In Silico Screening of SNP's in Prion Protein and Comparative Studies between Phytochemical Compounds that inhibit Prion Protein Causing Neuro-degenerative Diseases

M. Anitha[1], Mahendran Radha[2*], Dhanaya[3], Jeyabaskar Suganya[4]

[1]Department of Bioinformatics, School of Life Sciences, Research Scholar, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, India.

[2*]Department of Bioinformatics, School of Life Sciences, Professor & Head, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, India. E-mail: mahenradha@gmail.com, hodbioinfo@velsuniv.ac.in

[3]Department of Bioinformatics, School of Life Sciences, Student, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, India.

[4]Department of Bioinformatics, School of Life Sciences, Assistant Professor, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, India.

Abstract

The misfolded PrP (prion protein) aggregates and deposits in the brain as a sign of neurodegenerative diseases, which are very lethal. The prion-like prionoids lead to a variety of neurodegenerative diseases in humans as well as in animals. Prion protein is active in the brain and several other tissues which are made by receiving instructions from the PRNP gene. Prion diseases are progressive, fatal, attack the brain structure or additional neural cells, and lack effective treatment. Consequently, there is an imperative need to create new and more effective remedial strategies needs to combat these fatal diseases. The current work aim is to identify mutated SNP and its position which is responsible for the main cause of the disease. We have predicted the protein stability, Amino acid function, Secondary structure of the amino acid, Mutated prion protein Homology modeling, and analysis of Protein 3D structure. Through the literature survey, we have identified phytochemical compounds from medicinal plants which possess pharmacological activities to cure neurodegenerative diseases. And we have also retrieved a few currently used synthetic compounds which are used for the treatment of neurodegenerative disease. Both the set of compounds were further analyzed for Molecular docking against the target prion protein. The docking results and comparative docking studies reveal that the natural compounds exhibit the best binding energy than the synthetic compounds. Natural compounds are more effective in the treatment and cure of various neurodegenerative diseases without any side effects.

Keywords: Computational Analysis, Molecular Docking, Neurodegenerative Disease, Phytochemical, Prion Protein.

DOI: 10.47750/pnr.2022.13.S03.011

INTRODUCTION

Prion Protein (PrP) causes neurodegenerative disorders that are fatal and incurable and result in the progressive degeneration and death of nerve cells. [1]. this condition causes ataxias, which affect mobility, and dementias, which affect brain abilities [2]. Prions are thought to be the cause of, fatal familial insomnia (FFI), kuru and Prion protein (PrP), Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker syndrome (GSS), and its variant (vCJD). BSE, also known as "mad cow disease" in cattle, is believed to be the cause of CJD (Creutzfeldt-Jakob disease) in humans[3]. Amyloids are from the abnormal aggregates of prion protein and it accumulates in the infected tissue which cell apoptosis and tissue injury[4]. Other neurodegenerative diseases like Parkinson's disease and Alzheimer's disease are brought on by amyloids. [5]. Additionally, there is research that indicates prions may contribute to the development of prion-like diseases such as ALS (amyotrophic lateral sclerosis), Parkinson's disease, and Alzheimer's disease. The SNPs for Prion protein were retrieved using the NCBI database. Using various bioinformatics tools we have predicted Mutated SNP and disease-related nsSNP. Computational analysis has predicted the Mutated SNP of rsID rs74315410. Protein structure prediction was performed using HOPE[7]. Later after performing a thorough literature survey, we have identified 7 phytochemical compounds from various plants such as celastruspaniculatus, piper longum, piper nigurum, psidium gujava, sebasina grandiflora, Terminalia chebula, cuscuttareflexa, vitex

negundo, solanum nigurum, calotropis procera, zingiber officinale, Cynodon dactylon, Lawsonia inermis, Curcuma longa, cyperus scariosus, juglansregia, glycyrrhiz aglabra, eclipta alba[8, 9]. We have also retrieved synthetic compounds from a literature survey that is currently used as a drug for neurodegenerative disease[10]. We have carried out Molecular docking using Argus Lab software for both the phytochemical compounds and synthetic compounds with the target Protein. Through the comparative studies, we have analyzed the binding energy of both the Phytochemical and synthetic compounds to identify the best compound which could be used for the treatment of neurodegenerative diseases.

