Title: *In silico* analysis of the altered protein structure and functions caused by existing synaptic gene mutations in Indian population having neurological disorders

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# Equal contribution

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Abstract: Neurological disorders encompass a diverse range of conditions that affect individuals' cognitive, emotional, and social functioning. Though these disorders are multifactorial, genetic factors play a significant role in the pathogenesis. Synaptic gene mutations or synaptic protein dysfunction are shown to be closely associated with neuropathology, suggesting the term synaptopathies. These synaptic abnormalities affect the proper brain physiology, release, regulation of neurotransmitters, disrupting neuronal communication and leading to the manifestation of these disorders. Although significant progress has been made, this area still remains uncharted as the outlaw around mental health and exceeds the research work that has been done till date. This study aims at understanding the critical role of synaptic compartment by conducting comprehensive analysis of existing synaptic gene mutations responsible for the development of three significant disorders in the Indian population: autism spectrum disorder (ASD), epilepsy and schizophrenia. Our *in silico* analysis reveals, mutations in genes *RPL10*, *GABRA1* and *DRD2* corresponds to ASD, Epilepsy and Schizophrenia, respectively are predicted to be deleterious. Further, the proteins encoded by these mutated genes - Large ribosomal subunit protein uL16, Gamma-aminobutyric acid receptor subunit alpha-1 and D(2) dopamine receptor results in altered protein-protein interactions possibly resulting in disturbing different neuronal functions. Specifically, the disturbed interaction between D(2) dopamine receptor NCS1 might hamper the presynaptic functions such as neurotransmitter release. Together, our study helps to further understand the synaptic signalling in the context of the Indian population, which indeed potentiates the usage of model systems to identify novel therapeutic targets aiding to focus on developing personalised medications.

Keywords: Neurological disorders, ASD, Schizophrenia, Epilepsy, Indian Population.

Introduction: The brain is the master regulator of the body, with several signal transduction pathways critical in controlling all voluntary and involuntary actions, as well as other diverse biological functions. Human brain is an advanced network that also facilitates cognition, perception, judgement, memory and retrieval (Greeno et al., 1996). Synaptic communication is critical for transfer of information and these neurological disorders that manifest due to synaptic dysfunction are broadly termed synaptopathies (Lepeta et al., 2016). Understanding the shared origins and fundamental distinctions in disorders linked with synaptic dysfunction could lead to novel therapeutic approaches for neurological disorders. Genetic mutations in genes coding for proteins change protein structure and its function and may contribute to a phenotypic attribute of the disorder. As a result, studying synapses at the molecular level is critical in determining aetiology.

Single nucleotide polymorphism (SNP), one of the most common types of genetic diversity in the human genome, has an important role in phenotypic variation and individual differentiation within a species. SNPs can occur in both coding and noncoding areas of the genome, where SNPs in the coding area are classed as synonymous or non-synonymous (nsSNPs), which are further classified as missense or nonsense (Edwards et al., 2007). nsSNPs substitute protein residues, impacting protein stability, dynamics, physiological molecular interactions, formation of stable complexes with binding partners and even post translational modifications (Yang et al., 2019). Negative genetic variation can modify the electrostatic nature of protein surfaces, causing changes in stability or folding; this influences binding selectivity and affinity, compromising protein function. The identification of detrimental nsSNPs assists in the establishing of the likely biochemical pathway causing the disease and the creation of effective inhibitors to target the mutant proteins (Rong et al., 2021).

Introns, previously considered junk DNA, are non-coding sequences that flank exons, and are about 20 times larger than exons. During transcription, the removal of introns from precursor mRNA along with other post transcriptional modifications gives rise to mature mRNA that codes for functional protein (Vaz-Drago et al., 2017). Despite typically being excised, introns play a key role in protein function. Introns include highly conserved sequences that are fundamental to gene expression and diversity (Kelemen et al., 2013). A pseudo-exon, or an intronic region surrounded by apparent consensus splice sites, is now regarded to be a more prevalent source of sickness than previously thought (Grellscheid et al., 2006).

The study focuses on analysing the existing gene mutations linked to autism spectrum disorder (ASD), epilepsy and schizophrenia in India population and their effect on protein structure, function in mediating the synaptic signalling. ASD and schizophrenia are two different neurological conditions, with distinct phenotypic changes, yet display similarities at certain behaviours such as disturbed social cognition, altered neuroanatomy, disturbed GABAergic functioning and co-occurrence of epilepsy (Jutla et al., 2022; Gao and Penzes., 2015; Canitano and Pallagrosi., 2017; Keller et al., 2017; Cascella et al., 2009). We refine the synaptic genes from genes whose mutations are implicated in Indian populations. These synaptic genes are further compartmentalised into presynaptic and postsynaptic, to gain insight into the mechanisms by which these gene mutations alter synaptic function, more specifically synaptic protein function. By studying localised structural changes and its impact on the protein's orthosteric site and protein binding hot spots, we theorise on the mode of alteration of protein functions due to the SNPs, which may impact biochemical pathways in the synapse.

