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In Silico Analysis for DPYD Gene and the Effect of the Mutation on Dihydropyrimidine Dehydrogenase Enzyme

Asia M. Elrashid[1*], Mohamed Y. Basher[2], Ali I. A. Gharib[3], Mohamed A. I. Alfaki[4], Nawarz M. Mohammed[5], Ahmed M. Elmoselhy[6], Sahar G. Elbager[7] and Safinaz I. Khalil[8]

[1]Faculty of Science, Khartoum University, Sudan.
[2]Faculty of Veterinary Medicine, Khartoum University, Sudan.
[3]Faculty of Biotechnology, Omdurman Islamic University, Sudan.
[4]Faculty of Computer Science, Neelain University, Sudan.
[5]Faculty of Pharmacy, Omdurman Islamic University, Sudan.
[6]Faculty of Science, Alexandria University, Egypt.
[7]Faculty of Medical Laboratory Sciences, University of Medical Sciences and Technology (UMST), Sudan.
[8]Faculty of Medicine, University of Medical Sciences and Technology (UMST), Sudan.

Authors' contributions

This work was carried out in collaboration between all authors. Author AME designed the study, analysed and interpretated of data of the work and wrote the first draft of the manuscript. Authors MYB and SIK analysis and interpretation of data of the work and wrote the first draft of the manuscript. Authors AIAG, NMM and AME wrote the first draft of the manuscript. Authors MAIA and SIK supervised the work. Author SGE editied the final manuscript. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: Saharelbagir@ymail.com, alrashied@gmail.com;*

ABSTRACT

Aim: The aim of this study was to implement an in silico bioinformatics analysis for clinically observed missense variants in human DPYD gene to investigate the effect these variants on Dihydropyrimidine dehydrogenase enzyme 's structure and function.
Methods: The human DPYD gene was investigated in dbSNP/NCBI, 273238 SNPs were found; 99645 SNPs were Homo sapins; of which 534 were missense SNPs. Missense SNPs were selected for in silico analysis; SIFT, Polyphen2, SNPs & GO, Imutant 2.0, Mutation 3D , UCSF Chimera and HOPE were used to investigate the effect of SNPs on DPD protein's structure and function.
Results: 69 SNPs were found to be highly damaging for the protein by SIFT and Polyphen, of which 4 SNPs were observed to be associated to clinical presentations (M166V, V335L, I560S, D949V). These 69 SNPs were further analyzed by SNPs & GO, one SNP (D949V) was observed to be associated to clinical presentations. The 4 nsSNPs that observed to be associated to clinical presentations were further analyzed by I-Mutant, HOPE and chimera tools to predict their stability index and visualize wide and mutant residues in their protein 3D structure
Conclusions: We observe a range of structural and functional changes caused by single amino acid differences, including changes in protein structural, stability and binding properties associated with the 4nsSNPs (M166V, V335L, I560S, D949V). This can explain the variability in drug response and toxicity in patients who acquire these nsSNPs and treated with 5-FU.

Keywords: DPYD; 5 flurouracil; DPD; in silico analysis; SIFT; polyphen2; SNPs & GO; Imutant 2.0; Mutation 3D; UCSF Chimera; HOPE.

ABBREVIATIONS

3D : 3- Dimensional
5-FU : 5-Flurouracil
CPHmodels 3.2 Server : Protein homology modeling server
Db SNP : The Single Nucleotide Polymorphism Database
DPD : Dihydropyrimidine dehydrogenase deficiency
DPYD : Dihydropyrimidine dehydrogenase
FASTA : Text-based format
GO : Gene Oncology
HOPE : Have (y)Our Protein Explained
NCBI : National Center for Biotechnology Information
ns SNPs : Non-Synonymous Single Nucleotide Polymorphism Database
PDB : Protein Data Bank
Polyphen 2 : Polymorphism phenotyping V2
PSIC : Position Specific Independent Core Score
SIFT : Sorting Tolerant from Intolerant
SNP : Single Nucleotide Polymorphism Database
SVM : Support Vector machine

1. INTRODUCTION

The human DPYD gene located on chromosome 1p21.3 present as a single copy consists of 23 exsons, cover approximately 950 kb in length with 3 kb of coding sequence and an average intron size of about 43kb [1]. It encodes for dihydropyrimidine dehydrogenase (DPD) enzyme, an important catabolic enzyme in the metabolism of pyrimidines; thymine and uracil that occur naturally in the cell, beside cancer chemotherapy 5-fluorouracil (5-FU) drug [2].

