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In-silico modeling of EDNRB Protein Structure and its mutational analysis in Hirschsprung Disease

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ABSTRACT

Hirschsprung's disease is a developmental disorder characterized by an absence of enteric ganglia in the distal colon leading to a failure of innervation in the gastrointestinal tract. The endothelin-B receptor-mediated signaling pathway participate in the migration and differentiation of enteric ganglion cells.Mutation inendothelin receptor B gene (EDNRB), that encode a G protein- coupled transmembranous receptor protein causes Hirschsprung's disease. Different in- silico techniques were used to determine functional properties of EDNRB gene. The structure of EDNRB protein wasmodeled and validated. Protein structures with known missencemutation Trp276Cys, was modeled and submitted in PMDB. Changes in the interaction between EDNRB and EDN3were determined with wild type protein structure and mutated structure. A decrease in the protein stability and loss of interaction between the subunits was observed. This could have a significant impact on the disease progression.

KEYWORDS EDNRB, EDN3,Hirschsprung'sdisease, W276C

INTRODUCTION

Hirschsprung's disease is a congenital developmental disorder characterized by the absence of enteric ganglia in the distal colon that leads to a failure of innervations in the gastrointestinal tract, with a heritability of roughly 100% and multi factorial inheritance in most cases(Puffenberger EG. et al, 1994). Families with apparent monogenic inheritance, there is incomplete penetrance of disease-causing mutations and intra and inter-family variation of phenotype severity, suggesting that modifying genetic, stochastic or environmental factors are involved(Amiel J. et al, 1996). The length of the aganglionic intestinal segment is highly correlated to risk in siblings and to the ratio of affected females to males (Amiel J. et al, 2008). The incidence of Hirschsprung's disease is estimated to be 1 in 5000 live births(Bodian and Carter CO M. 1963).

Many genetic studies has demonstrated that HSCR is associated with mutations in eight different genes (EDNRB, EDN3, ECE1, SOX10, RET, GDNF, NRTN and ZFHX1B) (Chakravarti, A. &Lyonnet, S. 2001)

(Amiel, J. et al, 2001). Recent studies has suggested that RET and EDNRB are the primary genes implicated in the etiology of Hirschsprung's disease(Badner JA. et al, 1990). All eight genes participate in the early development of the enteric nervous system, and most act through two different biochemical pathways mediated by RET and EDNRB(Chakravarti, A. 1996). (Paul K. et al,2009).

The EDNRB gene encodes a 442 amino acid heptahelicalG protein-coupled receptor (Arai H et al, 1993) that equally binds EDN 1, 2 and 3 and is involved in the intracellular signaling pathway. (Edery P. et al.1996) Binding of EDNRB with its ligands, endothelin 1, 2 and 3 (EDN 1, 2 and 3) (Bidaud et al, 1997) (Sakurai T. et al, 1992)C triggers intracellular signal transduction events which lead to the activation of phospholipase Cb, plasmamembrane calcium channels, and non receptor tyrosine kinases (Baynash AG. et al, 1994).

A known G→T missense mutation(W276C) in exon 4 of EDNRB gene located to 13q22 that substitutes the highly conserved Trp-276 residue in the fifth trans membrane helix of the G protein-coupled receptor with a Cys residue (W276C). The penetrance of the Trp276Cys mutation is 74% in homozygotes and 21% in heterozygotes(Puffenberger EG. et al, 1994).

Hence, it was found fit to evaluate the influence of the muta-

tion (Trp276Cys) in the disease progression by predicting the molecular changes in the protein structure through in silico models generated with the help of standard bioinformatics tools. These mutations could be predicted to affect the disease progression due to protein instability in combination with allelic heterogeneity.

Materials and Methods:

Sequence analysis, phylogenetic study and motifidentification:

Protein sequences of EDNRB genewas retrieved from NCBI database (PruittKD.et al, 2007) Basic local alignment search tool (BLAST;http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for similarity search against various organism deposited in NCBI database. Phylogenetic classification was done using MEGA5.05 (KumarS. etal).The sequenced EDNRB domains were subjected to phylogenetic tree construction with NJ Method at 1000 bootstrap replication value. The conserved motifs present EDNRB gene was analyzed using MEME (Multiple EM forElicitation) (Bailey TimothyL. et al.2009)that was used to identify core-conserved motifs. Parameters have been set manually with number of 30 motifs, minimum motif width was 10 and maximum motif width was 100.

