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Original Research Article

Comprehensive Data Analysis of Single Nucleotide Polymorphism (Snps) in Human *FOXP3* Gene

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Abstract

Background: The *FOXP3* gene provides instructions for producing the forkhead box P3 (FOXP3) protein. The FOXP3 protein attaches (binds) to specific regions of DNA and helps control the activity of genes that are involved in regulating the immune system. ***Aim:*** This study intended to analyze SNPs in *FOXP3* gene in the coding region, 5`UTR and 3`UTR using bioinformatics tools and visualizing the 3D structure of the gene. ***Methods:*** SNPs were retrieved from NCBI, and the protein sequences were retrieved from UniprotKB. SIFT and Provean software was used to predict whether the nsSNPs were tolerated or deleterious. Polyphen- 2 for nsSNPs function prediction on the produced protein.I-mutant and Mupro software to check the protein stability, finally PHD software to see whether the nsSNPs were related to a disease or not.Regulomedb to predict the regulatory element for 5`UTR region. For the 3`UTR PolymiRTs database to predict the variation in microRNA (miRNA). FuncPred software to show the unknown function for the selected SNPs.TheI-TASSER to visualize the 3D structure. ***Results:*** A total of 205 SNPs were retrieved, 180 SNPs in the CDS region, 20 in 3`UTR, 5in 5`UTR. SIFT prediction resulted in 93 deleterious and 87 toleratednsSNPs, Proveanrevealed60 deleterious and 120 tolerated,Polyphen-2 resulted in 29 probably damaging,11 possiblydamaging, 3 benign and 2 no result. I-mutant showed 12nsSNPs increasing, and 25 decreasing the protein stability, MUpro resulted in 3nsSNPs increasing the protein stability and 42 decreasing the protein stability, PHD resulted in 17 nsSNPs as disease related and 28 as neutral. For noncoding SNPs, Regulomedb resulted in 11 SNPs, PolymiRTs result in 11, and FuncPred result. ***Conclusion****:* The analysis of *FOXP3* gene showed that from 205 nsSNPs only 11 SNPs are highly damaging and 20 SNPs found in the 3UTR region which predicts its function using different methods. Still bioinformatics software have their limitations, the results from this study may be convenient in future for further population based research activities and towards development of perfect medicines.

Keywords: *FOXP3* gene, FuncPred, I-Mutant, I-Tasser, MUpro, Polyphen-2, Provean, ProjctHope,PolymiRTs, SIFT, SNPs, Regulomedb.

INTRODUCTION

The FOXP3 gene provides instructions for producing the Forkheadbox P3 (FOXP3) protein [1]).The FOXP3 protein binds to specific regions of DNA and helps control the activity of genes that are involved in regulating the immune system [2]. On the basis of its role in controlling gene activity, the FOXP3 protein is called a transcription factor [3].This protein is essential for the production and normal function of T cells, which play an important role in preventing autoimmunity [3].The FOXP3 protein is found primarily in the thymus, where these regulatory T cells are produced [4].The gene have many clinical condition related to the gene mutations including, immunedysregulation, polyendocrinopathy, enetropathy, X-linked syndrome and type 1diabetes [5]. Defects in the number and function of Tregcellsintheperipheralbloodandlesionalskinaccentuate theroleofTregcellsinvitiligo pathogenesis [6]. The crucialmaster transcription factor that regulates the fate and identity of Tregcellswhichplayanimperativeroleinpreventingautoimmunityisforkhead box protein3 (FOXP3). This keyimmuno-regulatory FOXP3 gene belongs to for khead/winged- helix transcription factor family, locatedatXp11.23inhumansis1296bpinlengthandconsists of 11translatedexonsthatencodeaproteinwith431amino-acids.ItcontainsaprolinerichN- terminal repress or domain that suppressestheexpressionoftargetgenes,

azinc-finger (amino acid 200–223), andleucine-zippermotif (aminoacids240–261) that allow FOXP3homo-or hetero dimerizationandaconserved DNA bindingorkhead domain (aminoacid 338–421). FOXP3 geneis vital for the development, proliferation, and function of CD4+ CD25+ Tregcellsandthereforeessentialforthemaintenanceofimmunehomeostasis [7].

MATERIAL AND METHODS

In this study the SNPs were retrieved from the NCBI dbSNP Nov.2020 (https://www.ncbi.nlm.nih.gov/). Computational analyses were applied for the coding, 5'UTR and 3'UTR regions.

Coding region

SIFT: Sorting Intolerance from Tolerant (https://sift.bii.a-star.edu.sg/) it predicts whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids. (SIFT can be applied to naturally occurring non-synonymous polymorphisms and laboratory-induced missense mutations [8].

Provean

Protein Variation Effect Analyzer (http://provean.jcvi.org/index.php) is a software tool which predicts whether an amino acid substitution or indel has an impact on the biological function of a protein. [9].PROVEAN is useful for filtering sequence variants to identify non -synonymous or indel variants that are predicted to be functionally important.[9] The performance of Provean is comparable to popular tools such as SIFT or PolyPhen-2 (.A fast computation approach to obtain pairwise sequence alignment scores enabled the generation of precomputed PROVEAN predictions for 20 single AA substitutions and a single AA deletion at every amino acid position of all protein sequences in human and mouse [9].

Polyphen- 2: Polymorphism Phenotyping v2 (http://genetics.bwh.harvard.edu/pph2/) is a tool which predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations [10].

I-Mutant: It is a suite of Support Vector Machine based predictors integrated in a unique web server [11]. (http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi).

It offers the opportunity to predict automatically protein stability changes upon single-site mutations starting from protein sequence alone or protein structure when available [12]. Moreover it gives the possibility to predict human deleterious Single Nucleotide Polymorphism starting from the protein sequence alone [13]. Users can choice among three different predictors [14].

