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Homology Modeling and *in-silico* Characterization of 5-hydroxytryotamine Receptor-6 from *Homo sapiens*

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Abstract—*Schizophrenia is a chronic mental health disorder characterized by characterized by the classic tetrad of extreme delusions, hallucinations, disorganized speech or behavior, and impaired cognitive ability. The present aim of this investigation is to apply computational approaches to understand insights of a protein responsible for narcolepsy which is an autoimmune disease. We performed in silico analysis of the protein using bioinformatics tools and servers. The protein 5- hydroxytryotamine receptor -6 of gene HTR6, which was retrieved from NCBI. The physio-chemical properties performed suggested that protein is basic and hydrophobic protein but is structurally unstable in nature. The secondary structural analysis results revealed that among secondary structure elements random coils dominated. Homology modeling approach has been used to define the tertiary structure of the protein using SWISS-MODEL workspace. The homology modeling showed that the QMEAN score of the model was -3.93, and the sequence identity was 31.00%. 3-D model validation was done to verify the reliability of generated model revealing that 94.2 % residues were in favored regions. The study concludes that homology modeling can be stated as paramount method for protein structure prediction; that can be further analyzed for mutation prediction and docking studies against various drug molecules for the receptor protein responsible for Schizophrenia Thus, indicating that protein can act as potential drug target.*

Keywords*: In silico, Homology modeling, QMEAN, Schizophrenia, HTR6.*

1. INTRODUCTION

Neurological disorders are diseases of the brain, spine and the nerves that connect them. There are more than 600 diseases of the nervous system, such as brain tumors, epilepsy, parkinson's disease, huntington's disease, muscular dystrophy, schizophrenia and stroke as well as less familiar ones such as frontotemporal dementia. The causes vary from disease to disease such as diseases caused by faulty genes (muscular dystrophy and Huntington's disease); problems with the way the nervous system develops (spinal bifida); when nerve cells are damaged or die (Degenerative diseases- Parkinson's disease and Alzheimer's disease); diseases of the blood vessels that supply the brain (stroke), due to injuries in the spinal cord and brain, seizure disorders (epilepsy) etc. Neurological disorders can be categorized according to the primary location affected, the primary type of dysfunction involved, or the primary type of cause. The broadest division is between central nervous system disorders and peripheral nervous system disorders [1].

Schizophrenia is a complex, chronic mental health disorder characterized by an array of symptoms, including delusions, hallucinations, disorganized speech and impaired cognitive ability. The early onset of the disease, along with its chronic course, makes it a disabling disorder for many patients and their families [2]. Disability often results from both negative symptoms and cognitive symptoms such as impairments in attention, working memory etc. [3]. In addition, relapse may occur because of positive symptoms (suspiciousness, delusions, hallucinations) [2, 3]. The inherent heterogeneity of schizophrenia has resulted in a lack of consensus regarding the disorder's diagnostic criteria, etiology and pathophysiology [2,4]. The prevalence of the disorder seems to be equal in males and females, although the onset of symptoms occurs at an earlier age in males than in females [3]. Males tend to experience their first episode of schizophrenia in their early 20s, whereas women typically experience their first episode in their late 20s or early 30s [5]. The goals in treating schizophrenia include targeting symptoms, preventing relapse and increasing adaptive functioning so that the patient can be integrated back into the community. Pharmacotherapy is the mainstay of schizophrenia management but non-pharmacological treatments, such as psychotherapy is also effective [6]. Schizophrenia medications can cause a variety of other adverse effects causing complications in working of endocrine system, cardiovascular system and central nervous sytem. Systematic reviews show that despite its relatively low incidence (15.2/100 000), the prevalence of schizophrenia (7.2/1000) is relatively high, because it often starts in early adult life and becomes chronic [7].

Serotonin (5-hydroxytryptamine, 5-HT) is probably unique among the monoamines in that its effects are subserved by as many as 13 distinct heptahelical, G-protein-coupled receptors (GPCRs) and one (presumably a family of) ligand-gated ion channel(s). These receptors are divided into seven distinct classes (5-HT(1) to 5-HT(7)) largely on the basis of their structural and operational characteristics (5-hydeoxytryptamine-6 is one of them) [8]. In our present

investigation we modeled the sequence of 5-hydroxytryotamine receptor -6 protein and characterize it using various computational approaches.