Materials and Methods

We have retrieved 23 SNPs for Prion protein using the NCBI database[11]. The Deleterious nsSNP was predicted using the 23 SNPs obtained from the NCBI dbSNP database by SIFT-Sorting Intolerant from Tolerant tool[12]. The PolyPhen 2 tool was used to retrieve the damaging SNPs. [13]. Using Screening for non-acceptable Polymorphisms (SNAP), The Effect of SNPs was found[14]. The Deleterious SNPs were retrieved using the Protein Variation Effect Analyzer (PROVEAN tool) [15]. Five SNPs that cause disease were retrieved by the SNPs & GO tool. One SNP was predicted to cause disease by the PhD-SNP database. The mutated protein stability is predicted using the I-Mutant tool [17]. A protein molecule's amino acid positions were predicted using the ConSurf tool. [18]. to predict the surface accessibility and structure of amino acid residue in protein structure, we utilized the NetSurfP server [19].

A prion protein mutation HOPE was used to perform homology modeling and analysis of its three-dimensional structure[20]. After a detailed literature survey, we have identified 7 phytochemical compounds from various medicinal plants[21, 22]. The Synthetic compound Levodopa (L-dopa) is the most widely used neurotoxic agent and may be used to cure neurodegenerative disease[23, 24]. The Phytochemical compounds and synthetic compounds were further analyzed for Molecular docking using Argus Lab software with the target protein (PDB ID: 4KML) modeled by the HOPE tool. The active sites for the protein were retrieved using the Computed Atlas of Surface Topography of proteins (CastP tool). It finds, measures, and describes the voids inside proteins as well as the pockets on their surfaces. The concave regions of proteins that are typically associated with binding activities are these surface pockets and voids. [25]. Further comparative analysis was performed for the docking results of phytochemical compounds and Synthetic compounds to identify the best binding energy[26].

Results and Discussion

Table 1. Disease Related SNP by Phd-SNP

S. No	SNP ID	SNPs & GO Prediction	Residue Changes
1.	rs11538758	Neutral	P105L
2.	rs74315401	Neutral	P102L
3.	**rs74315410**	**Disease**	**G131V**
4.	rs74315413	Neutral	H187R
5.	rs267606980	Neutral	G127V

Table: 2 shows the mutated protein stability is predicted using the I-Mutant tool. Since the DDG value is 0.21 Kcal/mol which is -0.5<=DDG<=0.5 the protein has Neutral Stability.

Table 2. Protein Stability

S. No	SNP ID	Residue Change	DDG Value Prediction
1.	rs74315410	G131V	0.21kcal/mol

The Consurf results have predicted the highly conserved amino acid and the dark purple color in figure1 indicates that highly conserved region. The function of Glycine is buried residue which is given in Table 3.

Table 3. Amino Acid Function by Consurf

S. No	Position	Seq	Score	Color	B/e	Function
1.	131	G	-1.283	9	b	S

We forecast the surface accessibility and structure of amino acid residue in protein structure using the NetSurfP server. The orange color in Figure 1 shows that the secondary structure of the residue Glycine located at position 131 is Helix.

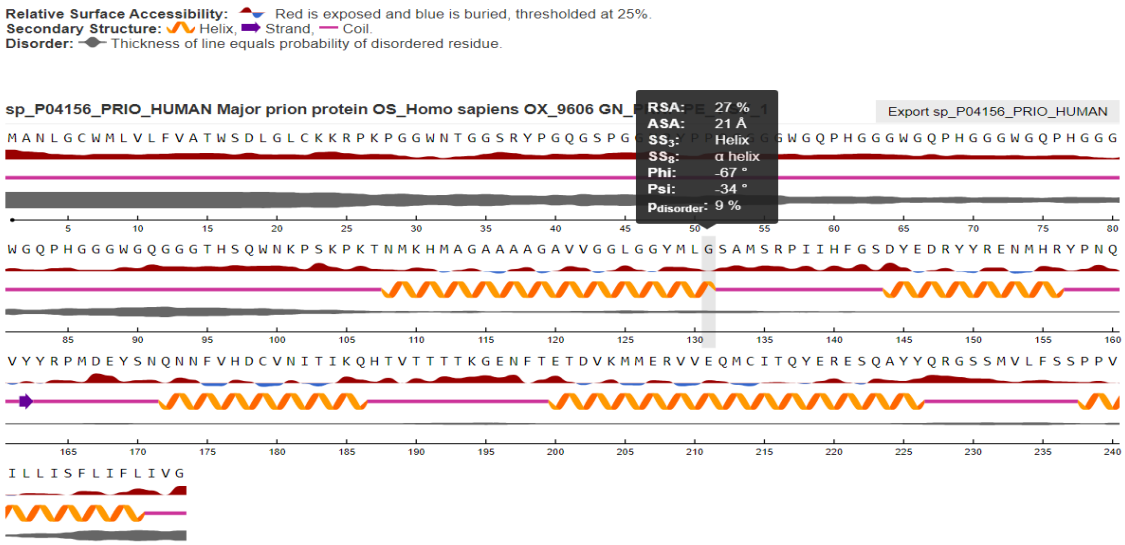

Figure 1: Netsurf Analysis Results

The 3-dimensional structure, mutation of amino acid, and the overview of protein in ribbon presentation and mutation are shown in Figure 2.

