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Research Article

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Molecular Modelling of nsSNPs in GABRA2 Gene In Epilepsy And Study Of Their Impact On Structure And Stability of GABRA2 Protein

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Abstract

Epilepsy is a neurological condition characterized by abrupt, unprovoked, and recurrent seizures that are unpredictable in frequency. The objective of this analysis was to identify novel nonsynonymous polymorphisms in the GABRA2 gene and determine their effect on protein structure and stability. Most pathogenic/deleterious nsSNPs were predicted using six different bioinformatic tools. Mutpred2, Mupro was used to check the impact of identified nsSNPS on protein structure and stability. The pathogenic score of SNPs was predicted using the FATHMM tool. The CONSURF webserver was used for conservation analysis of pathogenic SNPs.3-D structure of the protein was realized using SWISS-MODEL and residue position in protein visualized by Lite Mol. GeneMANIA and STRING database is used to predict the function of interlinked gene interactions. Out of 228 nsSNPs retrieved from the dbSNP database, we identified a novel missense variant, F285S (rs41310789) as most deleterious and its possible association with epilepsy syndrome focal epilepsy. Stability and conservation analysis results interpret rs41310789 present in evolutionarily conserved regions of a gene and affect its structure. This analysis provides information regarding the impact of nsSNPs that might affect the structure and activity of GABRA2 protein. Thus, these coding variants should be taken into scrutiny while genetic screening of epileptic patients.

Keywords: Computational Analysis, Seizures, Neurotransmitter, Coding Variants, GABA, Brain

Introduction

Epilepsy is characterized by frequent seizure, unusual sensation, and loss of awareness in some cases and cause different health-related problems [1]. Repeated epileptic seizures occur due to an imbalance between inhibitory and excitatory neurotransmitters in the brain [2]. According to a study of syndromes' global burden, epilepsy stands at second position worldwide [3]. Epilepsy is also a prevalent neurological disorder in Pakistan, accounting for 1% of the Pakistani population [4]. In Pakistani population, 9.99 over 1000 people have epilepsy. A higher number of cases belongs to older people over 30, i.e., 2 million individuals and 1/10th of the global burden of epilepsy in Pakistan [5]. Existing literature has provided comprehensive knowledge about the pathogenic mechanism of epilepsies, including variations in ion channels, abnormal release or uptake of neurotransmitters, and neuronal loss [6].

Gamma-Aminobutyric acid is a primary inhibitory neurotransmitter present in the human cerebrum. It acts through 3 classes of GABA receptors, ionotropic GABAA, GABAC, and metabolic GABAB, with unique biochemical and electrophysical properties. GABA receptors are ligand-gated chloride ion channels present all over the human brain [7].

GABRA2 gene plays a significant role in early infantile epileptic encephalopathy (EIEE). It is involved in the synaptic neurotransmission pathway and affects the GABAergic inhibition of excitatory and inhibitory neurons [8]. Mutations in the GABRA2 gene are implicated in several neurological disorders, including epilepsy. Multiple genome-wide association studies identified nsSNPs associated with an increased risk of epilepsy. Another study by Naama Orenstein et al. also positions GABRA2 as a potential candidate for Early-onset epileptic encephalopathy(EOEE) [8].

Mapping GABRA2 variants to protein structure showed they were located near the desensitization gate, critical regions for GABAergic receptors' proper functioning, and GABA energic synapse pathway is involved in epilepsy [9].

There is a bloom in bioinformatics tools during the sars covid-19 pandemic and increasingly used to analyze gene regulation and protein expression in molecular biology analysis [10]. The bioinformatics tools help us understand the role of nsSNPs in protein destabilization by predicting amino acid change in a protein sequence. Moreover, homology modelling can be performed to eval-

uate the crucial role of amino acid change on protein structure and its impacts on stability.

This study's main objective is to perform computational analysis to predict the most deleterious coding polymorphisms and identify their pathogenic effect on protein structure using different tools.

Materials and Methods

Data Source

All the literature reviewed related to epilepsy, GABRA2 gene,nsSNPs was searched using google scholar, science direct, Pubmed, and NCBI databases. We use keywords epilepsy, genetic association,nsSNPs, and human protein to search literature. As its a new study, we only select those publications that prove the association of nsSNPs in epilepsy.

Retrieval of SNPs

All the information about GABRA2 SNPs was gathered from dbSNP, a database established by National Center for Biotechnology Information (https://cutt.ly/2jotOe4). The amino acid sequence of the protein in FASTA format was collected from NCBI (NP_000798.2). Polymorphisms for GABRA2 were obtained from NCBI using different filters like missense, nonsynonymous, human, coding, nonsense, and intronic.

