COMPUTATIONAL MINING AND MOLECULAR DOCKING STUDIES ON SNP IN HUMAN FGF20 GENE AGAINST PARKINSON DISEASE

[1]Jeyabaskar Suganya, [*1]Mahendran Radha, [2]Abinaya B.
[1]Assistant Professor, [*1]Professor, [2]Student
[1]Department of Bioinformatics,
[1]School of Life Sciences, VISTAS, Pallavaram, Chennai-600117, Tamil Nadu, India.

ABSTRACT:

Parkinson Disease is the second neurodegenerative disease which mainly affects the motor system and cause of the disease is still unknown. Till today there were no proper medications for treating Parkinson Disease. The recent research on Parkinson Disease revealed that the Single Nucleotide Polymorphism (SNPs) which occur in the genes or protein was one of the main responsible for causing genetic PD. On detailed study of the genes which responsible for causing PD, the gene FGF20 is accountable for the depletion of dopamine in the brain. Dopamine is one of important neurotransmitter, which plays a vital brain functions by transmitting signals in between the neurons present in the brain. If any deficiency in the dopamine level in the brain lead to neurodegenerative disease (PD). In this current study, the attempt was made to analysis the SNP which is present in the FGF20 gene through *in silico* SNP Analysis and Prediction tools. The deleterious SNP present in the FGF20 gene was predicted by above tools and using Swiss PDB Viewer the mutated protein was reverted to normal protein. Further using Hex software, molecular docking was performed for normal protein against currently used drugs for Parkinson disease. On analyzing the docking interaction it was predicted that the normal protein shows best with the drugs. This study can be further implemented for the clinical trials to find out the treatment by altering the mutation in the gene and to determine the amount of dosage with this new protein-drug complex for safety level.

Keywords: Genetic Parkinson's disease, FGF20, SNP analysis, Swiss PDB viewer, Docking.

1. INTRODUCTION:

Parkinson's disease (PD) is a chronic degenerative disorder caused by destruction of dopamine producing neurons in substantia nigra of brain [1]. Parkinson disease is also called a parkinsonian syndrome due to genetic origin and idiopathic [2]. Clinical signs of Parkinson's are ruled out when about 80% of the dopamine-producing neurons are lost and rest of 20% is due to mutations in the genes regarding to the Genetic Parkinson disease [3]. The symptoms of Genetic Parkinson disease is same as idiopathic Parkinson disease which affects the functions of the bodies, including some of the routine activities which are controlled by the mechanism of dopamine [4]. In worldwide, it is estimated that around 6.3 millions of people, 2.4 million people were affected by Genetic Parkinson disease and rest were affected by the idiopathic and other types of Parkinson disease [5]. On statistical analysis, Genetic Parkinson Diseases were identified that 1 out of100 people are affected by Parkinson's before they reach 50 years [6].

The recent studies on PD exposed that disease could also be occurred due to sudden change or alteration in the specific genes which was responsible for the production of dopamine in the substantia nigra of the brain and if there was any reducing the production of the dopamine level could leads to parkinson's disease [7]. The further analysis it was recognized that Fibroblast growth factor (FGF) genes was one of the important gene coding protein were responsible for causing PD for most of the individuals [8].

The FGF gene was subdivided into 22 protein families with various biological and physiological deeds in human body and on scrutinizing FGF family it was recognized that FGF20 plays a major role in production of dopamine (neurotransmitters) neurons [9]. The survey on genetic PD revealed that there were strong genetic association has been identified between FGF20 gene and Parkinson's disease [10, 11]. FGF20 is 9.3 KB in size and located in the chromosome89. Researcher have found the genetic variation in the gene were mainly due to occurrences of Single nucleotide polymorphism (SNP) [12]. They also revealed that SNP may help in predicting an individual's response towards certain medications, its susceptibility to environmental factors thereby developing risk in causing particular genetic diseases [13, 14].

The currently available medications for PD are levodopa, amphetamine, amineptin, altropane and modafinil [15]. These drugs are normally increases the production dopamine level in the brain and specifically reduce the extracellular dopamine levels. These above prescribed drug activate the enzymes like COMT (catechol-O-methyltransferase), MAO (Monoamine oxidases) which increases the domain level in the brain cells. The enzyme COMT metabolizes or degrades dopamine and the enzyme MAO-B plays a vital role in breakdown of dopamine level in neuron [16]. These drugs also controls the symptoms of the PD, their by preventing many common side effects such as nausea, vomiting, and irregular heart rhythms [17].