MUpro: (http://mupro.proteomics.ics.uci.edu/) is a set of machine learning programs to predict how single-site amino acid mutation affects protein stability. We developed two machine learning methods: Support Vector Machines and Neural Networks. Both of them were trained on a large mutation dataset and show accuracy above 84% via 20 fold cross validation, which is better than other methods in the literature. One advantage of our methods is that they do not require tertiary structures to predict protein stability changes. Our experimental results show that the prediction accuracy using sequence information alone is comparable to that of using tertiary structures. So even you do not have protein tertiary structures available, you still can use this server to get rather accurate prediction. Of course, if you provide tertiary structures, our methods will take advantage of them and you might get slightly better predictions [15].

PHD-SNP: predictor of human single nucleotide polymorphism (https://snps.biofold.org/phd-snp/phd-snp.html) s based a SVM-based classifier [17]In the new version we developed a predictor based on a single SVM trained and tested on protein sequence and profile information [15]

HOPE (Have (y) Our Protein Explained) (http://www.cmbi.ru.) is an automatic mutant analysis server to analyze the structural effects of intended mutation. It provides the 3D structural visualization of mutated proteins, and gives the results by using UniProt and DAS prediction servers. The input for Project HOPE was the protein sequence and selection of the wild and mutant variants. The server predicts the output in the form of structural variation between mutant and wild type residues [18].

Non coding region

Regulomedb:(https://regulomedb.org/regulome-search/) is a database that annotates SNPs with known and predicted regulatory elements in the intergenic regions of the H. sapiens genome. Known and predicted regulatory DNA elements include regions of DNase hypersensitivity, binding sites of transcription factors, and promoter regions that have been biochemically characterized to regulate transcription. Sources of these data include public datasets from GEO, the ENCODE project, and published literature [19].

SNP function prediction: (Func Pred)

https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html). Th is a database web server in Asian population. This webserver helps in selecting SNPs for genetic association studies and consists of 3 pipelines ESE, EST finder and FAS – ESS. miRanda and miRBase. A user can query results for all the SNPs in a gene or chromosomal region or for a list of input SNPs for the

present study a list of SNP rsID was uploaded for analysis with default settings. The output was a list of SNPs with possible functional effect [20].

PolymiRTs:(http://compbio.uthsc.edu/miRSNP/) is an integrated database that systematically identifies DNA polymorphisms in miRNAs and miRNA target sites (PolymiRTs) it also elucidate the potential links of SNPs to molecular, physiological behavioral and disease phenotypes. The computational server was used in order to determine the effect of 3UTR SNPs of foxp3 gene in creating and abolishing functional consequences [21].

3D structure

Projct Hope: HOPE collects structural information from a series of sources, including calculations on the 3D protein structure, sequence annotations in Uniprot KB and prediction from the Reproof 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. HOPE will show the effect of that mutation in such a way that even those without a bioinformatics background can understand it. The fact that a 3D analysis of a mutated protein structure can contribute to a wide range of research fields is underlined by earlier projects in which the structural analysis was performed manually [22].

I-TASSER: Iterative Threading ASS Embly

(https://zhanglab.ccmb.med.umich.edu/I-TASSER/**)** is a hierarchical approach to protein structure prediction and structure-based function annotation [23]. It first identifies structural templates from the PDB by multiple threading approach LOMETS, with full-length atomic models constructed by iterative template-based fragment assembly simulations [24]. Function insights of the target are then derived by re-threading the 3D models through protein function database BioLiP. I-TASSER (as 'Zhang-Server') was ranked as the No 1 server for protein structure prediction in recent community-wide CASP7, CASP8, CASP9, CASP10, CASP11, CASP12, and CASP13 experiments. It was also ranked the best for function prediction in CASP9. The server is in active development with the goal to provide the most accurate protein structure and function predictions using state-of-the-art algorithms [25].

RESULTS

A total of 205 SNPs were retrieved, 180 SNPs in the coding CDS region, 20 in 3`UTR, 5in 5`UTR.

Coding Region

SIFT prediction resulted in 93 deleterious and 87 toleratedns SNPs, Proveanrevealed 60 deleterious and 120 tolerated (Figure 1).The double positive deleterious from the two previous software when submitted to Polyphen-2 resulted in 29 probably damaging, 11 possiblydamaging, 3 benign and 2 no result. I-mutant showed 12nsSNPs increasing, and 25 decreasing the protein stability, MUpro resulted in 3nsSNPs increasing the protein stability and 42 decreasing the protein stability (Figure 2). PHD resulted in 17 nsSNPs as disease related and 28 as neutral. The 17 SNPs that predicted to cause disease was investigated using project hope software so as to know more information about the mutations leading to disease. The results of Projct Hope were show in Table 1.