5-fluorouracil (5-FU) is chemotherapy drug widely used in the treatment of a broad spectrum of cancers including colorectal and breast cancer [3]. 5-FU exerts its anticancer effects through inhibition of thymidylate synthase (TS). Interrupting the action of TS enzyme blocks synthesis of the pyrimidine thymidine, which is required for DNA synthesis [4]. It has been demonstrated that more than 80% of the administered 5FU is catabolized by DPD [5].

Regarding DPYD gene and 5-FU there is a direct link or relationship between the individual's DPYD activity and the metabolism of 5-FU its self [6]. In 5-FU-based cancer chemotherapy, drug resistance highly vary among colorectal cancer patients limiting its clinical usage. Genetic differences among patients has been confirmed in many researches to contribute to variability in drug response [7-10]. Patients who have mutant allele in DPYD gene suffering from severe (lethal) toxicity after using 5-FU drug [7,8]. Moreover, the adverse effects of 5-FU are often lethal especially when this mutant reduce the DPD efficiency [9,10]. An estimated 10%–20% of treated patients develop serious, sometimes life-threatening, 5-FU toxicity [11]. These adverse events include mucosal, cutaneous, hematological, and digestive toxic side effects. It is also estimated that approximately 0.5% of patients die from these early toxic effects [12,13].

Out of more than 30 potentially relevant SNPs reported in the literature [14,15], we could determine the following four missense SNPs, (M166V, V335L, I560S, D949V) as most often implied in clinical DPD deficiency. Studies suggested when Aspartic acid changes to Valine in 949 position, it interferes with electron transport or cofactor binding and strongly associates with grade III and IV toxicity in a cohort of patients with colorectal cancer treated with 5-FU–based therapy [10], whereas Isoleucine change to serine in position 560, that point mutation due to destabilize the DPD protein [10]. When Isoleucine changes to Serine at position 560, then the enzyme activity will be inhibited by (12.5%-25%) and by more than 25%, when Aspatic acid changes to Valine at 949 position but still less than wild type [16].

Bioinformatics play an important role in almost all aspects of drug discovery, development and assessment. Bioinformatics resources (data bases and software) facilitate the understanding and prediction of the drug metabolism, elimination and also toxicity of drugs [17,18]. Identification of SNPs responsible for adverse drug reaction or toxicity, provide a basic information for physicians to setup a therapy toward reducing the risk of complications and improving patient quality of life.

In this study we applied different available computational tools to analyze the most missense SNPs implied in clinical DPD deficiency to investigate the effect of these variants on protein's structure and function.

2. MATERIALS AND METHODS

Different software; SIFT, polyphen-2, Imutant3.0, SNPs & GO and chimera were used to investigate the effect of SNPs mutations on DPD protein structure and function. Prediction of deleterious effect of non synonymous SNPs was done by SIFT and Polyphen-2 software. The association of missense SNPs with disease was done by SNPs & GO software. Prediction of stability changes was investigated in I mutant. The structural changes in 3D structure were analyzed using HOPE and Chimera software.

2.1 Datasets

SNPs located in DPYD gene were obtained from the database of SNPs (dbSNP); it is , a genetic variation database established by the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/SNP). In this study Homosapien missense SNPs had been selected and submitted to bioinformatics tools for further investigation.