In the current study, the candidate gene of PD which encoded protein was analyzed using Bioinformatics databases. The SNPs (mutation) present in the protein which is responsible for neurodegenerative disease was identified. The mutation was reverted to normal protein using Bioinformatics tools and further the targets were docked with current available drugs which are normally used to increase the dopamine levels.

2. MATERIALS AND METHODS:

Retrieving sequence of FGF 20:

The Gene FGF20 of Homo sapiens was identified from NCBI databases and the protein sequence of FGF20 of Homo sapiens was retrieved from SWISSPROT databases.

Analysis of FGF 20:

1. ProtParam Tool: (https://web.expasy.org/protparam/)

ProtParam is a tool for computing the various physical and chemical properties for a target protein. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY). The physiochemical parameters of the protein sequence were analyzed using PROTPARAM tool [18].

2. NCBI- dbSNP: (https://www.ncbi.nlm.nih.gov/projects/SNP/)

The database classifies target nucleotide sequence variations along with the following types and % composition of the database: single nucleotide substitutions (99.77%), small insertion/deletion polymorphisms (0.21%), invariant regions of sequence (0.02%) microsatellite repeats (0.001%), named variants and uncharacterized heterozygous assays (<0.001%). The dbSNP database shows the disease-causing clinical mutations as well as neutral polymorphisms which is present in the gene. The list of SNP'S (rs ID) present in the gene was predicted by submitting gene ID using NCBI- dbSNP [19].

3. SIFT dbSNP: (https://sift.bii.a-star.edu.sg/www/SIFT_dbSNP.html)

SIFT dbSNP predictions mutation for a list non synonymous SNPs (rsIDs) from NCBI's dbSNP (build 135) database. Either all BLAST hits can be used in the protein alignment or the top hit to each organism. The result of NCBI- dbSNP was submitted to SIFT dbSNP for the detection the amino acid substitution in the gene which affects the protein function [20].

Prediction non-synonymous SNP:

1. SIFT PREDICTION: (https://sift.bii.a-star.edu.sg/)

Sorting Intolerant from Tolerant (SIFT) is a tool that predict an amino acid substitution that influence the protein function which can be designate a substitution for further research . SIFT has been converted into one of the customary tools to distinguish missense variation. SIFT create an MSA and make use of sequence homology to compute the likelihood that an amino acid substitution will have an undesirable effect on protein function [20].

2. SNAP: (http://snpanalyzer.uthsc.edu/)

Screening for non-acceptable polymorphisms (SNAP) is a neural network-based method for the prediction of the functional property of non-synonymous SNPs. SNAP wants only sequence information as input and benefits from functional and structural annotations also. SNAP make use of various biophysical characteristics of the substitution, in addition to evolutionary information, to predict whether or not a mutation is prone to alter protein function [21].

3. POLYPHEN2: (http://genetics.bwh.harvard.edu/pph2/)

Polymorphism Phenotyping v2(PolyPhen-2), is a software and also a Web server, which uses structural and comparative evolutionary relationship to predict the possible impact of amino acid substitutions on the stability and function of human proteins. It performs functional annotation of single-nucleotide polymorphisms (SNPs), extracts protein sequence annotations and structural ascribe, and construct conservation profiles. It then guesstimates the probability of the missense mutation being damaging based on a combination of all these properties [22].

4. PANTHER: (http://www.snps3d.org/).

The Panther Evolutionary Analysis of SNPs computes substitution position-specific evolutionary conservation scores depends on alignments of evolutionarily related proteins to predict the pathogenicity. The alignments protein families are obtained from the PANTHER library of based on their Hidden Markov Models (HMMs). The substitution position-specific evolutionary conservation score describes the amino acid probability, in particular, positions among evolutionarily related sequences [23].

5. NsSNPAnalyzer: (http://www.pantherdb.org/tools/csnpScoreForm.jsp).

NsSNPAnalyzer is a tool to predict whether a nonsynonymous single nucleotide polymorphism (nsSNP) has a phenotypic effect. It also provides additional information about the SNP to make easy for the elucidation of result [24].

6. PhD-SNP: (snps.biofold.org/phd-snp/phd-snp.html)

PhD-SNP is a prediction method based on single-sequence and sequence profile based support vector machines trained on Swiss-Prot variants. The single-sequence SVM (SVM-Sequence) classifies the missense variant to be pathogenic or neutral based on the nature of the substitution and properties of the neighboring sequence environment. The profile-based SVM (SVM-Profile) utilizes sequence profile information taken from MSAs, and classifies the variant according to the ratio between the frequencies of the wild-type and substituted residue. A decision tree algorithm chooses which one of the two SVMs described above is to be used at each case based on the occurrence of wild-type and mutant amino acids at the given position [25].