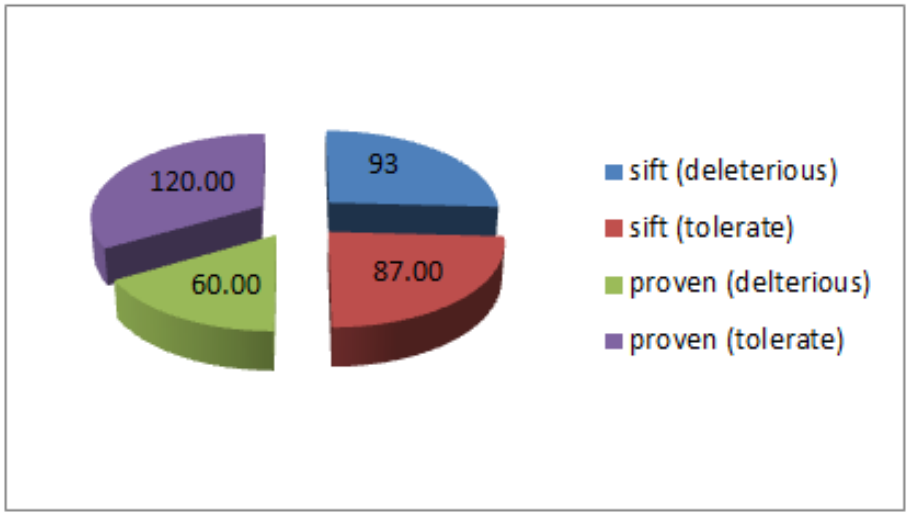

Fig-1: Showing the results of SIFT and Provean software

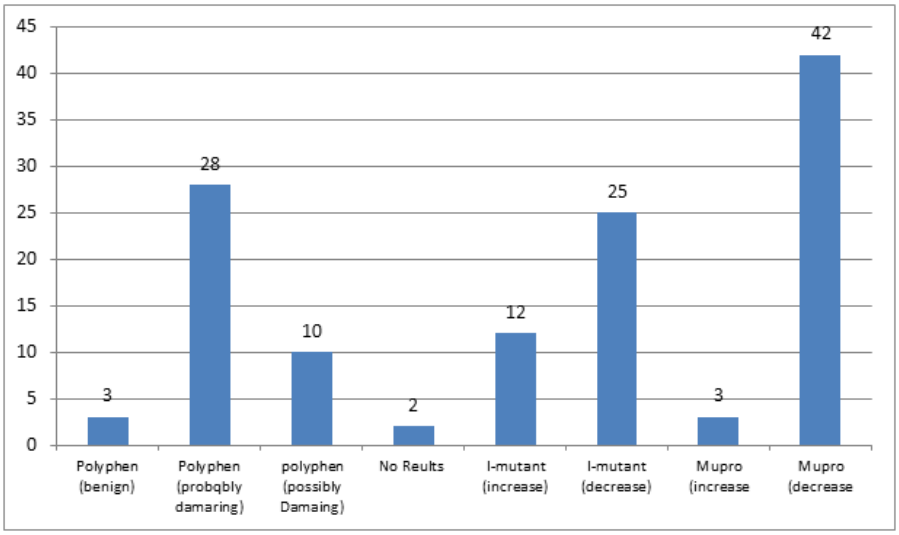

Fig-2: Showing the results of Polyphen-2, I-Mutant and MUpro software

Projct Hope results

The SNP rs2232369 (G220V) amino acid Glycine was converted into valine, the mutant reside valine is bigger than the wild type residue Glycine and the mutant type is more hydrophobic than the wild type. Only this residue type was found at this position. Mutation of a 100% conserved residue is usually damaging for the protein. The big size of mutant type lead to bumps the torsion angles for this residue are unusual. Only glycine is flexible enough to make these torsion angles, mutation into another residue will force the local backbone into an incorrect conformation and will disturb the local structure. This SNP was screened for multiple sclerosis (MS) patients [26] and resulted in the fact that there is no association for all patients. The results were shown in figure -3.

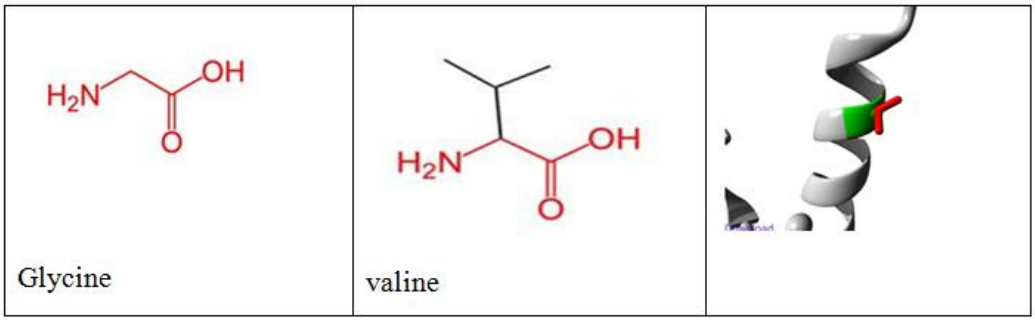

Fig-3: Wild. Mutant and 3D structure of rs2232369 (G220V)

The rs28935477 (R407W) the arginine converted into tryptophan and the mutant residue is bigger than wild and the wild type charge positive and mutant type is neutral and this lead to charge of the wild-type residue will be lost, this can cause loss of interactions with other molecules or residues the mutant is more hydrophobic than the wild type. Which can result in loss of hydrogen bonds and/or disturb correct folding? Mutation is 100% conserve in this position. The results were shown in figure-4.

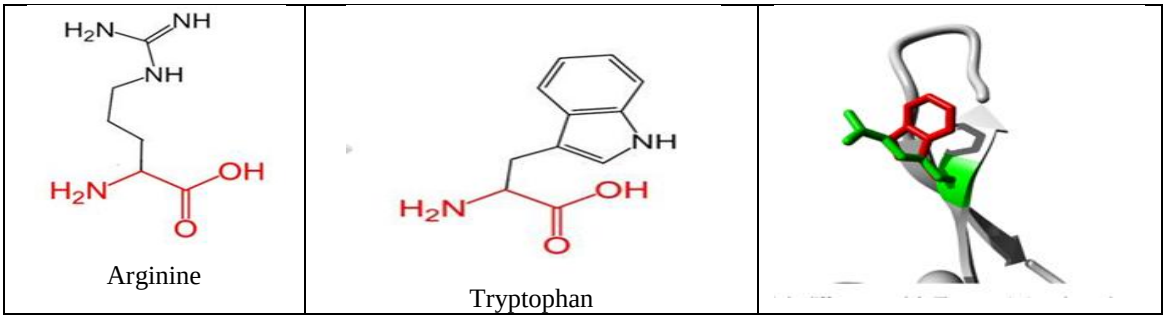

Fig-4: Wild. Mutant and 3D structure of rs28935477 (R407W)

The rs28935477 (R370W) also Arginine was converted into tryptophan, tryptophan is bigger than arginine, Arginine with positive charge and tryptophan is neutral tryptophan is also more hydrophobic than arginine. The mutated bigger size can disturb multimeric interaction also more hydrophobicity of arginine cause multimeric contacts affects. A mutation to "Histidine" was found at this position. The effect of this variant is annotated as: R -> H (in IPEX; mild phenotype; no loss of protein expression; impairs its ability to confer inhibitory function to regulatory T-cells; no loss of DNA-binding when associated with A-373) the mutation is located in a region with known splice variants, described as:

R -> H (in IPEX; mild phenotype; no loss of protein expression; impairs its ability to confer inhibitory function to regulatory T-cells; no loss of DNA-binding when associated with A-373). The wild-type residue is not conserved at this position. Another residue type was observed more often at this position in other homologous sequences. This means that other homologous proteins exist with that other residue type than with the wild-type residue in your protein sequence. Therefore the mutation is damaging and conserve. The results were shown in figure-5.

