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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Research Article

Keywords: Computational analysis, Seizures, Neurotransmitter, Coding variants, GABA, Brain

Posted Date: November 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1019527/v1>

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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 non-synonymous 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 databases were 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.

1. Introduction

Epilepsy is characterized by frequent seizure, unusual sensation, and loss of awareness in some cases and cause different health-related problems (Sirven, 2015). Repeated epileptic seizures occur due to an imbalance between inhibitory and excitatory neurotransmitters in the brain (Fisher et al., 2005). According to a study of syndromes' global burden, epilepsy stands at second position worldwide (Murray et al., 2012). All patients with seizures usually do not have epilepsy, but all patients with epilepsy experience seizures either repeated or frequent (Beghi, 2020). Epilepsy is the 4th most common neurological disorder and affects anyone regardless of age, gender, all races, and ethnicity (Asker, 2021). The definition of Epilepsy was recently updated by ILAE (International League against Epilepsy) "a short-term appearance of early signs and symptoms due to uncontrolled or synchronized neuronal action in the brain (Fisher et al., 2005). Diagnosis of epilepsy depends on the following criteria's: 1) More than two unconscionable or involuntary seizures or clusters of seizures happening in less than one day apart, (2) A single unconscionable or involuntary seizure that has a possibility of having another seizure or 60% chances of reoccurrence after two unconscionable seizures in ten years (Maloney et al., 2021) (3) Epilepsy syndrome are either genetic or idiopathic (unknown cause) (Falco-Walter et al., 2018). A severe form of epilepsy is present primarily in old age people and infants, and they are at high risk. Typically, brain injury is considered a cause of epilepsy in affected individuals, resulting from abnormal circuit activity in the brain. However, several other leading causes of epilepsy are genetics (Sun et al., 2021), brain abnormalities, developmental disorders, metabolic changes, immune system abnormalities, tumours, prenatal injury, and unknown causes. In about 50% of global cases, epilepsy causes are still unknown (Beghi et al., 2019). In minor cases, the cause of epilepsy is known. 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 (Fu et al., 2020).

According to World Health Organization, more than 3 million people in the US and 50 million people globally have epilepsy, making it one of the prevalent neurological conditions (Goldenberg, 2010). Higher incidence and

prevalence of epilepsy are present in developing countries; nearly 90% of individuals who reside there have epilepsy (Owolabi et al., 2020). Despite many new kinds of research, epilepsy remains a significant cause of mortality and disability globally. Due to several economic, ethnic, and social reasons, sub-Saharan Africa countries have many individuals living with epilepsy and bear an enormous disorder burden (Paul et al., 2012).

There is a considerable treatment gap in low and middle-income country residents because people often stigmatize epilepsy and do not get proper treatment (Thijs et al., 2019). Overall, 75% of epileptic patients did not get proper treatment due to poor social and economic situations in developing countries (Espinosa-Jovel et al., 2018). According to the WHO estimate, 70% of people live seizure-free life if adequately diagnosed and appropriately treated with antiepileptic drugs (Brodie et al., 2012). Epilepsy is also a prevalent neurological disorder in Pakistan, accounting for 1% of the Pakistani population (Iqbal et al., 2018). In the 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 (Siddiqui et al., 2015). A better health system will help reduce disease burden with cost-effective treatment, increased diagnostic capabilities, and drug supply (Megidido et al., 2016).

Gamma-Aminobutyric acid is a primary inhibitory neurotransmitter present in the human cerebrum. It acts through 3 classes of *GABA* receptors, ionotropic $GABA_A$, $GABA_C$, and metabolic $GABA_B$, with unique biochemical and electrophysical properties. In humans, GABA plays a crucial role in the development of the brain. $GABA_A$ receptors are ligand-gated chloride ion channels present all over the human brain (Arias et al., 2014). *GABRA2* is a protein-coding gene and highly expressed in the hippocampus and mediates synaptic transmission. *GABRA2* gene is positioned on the short p arm of Chr.no 4 and localized more precisely to the subtelomeric region of band 4p21. This gene has 14 exons, out of which nine exons are present in the coding region. A total of 8520 nucleotides are present in the whole *GABRA2* gene.

