown in Table 2.

Table 2. *Results of Polyphen-2 score and prediction for MPLgene.*

SNP	Amino Acid Change	Protein ID	Polyphen-2 Score	Polyphen-2 Prediction
rs6088	E168K	ENSP00000361548	0.994	Probably Damaging
rs28928907	R102P	ENSP00000361548	1	Probably Damaging
rs28928908	P275T	ENSP00000361548	1	Probably Damaging
rs113696793	D295G	ENSP00000361548	0.993	Probably Damaging
rs117656396	L265F	ENSP00000361548	0.024	Benign
rs121913611	R257C	ENSP00000361548	1	Probably Damaging
rs137952228	T183M	ENSP00000361548	0.996	Probably Damaging
rs139770726	E335K	ENSP00000361548	1	Probably Damaging
rs141311765	Y252H	ENSP00000361548	0.999	Probably Damaging
rs145714475	F245S	ENSP00000361548	0.987	Probably Damaging
rs147608148	S402P	ENSP00000361548	0.319	Benign
rs148276667	R75H	ENSP00000361548	0.999	Probably Damaging
rs149810307	V368L	ENSP00000361548	0.861	Possibly-Damming
rs200454070	R426Q	ENSP00000361548	1	Probably Damaging
rs201101813	D128Y	ENSP00000361548	0.998	Probably Damaging
rs202137992	P173A	ENSP00000361548	0.709	Possibly-Damming
rs202206935	L109F	ENSP00000361548	0.085	Benign

SNP	Amino Acid Change	Protein ID	Polyphen-2 Score	Polyphen-2 Prediction
rs267598614	E99K	ENSP00000361548	0.459	Possibly-Damming
rs267598615	E149K	ENSP00000361548	0.753	Possibly-Damming
rs369486165	E332K	ENSP00000361548	0.73	Possibly-Damming
rs371232858	A418T	ENSP00000361548	0.996	Probably Damaging
rs372164360	F315I	ENSP00000361548	0.999	Probably Damaging
rs373621350	R390C	ENSP00000361548	0.992	Probably Damaging

Using I-Mutant software 16 nsSNPs where decreasing the protein stability while 6 nsSNPs were increasing the protein stability. I- Mutant offer the opportunity to predict automatically protein stability change upon single-site mutation. The results were shown in Table 3.

Table 3. *Results of I-Mutant prediction, RI and DDG for MPLgene.*

SNP	Amino Acid Change	PROTEIN ID	PREDICTION	RI	DDG
rs6088	E168K	ENSP00000361548	Decreased	3	0.57
rs28928907	R102P	ENSP00000361548	Decreased	1	0.42
rs28928908	P275T	ENSP00000361548	Decreased	4	0.61
rs113696793	D295G	ENSP00000361548	Decreased	9	1.91
rs117656396	L265F	ENSP00000361548	Decreased	6	0.55
rs121913611	R257C	ENSP00000361548	Decreased	4	0.6
rs137952228	T183M	ENSP00000361548	Increased	1	0.1
rs139770726	E335K	ENSP00000361548	Decreased	5	1.29
rs141311765	Y252H	ENSP00000361548	Decreased	0	0.46
rs145714475	F245S	ENSP00000361548	Decreased	4	0.47
rs148276667	R75H	ENSP00000361548	Decreased	5	0.5
rs149810307	V368L	ENSP00000361548	Increased	2	0.1
rs200454070	R426Q	ENSP00000361548	Decreased	7	1.5
rs201101813	D128Y	ENSP00000361548	Increased	7	0.33
rs202137992	P173A	ENSP00000361548	Decreased	3	0.12
rs202206935	L109F	ENSP00000361548	Increased	2	0.03
rs267598614	E99K	ENSP00000361548	Decreased	6	1.8
rs267598615	E149K	ENSP00000361548	Decreased	3	0.62
rs369486165	E332K	ENSP00000361548	Increased	4	0.25
rs371232858	A418T	ENSP00000361548	Decreased	9	1.37
rs372164360	F315I	ENSP00000361548	Increased	0	0.71
rs373621350	R390C	ENSP00000361548	Decreased	6	0.59

To predict the capability of causing disease or being neutral, PHD software was used the results were show in Table 4.