Identification of Most Deleterious nsSNPs

We use six different bioinformatics tools to predict the effect of nsSNPs on GABRA2 protein structure. These tools include SIFT, PROVEAN, PolyPhen-2 PANTHER PhD-SNP, and SNP&GO (Table 1). We only include SNPs that are predicted as deleterious by these software [11-15].

Table 1: List of Tools Used to Predict Most Pathogenic nsSNPs

TOOL	THRESHOLD VALUE	PREDICTION	URLS
SIFT	< 0.05	Deleterious	https://sift-dna.org
PROVEAN	≤ 2.5	Deleterious	https://provean.jcvi.org/index.php
PolyPhen-2	1.0	Deleterious	https://genetics.bwh.harvard.edu/pph2/
PhD-SNP	< 0.5	Disease	https://snps.biofold.org/phd-snp/phd snp.html
SNP&GO	>0.5	Disease	https://snps-and-go.biocomp.unibo.it/snps-and-go/
PANTHER	N/A	Probably/Possibly damaging	https://www.pantherdb.org/tools/

Stability Analysis

We used two different bioinformatic tools to check the impact of pathogenic single nucleotide polymorphisms on protein structure and stability (Table 2). Mupro used artificial intelligence technology to predict the stability score, while Mutpred2 used 14 different molecular and structural functions to realize the impacts of nsSNPs on protein structure [16, 17].

Table 2: Algorithms Used to Check The Impact on Protein Stability

ALGORITHMS	PREDICT	URLS	REFERENCES
Mupro	Protein stability	https://www.ics.uci.edu/~baldig/mutation.html	(Saih et al., 2021)
Protein stability	Protein stability	http://mutpred.mutdb.org/	(Pejaver et al., 2020)

Disease Association Analysis

We use FATHMM to predict the phenotypic and molecular significance of single point mutations and their impact on the human genome. This software use hidden HMMs(Markov models) to predict the effect of both coding variants(missense or nsSNPs) and non-coding variants in the human genome [18, 19].

Conservation Analysis

Consurf was used to characterized variable and conserved areas of GABRA2. Fifteen years ago, the Consurf web software was developed to visualize evolutionarily conserved regions present in a protein sequence. This server predicts the degree of amino acid conservation [20, 21].

Three-Dimensional Structure of the Protein

3-D structure of GABRA2 protein was generated by SWISS-MODEL web server, and residue position in protein sequence was visualized by Lite Mol.GeneMANIA and STRING database is used to predict the function of interlinked gene interactions. GeneMANIA and STRING are online databases that predict genes' interactions with each other and their common associations like functional/molecular pathways, genetic interactions,co-expression, and other typical interactions between them. In the case of GABRA2, we found everyday neurotransmitter activity and neuron-neuron synaptic transmission with other genes [22-27].

Results

Distribution of SNPs

A total of 36912 SNPs present in human GABRA2 were retrieved from the dbSNP database. On other selection, 34375 SNPs were intronic variants,142 as synonymous, five pathogenic variants, and 228 as nonsynonymous SNPs (Figure 1). According to NCBI data, nonsynonymous SNPs contribute to only 0.62% of all the SNPs reported in the human GABRA2 gene.