GABRA2 gene plays a significant role in early infantile epileptic encephalopathy (EIEE). This disease affects newborns, usually within the first three months. This gene is involved in the synaptic neurotransmission pathway and affects the GABAergic inhibition of excitatory and inhibitory neurons. 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) (Orenstein et al., 2018).

Mapping *GABRA2* variants to protein structure showed they were located near the desensitization gate, critical regions for GABAergic receptors' proper functioning (Sanchis-Juan et al., 2020), and GABA energetic synapse pathway is involved in epilepsy.

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 (Mehmood, 2014). 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 was performed to evaluate 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.

2. Materials And Methods

2.1. 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.

2.2. 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 were collected from NCBI (NP_000798.2). Polymorphisms for *GABRA2* were obtained from NCBI using different filters like missense, non-synonymous, human, coding, nonsense, and intronic.

2.3. 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(Vaser et al., 2016), PROVEAN (Choi and Chan, 2015), PolyPhen-2(Adzhubei et al., 2010), PANTHER(Tang and Thomas, 2016), PhD-SNP, and SNP&GO(Thusberg et al., 2011) (Table 1). We only include SNPs that are predicted as deleterious by software.

2.4. 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 (Cheng et al., 2006) used artificial intelligence technology to predict the stability score, while Mutpred2(Pejaver et al., 2020) used 14 different molecular and structural functions to realize the impacts of nsSNPs on protein structure.

2.5. Disease association analysis

We use FATHMM(Hassan et al., 2019) 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(Shihab et al., 2013).

2.6. Conservation analysis

Consurf was used to characterize 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(Ashkenazy et al., 2010). This server predicts the degree of amino acid conservation(Ashkenazy et al., 2016).

2.7. Three-dimensional structure of the protein.

3-D structure of *GABRA2* protein was generated by SWISS-MODEL web server (Sharma et al., 2020), and residue position in protein sequence was visualized by Lite Mol(Xing et al., 2019).GeneMANIA and STRING database (Szkarczyk et al., 2021)is used to predict the function of interlinked gene interactions (Montejo et al., 2014). 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(Szkarczyk et al., 2019). In the case of *GABRA2*, we found everyday neurotransmitter activity and neuron-neuron synaptic transmission with other genes(Warde-Farley et al., 2010).

3. Results

3.1. 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 non-synonymous SNPs (Figure 1). According to NCBI data, non-synonymous SNPs contribute to only 0.62% of all the SNPs reported in the human *GABRA2* gene.

3.2. 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 non-synonymous 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.

3.2. 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).

3.3. 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).

3.4. 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 visualize mutant protein sequences and marked mutations on 3-D structure (Figure 3). The blue-coloured balls on 3D structures show the location of identified nsSNPs on protein.

3.5. STRING database networks

To better understand *GABRA2* gene function, it is vital to study their interactions with other genes. Consequently, Gene Mania 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).

4. 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 (Vallejos-Vidal et al., 2020). These substitutions are responsible for changing protein structure and function, affecting human health, and causing multiple disorders (Robert and Pelletier, 2018). 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 (Khatun et al., 2020). This type of analysis of nsSNPs is substantiating to refine the molecular characterization of nsSNPs that would contribute massive support in personalized medication development (Loganathan et al., 2021).

In a GWAS study, Butler & K identified a de novo missense variant present in *GABRA2* causing early-onset of epilepsy (Butler et al., 2018). Another study confirmed de novo missense mutation in *GABRA2* at chromosome no four by Sanger sequencing (Orenstein et al., 2018).

This insilico study aims to identify the role of novel nsSNPs identified using all computational tools and visualize their effect on protein structure and function (Gong et al., 2021). 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. Substitution of conserved amino acids disrupts protein's biological activities because they are located in biologically active domains of the protein (Miller and Kumar, 2001).

Homology modelling showed mutant protein type resembles 6huj.1.D template of *GABRA1* gene, which is closet analogue of *GABRA2* gene. A study done by Johannsson, Katrine et al. reported

GABRA1 mutations play a significant role in severe and benign epilepsy syndromes (Johannesen et al., 2016).

