ORIGINAL ARTICLE

Computational Analysis of Single Nucleotide Polymorphisms (SNPs) in Human *FLT3* Gene

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ABSTRACT

Background: **AML acute myeloid leukemia prevalence about 25%of adult leukemia. FLT3 gene mutations in about 30% of patients and associated with severe deterioration.**

Materials & Methods: **In this study, investigations of FLT3 were done computationally, SIFT and POLYPHEN-2 servers and more had been used.**

Results: **The pathological SNPs had been detected and confirmed to be 40 SNPs, 10 of them seeming to be most pathological and they scored (0,1) in sift and polyphen-2 servers respectively. The change in 3D structure had been explained for the ten pathological double-damaging SNPs which are proved to be most responsible SNPs of causing acute myeloid leukemia (AML).**

Conclusion: **Hopefully, this paper will provide informative subject to help future researchers investigate FLT3 for AML properly. This study is considered a distinctive one, because no in silico studies had been conducted regarding this subject before.**

KEY WORDS

AML acute myeloid leukemia, FLT3 FMS-like tyrosine kinase3, SIFT Sorting Intolerant From Tolerant, POLYPHEN

Polymorphism Phenotyping v2, DDGDeltaDeltaG, HOPE Have your Protein Explained

INTRODUCTION

Acute myeloid leukemia (AML) is a group of hematopoietic neoplasms that affect precursor cells committed to the myeloid line of cellular development, giving rise to granulocytic, monocytic, erythroid, or megakaryocytic elements. AML accounts for 25% of adult leukemia cases globally and has the lowest survival rate[1-3]. Despite numerous efforts to find a solution to this devastating disease, it remains challenging. In many cases, mutations in specific genes, including FLT3, are found in AML patients[4-8]. FLT3 gene mutations are the most common mutations in AML, occurring in about 30% of patients[9-11]. When the FLT3 gene is mutated, it becomes constantly activated, causing cancer cells to multiply rapidly compared to other AML patients[9-11].

FLT3 mutations are associated with a poor outcome and short survival rate. The FLT3 gene, located on chromosome 13(13q12), encodes a transmembrane tyrosine kinase receptor that stimulates cell proliferation upon activation[12]. Mutations in the FMS-like tyrosine kinase 3 gene, producing internal transmembrane duplications (FLT3/ITD) and constitutive activation of the FLT3 receptor tyrosine kinase, are quite common in AML, particularly in patients with normal karyotypes, and have been associated with poorer survival in children and in younger and older adults receiving intensive chemotherapy[13-16]. It has been proposed that FLT3/ITD mutational status is the primary predictor of outcome among patients with intermediate-risk AML by karyotype analysis[16]. FLT3 mutations are present in approximately 10 and 30 percent of patients with cytogenetically normal AML in the pediatric and adult populations, respectively, and concurrent abnormalities in other genes, such as NPM1, may influence the impact of the FLT3 mutation[17-19]. There are two main types of FLT3 mutations. The most common are internal tandem duplications of different lengths that result in ligand-independent activation of the FLT3 receptor and a proliferative signal. Alternatively, point mutations in the activating loop of the kinase domain of FLT3 may result in tyrosine kinase activation of FLT3 in 5 to

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Table 1: explains sift and polyphen-2 servers result