7. SNP3D: (http://www.SNPs3D.org)

SNPs3D is a web server which assigns molecular functional property of non-synonymous SNPs based on their structural and sequence analysis. It has three primary modules. One module recognizes which genes are responsible for a specified disease. Second module gives information about the interaction between sets of candidate genes. The third module examines the possible impact of non-synonymous SNPs on protein function [26].

8. LS-SNP/PDB: (https://sift.bii.a-star.edu.sg/www/SIFT_dbSNP.html)

Large scale annotation of coding nsSNP (LS-SNP) is a new web server for human non-synonymous (SNPs). It provides protein graphics along with UCSF Chimera molecular visualization software. LS-SNP/PDB interpret all human SNPs that produce an amino acid change in a protein structure by using features of their confined structural environment, reputed binding interactions and evolutionary conservation [27].

Protein and ligand Preparation:

The three dimensional structure of that mutated protein was retrieved by SNP3D database and the position of the snp is viewed in LS-SNP/PDB webserver. Current Drug which is used for Parkinson was identified from the Pubmed literature survey. These drugs show enhanced effects towards FGF20 for producing dopamine neurons. The three dimensional structure of the drugs was downloaded in sdf format using Pubchem.

1. PyMOL:

PyMOL is a potent and interactive molecular visualization software tool and used to view the protein, ligand. The three dimensional structure of Protein and Ligand were visualized using Pymol and saved in PDB file format for further analysis [28].

2. Swisspdbviewer:

Swiss pdb viewer is an interactive molecular graphics program for visualization and analysis of protein structures. Using Mutation and Energy minimization tool for the protein, mutation has been altered and Energy minimization was performed before and after mutation.

3. Active site identification of FGF 20: (http://sts.bioe.uic.edu/castp/index.html?2cpk)

The active sites of altered FGF 20 along with area and volume of binding pocket was carried out with Computed Atlas of Surface Topography of Proteins (CastP) database for finding out and calculate protein pockets , based on specific computational geometry process [29].

4. Docking studies using Hex 8.0.0 Cuba:

Docking of FGF20 against currently used drugs was performed with hex 8.0.0 Cuba docking software which is an interactive molecular graphics program and most commonly available software. The ligands are retrieved from the NCBI Pubchem database. All the parameters used in Hex software were selected by default. The parameters used for the docking process via HEX docking software were

1. FFT Mode Shape only
2. Correlation type 3D fast life
3. Grid Dimension 0.6
4. Receptor range 180
5. Ligands Range 180
6. Twist range 360
7. Distance Range 40

The Etotal indicates the binding score of the receptor and ligands. The docking results have been saved in PDB file format [30].

5. Visualization of docking interaction:

Argus lab: Docking interactions are visualized in Argus lab which is used for the building of the protein and visualization of the molecules and also to spot the clear interaction between protein and ligand [31].

3. RESULTS AND DISCUSSION:

Analysis of Sequence:

The protein sequence was retrieved from the Swiss-Prot database which contains 211 amino acid residues. The fasta file format of the protein is >sp|Q9NP95|FGF20_HUMAN Fibroblast growth factor 20. The physiochemical properties of the protein: molecular weight (23498.6), theoretical pI (8.89), aliphatic index (80.43), GRAVY - Grand average of hydropathicity (-0.455) were predicted by ProtParam database.

Analysis of SNPs:

From NCBI's Gene database the human FGF20 gene ID (26281) were identified and then submitted to dbSNP database which predicted 30 SNP (rs) IDs, from the predicted SNPs17 rs IDs were found to be non-synonyms SNPs which affects the protein functions.

Table 1: List of rs id's of nonsynonyms SNPs of FGF20 gene encoded protein

rs375846209	rs143400495	rs112951749	rs3793405	rs377326763	rs372796901
rs17550360	rs201317229	rs112761348	rs367661392	rs10089600	rs376283820
rs386543945	rs200152641	rs368189425	rs370758961	rs376046538	

Further these non-synonyms (ns) SNP was analyzed using Sift dbSNP to spot out whether the presence of these nsSNPs can affect the function and structure of the FGF20 gene. The database predicted that out of 17 rs IDs only 1 rs ID was damaging the structure and the function of protein.