2. METHODOLOGY

Sequence retrieval and functional characterization

The amino acid sequence of 5- hydroxytryotamine receptor -6 protein was retrieved from NCBI (http://www.ncbi.nlm.nih.gov). Target sequence of proteins present in human body, was downloaded in FASTA format with the accession number NP_000862.1. For the identification of transmembrane regions and hydropathicity, SOSUI server was used [9].

Physiochemical characterization

Functional annotation at the sequence level is performed. Particularly, amino acid compositions were computed using the Expasy's ProtParam [10] server for 5- hydroxytryotamine receptor -6 protein (http://us.expasy.org/tools/protparam.html). *In-silico* protein statistics were carried out with respect to several parameters such as grand instability index [11], average molecular weight, extinction coefficient [12], theoretical pI (isoelectric point), aliphatic index [13], -R and +R (total number of positive and negative residues), residues charge, grand averge hydropathicity (GRAVY)[10,14] , and half-life.

Secondary structure prediction

Various physical and chemical properties of protein sequence were required or predicting the primary structure. The Secondary structure feature was identified by using GORIV [15]. The secondary structure prediction is based on the information of their primary structure prediction. It is used to describe secondary structure features such as sequence length, alpha helix, beta turn and random coil etc.

Tertiary structure prediction

In order to generate three dimensional models, homology modeling approach was applied. The modeling of 3D structure of the sequences was executed by Swiss-Modeler [16-18] program.

Model visualization and evaluation

For visualization of three dimensional models, the Swiss-PDBViewer was used. Rampage server (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) was used for evaluating and assessing the accuracy of the model [19, 20].

3. RESULT AND DISCUSSION

The protein of 444 amino acids is having molecular weight of 46.9543 kilodaltons. The physicochemical properties (Table 1) and amino acid composition (Table 2) determine the fundamental properties of the protein.

Table 1: Physicochemical properties of 5-hydroxytryotamine receptor -6 from *Homo sapiens*.

Parameters	Value
Sequence length	440
Accession number	NP_000862.1
Molecular weight	50680.43
Theoretical pI (isoelctric point)	9.25
Aliphatic index	106.95
Instability index	45.95
Grand average of hydropathcity (GRAVY)	0.432955
Total number of atom	6696
Extinction coefficient	52940
Total number of negatively charged residues (Asp+Glu)	21
Total number of positively charged residues (Arg+Lys)	33

Table 2: Amino acid composition (in %) of hypo-certin receptor-2 of *Homo sapiens*.

Name of the Amino acid	Amino acid Three letter code (one letter code)	Value	Percentage%
Alanine	Ala (A)	51	11.6
Arginine	Arg (R)	27	6.1
Asparagines	Asn (N)	12	2.7
Aspartic Acid	Asp (D)	13	3.0
Cysteine	Cys (C)	14	3.2
Glutamine	Gln (Q)	12	2.7
Glutamic acid	Glu (E)	8	1.8
Glycine	Gly (G)	30	6.8
Histidine	His (H)	5	1.1
Isoleucine	Ile (I)	15	3.4
Leucine	Leu (L)	74	16.8
Lysine	Lys (K)	6	1.4
Methionine	Met (M)	10	2.3
Phenylalanne	Phe (F)	19	4.3
Proline	Pro (P)	44	10.0
Serine	Ser (S)	37	8.4
Threonine	Thr (T)	24	5.5
Tryptophan	Trp (W)	8	1.8
Tyrosine	Tyr (Y)	6	1.4
Valine	Val (V)	25	5.7

The isoelectronic point (or theoretical pI) is the pH at which the protein does not migrate in an electric field. The computed pI value that was less than 7 (pI<7) indicates that proteins were considered as acidic or greater than 7 (pI>7) reveals that proteins were basic in character. As, the pI value was greater than 7 (9.25) reveals that proteins were basic in character. The computed isoelectric point will be useful for developing buffer system for purification by isoelectric focusing method. Total numbers of negatively charged residues are lower than the total number of positively charged residues implies that these

proteins are having extracellular portion. The extinction coefficient of a protein as calculated by the program depends on the molar extinction coefficient of Tyrosine, Tryptophan and Cysteine residues. Extinction coefficient which depends on the molar extinction coefficient of Tyrosine, Tryptophan and Cysteine residues, its values for protein at 280 nm is 52940 $M^{-1}cm^{-1}$. The extinction coefficient can be used to calculate the concentration of a protein in solution.