SNP	REF ALLELE-ALT ALLELE	AMINO ACID CHANGE	PROTEIN ID	SIFT SCORE	SIFT PREDICTION	PSIC	Polyphen-2 prediction
rs201232010	T-A	D779V	ENSP00000241453	0.004	Deleterious	0.878	Possibly damaging
rs141942072	C-T	G493E	ENSP00000241453	0.021	Deleterious	0.825	Possibly damaging
rs201923726	C-T	R852H	ENSP00000370369	0	Deleterious	0.935	Possibly damaging
rs141185874	A-G	F349L	ENSP00000241453	0.041	Deleterious	0.936	Possibly damaging
rs200909894	T-C	M867V	ENSP00000438139	0.01	Deleterious	0.954	Possibly damaging
rs121913487	A-T	D835E	ENSP00000241453	0	Deleterious	0.976	Possibly damaging
rs121913232	G-C	I836M	ENSP00000370369	0.003	Deleterious	0.983	Possibly damaging
rs201504848	C-G	D868H	ENSP00000438139	0.049	Deleterious	0.986	Possibly damaging
rs62636526	C-G	V16L	ENSP00000241453	0	Deleterious	0.994	Possibly damaging
rs121909646	T-A	D835V	ENSP00000241453	0	Deleterious	0.999	Probably damaging
rs121913491	T-C	Y572C	ENSP00000241453	0	Deleterious	0.999	Probably damaging
rs117240821	G-C	P267R	ENSP00000370369	0.044	Deleterious	1	Probably damaging
rs375516435	A-T	S996T	ENSP00000370369	0	Deleterious	0.982	Probably damaging
rs142796469	G-C	P849R	ENSP00000438139	0	Deleterious	0.984	Probably damaging
rs376895552	C-A	G64W	ENSP00000370369	0.012	Deleterious	0.990	Probably damaging
rs201905189	G-A	T432M	ENSP00000370369	0.021	Deleterious	0.993	Probably damaging
rs139990734	C-T	V441M	ENSP00000241453	0.009	Deleterious	0.994	Probably damaging
rs147819024	A-T	W462R	ENSP00000241453	0.017	Deleterious	0.997	Probably damaging
rs201398123	T-C	T866A	ENSP00000241453	0	Deleterious	0.998	Probably damaging
rs200909894	T-C	M911V	ENSP00000370369	0.009	Deleterious	0.998	Probably damaging
rs201398123	T-C	T825A	ENSP00000438139	0	Deleterious	0.999	Probably damaging
rs372303125	G-A	A680V	ENSP00000241453	0	Deleterious	0.999	Probably damaging
rs370661880	T-A	I33F	ENSP00000438139	0	Deleterious	0.999	Probably damaging
rs370459694	A-G	I884T	ENSP00000370369	0.003	Deleterious	0.999	Probably damaging
rs141942072	C-A	G493V	ENSP00000241453	0.008	Deleterious	0.999	Probably damaging
rs142796469	G-C	P890R	ENSP00000241453	0	Deleterious	1.000	Probably damaging
rs142796469	G-C	P893R	ENSP00000370369	0	Deleterious	1.000	Probably damaging
rs201208287	C-A	A814S	ENSP00000241453	0	Deleterious	1.000	Probably damaging
rs201398123	T-C	T869A	ENSP00000370369	0	Deleterious	1.000	Probably damaging
rs201923726	C-T	R849H	ENSP00000241453	0	Deleterious	1.000	Probably damaging
rs201923726	C-T	R808H	ENSP00000438139	0	Deleterious	1.000	Probably damaging
rs368432815	A-T	D870E	ENSP00000241453	0	Deleterious	1.000	Probably damaging
rs368432815	A-T	D873E	ENSP00000370369	0	Deleterious	1.000	Probably damaging
rs368432815	A-T	D829E	ENSP00000438139	0	Deleterious	1.000	Probably damaging
rs376588714	T-C	Y842C	ENSP00000241453	0	Deleterious	1.000	Probably damaging
rs370459694	A-G	I881T	ENSP00000241453	0.003	Deleterious	1.000	Probably damaging
rs370459694	A-G	I840T	ENSP00000438139	0.003	Deleterious	1.000	Probably damaging
rs200909894	T-C	M908V	ENSP00000241453	0.01	Deleterious	1.000	Probably damaging
rs75849452	A-G	V897A	ENSP00000370369	0	Deleterious	1.000	Probably damaging

10 percent of patients[20-22].

Studies have shown that patients with FLT3-ITD have an estimated two-year progression-free survival rate of 20 percent and a four-year overall survival rate of approximately 20 percent. In contrast, the mutations of the tyrosine kinase domain of FLT3 do not appear to be associated with the same poor outcome as FLT3/ITD. Additional characterization of the various types of FLT3 mutations, as well as analyses of the clinical impact of differing ratios of mutated/wild-type variants on outcome, are in progress[23,24].