Molecular modeling and verification:

For three dimensional structure predictions, target proteins were taken for PDB BLAST searchto select corresponding template structures. Discovery studio version 3.1 (http://www.accelrys.com, Discovery Studio 3.1, 2011, AccelrysInc, San Diego, CA, USA) (JoshiRS. et al, 2013)was used for homology modeling of target proteins. Structureof EDNRB was modeled using Discovery Studio 3.1. The modeled structures were submitted in PMDB with PMDBID PM0079229. The secondary elements of predicted structure were determined using UCSF chimera and PDBSum server (PettersenEF.et al,2004). Finally, predicted 3D model was subjected to a series of tests for testing its internal consistency and reliability.The Quality of the model was checked with verify3D (Roland Luthy. et al, 1992), and Errat server (Colovos C. and YeatesTO.1993)

The stereo chemical properties based on backbone conformation were evaluated by inspection of Psi/Phi/Chi/Omega angle using Ramachandran plot of PDBSum database using PROCHECK server (MorrisAL. et al,1992).Structure refinement was done using RAMPAGE(LovellSC. et al, 2003).

Protein- Protein interaction:

Hex 6.3(Ritchie DWandVenkatraman V. et al, 2010) was used to determine the protein-protein interaction. Docking was done between the modeled structure of EDNRB and its ligand ET-3.Hex 6.3 tools were used for protein interaction study.Interaction obtained through docking study were visualized using Discovery studio version 3.1.

Result and Discussion:

Structure modeling-

Modeled Protein structure of EDNRB shows 2 sheets, 2 beta hair pins, 4 strands, 14 helices, 31 helix-helix interacs, 28 beta turns,7 gamma turns.(fig1)

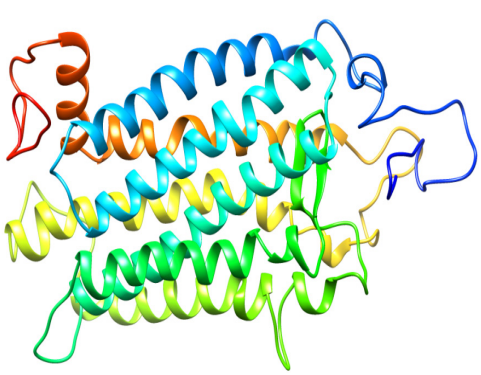


Fig1-3 D structure of EDNRB protein modeled by Esypred and swissmodel software With PMDB ID PM0079229

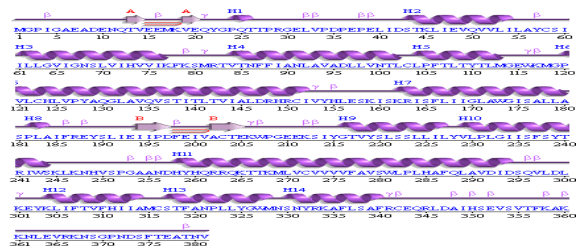


Fig2- Secondary structure of of EDNRB protein

Sequence analysis, phylogenetic study and motif identification:

Two major clusters were obtained from phylogenetic tree based on EDNRB sequences from selected species. Homo sapiens(NP852122.1) showing maximum similarity with Gorilla gorilla(XP004024824.1) Motif 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 16, 18 and 19 were conserved in all the species.

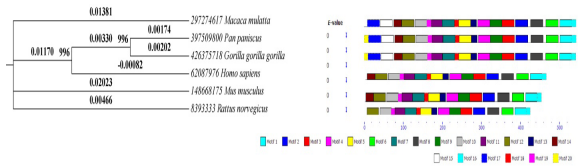


Figure 3: Phylo genetics tree based on EDNRB sequences from different species along with schematic distribution of respective conserved motifs; identified by means of MEME software.