5. Conclusion

In this study, we explored the disease-causing *GABRA2* gene by assessing the effect of non-synonymous 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.

Declarations

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Authors Contributions

All the authors contributed equally in study design and conception.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S., Sunyaev, S.R., 2010. A method and server for predicting damaging missense mutations. *Nat. Methods*. <https://doi.org/10.1038/nmeth0410-248>
2. Arias, A.J., Covault, J., Feinn, R., Pond, T., Yang, B.-Z., Ge, W., Oncken, C., Kranzler, H.R., 2014. A *GABRA2* Variant Is Associated with Increased Stimulation and Alcohol Alcohol. 49, 1–9. <https://doi.org/10.1093/alcalc/agt163>
3. 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 Res.* 44, W344–W350. <https://doi.org/10.1093/nar/gkw408>
4. Ashkenazy, H., Erez, E., Martz, E., Pupko, T., Ben-Tal, N., 2010. ConSurf 2010: calculating evolutionary conservation in sequence and structure of *Nucleic Acids Res.* 38, W529-33. <https://doi.org/10.1093/nar/gkq399>
5. Asker, A.U., 2021. Assessment of the Quality of Life in Patients with Epilepsy in the Populous Region of Baku. *Neuroscience* 5, 35–40.

6. Beghi, E., 2020. The Epidemiology of Epilepsy. *Neuroepidemiology* 54, 185–191.
<https://doi.org/10.1159/000503831>
7. Beghi, E., Giussani, G., Nichols, E., Abd-Allah, F., Abdela, J., Abdelalim, A., Abraha, H.N., Adib, M.G., Agrawal, S., Alahdab, F., Awasthi, A., Ayele, Y., Barboza, M.A., Belachew, A.B., Biadgo, B., Bijani, A., Bitew, H., Carvalho, F., Chaiah, Y., Daryani, A., Do, H.P., Dubey, M., Endries, A.Y.Y., Eskandarieh, S., Faro, A., Farzadfar, F., Fereshtehnejad, S.-M., Fernandes, E., Fijabi, D.O., Filip, I., Fischer, F., Gebre, A.K., Tsadik, A.G., Gebremichael, T.G., Gezae, K.E., Ghasemi-Kasman, M., Weldegewergs, K.G., Degefa, M.G., Gnedovskaya, E. V, Hagos, T.B., Haj-Mirzaian, Arvin, Haj-Mirzaian, Arya, Hassen, H.Y., Hay, S.I., Jakovljevic, M., Kasaeian, A., Kassa, T.D., Khader, Y.S., Khalil, I., Khan, E.A., Khubchandani, J., Kisa, A., Krohn, K.J., Kulkarni, C., Nirayo, Y.L., Mackay, M.T., Majdan, M., Majeed, A., Manhertz, T., Mehndiratta, M.M., Mekonen, T., Meles, H.G., Mengistu, G., Mohammed, S., Naghavi, M., Mokdad, A.H., Mustafa, G., Irvani, S.S.N., Nguyen, L.H., Nixon, M.R., Ogbo, F.A., Olagunju, A.T., Olagunju, T.O., Owolabi, M.O., Phillips, M.R., Pinilla-Monsalve, G.D., Qorbani, M., Radfar, A., Rafay, A., Rahimi-Movaghar, V., Reinig, N., Sachdev, P.S., Safari, H., Safari, S., Safiri, S., Sahraian, M.A., Samy, A.M., Sarvi, S., Sawhney, M., Shaikh, M.A., Sharif, M., Singh, G., Smith, M., Szoeki, C.E.I., Tabarés-Seisdedos, R., Temsah, M.-H., Temsah, O., Tortajada-Girbés, M., Tran, B.X., Tsegay, A.A.T., Ullah, I., Venketasubramanian, N., Westerman, R., Winkler, A.S., Yimer, E.M., Yonemoto, N., Feigin, V.L., Vos, T., Murray, C.J.L., 2019. Global, regional, and national burden of epilepsy, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 18, 357–375. [https://doi.org/10.1016/S1474-4422\(18\)30454-X](https://doi.org/10.1016/S1474-4422(18)30454-X)
8. Brodie, M.J., Barry, S.J.E., Bamagous, G.A., Norrie, J.D., Kwan, P., 2012. Patterns of treatment response in newly diagnosed epilepsy. *Neurology* 78, 1548 LP – 1554.
<https://doi.org/10.1212/WNL.0b013e3182563b19>
9. 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, 2392–2405. <https://doi.org/10.1093/brain/awy171>
10. Cheng, J., Randall, A., Baldi, P., 2006. Prediction of protein stability changes for single-site mutations using support Proteins 62, 1125–1132. <https://doi.org/10.1002/prot.20810>
11. Choi, Y., Chan, A.P., 2015. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics* 31, 2745–2747. <https://doi.org/10.1093/bioinformatics/btv195>
12. Espinosa-Jovel, C., Toledano, R., Aledo-Serrano, Á., García-Morales, I., Gil-Nagel, A., 2018. Epidemiological profile of epilepsy in low income populations. *Seizure* 56, 67–72.
<https://doi.org/https://doi.org/10.1016/j.seizure.2018.02.002>
13. Falco-Walter, J.J., Scheffer, I.E., Fisher, R.S., 2018. The new definition and classification of seizures and epilepsy. *Epilepsy Res.* 139, 73–79. <https://doi.org/https://doi.org/10.1016/j.eplepsyres.2017.11.015>
14. Fisher, R.S., van Emde Boas, W., Blume, W., Elger, C., Genton, P., Lee, P., Engel, J.J., 2005. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* 46, 470–472. <https://doi.org/10.1111/j.0013-9580.2005.66104.x>
15. 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. *Biomed. Pharmacother.* 131, 110658.
<https://doi.org/https://doi.org/10.1016/j.biopha.2020.110658>