Several clinical trials with different oral FLT3 tyrosine kinase inhibitors, including CEP701 (lestaurtinib), sunitinib maltase (SU11248), sorafenib, and PKC412 (Midostaurin), have been initiated, and evidence of anti-leukemic activity has been seen in phase 2 studies[25-31]. However, most responses were incomplete and transient. Given the heterogeneity in the outcomes of different subtypes of FLT3 mutated patients, further analysis of this subtype, as well as prospective studies, are crucial in determining their effects on prognosis and possible treatment recommendations. This article aims to analyze single nucleotide polymorphisms (SNPs) in the human FLT3 gene.

METHODOLGY

In this study, we aimed to detect all single nucleotide polymorphisms (SNPs) in the translated coding region of the FMS-like tyrosine kinase 3 gene. We retrieved SNP data from db-SNP NCBI and identified a total of 5801 SNPs in the human genome. Of these, we targeted the 214 SNPs located in the translated coding region for further analysis.

SIFT SERVER: To assess the potential impact of each SNP on protein function, we used the SIFT server. SIFT evaluates whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids. It is a widely used tool for predicting the functional consequences of naturally occurring nonsynonymous polymorphisms and laboratory-induced missense mutations[32].

Table 2: I-MUTANT and SNPs & GO results

SNP	AMINO ACID CHANGE	DDG	I MUTANT PREDICTI-ON	RI	PHD PREDICTI-ON	PHD RI	PHD PROBABILI-TY	SNP PREDICTI-ON	SNP RI	SNP PROBABI-LITY
rs201232010	D779V	0	Decrease	0	Disease	4	0.695	Neutral	2	0.396
rs141942072	G493E	-0.96	Decrease	4	Disease	2	0.622	Disease	4	0.701
rs201923726	R852H	-1.46	Decrease	9	Disease	7	0.846	Disease	1	0.56
rs141185874	F349L	-1.21	Decrease	5	Disease	2	0.612	Neutral	1	0.468
rs200909894	M86V	-0.85	Decrease	7	Disease	4	0.675	Disease	1	0.453
rs121913487	D835E	-0.82	Decrease	1	Disease	4	0.693	Neutral	1	0.445
rs121913232	I836M	-1.96	Decrease	9	Neutral	5	0.261	Neutral	8	0.123
rs201504848	D868H	-0.11	Decrease	2	Disease	9	0.935	Disease	7	0.827
rs121909646	D835V	-0.55	Decrease	5	Disease	6	0.788	Disease	1	0.535
rs121913491	Y572C	-0.8	Increase	2	Disease	3	0.633	Neutral	6	0.211
rs117240821	P267R	-0.79	Decrease	6	Neutral	1	0.432	Neutral	7	0.142
rs142796469	P849R	-1.32	Decrease	8	Disease	8	0.91	Disease	5	0.763
rs376895552	G64W	-0.21	Increase	0	Disease	8	0.916	Disease	5	0.771
rs201905189	T432M	-0.33	Decrease	4	Disease	8	0.915	Disease	5	0.771
rs139990734	V441M	-0.52	Decrease	2	Disease	0	0.51	Disease	4	0.682
rs147819024	W462R	-1.07	Decrease	8	Disease	7	0.846	Disease	1	0.557
rs201398123	T866A	-1.26	Decrease	7	Disease	7	0.848	Disease	1	0.564
rs200909894	M911V	-0.85	Decrease	7	Disease	6	0.817	Disease	1	0..550
rs201398123	T825A	-1.26	Decrease	7	Disease	3	0.655	Neutral	3	0.327
rs372303125	A680V	0.22	Increase	5	Disease	3	0.655	Neutral	3	0.327
rs370459694	I884T	-2.44	Decrease	9	Disease	2	0.591	Neutral	5	0.252
rs141942072	G493V	-0.92	Decrease	8	Neutral	4	0.295	Neutral	3	0.33
rs142796469	P890R	-1.32	Decrease	8	Disease	4	0.704	Disease	0	0.52
rs142796469	P893R	-1.32	Decrease	8	Disease	3	0.644	Neutral	3	0.363
rs201208287	A814S	-0.74	Decrease	9	Disease	7	0.867	Disease	1	0.569
rs201398123	T869A	-1.26	Decrease	7	Disease	7	0.867	Disease	1	0.569
rs201923726	R849H	-1.6	Decrease	9	Disease	7	0.867	Disease	1	0.569
rs201923726	R808H	-2.17	Decrease	1	Disease	5	0.762	Disease	0	0.523
rs368432815	D870E	-0.29	Increase	2	Disease	5	0.759	Disease	0	0.52
rs368432815	D873E	-0.29	Increase	2	Disease	6	0.778	Disease	1	0.535
rs368432815	D829E	-0.29	Increase	2	Disease	6	0.823	Disease	4	0.711
rs376588714	Y842C	-1.45	Decrease	4	Neutral	3	0.349	Neutral	5	0.273
rs370459694	I881T	-2.44	Decrease	9						
rs370459694	I840T	-2.44	Decrease	9						
rs200909894	M908V	-0.85	Decrease	7						
rs75849452	V897A				Neutral	8	0.244	Neutral	5	0.076
rs62636526	V16L	-1.34	Decrease	7	Disease	1	0.572	Neutral	4	0.306
rs375516435	S996T	0	Decrease	2	Disease	2	0.618	Disease	1	0.562
rs370661880	I33F	0.02	Increase	1	Disease	3	0.652	Neutral	3	0.327