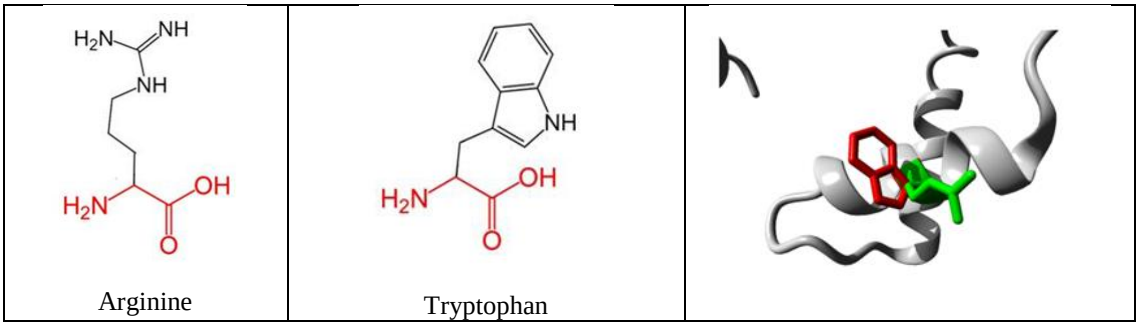

Fig-5: Wild. Mutant and 3D structure of rs28935477 (R370W)

rs122467170 (R384T) alanine into threonine with different position show the mutant type is bigger and wild type with more hydrophobicity This mutation matches a previously described variant, with the following description: A -> T (in IPEX; no loss of protein expression The mutation is located in a region with known splice variants. The results were shown in figure-6.

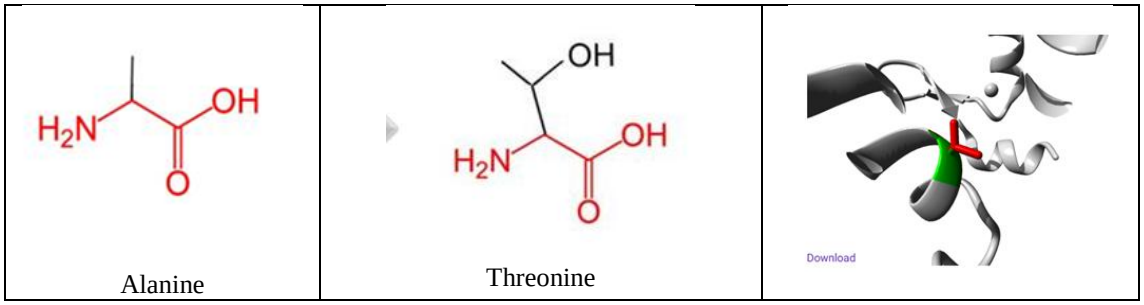

Fig-6: Wild. Mutant and 3D structure of rs122467170 (R384T)

Rs122467170 (A349T) also alanine into threonine in this different position the mutant residue is bigger than the wild type one the wild type is more hydrophobic and it show different interactions due to this mutation. The results were shown in figure-7.

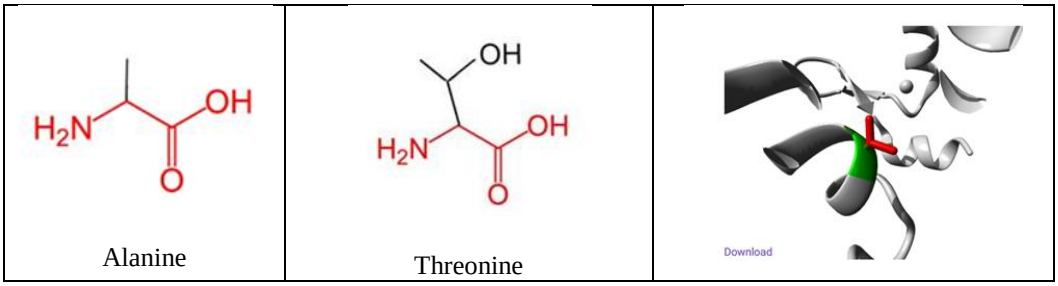

Fig-7: Wild. Mutant and 3D structure of rs122467170 (A349T)

Rs122467170 (E409T) which aspartic acid converted into threonine at this position the mutant residue is smaller than the wild one and the wild type charge is positive and the mutant one is neutral mutant type is more hydrophobic than the wild residue. The wild-type residue forms a hydrogen bond with Glutamic Acid at position 412. The size difference between wild-type and mutant residue makes that the new residue is not in the correct position to make the same hydrogen bond as the original wild-type residue did. The difference in hydrophobicity will affect hydrogen bond formation. The wild-type residue forms a salt bridge with: Arginine at position 414 and Lysine at position 415.

The difference in charge will disturb the ionic interaction made by the original, wild-type residue. The results were shown in figure-8.