Table 2: Predicted rs id's which affect the structure and function of protein

rs ids	Prediction	Warning	rs ids	Prediction	Warning
rs375846209	TOLERATED	-	rs143400495	TOLERATED	-
rs112951749	TOLERATED	-	rs3793405	TOLERATED	-
rs377326763	TOLERATED	-	rs372796901	TOLERATED	-
rs17550360	DAMAGING	*Low confidence*	rs201317229	TOLERATED	-
rs367661392	TOLERATED	-	rs10089600	TOLERATED	-
rs376283820	TOLERATED	-	rs386543945	TOLERATED	-
rs200152641	TOLERATED	-	rs368189425	TOLERATED	-
rs370758961	TOLERATED	-	rs376046538	TOLERATED	-
rs112761348	TOLERATED	-			

Predicted nsSNP:

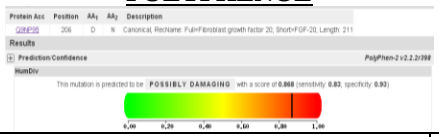
The nsSNP (rs17550360) were submitted to SIFT database which predicts the mutation in the gene. The database predicted 15 mutations in the protein sequence. They are as follows: D206N, Y204H, V195E, A179S, P175A, G162S, E136K, S135C, G116R, I110V, L98W, I79V, Q78E, S47R and Q20L. SNP predictions tools predicted the particular mutation which may alter the FGF20 protein structure and function there affecting the function of the FGF20 gene (Table 3). The result of SNP prediction tools, predicted that the mutation (D206A) was responsible for the alteration in protein structure and function. Further the3D structure of the FGF20 protein was retrieved using SNP3D and LS-SNP/PDB (Table 4).

Table 3: Result of SNP predictions tools.

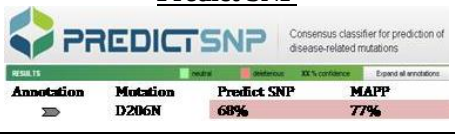
PANTHER

subPSEC	$P_{deleterious}$	substitution	MSA position	P_{wt}	$P_{substituted}$	NC
-3.06463	0.51615	D206N	194	0.68614	0.03281	1.459
-2.61628	0.40523	P175A	163	0.68346	0.03814	1.048
-5.23791	0.9036	G116R	103	0.87645	0.00567	2.22
-2.05464	0.27982	Q78E	62	0.48641	0.05122	1.048

POLYPHENOL



Predict SNP



PHD SNP

206	I	Neutral	1
206	W	Neutral	3
206	G	Neutral	1
206	P	Neutral	4
206	S	Neutral	3
206	C	Disease	4
206	H	Neutral	2
206	R	Neutral	1
206	K	Neutral	2
206	N	Neutral	6
206	D	Disease	8

SNAP

nsSNP	Prediction	Reliability Index	Expected Accuracy (%)
Q20L	Neutral	1	60
S47R	Neutral	5	89
Q78E	Neutral	3	78
I79V	Neutral	2	69
L98W	Neutral	0	53
I110V	Non-neutral	0	58
G116R	Non-neutral	4	82
S135C	Neutral	2	69
E136K	Non-neutral	4	82
G162S	Neutral	0	53
P175A	Non-neutral	0	58
A179S	Neutral	7	94
V195E	Non-neutral	0	58
Y204H	Neutral	3	78
D206N	Non-neutral	2	70

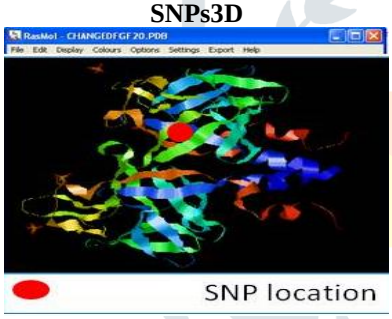
NS ANALYZER

nsSNPAnalyzer: predicting disease-associated nonsynonymous single nucleotide polymorphisms

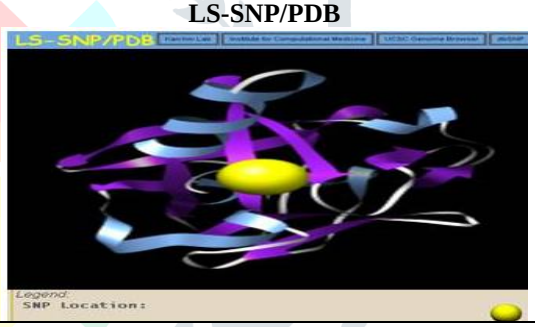
RESULT		
SNP	PHENOTYPE	SECONDARY
Q20L	Neutral	–
S47R	Neutral	–
Q78E	Neutral	S
I79V	Neutral	S
L98W	Disease	S
I110V	Neutral	S
G116R	Disease	C
S135C	Neutral	C
E135K	Neutral	C
G162S	Disease	C
P175A	Neutral	C
A179S	Neutral	–
V195E	Neutral	C
Y204H	Neutral	C
D206N	Disease	C