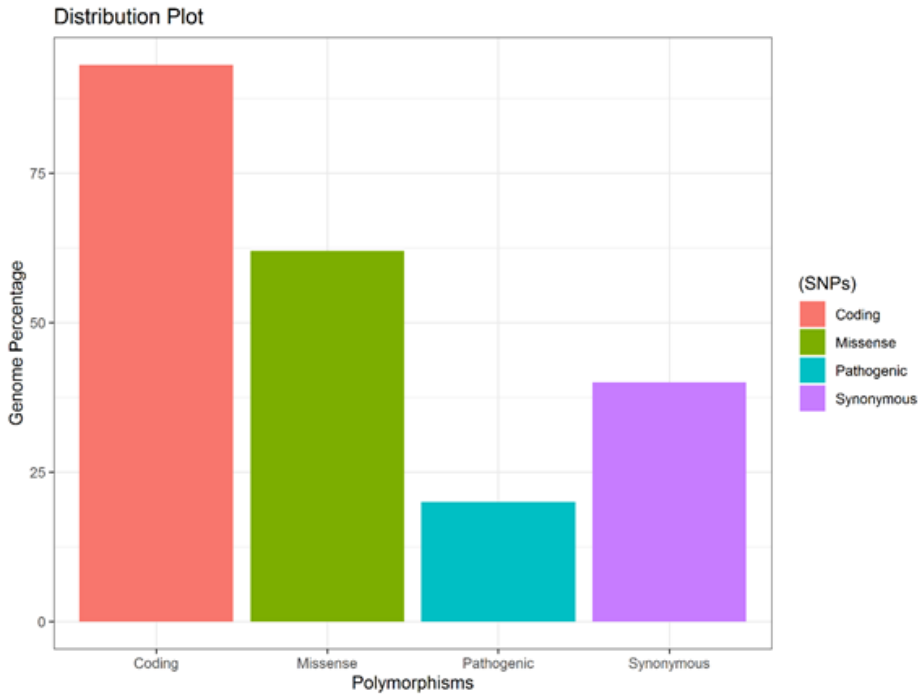


Figure 1: Distribution of GABRA2 SNPs Present in Human Genome

Prediction of Most Deleterious nsSNPs in *GABRA2*

Seven different algorithms, SIFT, PROVEAN, Polyphen, Panther, SNP&GO, PhD-SNP, and FATHMM, were applied to predict the impact of pathogenic nsSNPs on protein stability and structure. Fathmm also predicts specific epileptic syndromes associated with these SNPs (Table 3). Out of 228 nonsynonymous single nucleotide polymorphisms, only six were predicted as deleterious/disease/damaging, while FATHMM predicts only F285S as damaging, causing focal epilepsy. Out of these six nsSNPs, only F285S was predicted as most pathogenic.

Table 3: Output of nsSNPs Predict As Deleterious By All Algorithms

SNP id	Substitution	SIFT	PROVEAN	Polyphen	Panther	SNP&GO	PhD-SNP	FATHMM
rs17852044	V80A	T	D*	P.D	P.D	D	D	N/A
rs41305781	T43A	D*	D*	P.D	P.D	D	N	N/A
rs41310789	F285S	D*	D*	P.D	P.D	D	D	D!
rs74440199	T70I	D*	D*	P.D	P.D	N	D	N/A
rs76519302	P79S	D*	D*	P.D	P.D	N	D	N/A
rs199725032	F93C	D*	D*	P.D	P.D	D	D	N/A
D*: deleterious,P.D:probably damaging,N:neutral,D:disease,D!:damaging.								

Protein Stability Analysis

After finding the most deleterious nsSNP, we subjected it to Mupro and MutPred algorithms to find its impact on protein stability. Mupro uses a scale between 1 and -1 to give a predictability score and a score of fewer than 0 means protein stability decreases due to mutation, while a score of more than 0 means protein stability increases (Table 4).

Table 4: Stability Analysis Results

MUPRO SCORE	PREDICTION	MUTPRED SCORE	PREDICTION
-1.4209216	Decrease	0.946	Altered Ordered interface Altered Transmembrane protein A gain of Relative solvent accessibility A gain of O-linked glycosylation at F285. Altered Metal-binding.

MuPro is a web based server requires input as protein sequence along with original and substituted amino acid and their mutation position. The output comes as a score between 1 and -1.A score of less than 0 means protein stability decreases due to mutation, while a score of more than 0 means protein stability increases

Table 5: Protein Structure stability Prediction by MuPro.

db. SNP ID	Position	Wild type	New type	MUpro Prediction	Score
rs519972	407	A	T	DECREASE	-1.1659455
rs41305781	43	T	A	DECREASE	-0.6463223
rs200604169	394	P	L	DECREASE	-1.1240578
rs201337492	394	P	S	DECREASE	-1.8117133
rs371482725	7	I	V	DECREASE	-0.80389694
rs202063015	2	K	M	INCREASE	0.40566616
rs373038663	186	S	C	DECREASE	-0.75328135
rs370917838	366	A	T	DECREASE	-1.1659195
rs201873906	420	M	T	DECREASE	-2.3525122
rs201634979	119	S	N	DECREASE	-1.5025969
rs200987678	443	R	K	DECREASE	-0.74614166
rs200623602	195	T	A	DECREASE	-0.53655061
rs200515415	377	N	K	DECREASE	-0.48169931
rs200327122	10	M	T	DECREASE	-1.8330516
rs199725032	93	F	C	DECREASE	-1.4173713
rs199561756	133	K	R	DECREASE	-0.14886374
rs149542311	9	N	S	DECREASE	-1.0518561
rs147496964	382	P	L	INCREASE	0.16958276
rs143035942	360	V	I	DECREASE	0.041290255
rs139362120	10	M	I	DECREASE	-1.1098266
rs76519302	79	P	S	DECREASE	-1.338545
rs74611721	41	I	V	DECREASE	-1.1308063
rs74440199	70	T	I	DECREASE	-0.49642452
rs41310789	285	F	S	DECREASE	-1.4209216
rs41301819	16	V	L	DECREASE	-1.2993474
rs17852044	80	V	A	DECREASE	-0.74708111