16. Goldenberg, M.M., 2010. Overview of drugs used for epilepsy and seizures: etiology, diagnosis, and treatment. *P T* 35, 392–415.
17. Gong, T., Yang, L., Shen, F., Chen, H., Pan, Z., Zhang, Q., Jiang, Y., Zhong, F., Yang, P., Zhang, Y., 2021. Computational and Mass Spectrometry-Based Approach Identify Deleterious Mol. . <https://doi.org/10.3390/molecules26154653>
18. Hassan, M.S., Shaalan, A.A., Dessouky, M.I., Abdelnaiem, A.E., ElHefnawi, M., 2019. Evaluation of computational techniques for predicting single nucleotide variants pathogenicity. *Genomics* 111, 869–882. <https://doi.org/https://doi.org/10.1016/j.ygeno.2018.05.013>
19. Iqbal, M.J., Wasim, M., Rashid, U., Zeeshan, N., Ali, R., Nayyab, S., Habib, S., Manzoor, B., Zahid, N., 2018. generalized tonic clonic seizures and children with childhood absence epilepsy. *J. Chin. Med. Assoc.* 81, 665–669. <https://doi.org/10.1016/j.jcma.2018.05.001>
20. Johannesen, K., Marini, C., Pfeiffer, S., Møller, R.S., Dorn, T., Niturad, C.E., Gardella, E., Weber, Y., Søndergård, M., Hjalgrim, H., Nikanorova, M., Becker, F., Larsen, L.H.G., Dahl, H.A., Maier, O., Mei, D., Biskup, S., Klein, K.M., Reif, P.S., Rosenow, F., Elias, A.F., Hudson, C., Helbig, K.L., Schubert-Bast, S., Scordo, M.R., Craiu, D., Djémié, T., Hoffman-Zacharska, D., Caglayan, H., Helbig, I., Serratosa, J., Striano, P., De Jonghe, P., Weckhuysen, S., Suls, A., Muru, K., Talvik, I., Talvik, T., Muhle, H., Borggraefe, I., Rost, I., Guerrini, R., Lerche, H., Lemke, J.R., Rubboli, G., Maljevic, S., 2016. Phenotypic spectrum of GABRA1: From generalized epilepsies to severe epileptic encephalopathies. *Neurology* 87, 1140–1151. <https://doi.org/10.1212/WNL.0000000000003087>
21. Khatun, M.S., Shoombuatong, W., Hasan, M.M., Kurata, H., 2020. Evolution of Sequence-based Bioinformatics Tools for Protein-protein Interaction Prediction. *Curr. Genomics* 21, 454–463. <https://doi.org/10.2174/1389202921999200625103936>
22. 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. *J. Cell. Biochem.* 122, 1460–1474. <https://doi.org/https://doi.org/10.1002/jcb.30069>
23. Maloney, E.M., , É.J., Costello, D.J., 2021. Causes and classification of first unprovoked seizures and newly-diagnosed epilepsy in a defined geographical area- an all-comers analysis. *Seizure* 92, 118–127. <https://doi.org/https://doi.org/10.1016/j.seizure.2021.08.016>
24. Megiddo, I., Colson, A., Chisholm, D., Dua, T., Nandi, A., Laxminarayan, R., 2016. Health and economic benefits of public financing of epilepsy treatment in India: An agent-based simulation model. *Epilepsia* 57, 464–474. <https://doi.org/https://doi.org/10.1111/epi.13294>
25. Mehmood, M., 2014. Use of Bioinformatics Tools in Different Spheres of Life Sciences. *J. Data Mining Genomics Proteomics* 5. <https://doi.org/10.4172/2153-0602.1000158>
26. Miller, M.P., Kumar, S., 2001. Understanding human disease mutations through the use of interspecific genetic variation. *Hum. Mol. Genet.* 10, 2319–2328.
27. Montojo, J., Zuberi, K., Rodriguez, H., Bader, G.D., Morris, Q., 2014. GeneMANIA: Fast gene network construction and function prediction for Cytoscape. *F1000Research* 3, 153. <https://doi.org/10.12688/f1000research.4572.1>
28. Murray, C.J.L., Vos, T., Lozano, R., Naghavi, M., Flaxman, A.D., Michaud, C., Ezzati, M., Shibuya, K., Salomon, J.A., Abdalla, S., Aboyans, V., Abraham, J., Ackerman, I., Aggarwal, R., Ahn, S.Y., Ali, M.K., Alvarado, M.,