2.2 Prediction of Structural Impact of nsSNPs on Protein by SIFT Software

Sorting Tolerant from Intolerant (SIFT) is an online bioinformatics software that uses an algorithm to predict the effect of amino acid substitutions, resulting from Non-synonymous SNPs (nsSNPs), on protein function[19]. SIFT uses sequence homology to predict the effects of all possible substitutions at each position in the protein sequence. The threshold intolerance score for SNPs is 0.05 or less [20]. nsSNPs within dbSNP retrieved data were selected as an input for SIFT. Available at: (http://sift.jcvi.org/).

2.3 Prediction of Functional Modification of Coding nsSNPs by Polyphen-2

Polymorphism Phenotyping v2 (PolyPhen-2) is another bioinformatics server tool that predicts the damaging effects of missense mutations. PolyPhen searches for protein 3D structures and make multiple alignments of homologous sequences and amino acid contact in several protein databases and calculate position-specific independent count scores (PSIC) for each of two variants and then computes the PSIC scores difference between two variants, where the higher PSIC score difference indicates that the functional impact of amino acid substitution is likely to occur .PolyPhen-2 outcome can be one

of the following: probably damaging, possibly damaging, or benign, with score range from 0 to 1 [21].

Available:(http://genetics.bwh.harvard.edu/pph2/)

The input FASTA sequence of protein with the position of interest and the new residue were submitted to Polyphen to predict functional impact of mutations.

2.4 Prediction of Disease Associated Variations by SNPs&GO

Single Nucleotide Polymorphism Database (SNPs) & Gene Ontology (GO**)** is a support vector machine (SVM) based on the method to accurately predict the disease related mutations from protein sequence. FASTA sequence of whole protein is considered to be an input option and output will be the prediction results based on the discrimination among disease related and neutral variations of protein sequence. The probability score higher than 0.5 reveals the disease related effect of mutation on the parent protein function [22].

Available:(http://snps.biofold.org/snps-and-go/pages/method.html).

2.5 Analysis of nsSNPs' Impact on Protein Stability using I Mutant 2.0 Server

Change in protein stability disturbs both protein structure and protein function [23]. I-Mutant is a suite of support vector machine, based predictors integrated in a unique web server. It offers the opportunity to predict the protein stability changes upon single-site mutations. from the protein structure or sequence. [24]. The FASTA sequence of protein retrieved from UniProt is used as an input to predict the mutational effect on protein and stability RI value (reliability index) computed.

Available:(http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant 2.0.cgi.)

2.6 Automatic Protein Structural Analysis and Information Using HOPE Server

Automatic mutant analysis server can provide insight into the structural effects of a mutation. HOPE collects information from a wide range of information sources including calculations on the 3D coordinates of the protein by using sequence annotations from the UniProt database, and predictions by DAS services. Input method of Project HOPE carries the protein sequence and selection of Mutant variants. HOPE server predicts the output in the form of structural variation between mutant and wild type residues. HOPE builds a report with text, figures, and animations that is easy to use and understandable for medical researchers [25].
Available: (http://www.cmbi.ru.nl/hope/)

2.7 Modeling Amino Acid Substitution by UCSF Chimera Model Software

CPHmodels-3.2 server is a protein homology modeling prediction server, used to predict the 3D structure of proteins with an unknown 3D structure model. Modeling SNPs on the 3D structure of the proteins is a very helpful action in order to predict the effect that SNPs may cause on the structural level. The template recognition based on profile-profile alignment guided by secondary structure and exposure predictions [26]. Protein sequences requirements were submitted to CPH server to get the model as PDB file.

Available:http://www.cbs.dtu.dk/services/CPHmodels/.

The resultant PDB files were opened using Chimera program which was used to visualize the PDB structure.