MATERIALS AND METHODS:

Primary list of all mutated genes associated with neurological disorders like ASD, Epilepsy and Schizophrenia in the Indian population was gathered, based on PubMed and google scholar search. Using the SynGo database (Koopmans et al., 2019) the list of genes was segregated into synaptic and nonsynaptic genes. The synaptic genes were considered, and the non-synaptic genes were rejected, for further analysis. The list of genes was further classified into intronic and exonic variants, the exonic or the coding variants have synonymous and nonsynonymous which could contribute to the disease formation. To find out disease causing mutations, Position-Specific Independent Counts Scores (PSIC) tools such as PolyPhen2 and SIFT were used (Adzhubei et al., 2013). The higher the PSIC score, the higher the mutation strength. PolyPhen scores can be interpreted as follows: 0.0 to 0.15 - predicted to be benign; 0.15 to 1.0 - possibly damaging. 0.85 to 1.0 - confidently predicted to be damaging. SIFT (Sorting Intolerant From Tolerant) uses sequence homology to predict whether an amino acid substitution will affect protein function or not. The farther the SIFT score is from 1, the more damaging the mutation has been predicted to be. PolyPhen-2 and SIFT scores use the same range, 0.0 to 1.0, but with opposite intents. A variant with a PolyPhen-2 score of 0.0 is predicted to be benign, whereas a variant with a SIFT score of 1.0 is predicted to be benign.

We next compared different variants corresponding to a single synaptic gene, further compared all the synaptic genes to identify the most deleterious gene mutation per neurological condition. The nucleotide sequence of the respective gene variants that are disease causing, were taken from NCBI and the mutations were induced by altering the nucleotide sequence (SNP) according to the respective mutations.

Amino acid sequences were identified for the wild type (WT) and mutated gene encoded protein using mutation taster (Schwarz et al., 2014). These amino acid sequences were used to develop the protein structures using the Swiss model (Waterhouse et al., 2018). The protein structures were then analysed on the mutated site for both the WT and mutated protein using discovery studio. Furthermore, the list of interacting protein partners to the WT protein were prepared using the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) analysis tool (Szklarczyk et al., 2023), with highest confidence (0.900) as minimum required interaction score. Then to check for the binding differences between the WT protein and mutated proteins with their interaction partners Cluspro (Kozakov et al., 2017) was used.

RESULTS:

1. **Synaptic gene mutations implicated in the Indian population.**

Synaptic proteins regulate ion channel localisation, synaptic vesicle cycle and positional priming and other critical functions (Gundelfinger et al., 2012). Dysfunction in synaptic genes leads to synaptic protein dysregulation and function. Using the SynGO (cellular component) database, we narrow down on synaptic genes from the larger list of genes (supplementary table 1) whose mutations are implicated in the Indian population with neurological disorders (Figure1 and supplementary table 2). Indeed, except for epilepsy conditions, the synaptic genes are localised to both pre and post synaptic compartments in ASD and schizophrenia. Thus, by studying the mutations in these genes, we can understand the mechanism by which these mutations may contribute to the aetiology of synaptopathies mentioned above.

Intronic variants, despite not contributing to amino acid substitution or changes in the coding sequence, have been implicated in various disorders (Baskin et al., 2011). Some of these variants are present in linkage disequilibrium with other exonic variants, whereas other intronic mutations cause mis-splicing and pseudo-exons (Vaz-Drago et al., 2017). Thus, it is vital to study intronic variations parallel to exonic variants when establishing a roster of susceptibility genes for any disorder. Surprisingly, more than 50% of the mutations implicated in Schizophrenia, both synaptic and non-synaptic genes are intronic variants, whereas we did not observe this trend in case of ASD and epilepsy (Supplementary Figure 1 and supplementary table 3).