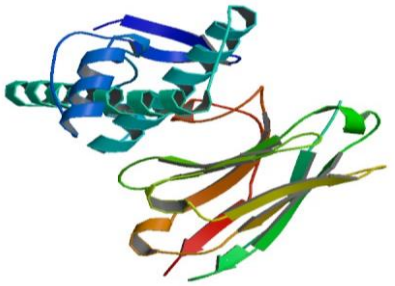

Figure 2: 3-Dimensional Structure of the Protein of PDB ID: 4KML

Amino acid and mutant amino acid graphic structures are represented below in Figure 3. Red represents the backbone, which is the same for each amino acid. Each amino acid's distinct side chain is colored black.

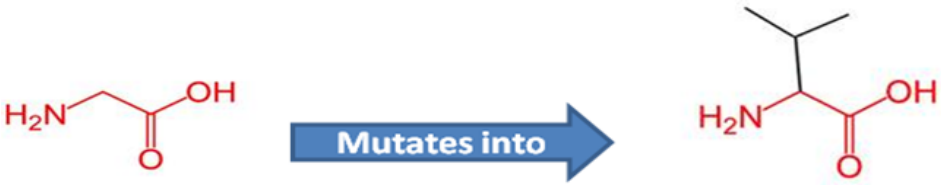

Figure 3: Graphical representation of Mutation

Proteins in figure 4 are colored according to their constituent elements: blue denotes α-helix, red denotes β-strand, green denotes turn, yellow denotes 3/10 helix, and random coil denotes cyan. When present, other molecules in the complex are indicated by grey color.

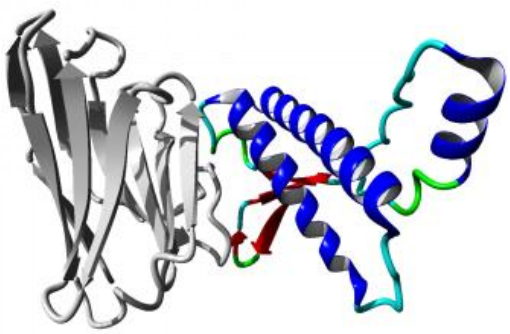

Figure 4: Protein 3D Structure

In Figure 5 the side chains of the natural and mutated residues are displayed and are colored red and green, respectively. The grey-colored part is the protein. It shows the natural side chain and the mutant side chain in alternation.

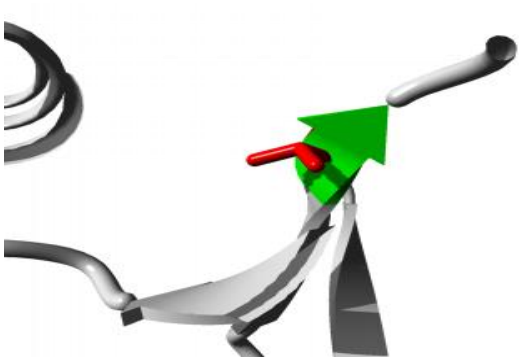

Figure 5: Overview of the Mutation

Table 4 shows the characteristics of the compounds that were obtained from (the PubChem) repository.

Table 4: Phytochemical and Synthetic Compounds

S. No	Natural Compounds	Pubchem ID
1.	2-propenoic acid	6581
2.	4-hydroxycinnamic	5328791
3.	Acetaminophen	1983
4.	Ferulic acid	445858
5.	Formononetin	5280378
6.	Liquiritigenin	114829
7.	Melilotic acid	873
S. No	**Synthetic Compound**	**Pubchem ID**
1.	Levodopa	6047

The active sites of the protein were predicted by the Cast-P tool and are displayed in Table 5.

Table 5: Protein Active Regions 4KML

Serial Number	Chain	SeqID	AA
1.	A	166	MET
2.	A	167	ASP
3.	A	168	GLU
4.	A	169	TYR
5.	A	170	SER
6.	A	171	ASN
7.	A	174	ASN
8.	A	175	PHE
9.	A	218	TYR
10.	A	225	TYR

The Comparative studies of docking results indicate that the phytochemical compound Melilotic acid shows the greatest docking score of -16.65 Kcal/mol when compared to Synthetic compound Levodopa (Table 6).