POLYPHEN-2: To further evaluate the potential impact of deleterious SNPs on protein structure and function, we utilized the PolyPhen-2 server. PolyPhen-2 is a tool that predicts the possible impact of an amino acid substitution on the structure and function of a human protein based on physical and comparative considerations. The tool evaluates the effect of the mutation qualitatively and categorizes it as benign, possibly damaging, or probably damaging based on pairs of false positive rate (FPR) thresholds. Mutations predicted to be probably damaging have higher confidence in the prediction than those predicted to be possibly damaging. Mutations with estimated false positive rates above a certain threshold are classified as benign[33].

I-MUTANT SERVER: We also measured the stability of proteins using the I-Mutant server, which is a neutral network-based predictor of protein stability changes upon a single point mutation from the protein structure. The server is used to calculate the free energy value DeltaDeltaG (ΔΔG) that represents the difference in unfolding Gibbs free energy values between the mutated protein and the wild type (in kcal/mol)[34].

SNPs&GO SERVER: To predict disease-associated variations from protein sequence and structure, we utilized the SNPs&GO server. SNPs&GO is an accurate method that predicts whether a variation is disease-related or not by exploiting the corresponding protein functional annotation. This server collects information derived from the protein and combines it with other databases, including the UniProt database, HSSP, and DAS-servers, to provide a comprehensive report[35].

PROJECT HOPE CMBI: Finally, we confirmed our findings using the report generated by the PROJECT HOPE CMBI tool. This tool collects information from multiple sources, including the protein 3D-structure or homology model, the UniProt database, HSSP, and DAS-servers. All information is stored in a database and can be combined using a decision schedule. HOPE provides a detailed report that explains the effect of a point mutation on the 3D-structure and function of the protein[36].