Motif	With	Best possible match
1	30	CWCQSFEEKQSLEEKQSCLKFKANDHGYDN
2	30	FYTLMTCEMLRKKSGMQIALNDHLKQRREV
3	30	HPVQKTAFMQFYKTAKDWWLFSFYFCLPLA
4	30	DRYRAVASWSRIKGIGVPKWTAVEIVLIWV
5	30	HIVIDIPINVYKLLAEDWPFGAEMCKLVPF
6	30	DYIGINMASLNSCINPIALYLVSKRFKNCF
7	30	CLVFVLGIIGNSTLLRIIYKNKCMRNGPNI
8	30	LVFALCWLPLHLSRILKLTLYNQNDPNRCE
9	30	VSVVLAVPEAIGFDIITMDYKGSYLRICML
10	30	QTAEIMTPPTKTLWPKGSNASLARSLAPAE
11	26	PRTISPPPCQGPIEIKETFKYINTVV
12	30	CGRALVALVLACGLSRIWGEERGFPPDRAT
13	14	IQKASVGITVLSLC
14	19	RSGHRTPSGAGSSMQPPPS
15	30	PRAGWNPAGFRVPGRWSPFVALHLVCQIRE
16	10	FRSSNKYSSS
17	30	TPSKRWRLHCLAFSQRFVRARPACSSREAC
18	10	IASLALGDLL
19	10	VPKGDRTAGS
20	10	MNKSTCLMAA

Table1: Multilevel consensus sequences of EDNRB for motifs among different species.

Physiochemical analysis

Analysis of physiochemical properties of the EDNRB protein showed that it is rich in Leucine (12.9%), Serine (8.1%), and Alanine (7.2%). EDNRB protein is mainly hydrophobic in nature. The instability index was above 40 for EDNRB protein indicating that it is highly unstable. This hydrophobic nature facilitate in determination of the structural properties of EDNRB protein.

Molecular modeling and verification:

RAMPAGE(Fig 4) study of predicted model of EDNRB showedthat 88.6% of total residues in the most favored regions and 7.5% of residues in additional allowed regions. 3.9% residues were found in generously allowed regions and no residues were found in disallowed region for EDNRB protein. The model having more than 90% in favored region is best model; based on these findings we have predicted that the used method in present study is reliable.In Fig 5.The quali-

ty factor checked by Errat server was 52.125% for EDNRB and suggests that the models having good quality. In Fig 6 plot show, Verify 3D score lied between 0.55 –(-0.47) for EDNRB proteinThe amino acids exist in between the marked region. Hence we can say that the modeled structure is good.