Anderson, H.R., Anderson, L.M., Andrews, K.G., Atkinson, C., Baddour, L.M., Bahalim, A.N., Barker-Collo, S., Barrero, L.H., Bartels, D.H., Basáñez, M.-G., Baxter, A., Bell, M.L., Benjamin, E.J., Bennett, D., Bernabé, E., Bhalla, K., Bhandari, B., Bikbov, B., Bin Abdulhak, A., Birbeck, G., Black, J.A., Blencowe, H., Blore, J.D., Blyth, F., Bolliger, I., Bonaventure, A., Boufous, S., Bourne, R., Boussinesq, M., Braithwaite, T., Brayne, C., Bridgett, L., Brooker, S., Brooks, P., Brugha, T.S., Bryan-Hancock, C., Bucello, C., Buchbinder, R., Buckle, G., Budke, C.M., Burch, M., Burney, P., Burstein, R., Calabria, B., Campbell, B., Canter, C.E., Carabin, H., Carapetis, J., Carmona, L., Cella, C., Charlson, F., Chen, H., Cheng, A.T.-A., Chou, D., Chugh, S.S., Coffeng, L.E., Colan, S.D., Colquhoun, S., Colson, K.E., Condon, J., Connor, M.D., Cooper, L.T., Corriere, M., Cortinovis, M., de Vaccaro, K.C., Couser, W., Cowie, B.C., Criqui, M.H., Cross, M., Dabhadkar, K.C., Dahiya, M., Dahodwala, N., Damsere-Derry, J., Danaei, G., Davis, A., De Leo, D., Degenhardt, L., Dellavalle, R., Delossantos, A., Denenberg, J., Derrett, S., Des Jarlais, D.C., Dharmaratne, S.D., Dherani, M., Diaz-Torne, C., Dolk, H., Dorsey, E.R., Driscoll, T., Duber, H., Ebel, B., Edmond, K., Elbaz, A., Ali, S.E., Erskine, H., Erwin, P.J., Espindola, P., Ewoigbokhan, S.E., Farzadfar, F., Feigin, V., Felson, D.T., Ferrari, A., Ferri, C.P., Fèvre, E.M., Finucane, M.M., Flaxman, S., Flood, L., Foreman, K., Forouzanfar, M.H., Fowkes, F.G.R., Fransen, M., Freeman, M.K., Gabbe, B.J., Gabriel, S.E., Gakidou, E., Ganatra, H.A., Garcia, B., Gaspari, F., Gillum, R.F., Gmel, G., Gonzalez-Medina, D., Gosselin, R., Grainger, R., Grant, B., Groeger, J., Guillemin, F., Gunnell, D., Gupta, R., Haagsma, J., Hagan, H., Halasa, Y.A., Hall, W., Haring, D., Haro, J.M., Harrison, J.E., Havmoeller, R., Hay, R.J., Higashi, H., Hill, C., Hoen, B., Hoffman, H., Hotez, P.J., Hoy, D., Huang, J.J., Ibeanusi, S.E., Jacobsen, K.H., James, S.L., Jarvis, D., Jasrasaria, R., Jayaraman, S., Johns, N., Jonas, J.B., Karthikeyan, G., Kassebaum, N., Kawakami, N., Keren, A., Khoo, J.-P., King, C.H., Knowlton, L.M., Kobusingye, O., Koranteng, A., Krishnamurthi, R., Laden, F., Lalloo, R., Laslett, L.L., Lathlean, T., Leasher, J.L., Lee, Y.Y., Leigh, J., Levinson, D., Lim, S.S., Limb, E., Lin, J.K., Lipnick, M., Lipshultz, S.E., Liu, W., Loane, M., Ohno, S.L., Lyons, R., Mabweijano, J., MacIntyre, M.F., Malekzadeh, R., Mallinger, L., Manivannan, S., Marcenes, W., March, L., Margolis, D.J., Marks, G.B., Marks, R., Matsumori, A., Matzopoulos, R., Mayosi, B.M., McAnulty, J.H., McDermott, M.M., McGill, N., McGrath, J., Medina-Mora, M.E., Meltzer, M., Mensah, G.A., Merriman, T.R., Meyer, A.