Table 4. *Results of PHD prediction, RI and Probability for MPLgene.*

SNP	Amino Acid Change	Protein ID	Prediction	RI	Probability
rs6088	E168K	ENSP00000361548	Disease	3	0.646
rs28928907	R102P	ENSP00000361548	Disease	6	0.797
rs28928908	P275T	ENSP00000361548	Disease	1	0.46
rs113696793	D295G	ENSP00000361548	Disease	5	0.7
rs117656396	L265F	ENSP00000361548	Neutral	7	0.14
rs121913611	R257C	ENSP00000361548	Disease	3	0.659
rs137952228	T183M	ENSP00000361548	Neutral	5	0.232
rs139770726	E335K	ENSP00000361548	Neutral	0	0.497
rs141311765	Y252H	ENSP00000361548	Disease	0	0.502
rs145714475	F245S	ENSP00000361548	Disease	0	0.455
rs148276667	R75H	ENSP00000361548	Disease	3	0.674
rs149810307	V368L	ENSP00000361548	Neutral	4	0.316
rs200454070	R426Q	ENSP00000361548	Neutral	5	0.236
rs201101813	D128Y	ENSP00000361548	Disease	3	0.654
rs202137992	P173A	ENSP00000361548	Disease	2	0.606
rs202206935	L109F	ENSP00000361548	Neutral	3	0.361
rs267598614	E99K	ENSP00000361548	Disease	3	0.637
rs267598615	E149K	ENSP00000361548	Disease	1	0.567
rs369486165	E332K	ENSP00000361548	Neutral	6	0.184
rs371232858	A418T	ENSP00000361548	Neutral	3	0.372
rs372164360	F315I	ENSP00000361548	Neutral	2	0.399
rs373621350	R390C	ENSP00000361548	Disease	1	0.545

Classification the nsSNPs to disease and neutral through the SNP & Go prediction tool revealed 20 nsSNPs as Neutral and only one was as disease related nsSNP (rs28928907/ R102P). The table 5 below showed the results

Table 5. *Results of SNP & GO prediction, RI and Probability for MPLgene.*

SNP	Amino Acid Change	Protein ID	Prediction	RI	Probability
rs6088	E168K	ENSP00000361548	Neutral	5	0.262
rs28928907	R102P	ENSP00000361548	Disease	1	0.53
rs28928908	P275T	ENSP00000361548	Neutral	9	0.054
rs113696793	D295G	ENSP00000361548	Neutral	1	0.465
rs117656396	L265F	ENSP00000361548	Neutral	9	0.026
rs121913611	R257C	ENSP00000361548	Neural	5	0.275
rs137952228	T183M	ENSP00000361548	Neutral	9	0.06
rs139770726	E335K	ENSP00000361548	Neutral	8	0.099
rs141311765	Y252H	ENSP00000361548	Neutral	8	0.098
rs148276667	R75H	ENSP00000361548	Neutral	3	0.355
rs149810307	V368L	ENSP00000361548	Neutral	8	0.086
rs200454070	R426Q	ENSP00000361548	Neutral	7	0.134
rs201101813	D128Y	ENSP00000361548	Neutral	0	0.48
rs202137992	P173A	ENSP00000361548	Neutral	8	0.124
rs202206935	L109F	ENSP00000361548	Neutral	8	0.093
rs267598614	E99K	ENSP00000361548	Neutral	4	0.289
rs267598615	E149K	ENSP00000361548	Neutral	5	0.245
rs369486165	E332K	ENSP00000361548	Neutral	9	0.049
rs371232858	A418T	ENSP00000361548	Neutral	9	0.071
rs372164360	F315I	ENSP00000361548	Neutral	8	0.119
rs373621350	R390C	ENSP00000361548	Neutral	6	0.204

Project-Hope results

The prediction of the mutation of Arginine into a Proline at position 102. (rs28928907) was shown in Figure 1. Each amino acid has its own specific size, charge, and hydrophobicity-value. The original wild-type residue and newly introduced mutant residue often differ in these properties. The mutant residue is smaller than the wild-type residue. The wild-type residue charge was POSITIVE; the mutant residue charge is NEUTRAL. The wild-type residue is more hydrophobic than the mutant residue

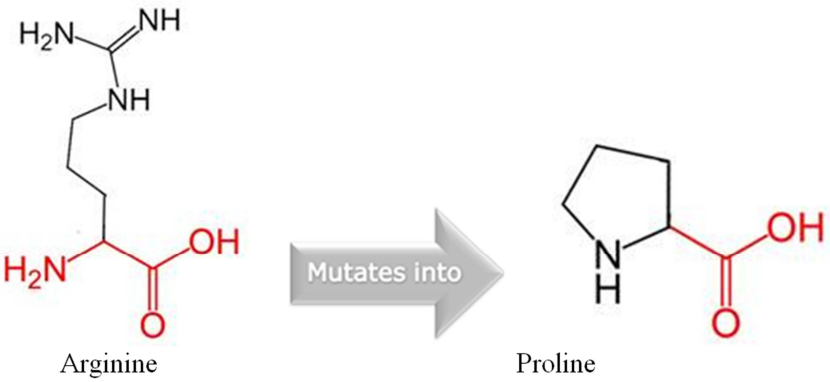

Figure 1. *The schematic structures of the original (left) and the mutant (right) amino acid. The backbone, which is the same for each amino acid, is colored red. The side chain, unique for each amino acid, is colored black.*

For the prediction of the mutation P275T (rs28928908), each amino acid has its own specific size, charge, and hydrophobicity-value. The original wild-type residue and newly introduced mutant residue often differ in these properties. The wild-type residue is more hydrophobic than the mutant residue. The results were shown in Figure 2.