Table 6: Impact of Substitutions on Protein Structure

a.a Substitution	Mupred score	Effect on Protein structure
V135A	0.628	Altered Ordered interface(P=0.28) Loss of Relative solvent accessibility(P=0.26) Loss of Strand(P=0.26) The gain of B-factor(P=0.24) Altered Metal-binding(P=0.23) Altered DNA binding(P=0.23) Loss of Allosteric site at T140(P=0.21) Altered Transmembrane protein(P=0.20) A gain of Methylation at K133(P=0.11) Altered Stability(P=0.10)

T43A	0.874	Altered Transmembrane protein(P=0.24) The gain of N-linked glycosylation at N38(P=0.14)
F285S	0.946	Altered Ordered interface(P=0.38) Altered Transmembrane protein(P=0.30) A gain of Relative solvent accessibility(P=0.25) A gain of O-linked glycosylation at F285(P= 0.23) Altered Metal-binding(P=0.12)
T70I	0.912	Altered Ordered interface(P=0.35) Altered Disordered interface (P=0.29) Altered Transmembrane protein(P=0.21)
P79S	0.881	Altered Ordered interface(P=0.28) The gain of Relative solvent accessibility(P=0.28) Altered Transmembrane protein(P=0.23)
K133R	0.727	Loss of Relative solvent accessibility(P=0.26) Altered Metal binding(P=0.25) Altered Transmembrane protein(P=0.17) Altered DNA binding(P=0.15)
F93C	0.936	Altered Ordered interface(P=0.33) Altered Metal-binding(P=0.30) Altered DNA binding(P=0.22) The gain of Allosteric site at D90(P=0.19) Altered Transmembrane protein(P=0.16) A gain of Pyrrolidone carboxylic acid at Q95(P=0.06)
A375T	0.591	Loss of Ubiquitylation at K380(P=0.19) Loss of GPI-anchor amidation at N373(P=0.02) Loss of Sulfation at Y374(P=0.02) The gain of N-linked glycosylation at N377(P=0.01)
V424D	0.857	Altered Transmembrane protein(P=0.34) Altered Disordered interface(P=0.30) The gain of Allosteric site at R422(P=0.25) Altered Ordered interface(P=0.24) Altered DNA binding(P=0.23)
N377K	0.667	A gain of Ubiquitylation at N377(P=0.20) The gain of GPI-anchor amidation at N373(P=0.02) Loss of Sulfation at Y374(P=0.02) Loss of N-linked glycosylation at N377(P=0.01)
T195A	0.516	Loss of Relative solvent accessibility(P=0.28) Altered Transmembrane protein(P=0.27) Altered Ordered interface(P=0.26) Altered Metal-binding(P=0.04)
R443K	0.785	Altered Ordered interface(P=0.26) Altered Transmembrane protein(P=0.25) The gain of Allosteric site at Y440(P=0.22)
S119N	0.639	Loss of Allosteric site at W122(P=0.26) Altered Transmembrane protein(P=0.18)
M420T	0.824	Altered Transmembrane protein(P=0.26) A gain of Allosteric site at R422(P=0.26) Altered Stability(P=0.20) Altered DNA binding(P=0.19) Loss of Acetylation at K416(P=0.19)

Table 7: Association of Other Genes In Epilepsy

GENE	Extended Form	Disease association
GABRA5	Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha5	• Epileptic Encephalopathy, Early Infantile, 79 • childhood absence epilepsy
GABRB2	Gamma-Aminobutyric Acid Type A Receptor Subunit Beta2	• Undetermined Early-Onset Epileptic Encephalopathy • Epileptic Encephalopathy, Infantile or Early Childhood, 2
GABRA4	Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha4	• Status Epilepticus
GABRG2	Gamma-Aminobutyric Acid Type A Receptor Subunit Gamma2	• Generalized Epilepsy with Febrile Seizures Plus (GEFS+) • Benign Epilepsy with Centrotemporal Spikes (BRE) Epileptic Encephalopathy, Early Infantile, 74 (EIEE74) • Febrile Seizures, Familial, 8 (FEB8)
KCNJ3	Potassium Inwardly Rectifying Channel Subfamily J Member 3	• Epilepsy syndrome
DNM1	Dynamin 1	• Visual Epilepsy • Epileptic Encephalopathy, Early Infantile, 31 (EIEE31)

Conservation Analysis

Certain variations in the human genome cause multiple diseases and affect human health. Consurf is the webserver used for predicting conserved evolutionary domains in a protein sequence. In order to check our novel nsSNP conservation frequency, we manipulated the original protein structure and subjected it to a consurf web server. The analysis results showed that F285S is present in a conserved region and a buried residue(Figure 2).