-C., Miglioli, V., Miller, M., Miller, T.R., Mitchell, P.B., Mock, C., Mocumbi, A.O., Moffitt, T.E., Mokdad, A.A., Monasta, L., Montico, M., Moradi-Lakeh, M., Moran, A., Morawska, L., Mori, R., Murdoch, M.E., Mwaniki, M.K., Naidoo, K., Nair, M.N., Naldi, L., Narayan, K.M.V., Nelson, P.K., Nelson, R.G., Nevitt, M.C., Newton, C.R., Nolte, S., Norman, P., Norman, R., O'Donnell, M., O'Donnell, M., O'Hanlon, S., Olives, C., Omer, S.B., Ortblad, K., Osborne, R., Ozgediz, D., Page, A., Pahari, B., Pandian, J.D., Rivero, A.P., Patten, S.B., Pearce, N., Padilla, R.P., Perez-Ruiz, F., Perico, N., Pesudovs, K., Phillips, D., Phillips, M.R., Pierce, K., Pion, S., Polanczyk, G. V., Polinder, S., Pope, C.A., Popova, S., Porrini, E., Pourmalek, F., Prince, M., Pullan, R.L., Ramaiah, K.D., Ranganathan, D., Razavi, H., Regan, M., Rehm, J.T., Rein, D.B., Remuzzi, G., Richardson, K., Rivara, F.P., Roberts, T., Robinson, C., De León, F.R., Ronfani, L., Room, R., Rosenfeld, L.C., Rushton, L., Sacco, R.L., Saha, S., Sampson, U., Sanchez-Riera, L., Sanman, E., Schwebel, D.C., Scott, J.G., Segui-Gomez, M., Shahrzad, S., Shepard, D.S., Shin, H., Shivakoti, R., Singh, D., Singh, G.M., Singh, J.A., Singleton, J., Sleet, D.A., Sliwa, K., Smith, E., Smith, J.L., Stapelberg, N.J.C., Steer, A., Steiner, T., Stolk, W.A., Stovner, L.J., Sudfeld, C., Syed, S., Tamburlini, G., Tavakkoli, M., Taylor, H.R., Taylor, J.A., Taylor, W.J., Thomas, B., Thomson, W.M., Thurston, G.D., Tleyjeh, I.M., Tonelli, M., Towbin, J.A., Truelsén, T., Tsilimbaris, M.K., Ubeda, C., Undurraga, E.A., van der Werf, M.J., van Os, J., Vavilala, M.S., Venketasubramanian, N., Wang, M., Wang, W., Watt, K., Weatherall, D.J., Weinstock, M.A., Weintraub, R., Weisskopf, M.G., Weissman, M.M., White, R.A., Whiteford, H., Wiebe, N., Wiersma, S.T., Wilkinson, J.D., Williams, H.C., Williams, S.R.M., Witt, E., Wolfe, F., Woolf, A.D., Wulf, S., Yeh, P.-H., Zaidi, A.K.M., Zheng, Z.-J., Zonies, D., Lopez, A.D., AlMazroa, M.A., Memish, Z.A., 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. *Lancet* (London, England) 380, 2197–2223. [https://doi.org/10.1016/s0140-6736\(12\)61689-4](https://doi.org/10.1016/s0140-6736(12)61689-4)
29. Orenstein, N., Goldberg-Stern, H., Straussberg, R., Bazak, L., Weisz Hubshman, M., Kropach, N., Gilad, O., Scheuerman, O., Dory, Y., Kraus, D., Tzur, S., Magal, N., Kilim, Y., Shkalim Zemer, V., Basel-Salmon, L., 2018. A de novo GABRA2 missense mutation in severe early-onset epileptic encephalopathy with a choreiform movement disorder. *Eur. J. Paediatr. Neurol.* 22, 516–524. <https://doi.org/https://doi.org/10.1016/j.ejpn.2017.12.017>