UCSF Chimera is a highly extensible program for interactive visualization and analysis of molecular structures and related data. Chimera software was used to scan the 3D (three-dimensional) structure of specific protein, and hence modifies the original amino acid with the mutated one to see the impact that can be produced. The outcome is then a graphic model depicting the mutation. Chimera (version 1.8) currently. Available:(http://www.cgl.ucsf.edu/chimera)

3. RESULTS AND DISCUSSION

3.1 Retrieval of SNPs

The human DPYD gene investigated in this work were retrieved from the NCBI dbSNP database (http://ncbi.nlm.nih.gov/). DPYD gene was containing a total of 273238 SNPs; 99645 SNPs were Homosapien ; of which 534 were missense SNPs. Initially missense coding SNPs were selected for our investigation.

3.2 Predicted Results by SIFT, PolyPhen, and SNAP& GO

Coding SNPs were analyzed using SIFT and Polyphen software. Batch nsSNPs (rs-IDs) were submitted to SIFT and Polyphen server. Out of 534 , 69 SNPs were predicted to be damaging by both servers. Out this 69 SNPs, four SNPs were observed to be associated with clinical presentations ; rs2297595 (M166V), rs55886062 (I560S), rs67376798 (D949V), rs72549306 (V335L).

SNPs that predicted to be deleterious with SIFT, Polyphen; were submitted to SNP&GO, 20 nsSNP (K→F, I→T, K→N, S→R, Y→C, R→W, L→S,R→C, S→L, T→I, L→W, A→D, P→S, G→R, D→N, I→N, D→V, K→E, H→Q, V→F) were predicted to be associated with disease. One SNP, rs67376798 (D949V) was observed to be associated to clinical presentations (Table 1).

The differences in prediction capabilities refer to the fact that every prediction algorithm uses different sets of sequences and alignments.

The 4 nsSNPs that observed to be associated to clinical presentations were selected to predict their stability index and visualize wide and mutant residues in their protein 3D structure .

3.3 Prediction of of nsSNPs Impact on the Protein Stability by I-Mutant 2.0 Server

We submitted independently the four proteins sequence containing missense SNPs which observed to be associated to clinical presentations due to effect of mutation on DPD activity to I-Mutant 2.0 server, all 4 SNPs (rs2297595, rs55886062, rs67376798, rs72549306) were predicted to decrease protein stability, these results demonstrated that all proteins stability were changed due to SNPs alteration (Table 2).

This finding reveal that, the DPYD mutation associated with decrease in DPD capacity to degrade 5FU resulting in excess drug accumulation and therefore toxicity and death, hence the genetic screening for the presence of these mutations in all patients who will receive a 5-FU–containing regimens is essential to reduce the risk of the severe toxicities associated with 5-FU and avoided toxic death due to this drug.

3.4 Protein 3D Structure Modeling by Chimera and HOPE

Protein sequences containing the nsSNPs were again submitted to CPH 3.2 server and HOPE to get the protein 3D structure model, then the CPH 3.2 resultant PDB files were opened using Chimera program to browse and respectively locate the 3D structure of the each protein and to alter the native amino acid with a mutated one, the final outcome was a graphic model depicting the mutation.

rs2297595 (M166V) results in a change in the amino acid Methionine into Valine at position 166. The mutant residue is smaller than the wild-type residue. The mutant will cause an empty space in the core of the protein. This mutant is located in a domain that is important for binding of other molecules (Fig. 1-a/1-c) and (Fig. 1-b/1-d).

'rs55886062 (1560S) in a change in the amino acid leucine into Serine at position 560. The mutant is bigger than the wild-type, the charge of which was neutral, while that of the mutant residue was negative. The wild-type residue is more hydrophobic than the mutant residue. The mutated residue is located in a domain that is important for the activity of the protein and in contact with residues in another domain (Fig. 2-a/2-c) and (Fig. 2-b/2-d).

rs67376798 (D949V) results in a change in the amino acid Aspartic acid into Valine at position 949. There is a difference in charge between the wild-type and mutant amino acid. The charge of the wild-type is lost by this mutation, this can cause loss of interaction with other molecules. The mutent residues is smaller than the wild-type this will cause a possible loss of external interactions. The hydrophobicity of the wild-type and mutant differs. (Fig. 3-a/3-c) and Fig. 3-b/3-d)

rs72549306 (V335L) results in a change in the amino acid Valine into leucine at position 335.The mutant residue is bigger than the wild-type residue. The mutated residue is located in a domain that is important for the activity of the protein and in contact with another domain that is also affect protein function. The wild-type residue was buried in the core of the protein, The mutant residue is bigger and probably will not fit. (Fig. 4-a/4-c) and (Fig. 4-b/4-d).