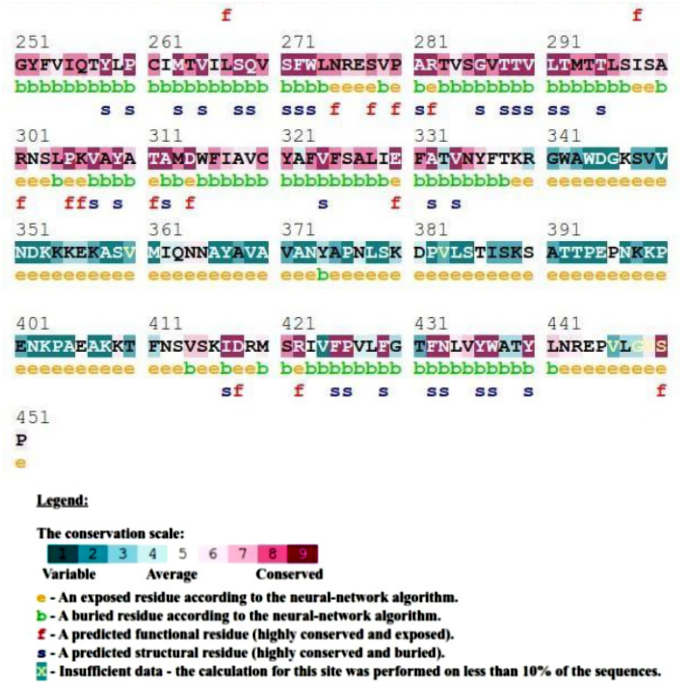


Figure 2: Prediction Of Evolutionary Conserved Sites in GABRA2 Gene by CONSURF Database

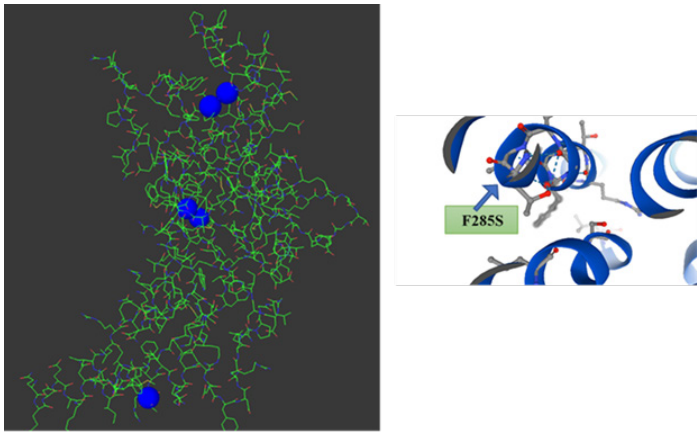


Figure 3: Location of Mutations on Mutant Protein Structure and Location of F285S Variant on Alpha Helix of Protein

Structural Modeling and Comparison of Variants

The SWISS-MODEL database generated several models for both native and mutant types of protein. We choose 6huj.1.D template for native and 6i53.1.B for mutant protein sequence. Lite Mol was used to visualizing mutant protein sequences and marked mutations on 3-D structure (Figure 3). The blue-colored balls on 3D structures show the location of identified nsSNPs on protein.

String Database Networks

To better understand GABRA2 gene function, it is vital to study their interactions with other genes. Consequently, GeneMania and STRING databases were used to scan genes that interact with GABRA2 protein.GABRA2 gene shows direct interactions with NSF, UBQLN1, GABRA3, GABRA4, GABRA5, and GABARAP gene, respectively (Figure 4).

