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Identification of the mutated sites present in the transmembrane regions of SCN1A_HUMAN (Sodium Voltage-Gated Channel Alpha Subunit 1) using Insilico techniques

Balaji Munivelan*

ABS Geno-informatics Pvt. ltd, Chennai-600005, Tamil Nadu, India

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ABSTRACT

Mutations in numerous genes which encode for voltage-gated sodium channels give rise to various epilepsy syndromes in humans. Our research investigation mainly focuses on the identification of the integral membrane protein of the SCN1A (Sodium Voltage-Gated Channel Alpha Subunit 1) in humans. Secondary, we focus on the transmembrane membrane (TP) amino acids directly involved in the epilepsy-involved mutated regions. Using Insilico protocols, we identify the TP proteins and amino acids and elucidate the Transmembrane Helix and the inside and outside amino acids regions of the SCN1A. With the help of Insilico proteomics server, the amino acids in the mutated regions involved in the TP were identified. Finally, 3D structure prediction was performed using homology modelling server and the modelled structure was cross validated for the TP and validated. The identified results were validated using molecular visualization tools. We prove that the mutated amino acids are present in the outer membrane of the TP regions. Thus, the outer membrane of sodium channel and the amino acids present in the outer membrane (T875M, R859C, and R1648H) play a vital role in Structure-Based Drug Designing and Drug Docking studies.

*Corresponding Author

Name: Balaji Munivelan
Phone: 9444-345-906
Email: bioinfobalaji@gmail.com

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INTRODUCTION

In our research work, we primarily focus on the Transmembrane helix regions directly involved in mutated amino acid regions of sodium channel gene. These amino acid regions play a vital role in sodium channel-related disorders. The most commonly mutated human epilepsy gene is SCN1A. About 900 different mutations are reported in various families. SCN1A gene codes for the α-subunit of the neuronal voltage-gated sodium ion channel, type1 (NaV 1.1), and is expressed in the central and peripheral nervous systems and in cardiac myocytes. [1, 2] . The first reported mutations in the SCN1A gene were in two families with generalized epilepsy with febrile seizures plus (GEFS+), while subsequent studies have shown that a majority (>80%) of the mutations are associated with the Dravet syndrome (DS) . Using advanced Insilico techniques, we found out the potential mutated amino acids which coincide with the integral membrane proteins (Inside-Outside Transmembrane Helix domains). Simultaneously, we also predicted the 3D structure and its effects [3–5] .

METHODOLOGY

Sequence Retrieval System

The SCN1A protein sequence (UniProt ID: P35498) was retrieved from UniProt database in order to perform protein modelling and predict the Transmembrane helix domains [6–8] .

Protein Modelling and 3D Visualizations

Protein modelling studies were done using an automated homology modelling server called CPH 3.0 server [7,8]. The modelled SCN1A protein 3D structure was viewed with the help of the Molecular visualization tool, Discovery studio. Using Discovery studio software we view the complete intra molecular regions of the mutated amino acids and the integral protein information [9–13] .

Trans-membrane Helix region prediction

The identified transmembrane helix domains were validated using TMHMM [9,10,11] tools in order to examine the outer and inner layers of the transmembrane amino acids present in the SCN1A protein.

RESULTS AND DISCUSSION

(This gene encodes a sodium channel alpha subunit, which contains four homologous domains, each of which comprises of six transmembrane regions.)

nt nucleotide , aa amino acids , OMIM : Online Mendelian Inheritance in Man® Chr. Loc - Chromosome location

The action potential in muscle cells and neurons are mainly involved in voltage-dependent sodium channels which are heteromeric complexes which help in controlling the sodium exchange between the intracellular and extracellular spaces. Each sodium channel comprises of a large glycosylated alpha subunit and two smaller beta subunits (Table 1). This gene encodes a sodium channel alpha subunit, which contains four homologous domains. Each of these domains is comprised of six transmembrane regions [14–17] .