Table 1. Predicted Results by SIFT, PolyPhen, and SNAP & GO

SNPs RS Number	Amino acids change	SIFT result	Polyphen result	SNP& GO result	SIFT Score	Ployphen score	SNP & GO score
rs367619008	K63E	DELETERIOUS	probably damaging	Disease	0	1	0.657
rs375990187	I97T	DELETERIOUS	probably damaging	Disease	0.003	1	0.596
rs370615432	K114N	DELETERIOUS	probably damaging	Disease	0.002	**1**	0.506
rs72549308	S201R	DELETERIOUS	probably damaging	Disease	0.007	**0.999**	0.724
rs72549307	Y211C	DELETERIOUS	probably damaging	Disease	0.026	**0.999**	0.689
rs1801266	R235W	DELETERIOUS	probably damaging	Disease	0.003	1	0.654
rs150437414	L310S	DELETERIOUS	probably damaging	Disease	0.009	1	0.689
rs143154602	R353C	DELETERIOUS	probably damaging	Disease	0.001	1	0.815
rs72549304	S492L	DELETERIOUS	probably damaging	Disease	0.004	1	0.511
rs112766203	T760I	DELETERIOUS	probably damaging	Disease	0.004	1	0.563
rs200643089	L775W	DELETERIOUS	probably damaging	Disease	0.022	**0.987**	0.657
rs374825099	A777D	DELETERIOUS	probably damaging	Disease	0.005	**0.999**	0.897
rs267598785	P789S	DELETERIOUS	probably damaging	Disease	0.03	1	0.601
rs147800606	G795R	DELETERIOUS	probably damaging	Disease	0	**0.998**	0.83
rs199777072	D829N	DELETERIOUS	probably damaging	Disease	0.004		0.637
rs372307932	I948N	DELETERIOUS	probably damaging	Disease	0.01	**0.968**	0.693
rs67376798	D949V	DELETERIOUS	probably damaging	Disease	0.004	**0.827**	0.839
rs141044036	K958E	DELETERIOUS	probably damaging	Disease	0.001	0.998	0.753
rs201268750	H978Q	DELETERIOUS	probably damaging	Disease	0.039	0.923	0.541
rs1801268	V995F	DELETERIOUS	probably damaging	Disease	0.011	0.591	0.726

Table 2. Prediction result of I-Mutant software

SNP ID	Amino acid Position	WT	MT	pH	Temp (°C)	Stability	RI
rs2297595	166	M	V	7.0	25	Decrease	7
rs55886062	560	I	S	7.0	25	Decrease	9
rs67376798	949	D	V	7.0	25	Decrease	1
rs72549306	335	V	L	7.0	25	Decrease	8