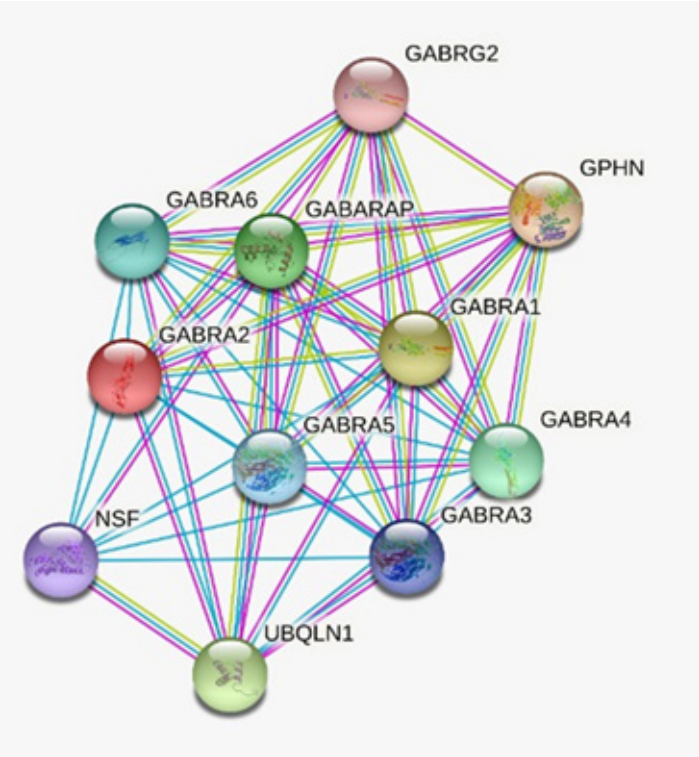


Figure 4: String Database Showed GABRA2 Functionally Interacts With Other Genes

Discussion

Although most single nucleotide polymorphisms were reported in intronic regions of the GABRA2 gene, in this analysis, we reported a missense variant of GABRA2 present in the coding region. SNPs are single nucleotide substitutions found in specific locations impacting the exonic regions of the human genome [28]. These substitutions are responsible for changing protein structure and function, affecting human health, and causing multiple disorders [29]. These single nucleotide polymorphisms cause less than 1% genetic change in the overall global population. We can easily access the number and molecular sequence of these SNPs stored in computational databases.

Using bioinformatics tools and algorithms helps us quickly design and execute an analysis without laborious and time-consuming experimental work [30]. This type of analysis of nsSNPs is substantiating to refine the molecular characterization of nsSNPs that would contribute massive support in personalized medication development [31].

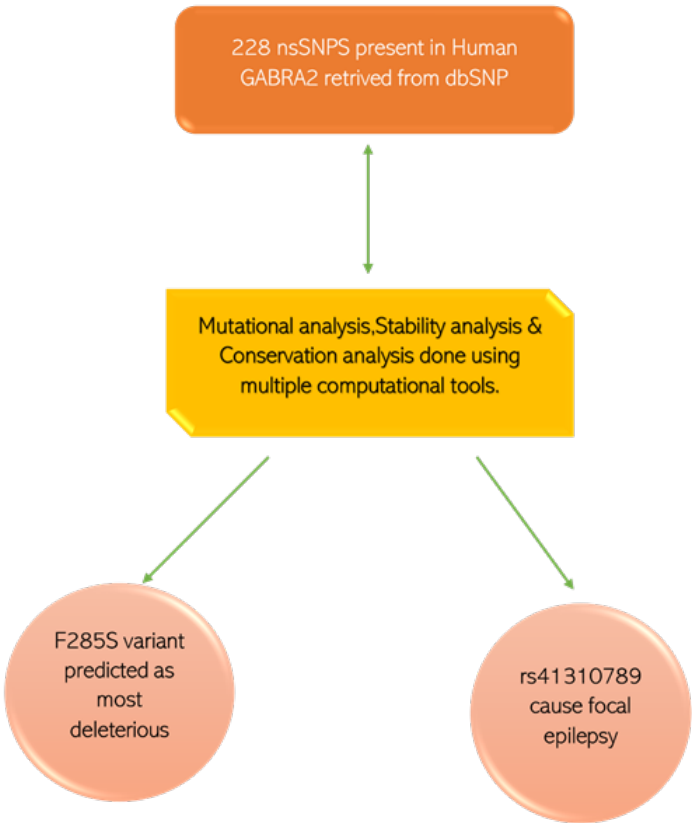
In a GWAS study, Butler & K identified a de novo missense variant present in GABRA2 causing early-onset of epilepsy [32]. Another study confirmed de novo missense mutation in GABRA2 at chromosome no four by Sanger sequencing [8].

This insilico study aims to identified the role of novel nsSNPs identified using all computational tools and visualize their effect on protein structure and function [34]. We collected all the GABRA2 gene SNPs from the dbSNP database and subjected only nsSNPs to all seven bioinformatics tools.Total six missense variants (V80A,T43A,F285S,T70I,P79S,F93) were predicted as pathogenic by all tools.While most of them picked F285S as the most deleterious nsSNP in gene sequence and caused a decrease in protein stability upon nucleotide substitution in the human genome.As a result of a change in amino acid F285S variant cause focal epilepsy.

The results of conservation analysis show that this variant is a buried residue and present in a conserved domain of the GABRA2 protein sequence. Substituation of conserved amino acids disrupts protein's biological activities because they are located in biologically active domains of the protein [34].

Homology modelling showed mutant protein type resembles 6huj.1.D template of GABRA1 gene, which is closet analog of GABRA2 gene. A study done by Johannesen, Katrine et al. reported GABRA1 mutations play a significant role in severe and benign epilepsy syndromes [35].

Graphical Abstract



Conclusion

In this study, we explored the disease-causing GABRA2 gene by assessing the effect of nonsynonymous SNPs by various online computational servers. There are 36912 single nucleotide polymorphisms within a GABRA2 gene,228 identified as nsSNPs,34375

as intronic, five pathogenic, and 142 as synonymous. Initially we choose six SNPs rs17852044, rs41305781, rs41310789, rs74440199, rs76519302, rs199725032 to select the most deleterious nsSNPs and to increase the precision of the analysis.Among these six, only one missense variant that is rs41310789, was found to be most pathogenic. This predicted nsSNPs were both causing epilepsy syndrome, focal epilepsy, and certain CNS disorders or cognitive diseases.Moreover, a wet lab molecular and functional analysis is necessary further to elucidate these nsSNPs in epilepsy and its related disorders [36-38].