Tars-Membrane Helix Prediction

TMHMM result

P35498-1_SCN1A_HUMAN Length: 2009

P35498-1_SCN1A_HUMAN Number of predicted TMHs: 19

P35498-1_SCN1A_HUMAN Exp number of AAs in TMHs: 478.28553

P35498-1_SCN1A_HUMAN Exp number, first 60 AAs: 0.00079

P35498-1_SCN1A_HUMAN Total prob of N-in: 0.35740

P35498-1_SCN1A_HUMAN TMHMM2.0 inside 1 124

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 125 147

P35498-1_SCN1A_HUMAN TMHMM2.0 outside 148 189

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 190 212

P35498-1_SCN1A_HUMAN TMHMM2.0 inside 213 218

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 219 241

P35498-1_SCN1A_HUMAN TMHMM2.0 outside 242 250

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 251 273

P35498-1_SCN1A_HUMAN TMHMM2.0 inside 274 400

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 401 423

P35498-1_SCN1A_HUMAN TMHMM2.0 outside 424 763

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 764 786

P35498-1_SCN1A_HUMAN TMHMM2.0 inside 787 798

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 799 821

P35498-1_SCN1A_HUMAN TMHMM2.0 outside 822 885

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 886 908

P35498-1_SCN1A_HUMAN TMHMM2.0 inside 909 968

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 969 991

P35498-1_SCN1A_HUMAN TMHMM2.0 outside 992 1217

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 1218 1237

P35498-1_SCN1A_HUMAN TMHMM2.0 inside 1238 1257

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 1258 1280

P35498-1_SCN1A_HUMAN TMHMM2.0 outside 1281 1346

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 1347 1369

P35498-1_SCN1A_HUMAN TMHMM2.0 inside 1370 1417

Table 1: Protein target summary

Gene name	Protein name	Chr.loc	Gene ID	MIM: ID	UniProt :ID	Nucleotide length	Protein length
SCN1A	sodium voltage-gated channel alpha subunit 1	2	6323	182389	P35498	6030 nt	2009 aa

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 1418 1440

P35498-1_SCN1A_HUMAN TMHMM2.0 outside 1441 1459

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 1460 1482

P35498-1_SCN1A_HUMAN TMHMM2.0 inside 1483 1541

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 1542 1561

P35498-1_SCN1A_HUMAN TMHMM2.0 outside 1562 1570

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 1571 1593

P35498-1_SCN1A_HUMAN TMHMM2.0 inside 1594 1599

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 1600 1622

P35498-1_SCN1A_HUMAN TMHMM2.0 outside 1623 1672

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 1673 1695

P35498-1_SCN1A_HUMAN TMHMM2.0 inside 1696 1762

P35498-1_SCN1A_HUMAN TMHMM2.0 TMhelix 1763 1785

P35498-1_SCN1A_HUMAN TMHMM2.0 outside 1786 2009

In the above picture, yellow represents the transmembrane domains present in the outer layer of the sodium channel, blue represents the transmembrane domains present in the inner layer of the sodium channel, and green represents the TMhelix domains present in the surface of the sodium channel. (Figure 1)

The above graphical picture shows the membrane proteins present in the various regions along with their respective protein sequence positions. (Figure 1)

SCN1A mutations are directly influenced by the functional effect of the protein. For example, these mutations (R1648H, W1204R, and T1875M in tsA201) play a vital role in the intracellular activity of sodium channel. It mainly affects the imbalance of sodium channel activity.[12] SCN1A mutations are directly influenced by the functional effect of the protein. For example, these mutations (R1648H, W1204R, and T1875M in tsA201) play a vital role in the intracellular activity of sodium channel. It mainly affects the imbalance of sodium channel activity. Our results given in graphical form shows that the mutated amino acids R1648H, W1204R, and T1875M are present in the outer membrane of sodium channel Epilepsy Syndromes Caused by SCN1A Mutations

Numerous clinical research data on sodium channel have reported that the amino acids R1648H, W1204R, and T1875M in mutated form play a role in deactivation of sodium channel. Hence, our findings show that the mutated amino acids present in the outer layer would play a significant role in structure-based drug designing and novel drug docking.

Molecular Effects of SCN1A Mutations

The clinical experimental effects of several SCN1A GEFS+ and DS mutations have been investigated using heterologous expression systems. The locations of the mutations for which functional data are mentioned in this review are shown in Figure 2. We distinguished the effects of five GEFS+ mutations (R859C, T875M, W1204R, R1648H and D1866Y) by expression in Xenopus oocytes,.