Instability index relies upon the occurrence of certain dipeptides along the length of the protein to distinguish between the unstable and stable protein. If the index is less than 40, it is probably stable in the test tube. If the value is greater than 40, it is probably not stable. Instability index 45.97 indicates the stability of the protein is low. The aliphatic index refers to the relative volume of a protein that is occupied by aliphatic side chains and contributes to the increased thermo stability of protein. The aliphatic index of a protein is a measure of the relative volume occupied by aliphatic side chain of the following amino acids *viz.,* alanine, valine, leucine and isoleucine. Higher value of aliphatic index (106.95) shows that protein is stable for wide range of temperature. Grand average of hydropathicity (GRAVY) index indicates the solubility of proteins: a positive GRAVY value indicates that proteins are hydrophobic in nature whereas a negative GRAVY value indicates more surface accessibility of the protein to interact with water (hydrophilic in nature). The results of primary structure analyses suggest that protein is hydrophobic in nature due to the presence of high content of non-polar residues (Val, Ala, Leu, etc.) because of having low GRAVY index.

From table 3, the presence of transmembrane proteins was predicted. It is an automated server which predict's transmembrane regions. It can be analyzed that this amino acid sequence is a membrane protein which consist of 8 transmembrane helices.

Table 3: Transmembrane Region prediction of 5-hydroxytryotamine receptor -6 from *Homo sapiens*.

N terminal	Transmembrane region	C terminal	Type	length
29	VAAALCVVIALT AAANSLLIALI	51	Primary	23
64	FLVSLFTSDLMV GLVVMPPAMLN	86	Secondary	23
102	WTAFDVMCCSA SILNLCLISLDR	124	Primary	23
142	RALALVLGAWS LAALASFLPLLL	164	Primary	23
184	ASLPFVLVASGL TFFLPSGAICF	206	Primary	23
211	ILLAARKQAVQV ASLTTGMASQA	233	Primary	23
275	GMFFVTWLPFFV ANIVQAVCDCI	297	Primary	23
303	DVLTWLGYCNS TMNPIIYPLFMR	325	Secondary	23

The secondary structural features were predicted by the GORIV server which indicates that selected protein and the result suggests that random coils are dominated to over extended strands and alpha helix in the provided protein (Table 4).

Table 4: Secondary structure prediction of 5-hydroxytryotamine receptor -6 from *Homo sapiens*.

Type	No. of Amino Acid	Percentage composition
Alpha helix (Hh)	161	36.59%
Extended strand (Ee)	52	11.82%
Random coil (Cc)	227	51.59%

The 3-dimensional structure was selected based on the highest sequence identity, lowest e-value, maximum query coverage, bit score and higher resolution of the template structure. The homology modeling showed that the QMEAN score [21] of the model was -3.93, and the sequence identity was 31%, sequence similarity was 0.36 and sequence coverage was 0.70. The result of QMEAN and GMQE scores were -3.93 and 0.50, respectively. Further, 97.1 % residues were in favored region and 2.6% residues were in allowed regions which shows that this model is extremely good model (Figure 1).

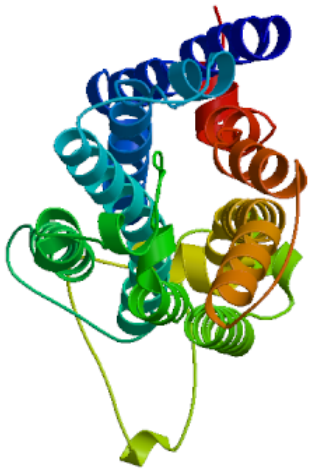

Fig. 1: 3D-model of 5-hydroxytryotamine receptor -6 from *Homo sapiens*.

4. CONCLUSION

In present investigation, amino acid sequences of 5-hydroxytryotamine receptor -6 from *Homo sapiens* have been characterized which is responsible for Schizophrenia. The primary analysis reveals that the protein is hydrophilic and expected to be stable over wide range of temperature. Secondary structure analysis established, random coils were the dominating followed by alpha helix, extended strand and beta turns. The model generated from homology modeling is reliable. The present investigation will provide insights about 3-D structure of protein which will further aid in formulating their uses in academics and industries.

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