Table 4: The protein structure were retrieved from SNP3D and LS-SNP/PDB database

SNPs3D



LS-SNP/PDB



Protein Preparation:

Using PyMol, the sequence and structure of FGF20 were viewed and observed the presence of Aspartic Acid in 206th position of mutated protein. In Swiss-Pdb Viewer the energy minimization was performed for the mutated protein using the energy minimization tool for finding an arrangement in space of a collection of atoms and Pair disorted geometries by moving atoms to release internal constraints. By Mutation tool, the mutated amino acid Aspartic Acid (ASP) was reverted to the normal amino acid Asparagine (ASN) and once again the energy minimization was performed for reverted protein (Table 5). On analyzing the result of energy, it was predicted that the reverted protein had a proper arrangement of atoms when compared with mutated protein. The 3D structure of mutated protein and reverted protein were saved in pdb file format for further analysis. The catalytic site of the proteins were predicted using CastP database and best ligand binding site was observed to be at pocket no. 47 containing 26 amino acid residues (Table 6).

Table 5: Sequence and structure of FGF20 Protein

Mutated protein of FGF 20	Single amino acid modified protein of FGF20
MAPLAEVGGFLGGLEGLGQQVGSHFLLPP AGERPPLLGERRSAAERSARGGPGAAQLA HLHGILRRRQLYCRTGFHLQILPDGSVQGT RQDHSLFGILEFISVAVGLVSIRGVDSGLYL GMNDKGELYGSEKLTSECIFREQFEENWY NTYSSNIYKHGDTGRRYFVALNKDGTPRD GARSKRHQKFTHFLPRPVDPERVPELYK**D** LLMYT	MAPLAEVGGFLGGLEGLGQQVGSHFLLPP AGERPPLLGERRSAAERSARGGPGAAQLA HLHGILRRRQLYCRTGFHLQILPDGSVQGT RQDHSLFGILEFISVAVGLVSIRGVDSGLYL GMNDKGELYGSEKLTSECIFREQFEENWY NTYSSNIYKHGDTGRRYFVALNKDGTPRD GARSKRHQKFTHFLPRPVDPERVPELYK**N** LLMYT

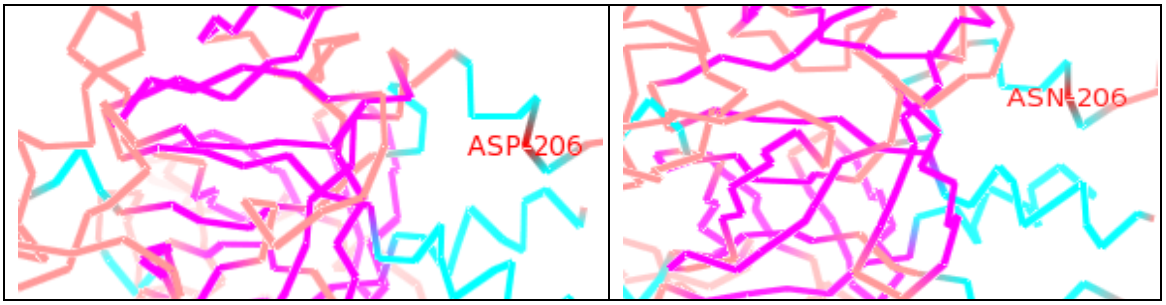
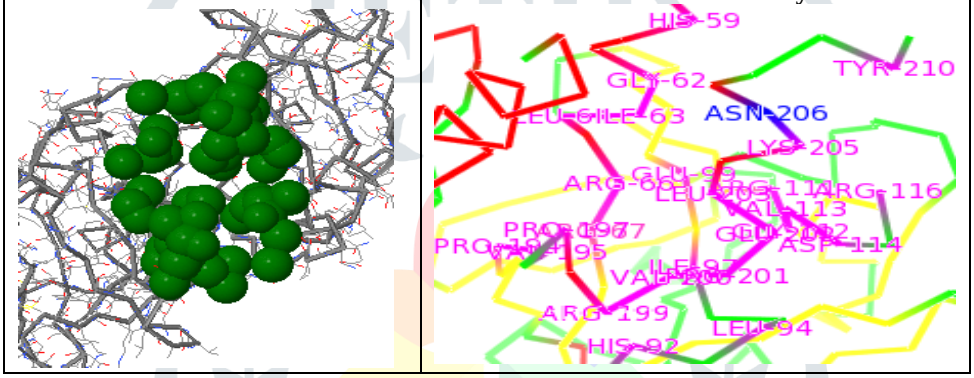


Table 6: Active site of FGF20 protein predicted by Cast P and viewed in PyMol.