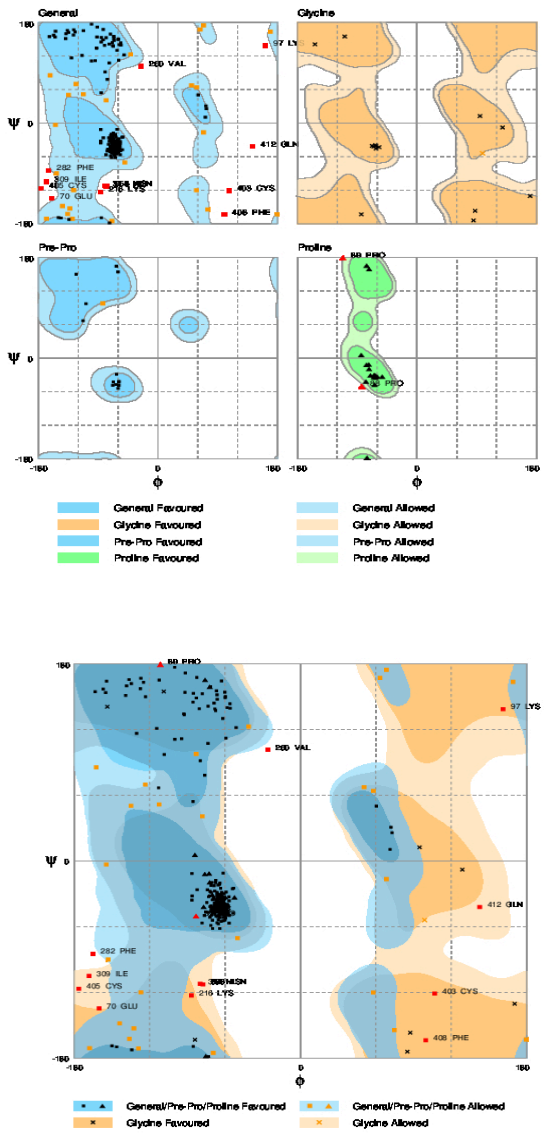


Figure 4: Rmachandran plot of modeled structure of EDNRB Protein

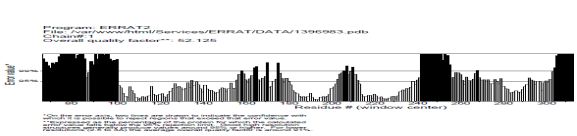


Figure 5: The quality factor of EDNRB Protein structure checked by Errat server

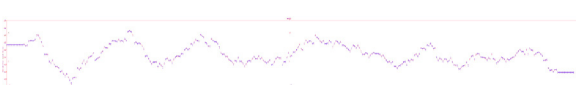
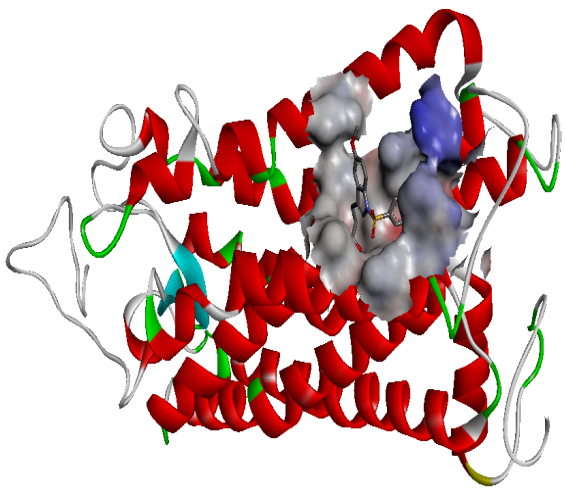


Figure 6: EDNRB Protein structure checked by Verify 3D

Protein-Protein interaction

EDNRB normal interaction	EDNRB mutated (W276C) interactions
LEU190, SER191, CYS193, ALA194, LEU195, ILE197, ASP198, ARG201, ILE212, TRP217, VAL220, GLU221, LEU224, ILE225, ILE288	LEU190, ALA194, GLU221, ILE222, , LEU224, ILE225, VAL227, VAL228, LEU284, PRO285, VAL231, ILE288

Table2: Docking interactions of normal EDNRB and EDNRB mutated (w276c) with EDN3.