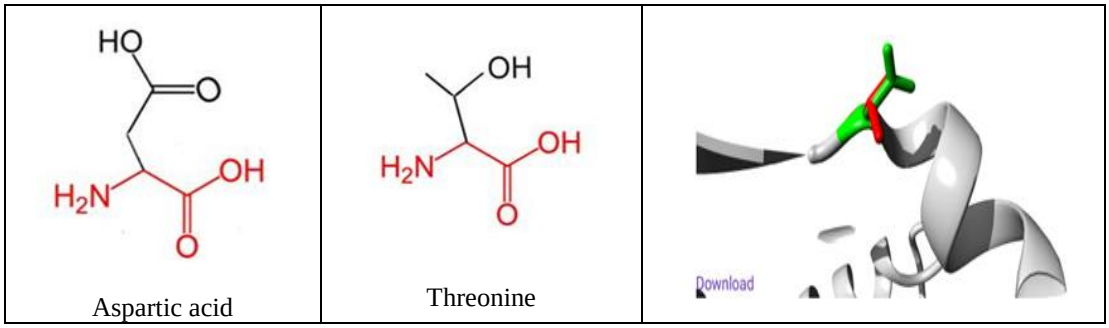

Fig-8: Wild. Mutant and 3D structure of rs122467170 (E409T)

Rs145368924 (R427W) the Arginine mutated into tryptophan the mutated is bigger than the wild residue wild type is positive were the mutant one is neutral the mutant is more hydrophobic than the wild one and mutation with highly conserve. The results were shown in figure-9.

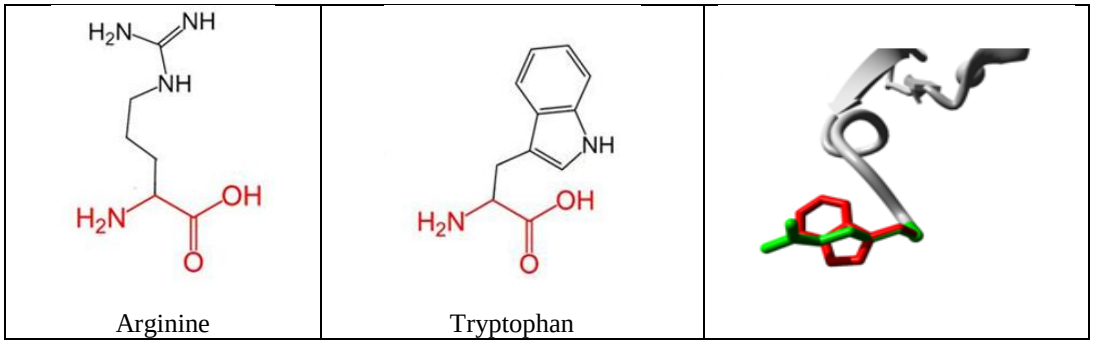

Fig-9: Wild. Mutant and 3D structure of rs145368924 (R427W

Rs145368924 (K382W) also lysine into tryptophan at this position the mutant type is bigger than the wild type , wild type is positive and the mutant type is neutral the mutant is more hydrophobic than the wild residue. The results were shown in figure 10.

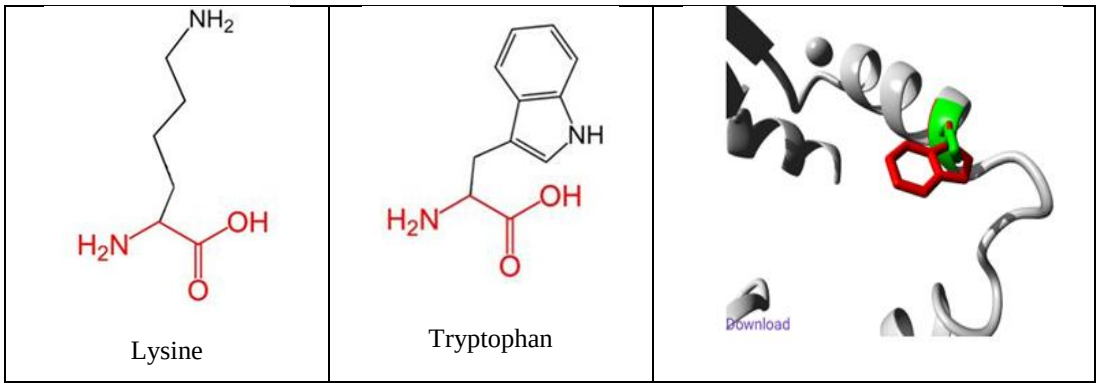

Fig-10): Wild. Mutant and 3D structure of rs145368924 (K382W)

Rs145368924 (L417W) Phenylalanine is converted into tryptophan at this position the mutant residue is bigger than the wild residue In the PDB file used for this analysis the mutated residue is involved in

a multimeric contact. (Note that PDB files sometimes contain crystallographic contacts that are not biologically relevant.). The mutation introduces a bigger residue at this position; this can disturb the multimeric interactions. The results were shown in figure-11.

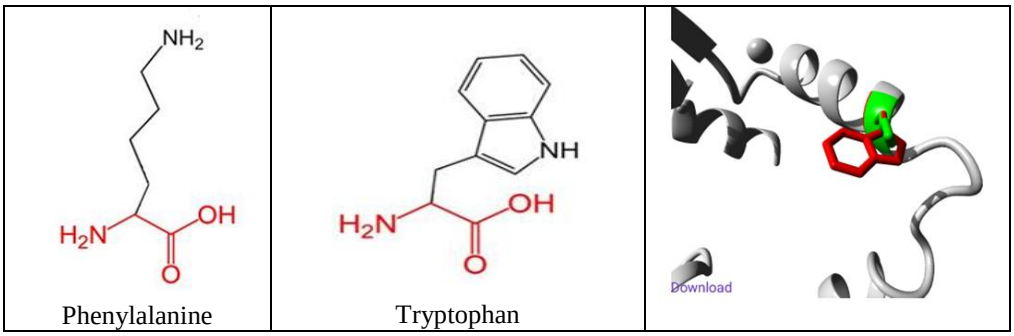

Fig-11: Wild. Mutant and 3D structure of rs145368924 (L417W)

Rs145368924 (F390W) lysine is converted into tryptophan the mutant one is bigger than the wild residue and the wild residue is positive were the mutant one is neutral the mutant is more hydrophobic than the wild one. The results were shown in figure-12.