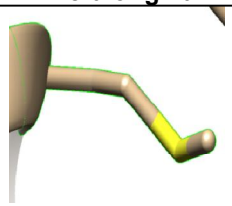
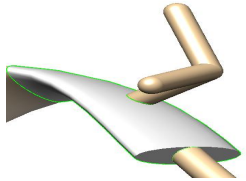
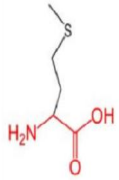
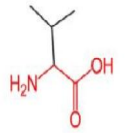
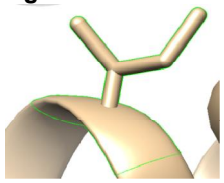
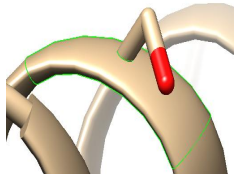
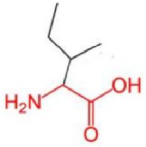
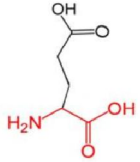
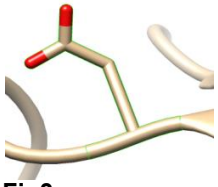
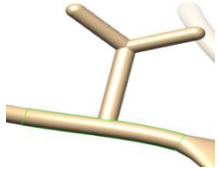
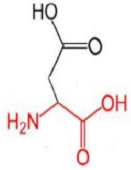
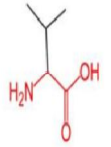
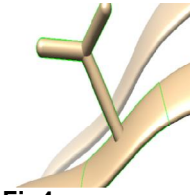
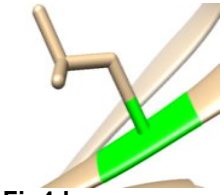
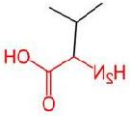
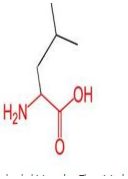
S\NP ID	Chimera original	Chimera mutant	HOPE original	HOPE mutant
rs2297595 (M166V)	 Fig1-a	 Fig1-b	 Fig1-c	 Fig1-d
rs55886062 (I560S)	 Fig2-a	 Fig2-b	 Fig2-c	 Fig2-d
rs67376798 (D949V)	 Fig3-a	 Fig3-b	 Fig3-c	 Fig3-d
rs72549306 (V335L)	 Fig4-a	 Fig4-b	 Fig4-c	 Fig4-d

Fig. 1-4. Schematic structures of the original (a) and the mutant (b) amino acid by Chimera and HOPE

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. In addition the effect of mutant residue on the binding site between different substrates or cofactors and protein also affect in the stability of the protein.

The mutant site for protein plays an important role in how this mutation affects the protein stability. mutant residue can be located on the surface of the protein so mutation can disturb interactions with other parts of the protein, or located in a domain that is important for binding of other molecules, like M166V SNP, and important for activity of the protein and contact with residues in another domain like I560S SNP. Also the wild-type residue can be located in preferred secondary structure of protein , a β-strand. The mutant residue prefers to be in another secondary structure, therefore the local conformation will be slightly destabilized Like V335L SNP.

When the mutant residue is bigger than the wild-type residue, this makes the new residue not in the correct position to make the same hydrogen bond as the original wild-type residue did, like I560S SNP. This mutated residue probably will not fit in the core of the protein. This affects the stability of the protein too. Sometimes mutant residue is smaller than the wild-type residue like M166V and D949V SNPs, thus mutant will cause an empty space in the core of the protein and causeing a possible loss of external interactions.

The hydrophobicity of the Wild-type and mutant residue can differ, like D949V. The charge of the wild-type may be lost by mutation. This can cause loss of interaction with other molecules and this happens, when there is a difference in charge between the wild-type and mutant amino acid. In return when the mutant residue charge is negative and wild-type residue was neutral, The wild-type residue then more hydrophobic than the mutant residue because the new amino acid charge is negative instead of neutral, like I560S SNP.

4. CONCLUSION

This study focus on the most missense SNPs implied in clinical DPD deficiency to investigate the impact of this genetic variation on 3D dimensional structure and function of DPD protein. We observe a range of structural and functional changes caused by single amino acid differences, including changes in protein structural, stability and binding properties associated with the 4nsSNPs (M166V, V335L, I560S, D949V). This can explain the variability in drug response and toxicity in patients who acquire this nsSNPs and treated with 5-FU. Consequently, identification and analysis of nsSNPs in DPYD gene may help in understanding their effects on DPD enzyme and their association with diseases and drug response also could help in the development of new medical testing markers and individualized medication treatment to reduce the risk of early severe life-threatening toxicities and death associated with 5-FU–based therapy.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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