Various literature information clearly prove that the mutations are directly involved the dysfunction of the sodium channel membrane. So, our aim is to find out how the sodium channel membranes are involved in mutation. The modifications due to R1648H and W1204R are predicted to enhance sodium channel function and neuronal excitability, whereas the modifications due to R859C and T875M should reduce channel function and neuronal excitability. The inhibition of the outer membrane amino acids would down-regulate the sodium channel proteins which helps in reducing the epileptic effect in patients.(Figure 2)

Epilepsy-Causing Mutations in SCN1A

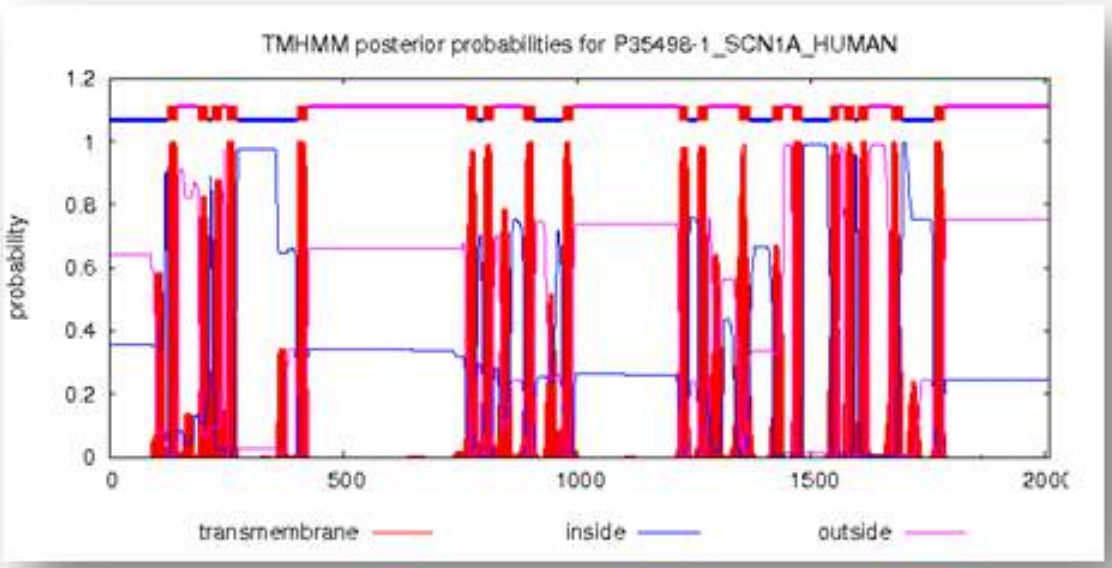

Figure 1: TMHMM result

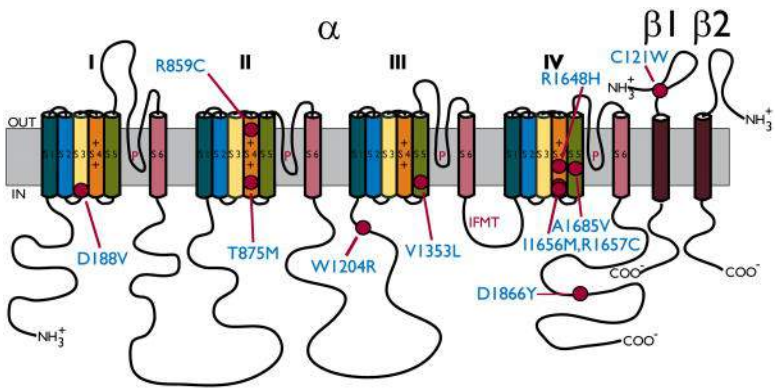

Figure 2: Sodium channel membrane

The diagram clearly shows the $Na_v1.1$ sodium channel α subunit and associated $\beta 1$ and $\beta 2$ subunits which are involved in membrane transport domains and the mutated amino acids responsible for channel inactivation. A complete list of the epilepsy-causing mutations in SCN1A can be found at Andrew Escayg1 and Alan L. Goldi (2010) (Figure 3).

Yellow colour indicates the outer membrane of sodium channel. The mutated amino acids in the outer membrane are present in the above ranges(Table 2) .