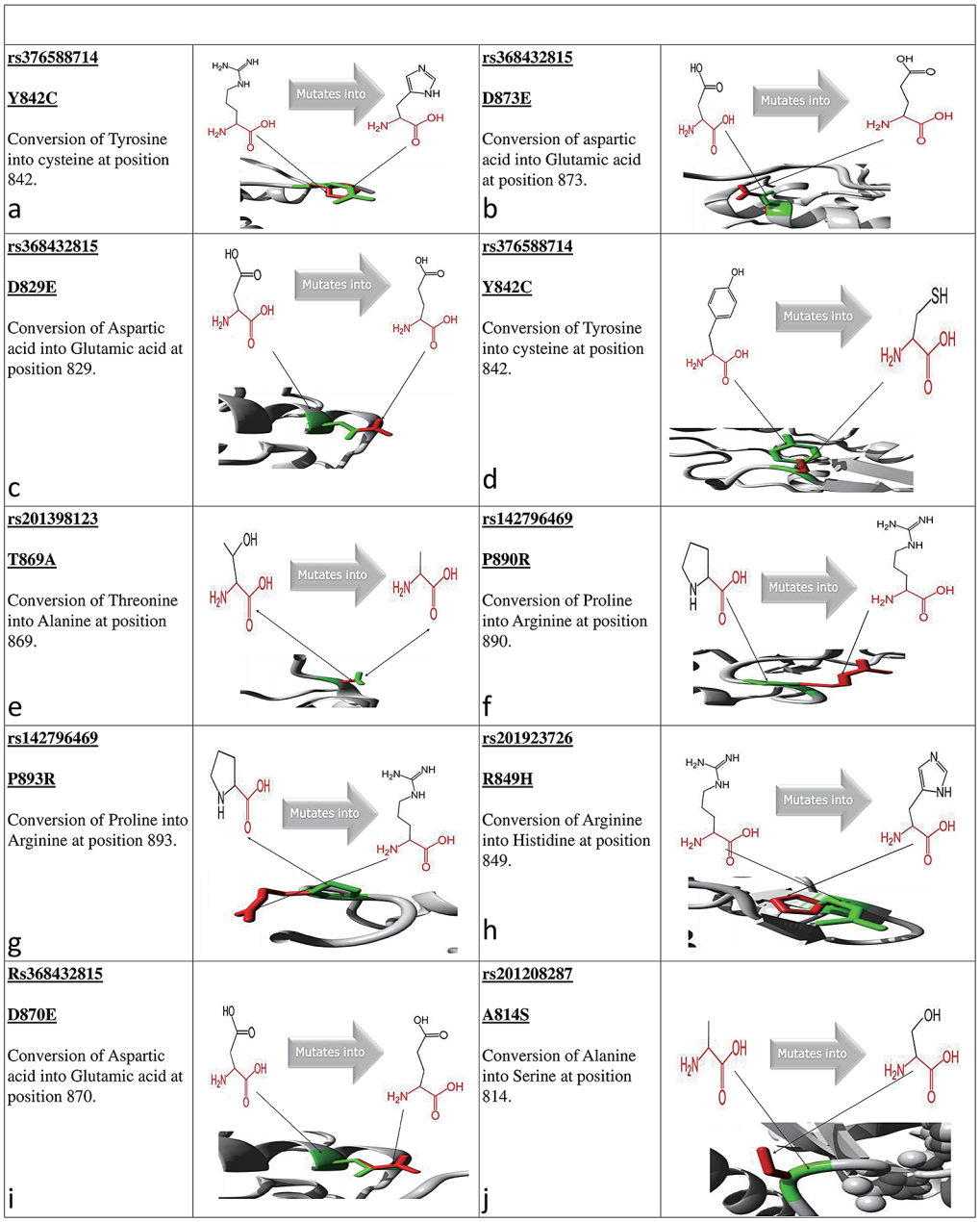

Figure 1: The change in 3D structure using Project Hope CMBI

RESULT AND DISSCUSION

Approximately 5801 SNPs of fms-like tyrosine kinase (*FLT3*) had been detected in dbSNP-NCBI and submitted as group to **sift** and **polyphen-2 servers** the results explain the degree of tolerance and protein damage that occurs upon mutation. 46 SNPs had been found Deleterious from SIFT server. They were submitted to polyphen-2 server and five SNPs were found to be possibly damaging, 33 SNPs were probably damaging and seven SNPs were benign (Table-1).

Proteins stability and disease association investigated by I-MUTANT and SNP &GO servers

A total number of forty Deleterious SNPs had been submitted to I-MUTANT SERVER and six were found with increased protein stability and the rest of the SNPs had decreased protein stability (Table-2). SNP&GO result contained (PHD, SNPs & GO Prediction and their probabilities respectively).

Functional testing

Double-positive damaging SNPs according to sift and polyphen-2 servers with score (0, 1) respectively were exposed to project hope software. The result showed 3D structures of the new residue proteins. Here the results discuss the conformational changes. All wild types and new residues are shown in (Figure 1) wild types are marked green while mutant types marked red, and change in physiochemical parameters also explained in (Figure 1).

C/T single nucleotide variation (rs201923726) led to conversion of arginine into histidine at position 808. The mutant residue is smaller than the wild-type residue. That might lead to loss/reduction of interactions and thus reduction of protein function. The mutation is located within a domain, annotated in UniProt as Protein kinase. It introduces an amino acid with different properties, which can disturb this domain and abolish its function.