Figure 2. *The schematic structures of the original (left) and the mutant (right) amino acid. The backbone, which is the same for each amino acid, is colored red. The side chain, unique for each amino acid, is colored black.*

Using GeneMANIA software it predicts the associated genes with *MPL* gene, results were shown in Figure 3 and the co-expressed and shared domain with, *MPL* gene, results were shown in Table 6.

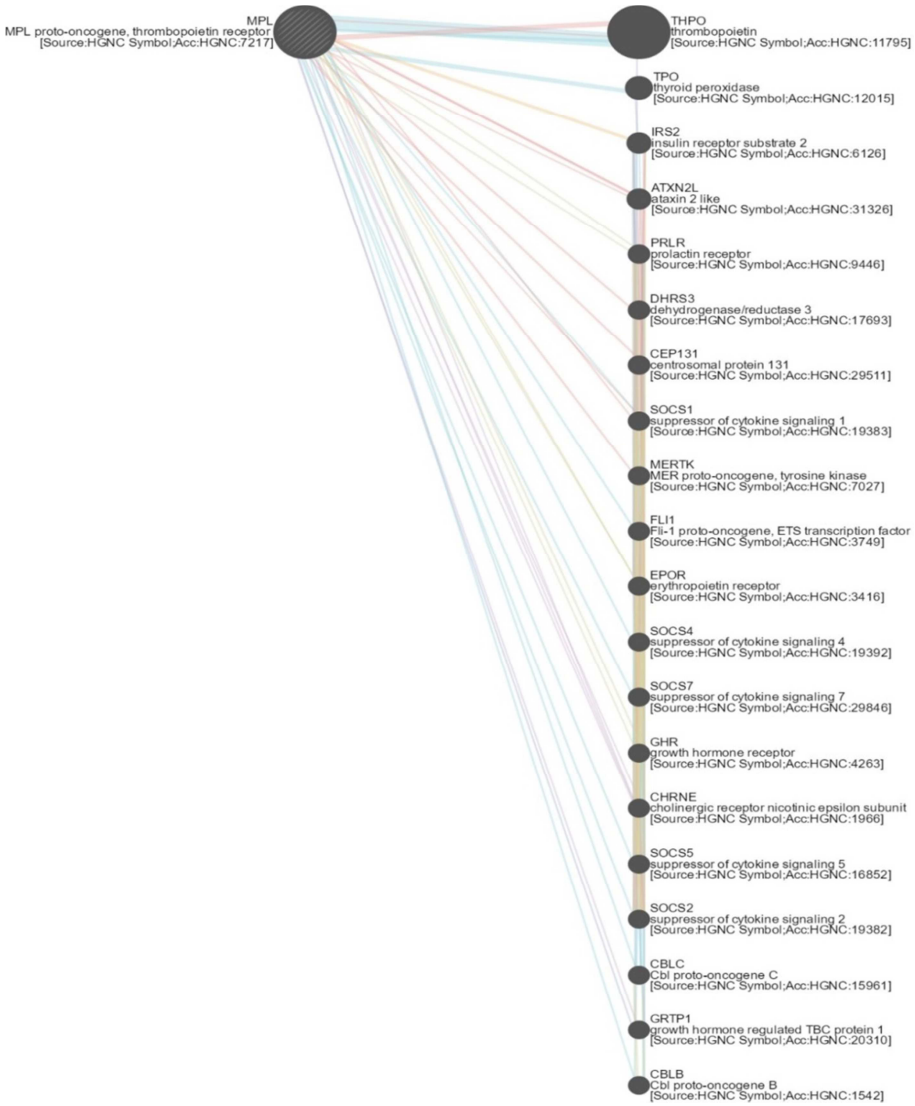

Figure 3. *GeneMANIA showing the MPL and associated genes.*

Table 6. *The co-expressed and shared domain with MPL gene by GeneMANIA.*

Gene	Description	Co Expression	Shared Domain
MPL	MPLproto-oncogene, thrombopoietinreceptor [Source: HGNCSymbol; Acc: HGNC: 7217]		
THPO	thrombopoietin [Source: HGNC Symbol; Acc: HGNC: 11795]	No	No
TPO	thyroid peroxidase [Source: HGNC Symbol; Acc: HGNC: 12015]	Yes	No
IRS2	insulin receptor substrate 2 [Source: HGNC Symbol; Acc: HGNC: 6126]	Yes	No
ATXN2L	ataxin 2 like [Source: HGNC Symbol; Acc: HGNC: 31326]	Yes	No
PRLR	prolactin receptor [Source: HGNC Symbol; Acc: HGNC: 9446]	Yes	Yes
DHRS3	dehydrogenase/reductase 3 [Source: HGNC Symbol; Acc: HGNC: 17693]	Yes	No
CEP131	centrosomal protein 131 [Source: HGNC Symbol; Acc: HGNC: 29511]	Yes	No
SOCS1	suppressor of cytokine signaling 1 [Source: HGNC Symbol; Acc: HGNC: 19383]	Yes	Yes
MERTK	MER proto-oncogene, tyrosine kinase [Source: HGNCSymbol; Acc: HGNC: 7027]	Yes	No
FLI1	Fli-1 proto-oncogene, ETS transcription factor [Source: HGNC Symbol; Acc: HGNC: 3749]	Yes	No
EPOR	erythropoietin receptor [Source: HGNC Symbol; Acc: HGNC: 3416]	Yes	Yes
SOCS4	suppressor of cytokine signaling 4 [Source: HGNC Symbol; Acc: HGNC: 19392]	No	Yes
SOCS7	suppressor of cytokine signaling 7 [Source: HGNC Symbol; Acc: HGNC: 29846]	Yes	Yes
GHR	growth hormone receptor [Source: HGNC Symbol; Acc: HGNC: 4263]	Yes	Yes
CHRNE	Cholinergic receptor nicotinic epsilon subunit [Source: HGNCSymbol; Acc: HGNC: 1966]	Yes	No
SOCS5	suppressor of cytokine signaling 5 [Source: HGNC Symbol; Acc: HGNC: 16852]	Yes	Yes
SOCS2	suppressor of cytokine signaling 2 [Source: HGNC Symbol; Acc: HGNC: 19382]	Yes	Yes
CBLC	Cbl proto-oncogene C [Source: HGNC Symbol; Acc: HGNC: 15961]	NO	Yes
GRTP1	growth hormone regulated TBC protein 1 [Source: HGNCSymbol; Acc: HGNC: 20310]	Yes	No
CBLB	Cbl proto-oncogene B [Source: HGNC Symbol; Acc: HGNC: 1542]	NO	Yes

4. Discussion