 30. 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 Neurol. Scand.* 142, 3–13. <https://doi.org/10.1111/ane.13246>
 31. Paul, A., Adeloye, D., George-Carey, R., Kolčić, I., Grant, L., Chan, K.Y., 2012. An estimate of the prevalence of epilepsy in Sub-Saharan Africa: A systematic analysis. *J. Glob. Health* 2, 20405. <https://doi.org/10.7189/jogh.02.020405>
 32. Pejaver, V., Urresti, J., Lugo-Martinez, J., Pagel, K.A., Lin, G.N., Nam, H.-J., Mort, M., Cooper, D.N., Sebat, J., Iakoucheva, L.M., Mooney, S.D., Radivojac, P., 2020. Inferring the molecular and phenotypic impact of amino acid variants with MutPred2. *Nat. Commun.* 11, 5918. <https://doi.org/10.1038/s41467-020-19669-x>
 33. Robert, F., Pelletier, J., 2018. Exploring the Impact of Single-Nucleotide Polymorphisms on Translation.
 34. 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 Res. Int.* 2021, 6685840. <https://doi.org/10.1155/2021/6685840>
 35. Sanchis-Juan, A., Hasenahuer, M.A., Baker, J.A., McTague, A., Barwick, K., Kurian, M.A., Duarte, S.T., BioResource, N., Carss, K.J., Thornton, J., Raymond, F.L., 2020. Structural analysis of pathogenic missense mutations in GABRA2 and identification of a novel de novo variant in the desensitization gate. *Mol. Genet. Genomic Med.* 8, e1106. <https://doi.org/10.1002/mgg3.1106>
 36. Sharma, D., Saini, A., Singh, D., 2020. SARS-CoV-2 whole-genome classification analysis, protein homology modelling and elucidation of associated protein functions. <https://doi.org/10.31219/osf.io/9afp3>
 37. Shihab, H.A., Gough, J., Cooper, D.N., Day, I.N.M., Gaunt, T.R., 2013. Predicting the functional consequences of cancer-associated amino acid substitutions. *Bioinformatics* 29, 1504–1510. <https://doi.org/10.1093/bioinformatics/btt182>
 38. Siddiqui, F., Sultan, T., Mustafa, S., Siddiqui, S., Ali, S., Malik, A., Sajjad, Z., Barech, S., Jooma, R., 2015. Epilepsy in pakistan: national guidelines for clinicians. *Pakistan J. Neurol. Sci.* 10.
 39. Sirven, J.I., 2015. Epilepsy: A Spectrum Disorder. *Cold Spring Harb. Perspect. Med.* 5, a022848. <https://doi.org/10.1101/cshperspect.a022848>
 40. Sun, Y., Lu, L., Li, L., Wang, J., 2021. An advance about the genetic causes of epilepsy, in: *E3S Web of Conferences*. EDP Sciences, p. 3068.
 41. Szklarczyk, D., Gable, A.L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N.T., Morris, J.H., Bork, P., Jensen, L.J., Mering, C. von, 2019. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* 47, D607–D613. <https://doi.org/10.1093/nar/gky1131>