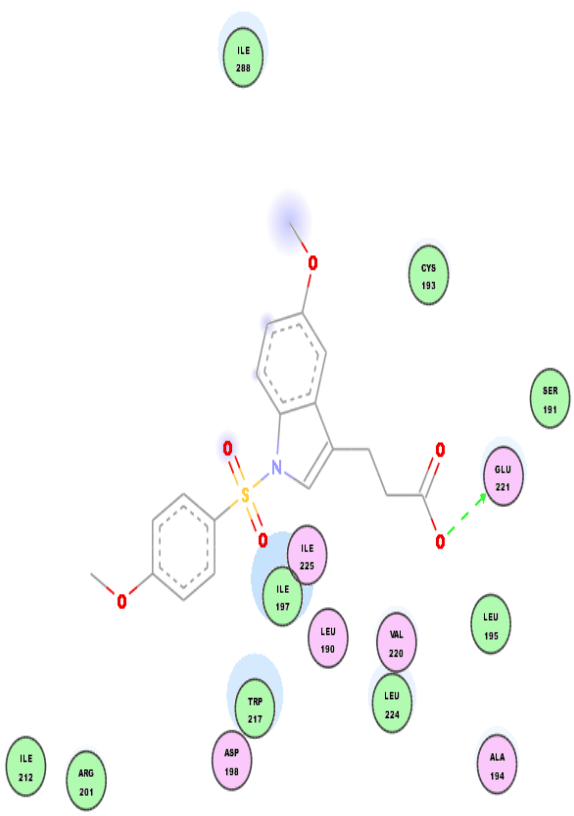


Figure 7: Interaction of Wild type EDNRB with EDN3

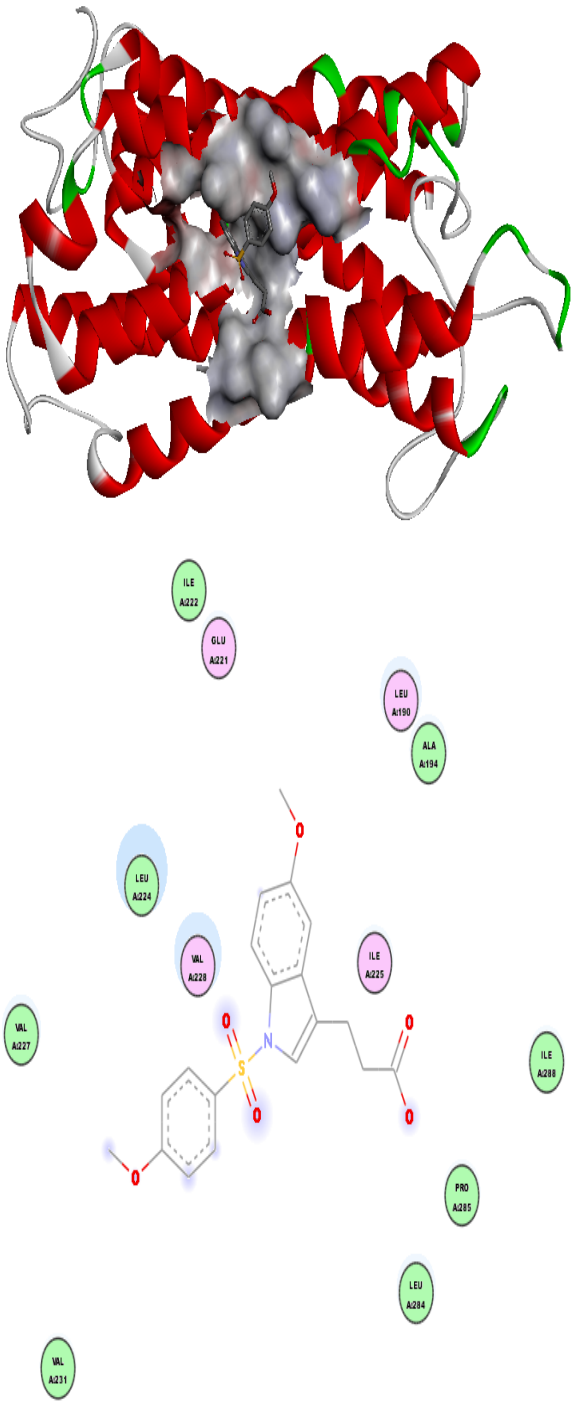


Figure 8: Interaction of mutated EDNRB (W276C) with EDN3

Discussion

The study was done to determine the sequential and structural aspect of EDNRB gene in Homo sapiens using computational tools. Phylogenetic tree results outlined the clustering of EDNRB gene of Homo sapiens alongwith different member of mammalian family.Phylogenetic study and meme analysis revealed that motif 15, 17 and 20 were absent in Homo sapiens and MusMusculus revealing that Homo sapiens show maximum similarity with Mus musculus.In HSCR patients a G>T missense mutation in EDNRB exon 4 that substitutes the highly conserved Trp-276 residue in the fifth trans-membrane helix of the G protein-coupled receptor with a Cys residue (W276C)(Puffenberger EG. et al, 1994).The change at residue 276 Tryptophan to cysteine due to missense mutation does not affect the overall confirmation of the protein.Themissence mutation occur in 8 helix but does not disturb the stability the α helical secondary structure, however the two residues trp, cys differ in both size and nature, as trp is non polar in nature and contain OH side group, and cys is polar in nature and contain sulphur side group and results in change in protein confirmation. This interaction study helps us in determining that in normal interaction of EDNRB with EDN3 interaction are observed at positions LEU190, SER191, CYS193, ALA194, LEU195, ILE197, ASP198, ARG201, ILE212, TRP217, VAL220, GLU221, LEU224, ILE225, ILE288. However interaction study of ET3 with mutated EDNRB (W276C) results in interaction at positions LEU190, ALA194, GLU221, ILE222, LEU224, ILE225, VAL227, VAL228, LEU284, PRO285, VAL231, ILE288. The mutation at W276C is intolerant. Trp is a non polar amino acid and cysteine is polar in nature. The change of amino acid from non-polar to polar would affect the interaction of EDNRB with ET3 ligand.The mutant protein has a different binding site then those of normal protein structure. During the study less interactions were observed in mutant protein structure. The interactions were in hydrophobic amino acids. EDNRB is hydrophobic in nature and in native protein ET3 binds at LEU195, ILE197, ASP198, ARG201, ILE212, TRP217, VAL220 in which ASP198 is acidic amino acid and ARG201 is basic amino acid. However in mutant structure the interaction was observed at positions VAL227, VAL228, LEU284, PRO285, VAL231 and all are hydrophobic in nature. Also the interaction observed was at different position than in native protein.

We can conclude that change in position of interaction would not lead to normal interaction of protein (EDNRB) with ligand (EDN3). Hence, we can predict that distorted interaction would lead to Hirshprung disease.

Further functional studies need to be done to establish protein turnover in wild type and mutant cell lines in order to validate the hypothesis that the protein instability induced due to the mutations and allelic heterogeneity are responsible for the disease progression an severity.

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