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References

1. Sirven, J. I. (2015). Epilepsy: a spectrum disorder. Cold Spring Harbor perspectives in medicine, 5(9), a022848.
2. Fisher, R. S., Boas, W. V. E., Blume, W., Elger, C., Genton, P., Lee, P., & Engel Jr, J. (2005). Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia, 46(4), 470-472.
3. Murray, C. J., Vos, T., Lozano, R., Naghavi, M., Flaxman, A. D., Michaud, C., ... & Haring, D. (2012). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. The lancet, 380(9859), 2197-2223.
4. Iqbal, M. J., Wasim, M., Rashid, U., Zeeshan, N., Ali, R., Nayyab, S., ... & Zahid, N. (2018). Mutational screening of GABRG2 gene in Pakistani population of Punjab with generalized tonic clonic seizures and children with childhood absence epilepsy. Journal of the Chinese Medical Association, 81(8), 665-669.
5. Siddiqui, F., Sultan, T., Mustafa, S., Siddiqui, S., Ali, S., Malik, A., & Jooma, R. (2015). Epilepsy in Pakistan: national guidelines for clinicians. Pakistan Journal of Neurological Sciences (PJNS), 10(3), 47-62.
6. Fu, J., Tao, T., Li, Z., Chen, Y., Li, J., & Peng, L. (2020). The roles of ER stress in epilepsy: Molecular mechanisms and therapeutic implications. Biomedicine & Pharmacotherapy, 131, 110658.
7. Arias, A. J., Covault, J., Feinn, R., Pond, T., Yang, B. Z., Ge, W., ... & Kranzler, H. R. (2014). A GABRA2 variant is associated with increased stimulation and 'high'following alcohol administration. Alcohol and Alcoholism, 49(1), 1-9.
8. Orenstein, N., Goldberg-Stern, H., Straussberg, R., Bazak, L., Hubshman, M. W., Kropach, N., ... & Basel-Salmon, L. (2018). A de novo GABRA2 missense mutation in severe early-onset epileptic encephalopathy with a choreiform movement disorder. European journal of paediatric neurology, 22(3), 516-524.
9. Sanchis-Juan, A., Hasenahuer, M. A., Baker, J. A., McTague, A., Barwick, K., Kurian, M. A., ... & Raymond, F. L. (2020). Structural analysis of pathogenic missense mutations in GABRA2 and identification of a novel de novo variant in the desensitization gate. Molecular genetics & genomic medicine, 8(7), e1106.
10. Mehmood, M. A., Sehar, U., & Ahmad, N. (2014). Use of bioinformatics tools in different spheres of life sciences. Journal of Data Mining in Genomics & Proteomics, 5(2), 1.
11. Vaser, R., Adusumalli, S., Leng, S. N., Sikic, M., & Ng, P. C. (2016). SIFT missense predictions for genomes. Nature protocols, 11(1), 1-9.
12. Choi, Y., & Chan, A. P. (2015). PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics, 31(16), 2745-2747.
13. Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P., ... & Sunyaev, S. R. (2010). A method and server for predicting damaging missense mutations. Nature methods, 7(4), 248-249.
14. Tang, H., & Thomas, P. D. (2016). PANTHER-PSEP: predicting disease-causing genetic variants using position-specific evolutionary preservation. Bioinformatics, 32(14), 2230-2232.
15. Thusberg, J., Olatubosun, A., & Vihinen, M. (2011). Performance of mutation pathogenicity prediction methods on missense variants. Human mutation, 32(4), 358-368.
16. Cheng, J., Randall, A., & Baldi, P. (2006). Prediction of protein stability changes for single-site mutations using support vector machines. Proteins: Structure, Function, and Bioinformatics, 62(4), 1125-1132.
17. Pejaver, V., Urresti, J., Lugo-Martinez, J., Pagel, K. A., Lin, G. N., Nam, H. J., ... & Radivojac, P. (2020). Inferring the molecular and phenotypic impact of amino acid variants with MutPred2. Nature communications, 11(1), 1-13.
18. Hassan, M. S., Shaalan, A. A., Dessouky, M. I., Abdelnaiem, A. E., & ElHefnawi, M. (2019). Evaluation of computational techniques for predicting non-synonymous single nucleotide variants pathogenicity. Genomics, 111(4), 869-882.
19. Shihab, H. A., Gough, J., Cooper, D. N., Day, I. N., & Gaunt, T. R. (2013). Predicting the functional consequences of cancer-associated amino acid substitutions. Bioinformatics, 29(12), 1504-1510.
20. Ashkenazy, H., Erez, E., Martz, E., Pupko, T., & Ben-Tal, N. (2010). ConSurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. Nucleic acids research, 38(suppl_2), W529-W533.
21. Ashkenazy, H., Abadi, S., Martz, E., Chay, O., Mayrose, I., Pupko, T., & Ben-Tal, N. (2016). ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. Nucleic acids research, 44(W1), W344-W350.
22. Sharma, D., Saini, A., & Singh, D. D. (2020). SARS-CoV-2 whole-genome classification analysis, protein homology modelling and elucidation of associated protein functions.
23. Xing, J. Y., Zhang, Y. J., Jing, W. Q., Li, W. L., & Yu, Y. M. (2019). Molecular characterization, expression patterns and