42. Szklarczyk, D., Gable, A.L., Nastou, K.C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N.T., Legeay, M., Fang, T., Bork, P., Jensen, L.J., von Mering, C., 2021. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.* 49, D605–D612. <https://doi.org/10.1093/nar/gkaa1074>
43. Tang, H., Thomas, P.D., 2016. PANTHER-PSEP: predicting disease-causing genetic variants using position-specific evolutionary preservation. *Bioinformatics* 32, 2230–2232. <https://doi.org/10.1093/bioinformatics/btw222>
44. , T.J., Sander, J.W., 2019. Epilepsy in adults. *Lancet* 393, 689–701. [https://doi.org/https://doi.org/10.1016/S0140-6736\(18\)32596-0](https://doi.org/https://doi.org/10.1016/S0140-6736(18)32596-0)
45. Thusberg, J., Olatubosun, A., Vihinen, M., 2011. Performance of mutation pathogenicity prediction methods on missense variants. *Hum. Mutat.* 32, 358–368. <https://doi.org/10.1002/humu.21445>
46. Vallejos-Vidal, E., Reyes-Cerpa, S., Rivas-Pardo, J.A., Maisey, K., Yáñez, J.M., Valenzuela, H., Cea, P.A., Castro-Fernandez, V., Tort, L., Sandino, A.M., Imarai, M., 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*).
47. Vaser, R., Adusumalli, S., Leng, S.N., Sikic, M., Ng, P.C., 2016. SIFT missense predictions for genomes. *Nat. Protoc.* 11, 1.
48. Warde-Farley, D., Donaldson, S.L., Comes, O., Zuberi, K., Badrawi, R., Chao, P., Franz, M., Grouios, C., Kazi, F., Lopes, C.T., Maitland, A., Mostafavi, S., Montojo, J., Shao, Q., Wright, G., Bader, G.D., Morris, Q., 2010. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res.* 38, W214–W220. <https://doi.org/10.1093/nar/gkq537>
49. Xing, J., Zhang, Y., Jing, W., Li, W.L., Yu, Y., 2019. MOLECULAR CHARACTERIZATION, EXPRESSION PATTERNS AND METHYLATION ANALYSIS OF ATP-BINDING CASSETTE SUB-FAMILY G MEMBER 2 (ABCG2) IN BROILERS. <https://doi.org/10.13140/RG.2.2.30637.54242>

Tables

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/

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)
			(Pejaver et al., 2020)
MutPred	Protein stability	http://mutpred.mutdb.org/	

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.

Table 4: Stability analysis results.

Mupro Score	Prediction	MutPred Score	Prediction
-1.4209216	Decrease	0.946	<ul style="list-style-type: none"> • Altered Ordered interface • Altered Transmembrane protein • A gain of Relative solvent accessibility • A gain of O-linked glycosylation at F285. • Altered Metal-binding.

Figures

Figure 1

Distribution of GABRA2 SNPs present in human genome.

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.

Figure 4

STRING database showed GABRA2 functionally interacts with other genes.