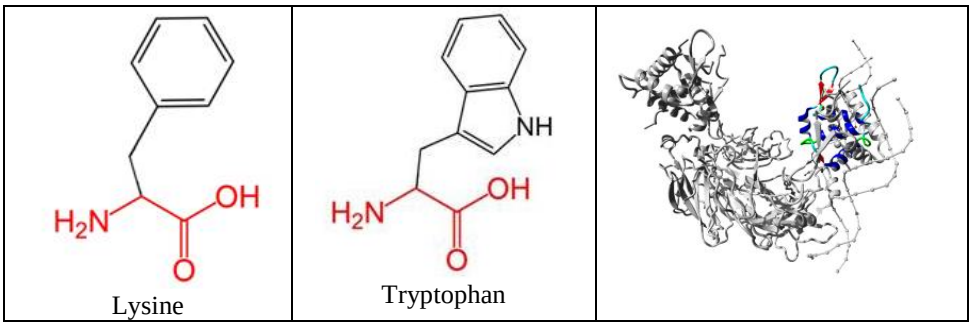

Fig-12: Wild. Mutant and 3D structure of rs145368924 (F390W)

rs145368924 (K382W) Glutamic acid is converted into Tryptophan the mutant residue is bigger than the wild residue wild type is positive were the mutant type is neutral the mutant residue is more hydrophobic than the wild residue the mutation is highly conserve at this position.

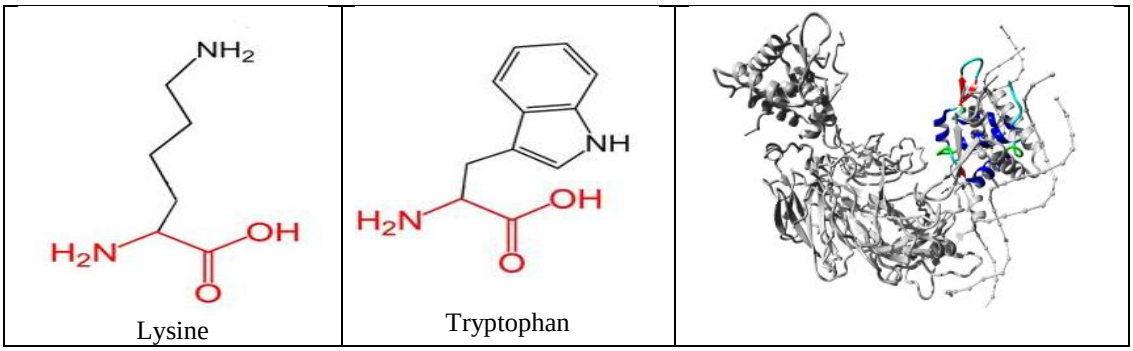

Fig-13: wild. Mutant and 3D structure of rs145368924 (K382W)

Rs200928046 (E272W) glutamic acid converted into tryptophan at this position the mutant type is bigger than the wild type and the wild type is positive in charge but the mutant residue is neutral the mutant residue is more hydrophobic than the wild one.

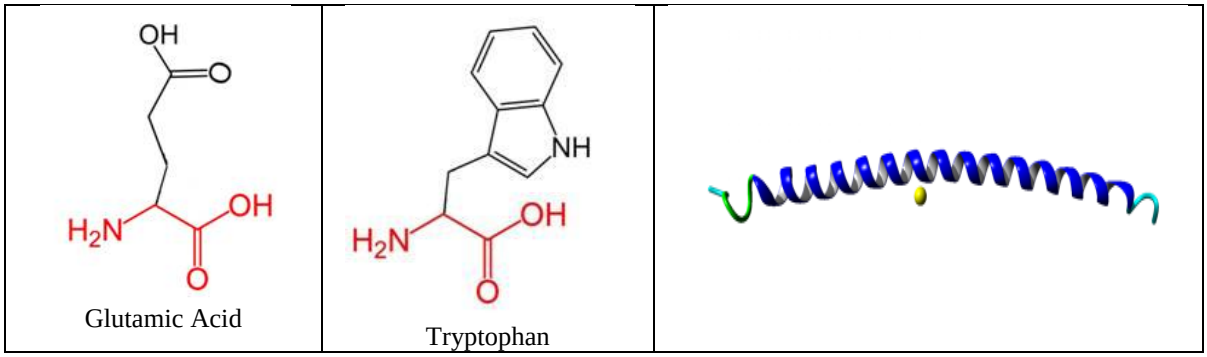

Fig-14): wild. Mutant and 3D structure of rs200928046 (E272W)

Rs368066149 (C154Y) cysteine is converted into tyrosine the mutant type is bigger than the wild and the wild residue is more hydrophobic than the mutant residue and the mutation located in highly conserve position.

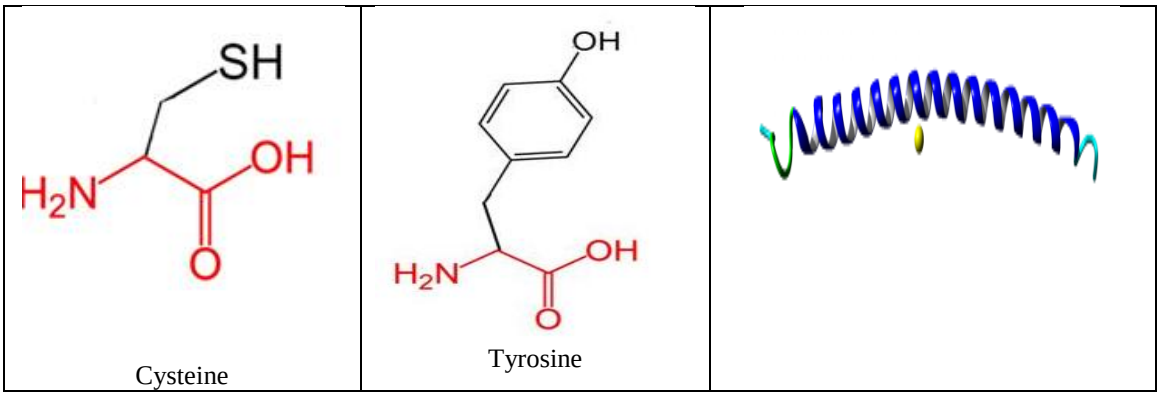

Fig-15: Wild. Mutant and 3D structure of rs368066149 (C154Y)

Rs368066149 (C204Y) also Cysteine converted into tyrosine at this position the mutant is bigger than the wild residue and the wild is more hydrophobic than the mutant type also mutation located in highly conserve position.