Table 6: Docking Results

S. No	Natural Compounds	Binding energy Kcal/mol
1.	2-propenoic acid	-5.20
2.	4-hydroxycinnamic	-7.22
3.	Acetaminophen	-9.90
4.	Ferulic acid	-10.49
5.	Formononetin	-12.75
6.	Liquiritigenin	-15.99
7.	**Melilotic acid**	**-16.65**
S. No	**Synthetic Compound**	**Binding energy Kcal/mol**
1.	Levodopa	-11.56

Figure 6 and Figure 7 show a graphical representation of docking results and Binding energy.

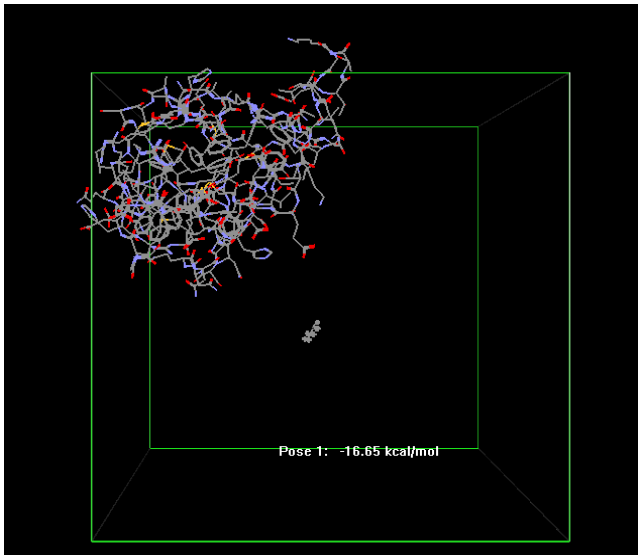

Figure 6: Docking Result of Melilotic Acid and Binding Energy: -16.65 Kcal/mol

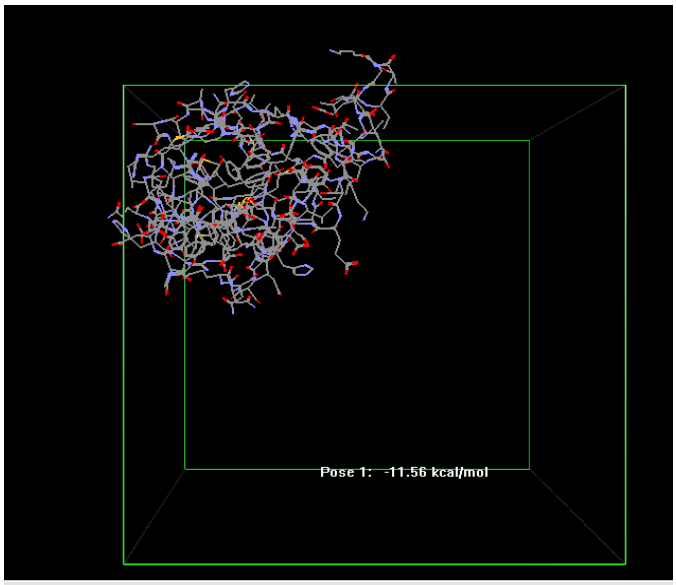

Figure 7: Docking Result of Levodopa and Binding Energy: -11.56 Kcal/mol

23 SNPs for Prion protein were found using the (National Center for Biotechnology Information) repository. The 23 SNPs were investigated to predict the Damaging nsSNP using the SIFT tool. Here we have predicted 15 Deleterious SNPs. POLYPHEN predicted 14 SNPs.We have predicted 12 Effect SNPs using the SNAP database. We have predicted only five deleterious SNPs with the PROVEAN tool. From analyzing SNPs using the Phd-SNP tool, after all, we have predicted only one SNP (rs74315410) causing disease. Here (G131V) Glycine is mutated into Valine at the position of 131. We have a predicted model of the mutated protein. The target protein's exact 3D structure is not known. Nevertheless, using a sequence homology structure, HOPE can create a model of our target protein. As a possible modeling template, HOPE identified PDB: 4KML. After a detailed literature survey, we have identified 7 phytochemical compounds from the medicinal plants and also identified the Synthetic compound "Levodopa" which is currently used as a drug for treating Neurodegenerative disease. The Phytochemical compounds and synthetic compounds were analyzed for Molecular docking studies using Argus Lab software with the protein (PDB ID: 4KML). Compared with the natural compound and synthetic compound Melilotic acid hits the highest binding energy (-16.65 Kcal/mol).

Conclusion

Thus the result obtained from the current study indicates that Neurodegenerative disease is caused by the Prion protein due to the mutation of G131V residue. And by comparing both binding energies of phytochemicals and synthetic compounds, we identified that the phytochemical compound: Melilotic acid shows the best binding energy and it has more effect to cure Neurodegenerative disease. Hence we can recommend that further clinical and in vitro studies should be carried out and this could lead to the discovery of novel potential drugs against several neurodegenerative diseases without any side effects.

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