Secondary structure of SCN1A: Red represents helix, blue represents sheets and white represents the coiled regions viewed using Discovery studio software

Secondary structure of SCN1A: Red represents helix, blue represents sheets, white represents the coiled

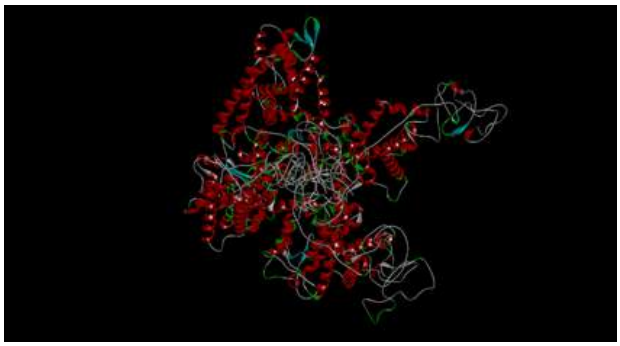

Figure 3: 3D structure of SCN1A

regions and yellow represents the transmembrane helix domains (1623 to 1672), viewed using Discovery studio software(Figure 4)

Secondary structure of SCN1A: Red represents helix, blue represents sheets, white represents the coiled

Table 2: Prediction of transmembrane regions

Gene name	Protein name	Integral membrane region	Functional Effects of SCN1A Mutations
SCN1A	sodium voltage-gated channel alpha subunit 1	Outside 822 to 885	T875M
		Outside 822 to 885	R859C
		Outside 1623 to 1672	R1648H

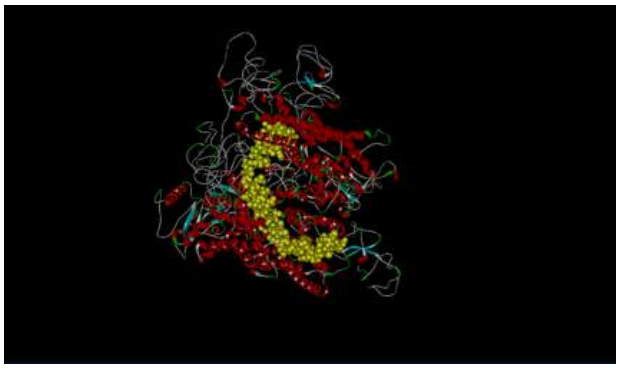

Figure 4: 3D structure of SCN1A

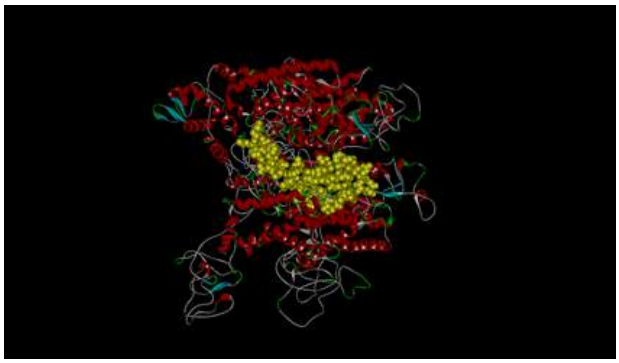

Figure 5: 3D structure of SCN1A

regions and yellow represents the transmembrane helix domains (822 to 885), viewed using Discovery studio software(Figure 5)

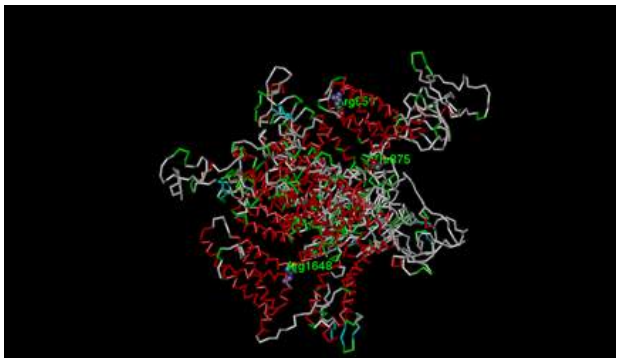

Figure 6: 3D structure of SCN1A

Secondary structure of SCN1A: Red represents helix, blue represents sheets, white represents the coiled regions and green represents the mutated amino acid positions viewed using Discovery studio software(Figure 6)

CONCLUSION

Several recent therapeutic targets are found in transmembrane domains and proteins. In conclusion, our results clearly elucidate that the proteins present in the outer membrane of the sodium channel play a potential role in drug binding. This was proved using 3D structure-based predictions. Hence, the outer membrane of sodium channel and the amino acids present in this outer membrane (T875M, R859C, and R1648H) significantly contribute to structure-based drug designing and drug docking studies.

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CONFLICT OF INTEREST

Authors declared no conflict of interest.

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ABOUT AUTHORS

Balaji Munivelan

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