Amino acid	No of binding pocket	Position of binding pocket	Amino acid	No of binding pocket	Position of binding pocket
Arg	5	66,67,111,116,119	Leu	3	64,94,203
Asp	1	114	Lys	1	205
Asn	1	206	Pro	3	194,197,201
Glu	2	62,112	Val	3	113,195,200
His	2	59,92	Tyr	1	210.
Ile	2	59,92			

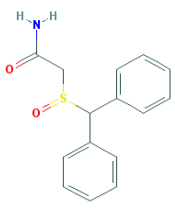
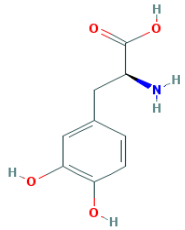
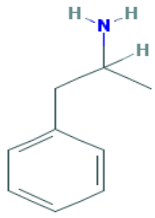
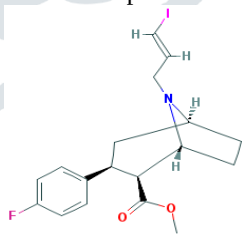
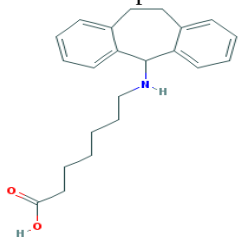
Active site showed in CastP **Active site Viewed in PyMol**



Ligands Preparation:

The drugs (ligand) which increase the dopamine level in the brain were retrieved using NCBI PubMed literatures. The 2D structure were downloaded in SDF file format from NCBI PubChem database and saved in Pdb format using a PyMol tool for further docking studies.

Table 7: The 2D structure of five Drug molecules retrieved from Pubchem

Modafinil	Levodopa	Amphetamine	Altropane	Amineptin
				

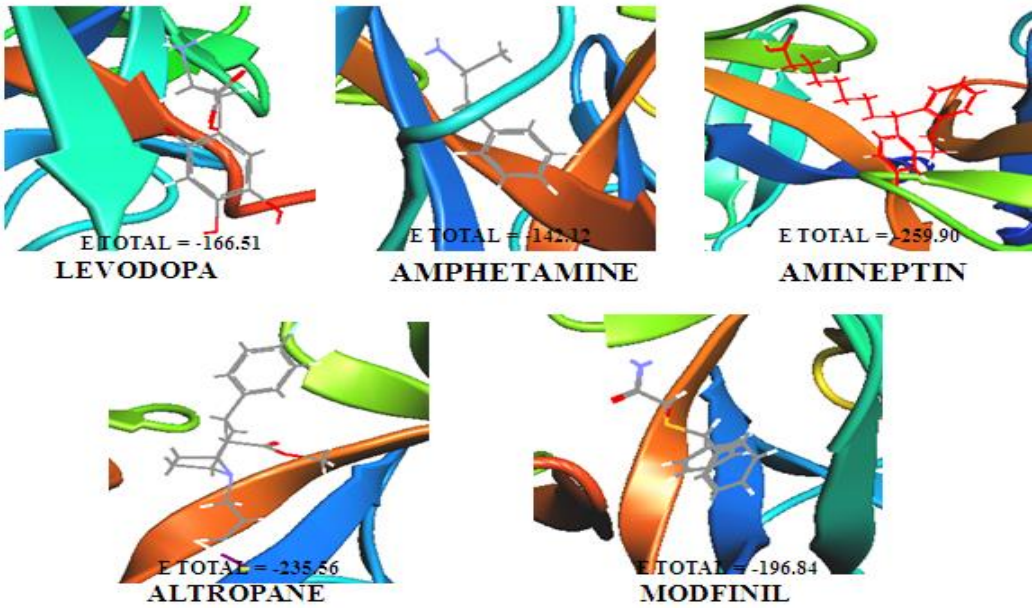
Docking:

For docking studies, the results were tabulated between FGF20 proteins and five ligands. Each protein-ligand interactions showed different Energy value (Etotal) in HEX software (Table 8). On further analyzing the docking result between two proteins it was confirmed that the reverted protein showed the binding interaction with the ligands than the mutated protein and also predicted that all five ligands are binding to the 206th position of ASN amino acid in the protein structure. The best pose of reverted protein and ligand interaction were viewed and saved in Argus lab file format (Figure 1). From the current studies it was predicted that the reverted protein exhibited the stronger the binding interaction with the currently available drugs for parkinson diseases.