A/T single nucleotide mutation (rs368432815) conversion of aspartic acid into glutamic acid at position 873 led to increased size of mutant type. The mutation is also located in the protein kinase domain. It produces an amino acid with different properties, affecting normal protein

function. (rs368432815) mutation of aspartic acid into glutamic acid at position 829 has resulted in a bigger mutant type than the wild one. The mutation as well as the previous ones occurs in the protein kinase domain. The large sized amino acid has different properties than the wild one leading to a different structure of the protein and effect on the function.

C/T single nucleotide mutation (rs376588714) transformation from a tyrosine into a cysteine at position 842 gives a mutant type, which is smaller and more hydrophobic than the wild residue. Mutation located on protein kinase domain. And affects protein structure (disturb in the correct folding due to loss of the hydrogen bonds) and function as well as the signal transduction between the neighboring domains due to the empty space made caused by the smaller amino acid.

C/Tmutation (rs201398123) conversion of threonine into alanine at position 869. The later has a smaller size than the wild typeand an increased level of hydrophobicity, which affects hydrogen bond formation thus, protein functions and signal transduction. Mutation similarly to previous ones located in protein kinase domain.

C/G single nucleotide polymorphism (rs142796469) conversion of Proline into Arginine at position 890. The mutant residue is bigger in the size than the wild residue, more hydrophobic, and positively charged while the wild residue was neutral. The mutant residue is located near a highly conservative domain named protein kinase-like domain with an unknown function. It's located on the surface of the protein and can affect protein interactions with other molecules.

C/G single-nucleotide variation (rs142796469) shows another conversion of Proline into Arginine at position 893 in a SNP. Size of mutant type is bigger and is more hydrophobic than the wild type that leads to direct effect on the protein interactions. In addition, it is positively charged while the wild residue is neutral. Mutation is located on the surface of an unknown domain.

A/T single nucleotide variation (rs368432815) conversion of arginine into histidine at position 849. Mutant residue is smaller than the wild one. It is neutral while the wild one was positively charged. Mutation is located on protein kinase domain. Thus, it is not conserved. The interactions between domains could be quite disturbed by the mutation affecting the protein function.

A/T single nucleotide mutation (rs368432815) transformation of Aspartic acid into Glutamic acid at position 870. The wild-type residue is much conserved and is located near a highly conserved region. The mutant residue is bigger and probably will not fit burying the wild residue in the core of the protein and changing its reactions.

A/G/T single-nucleotide variation (rs201208287) substitution of alanine into serine at position 814. The mutant residue is bigger than the wild-type residue but in contrast, the wild-type residue is more hydrophobic than the mutant residue.

Mutation is located in protein kinase-like domain. Moreover, the protein loses its hydrophobic activity due to entrapment of wild type at the core of the protein.

This mutation ID (**rs201398123**) did not show results on polyphen-2 software, I-Mutant software and project hope due to absence of the protein sequence on all databases (Expasy, UniProt, and NCBI). Also the following IDs(**rs200909894,rs370459694**)did not show results in SNPs& GO for unknown reason. The reference sequence (**rs75849452**) SNP have no identical sequences, I-MUTANT server result. However, ENSEMBL Database Suggested position [A894V] instead of [A987V].

CONCLUSION

In summary, computational tools can be useful and straightforward in identifying and validating gene SNPs. The FLT3 gene has been widely reported as a causative factor in acute myeloid leukemia. In this study, we used in silico analysis to investigate 214 SNPs in the transcript region of FLT3. SIFT server was utilized to determine the functional effects of the 46 deleterious and 167 tolerated SNPs. Further analysis was conducted using POLYPHEN-2 server, which identified 6 benign, 5 possibly damaging, and 35 probably damaging SNPs. Double positive damaging SNPs, which have scores of 0 and 1 on SIFT and POLYPHEN-2 servers, respectively, were found to have an impact on protein function and were evaluated for their physiochemical parameters and changes in 3D structure using PROJECT HOPE CMBI. A limitation of this study is the exclusive use of computational tools and reliance on the sample size available in the NCBI database

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