MPL gene has a vital role in human body and it is co-expressed with 16 genes and shared domain with 10 genes. *MPL* gene was investigated in NCBI database (http://www.ncbi.nlm.nih.gov/). It contains 2399 SNPswith197SNPs in the coding region. Non synonymous SNPs were analyzed by SIFT software, and only 23 SNPs predicted to be deleterious. These deleterious SNPs were analyzed using Polyphen-2 software to predict the damaging SNPs; only 20 SNPs were predicted to be deleterious in both software. I-mutant software was used to evaluate the degree of stability due to mutation. The present study found that 16 SNPs were scored to decrease the stability of the protein, While only seven SNPs had increased in the stability of the protein. Moreover, PhD was another promising software used beside the I-Mutant, 12 SNPs were sorted as a disease related, while 10 SNPs were neutral, but there was one SNP obtained no result from the database (rs147608148). For more confirmation for the mutation the study used SNP & Go software that only one SNP (rs28928907) was reported as a disease related, while the others SNPs were reported as a neutral. The Project Hope software was used to detect the effect the mutation on the protein structure and function many of the SNPs found were affecting these properties as illustrated by rs28928907 and rs28928908 as shown in figure 1and 2. Moreover a study done in 2011 suggested that *MPL* W515K/L was negative ET cases, and no mutations detected previously proposed as potential candidate drivers [19], and this findings might suggest why this SNPs was not detected as a pathogenic SNPs in this study, so another candidates SNPs or mutations may be studied as a target in the future for the ET cases. The quest for the full complement of driver mutations in ET therefore remains open.

5. Conclusions

The MPL gene plays important roles in cell signaling and proliferation. Mutations in this gene confer constitutive activation of the JAK-STAT pathway and other pathways promoting differentiation and proliferation of different lineage. The available data from the NCBI dbSNP database for *MPL* gene has been analyzed through several SNP analyzing tools and the predicted deleterious SNPs were evaluated for their deleterious effect on the protein function and stability. The study concluded that only one nsSNP which is the Arginine into a Proline at position 102. (rs28928907) is of high value to be suggested as diagnostic nsSNP for *MPL* gene.

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