Table 8: Shows the docking result of FGF20 protein with 5 ligands in Hex software.

S.No	Name of the Drug	Energy value (E Total) of Single amino acid modified protein	Energy value (E Total) of Mutated protein
1.	Levodopa	-166.51	-113.60
2.	Amphetamine	-142.12	-108.61
3.	Amineptin	-259.90	-198.11
4.	Altropane	-235.56	-189.03
5.	Modafinil	-196.84	-124.82

Figure 1: Docking result of drugs with FGF20 protein is viewed in Argus Lab.



4. CONCLUSION:

Bioinformatics provides the strong evidence for the linkage of protein coding SNPs which cause human genetic Parkinson disease. The further impact of bioinformatics on SNP-related research revealed that the presence of protein coding SNPs in the gene of FGF20 was one of the risk factors for the genetic Parkinson's disease. On analyzing the gene of FGF20 it was identified that mutation occurred in the protein sequence alter the function of the protein which is responsible for causing the disease. Using SNP analyzing and prediction tools, predicted that mutation occurred on the 206th position in the protein sequences causing disease when compared with other non-synomys SNPs. The amino acid Asparagine was misplaced by Aspartic acid in the 206th position of FGF 20 protein sequence. The mutation in the protein sequence was reverted and repaired its distorted geometries by SwissPDB Viewer to make the protein function normally. Mutated protein and Single amino acid modified protein was docked using Hex Software with 5 drugs which were currently in using for Parkinson's disease to increase dopamine level. The docking result shows the best binding interaction between Single amino acid modified protein FGF20 and five drugs when compared with mutated protein. Further some clinical trials are needed to be carried out to confirm that this disease can be treated well by altering the mutation in the FGF20 gene and also for the safety usage of drug dosage towards Parkinson disease.

5. CONFLICT OF INTEREST:

The authors declare they have no competing interests.

6. ACKNOWLEDGEMENT:

We acknowledge Vels Institute of Science, Technology and Advanced Studies (VISTAS) for providing us with required infrastructure and support system needed.

7. REFERENCE:

[1] Garrett E Alexander. Biology of Parkinson's disease. 2004. Pathogenesis and pathophysiology of a multisystem neurodegenerative disorder. Dialogues in Clinical Neuroscience, 6(3): 259-280.

[2] Christian Wider, Owen A Ross, and Zbigniew K Wszolek. 2010. Genetics of Parkinson disease and essential tremor. Curr Opin Neurol , 23(4): 388-393.

[3] Sachiko Murase and Ronald D McKay. 2006. A Specific Survival Response in Dopamine Neurons at Most Risk in Parkinson's disease. The Journal of Neuroscience, 26(38): 9750-9760.

[4] WilliamDauer. 2003. Parkinson's disease: Mechanisms and Models. Neuron, 39: 889-909.

[5] Ayano G. Parkinson's Disease. 2016. A Concise Overview of Etiology, Epidemiology, Diagnosis, Comorbidity and Management. J Neurol Disord, 4(6): 298.

[6] George DeMaagd and Ashok Philip. 2015. Parkinson's disease and Its Management. Part 1 disease entity, risk factors, pathophysiology, clinical presentation and diagnosis. Pharmacy and Therapeutics (P&T), 40(8): 504-510.

[7] Davie C A. 2008. A review of Parkinson's disease. British Medical Bulletin, 86(1): 109-127,

[8] Philippe Rizek, Niraj Kumar and Mandar S Jog. 2016. An update on the diagnosis and treatment of Parkinson disease. CMAJ, 188(16): 1157-1165.

[9] Itoh N and Ohta H. 2013. Roles of FGF20 in dopaminergic neurons and Parkinson's disease. Front Mol Neurosci, 6: 15.

[10] Eugene L Boshoff, Edward.JR Fletcher and Susan Duty. 2018. Fibroblast growth factor 20 is protective towards dopaminergic neurons *in vivo* in a paracrine manner. Neuropharmacology, 137: 156-163.

[11] Andrew S Allen and Glen A Satten. 2010. SNPs in CAST are associated with Parkinson disease: A confirmation study. Am J Med Genet B Neuropsychiatr Genet, 153B (4): 973-979.