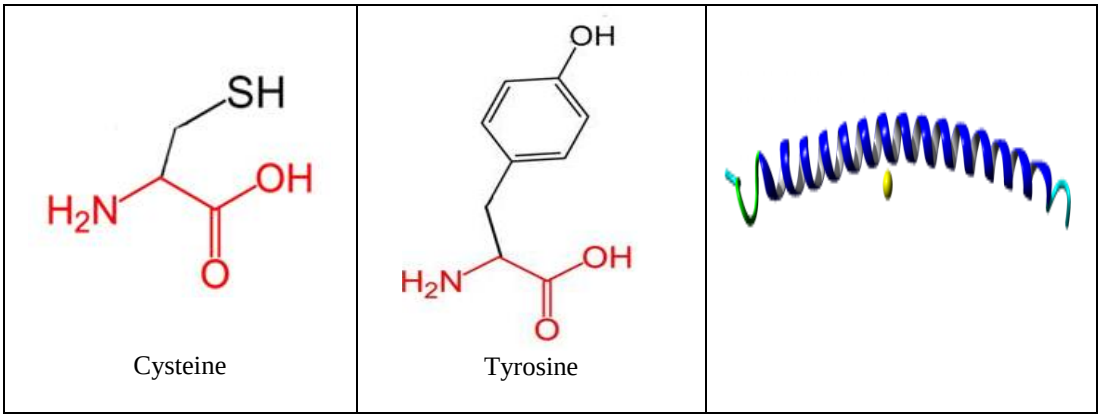

Fig-16: Wild. Mutant and 3D structure of rs368066149 (C204Y)

Rs369332983 (L391K) leucine converted into lysine the mutant type is bigger than the wild one and the wild residue is neutral were the mutant one is positive, the wild residue is more hydrophobic than the mutant residue The mutated residue is not in contact with a metal, however, one of the neighboring residues does make a metal-contact that might be affected by the mutation in its vicinity.

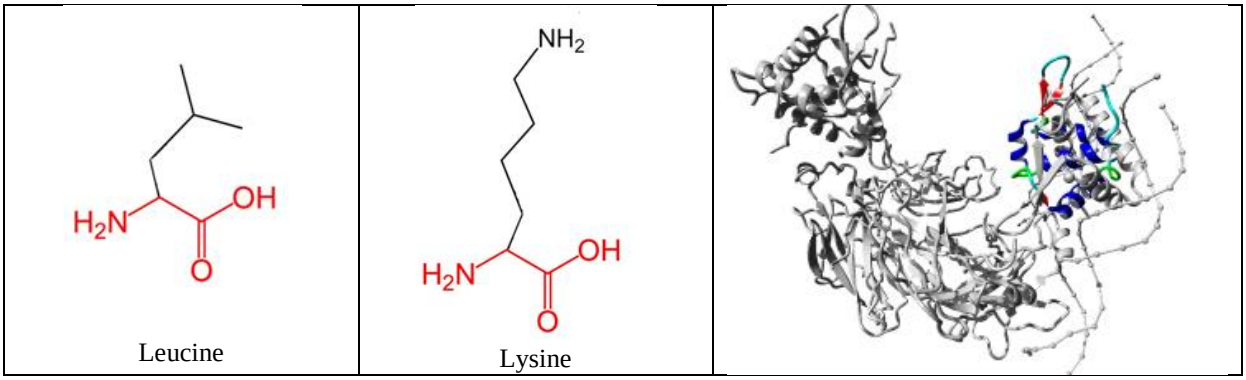

Fig-17: wild. Mutant and 3D structure of rs369332983 (L391K)

Rs373242883 (W224V) Tryptophan is converted into valine the mutant type is smaller than wild type and the mutation located in very high conserve position. The results were shown in figure-18.

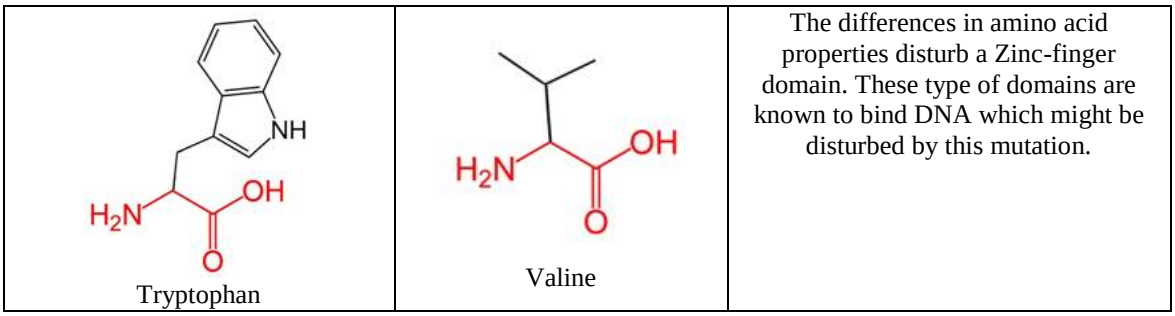

Fig-18: wild. Mutant and 3D structure of rs373242883 (W224V)

Non- coding region

The total SNPs of FOXP3 gene in the 3`UTR region were found to be 21, using Regulomedb database 11 SNPs rank 5 and score range between 0.0 to 0.5 with TF binding and DNase peak that all 11 SNPs in category 5 (TF binding or DNase peak).The results were shown in table 2.