methylation analysis of ATP-binding cassette sub-family G member 2 (ABCG2) in broilers. JAPS: Journal of Animal & Plant Sciences, 29(1).
24. Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., ... & von Mering, C. (2021). The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic acids research, 49(D1), D605-D612.
25. Montojo, J., Zuberi, K., Rodriguez, H., Bader, G. D., & Morris, Q. (2014). GeneMANIA: Fast gene network construction and function prediction for Cytoscape. F1000Research, 3.
26. Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., ... & Mering, C. V. (2019). STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic acids research, 47(D1), D607-D613.
27. Warde-Farley, D., Donaldson, S. L., Comes, O., Zuberi, K., Badrawi, R., Chao, P., ... & Morris, Q. (2010). The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic acids research, 38(suppl_2), W214-W220.
28. Vallejos-Vidal, E., Reyes-Cerpa, S., Rivas-Pardo, J. A., Maisey, K., Yáñez, J. M., Valenzuela, H., ... & Reyes-López, F. E. (2020). Single-nucleotide polymorphisms (SNP) mining and their effect on the tridimensional protein structure prediction in a set of immunity-related expressed sequence tags (EST) in Atlantic salmon (Salmo salar). Frontiers in Genetics, 10, 1406.
29. Robert, F., & Pelletier, J. (2018). Exploring the impact of single-nucleotide polymorphisms on translation. Frontiers in genetics, 9, 507.
30. Khatun, M., Shoombuatong, W., Hasan, M., & Kurata, H. (2020). Evolution of sequence-based bioinformatics tools for protein-protein interaction prediction. Current Genomics, 21(6), 454-463.
31. Loganathan, L., Kuriakose, B. B., Mushfiq, S., & Muthusamy, K. (2021). Mechanistic insights on nsSNPs on binding site of renin and cytochrome P450 proteins: A computational perceptual study for pharmacogenomics evaluation. Journal of Cellular Biochemistry, 122(10), 1460-1474.
32. Butler, K. M., Moody, O. A., Schuler, E., Coryell, J., Alexander, J. J., Jenkins, A., & Escayg, A. (2018). De novo variants in GABRA2 and GABRA5 alter receptor function and contribute to early-onset epilepsy. Brain, 141(8), 2392-2405.
33. Gong, T., Yang, L., Shen, F., Chen, H., Pan, Z., Zhang, Q., ... & Zhang, Y. (2021). Computational and Mass Spectrometry-Based Approach Identify Deleterious Non-Synonymous Single Nucleotide Polymorphisms (nsSNPs) in JMJD6. Molecules, 26(15), 4653.
34. Miller, M. P., & Kumar, S. (2001). Understanding human disease mutations through the use of interspecific genetic variation. Human molecular genetics, 10(21), 2319-2328.
35. Johannesen, K., Marini, C., Pfeffer, S., Møller, R. S., Dorn, T., Niturad, C. E., ... & Maljevic, S. (2016). Phenotypic spectrum of GABRA1: from generalized epilepsies to severe epileptic encephalopathies. Neurology, 87(11), 1140-1151.
36. Owolabi, L. F., Owolabi, S. D., Adamu, B., Jibo, A. M., & Alhaji, I. D. (2020). Epilepsy treatment gap in Sub-Saharan Africa: Meta-analysis of community-based studies. Acta Neurologica Scandinavica, 142(1), 3-13.
37. Saih, A., Baba, H., Bouqdayr, M., Ghazal, H., Hamdi, S., Kettani, A., & Wakrim, L. (2021). In Silico Analysis of High-Risk Missense Variants in Human ACE2 Gene and Susceptibility to SARS-CoV-2 Infection. BioMed research international.
38. Thijs, R. D., Surges, R., O'Brien, T. J., & Sander, J. W. (2019). Epilepsy in adults. The Lancet, 393(10172), 689-701.