[12] Steven Pierce and Gerhard A Coetzee. 2017. Parkinson's disease-associated genetic variation is linked to quantitative expression of inflammatory genes. PLoS One, 12(4): e0175882.

[13] Christine Klein and Ana Westenberger. 2012. Genetics of Parkinson's disease. Cold Spring Harb Perspect Med. 2(1): a008888.

[14] Gröger A, Kolb R, Schäfer R, Klose U. 2014. Dopamine Reduction in the Substantia Nigra of Parkinson's Disease Patients Confirmed by *In Vivo* Magnetic Resonance Spectroscopic Imaging. PLoS ONE, 9(1): e84081.

[15] Joseph Jankovic and L Giselle Aguilar. 2008. Current approaches to the treatment of Parkinson's disease. Neuropsychiatr Dis Treat, 4(4): 743-757.

[16] Amos D Korczyn. 2004. Drug treatment for parkinson's disease. Dialogues Clin Neurosci, 6(3): 315-322.

[17] Shin Hisahara and Shun Shimohama. 2011. Dopamine Receptors and Parkinson's disease. International Journal of Medicinal Chemistry, Article ID403039.

[18] Subhamay Panda and Goutam Chandra. 2012. Physicochemical characterization and functional analysis of some snake venom toxin proteins and related non-toxin proteins of other chordates. Bioinformation, 8(18): 891-896.

[19] Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, Sirotkin K. 2001. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res, 29 (1): 308-311.

[20] Ngak-Leng Sim, Prateek Kumar, Jing Hu, Steven Henikoff, Georg Schneider and Pauline C. 2012. SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Res, 40: W452-W457.

[21] Andrew D Johnson, Robert E Handsaker, Sara L Pulit, Marcia M Nizzari, Christopher J ODonnell and Paul IW de Bakker. 2008. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics, 24(24): 2938-2939.

[22] Corinna Ernst, Eric Hahnen, Christoph Engel, Michael Nothnagel, Jonas Weber, Rita K. Schmutzler and Jan Hauke. 2018. Performance of *in silico* prediction tools for the classification of rare BRCA1/2 missense variants in clinical diagnostics. BMC Med Genomics, 11: 35.

[23] Thomas PD, Kejariwal A, Campbell MJ, Mi H, Diemer K, Guo N, Ladunga I, Ulitsky-Lazareva B, Muruganujan A, Rabkin S, Vandergriff JA and Doremieux O. PANTHER. 2003. A browsable database of gene products organized by biological function, using curated protein family and subfamily classification. Nucleic Acids Res, 31(1): 334-41.

[24] Bao L1, Zhou M and Cui Y. 2005. NsSNPAnalyzer: Identifying disease-associated nonsynonymous single nucleotide polymorphisms. Nucleic Acids Res, 33: W480-2.

[25] Yip YL, Scheib H, Diemand AV, Gattiker A, Famiglietti LM, Gasteiger E, Bairoch A. 2004. The Swiss-Prot variant page and the ModSNP database: a resource for sequence and structure information on human protein variants, Hum Mutat, 23: 464-470.

[26] Peng Yue, Eugene Melamud and John Moult. 2006. SNPs3D: Candidate gene and SNP selection for association studies. BMC Bioinformatics, 7: 166.

[27] Ryan M1, Diekhans M, Lien S, Liu Y and Karchin R. 2009. LS-SNP/PDB: annotated non-synonymous SNPs mapped to Protein Data Bank structures. Bioinformatics. 25 (11): 1431-2.

[28] Jeyabaskar Suganya, Mahendran Radha, Sharanya Manoharan and Vasudevan Poornima. 2018. *In-Silico* analysis of SNPS from CAMP-GEFII gene associated with polycystic ovarian syndrome. IJPSR, 9(12): 5216-5220.

[29] Andrew Binkowski T, Shapor Naghibzadeh, and Jie Lianga. 2003. CASTp: Computed Atlas of Surface Topography of proteins. Nucleic Acids Res, 31 (13): 3352-3355.

[30] Gary Macindoe, Lazaros Mavridis, Vishwesh Venkatraman, Marie-Dominique Devignes and David W. Ritchie. 2010. HexServer: an FFT-based protein docking server powered by graphics processors. Nucleic Acids Res, 38: W445–W449.

[31] Suganya Jeyabaskar, Radha Mahendran. 2016. *In silico* QSAR and Molecular Docking Studies of Selected Medicinal Plant Compounds against NS5 & NS3 Protein of Dengue Virus: A Comparative Approach. Int J Pharm Bio Sci, 7(3): 1135-1144.