Table-2: Results of Regulomedb database for FOXP3 gene in the 3`UTR region

SNPs	Non conserve	rank	score	Description
rs11798996	UTR_3	5	0.02119	TF binding +Dnase 9 peaks
rs55827755	UTR_3	5	0.13454	TF binding +Dnase 15 peaks
rs56066773	UTR_3	5	0.39299	TF binding +Dnase 41 peaks
rs56066773	UTR_3	5	0.39299	
rs56159317	UTR_3	5	0.13454	TF binding +Dnase 18 peaks
rs56159317	UTR_3	5	0.13454	
rs56232250	UTR_3	5	0.13454	TF binding +Dnase 15 peaks
rs56232250	UTR_3	5	0.13454	
rs56355628	UTR_3	5	0.51006	TF binding +Dnase 22 peaks
rs56355628	UTR_3	5	0.51006	
rs148013438	UTR_3	5	18068	TF binding +Dnase 17 peaks
rs148013438	UTR_3	5	0.18068	
rs181219991	UTR_3	5	0.13454	TF binding +Dnase 15 peaks
rs181219991	UTR_3	5	0.13454	
rs181219991	UTR_3	5	0.13454	
rs186528542	UTR_3	5	0.13454	TF binding +Dnase 16 peaks
rs186528542	UTR_3	5	0.13454	
rs199701533	UTR_3	5	0	TF binding +Dnase 21 peaks
rs374374507	UTR_3	5	0.13454	TF binding +Dnase 12 peaks

Using Polymi RTs software the SNP rs199701533 with location 49106747 in mRNA transcript, the SNP cant form a G:U wobble base pair with mi RNA, this SNP with ancestor allele C and two C and T allele in mRNA, the miRD ID is link to miRBase which have 11 in number, the conservation in

allele C there are2, 3, 8 and 12 and in allele T 3, 8, 21 this conservation number show us the occurrence of the miRNA site in other vertebrate genome which in this study is human. The allele C have function class D that derived allele disrupt a conserved miRNA site (ancestral allele with support >=2) and the T allele have class C that show the ancestral allele create a new miRNA site. The predicted miRNA target site with no experimental support.

The FuncPred database showed 11 SNPs presenting their function category and description show the result of Func Pred. Using FuncPred software from 14 SNPs that represents the 5 and 3UTR SNPs the total of 6 SNPs were presented all are located in chromosome X with no effect due to its position in non-transcription factors binding site (TFBS). The rs11798996 SNP are located in splicing site that is located at 2 base pair of intron – exon junction region, exonic splicing enhancer (ESE), or exonic splicing silencer (ESS) which it may disrupt splicing activity and cause alternative splicing, also this SNP abolish domain. All SNPs cause miRNA that can inhibit protein translation through binding to the end of a messenger RNA. Also using Sanger method of miRNA showed three SNPs only from the total of 6 SNPs and they are rs11798996, rs55827755 and rs56355628. Thers55827755, rs56159317 and rs56232250 SNPs were found in nearby gene CCDC22 of foxp3 gene. The conservation score showed 0.005 that mean the SNPs are in non-coding region because higher score are for coding one. The rs11798996 SNP have regulatory potential score of 0.072699 that showed present of SNP in non-coding region because high regulatory potential score show the location of SNP in coding region.

Modeling of FOXP3 gene

Using I-TASSER the sequence of the protein was modeled, the result shows the 3D structure of protein representing the modeling side (Figure 19).

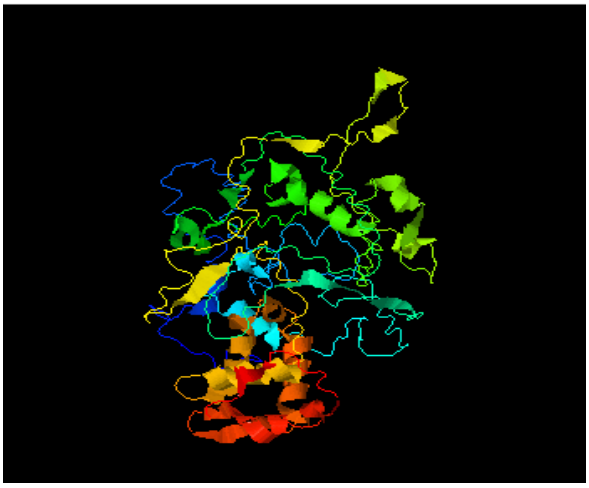

Fig-19: Modeling of FOXP3 gene using I-TASSER

In this study the total deleterious SNPs were 17. The rs2232369 had been studied in relation to multiple sclerosis disease in the FOXP3 gene but the result showed that no relation was detected[27], also rs28935477 and rs122467170 were found to be pathogenic and had a relation with insulin dependent diabetes mellitus [28]. The other 5 nsSNPs were not studied before and their relationdiabetes as an autoimmune disease was not clarified. These SNPs were considered as novel according to this study.

In the non-coding regions the SNP rs2232369rs56066773 in the 3`UTR was studied for its relation torheumatoid arthritis which is an autoimmune disease in which the number of or function of regulatory T cell (Treg) wasimpaired; FOXP3 is one of the major factor of Tregfunction. ThemicroRNA which binds to 3`UTR.A nucleotide substitution in the sequence of the target site of microRNA can affect the regulation of microRNA. Polymorphisms of rs56066773 and rs56232250 in the 3´UTR of gene FOXP3 can berelated to rheumatoid arthritis through the target gene. The mentioned study was aimed to investigate the relationship between two polymorphisms with rheumatoid arthritis and they found no significance relation between two polymorphisms and the disease[29].The SNP rs148013438 was been studied in astudy that link between autoimmune disease and allergy in children because sometimes the two disease can develop in the same patient the study resulted in frequent appearance of the SNP in numerous male patients sample indication of linking between the two disease [30].

CONCLUSION

The analysis of FOXP3 gene showed that from 180nsSNPs only 17 SNPs were highly damaging affecting the protein structure, function and stability. For the expression 20 SNPs were found in the 3`UTR region which predicts its function using different methods. Still bioinformatics software have their limitations, the results from this study may be convenient in future for further population based research activities and towards development of perfect medicines.

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