2. Severity of impact of mutations on protein structure

To determine the strength of the genetic mutations' contribution to the disorders, we used PSIC scores to narrow down to potentially highly damaging mutations, which allows us to further research the mechanism by which these specific mutations contribute to their associated disorders. Through the scores of both PolyPhen and SIFT, we have concluded that the three genes *RPL10*, *GABRA1*, *DRD2* and their mutations associated with ASD, epilepsy and schizophrenia, respectively, are of highest impact potential (Table 1). All the three genes are predicted to be damaging or deleterious based on the PolyPhen and SIFT scores. Among the three genes (where *RPL10* encodes for Large ribosomal subunit protein uL16, *GABRA1* encodes Gamma-aminobutyric acid receptor subunit alpha-1, *DRD2* codes for and D(2) dopamine receptor). *GABRA1* and *DRD2* encoded proteins are vital in neurotransmission related signal transduction pathways and are highly impaired in the diseases they are associated with, whereas *RPL10* codes for a ribosomal protein critical for the assembly of the polysomes (Noble, 2000; Maillard et al., 2022; Klauck et al., 2006).

3. Impact of mutation on protein-protein interaction and function

First, we sought to visualise the structural differences in proteins coded by mutated genes in comparison with WT (Figure 2). Visualisation of the putative structures helps us understand the impact of the SNPs chosen for study on the obvious structural changes in the proteins coded by these genes' mutations. Further, we analysed the sequence identity, which provides information about the similarity in sequence identity between the mutated protein structure formed, and the template of amino acids, i.e., WT. The sequence identity for the aforementioned proteins are as follows: Large ribosomal subunit protein uL16 - 98.9%**,** Gamma-aminobutyric acid receptor subunit alpha-1 - 99.07%, and D(2) dopamine receptor - 99.77%.

A protein's interactions with the other proteins in its vicinity plays a key role in predicting the function of target protein and drug ability of molecules. The majority of genes and encoded proteins' interactions drive the cellular machinery and identifying these interactions is pivotal to establishing specific physiological process pathways. We first identified the interaction partners of each of the identified proteins in WT condition (Figure. 3), using the STRING tool. From this list, we have assessed most affected "interacting partner-POI (protein of interest) interaction" (Table 2). In case of ASD, the interaction between *RPL19* and the Large ribosomal subunit protein uL16 is highly affected (difference in weighted scores between WT and mutated conditions is -48.6), which might result in disruption of polysome formation. For epilepsy, the affected interaction is between *GABBR2* and Gamma-aminobutyric acid receptor subunit alpha-1 (difference in weighted scores is -375.7). We further identified that, the strongly affected interaction is between *NCS1* and D(2) dopamine receptor, which belongs to schizophrenia (difference in weighted scores is 890.1) (Table 2). *NCS-1* encodes Neuronal calcium sensor 1 protein, which is a highly conserved EF hand calcium binding protein, a critical dopamine receptor interacting protein that has a variety of

neuronal functions like membrane traffic, cell survival, and ion channel and receptor signalling. Neuronal calcium sensor 1 and D(2) dopamine receptors colocalize at sites of synaptic activity and this interaction has shown to attenuate ligand-induced D(2) dopamine receptor internalisation and receptor phosphorylation, enhancing D(2) dopamine receptor signalling (Cravchik et al., 1996; Kabbani et al., 2002). Altogether our analysis and previous studies predict a strong influence of genetic mutations on protein-protein interactions thus affecting the neuronal functions.

Discussion:

Our current study elucidates the impact of existing single nucleotide polymorphisms on the overall structure and function of proteins coded by genes whose mutations are implicated in ethnically Indian populations having neurological disorders. Our bioinformatic analysis demonstrated that known mutations in genes *RPL10*, *GABRA1* and *DRD2* that are associated with ASD, epilepsy and schizophrenia respectively, are the most potentially damaging ones with respect to the protein structure and subsequent protein functions (Figure. 2). Interestingly, all the three candidates - Large ribosomal subunit protein uL16 (coded by *RPL10*), Gamma-Aminobutyric Acid Receptor Subunit alpha-1 (coded by *GABRA1*) and D(2) dopamine receptor (coded by *DRD2*), - are essential for optimum neurological function in the disorders that their mutations have been correlated in. Surprisingly, we also found that most of the synaptic genes' mutations in ASD and epilepsy are more common in exonic regions, whereas most of the synaptic gene mutations linked to schizophrenia are localised to intronic regions (Supplementary Figure 1).

Protein-protein interactions are vital for cellular functions, and disruptions in these interactions can lead to various disorders. For example, in ASD, PTEN-AKAP8L interaction is said to affect neuronal growth, and both proteins are POI for ASD aetiology (Pintacuda et al., 2023). By

investigating these interactions, we gain insights into disease causes and identify potential drug targets, offering hope for more effective treatments. Our results reveal, proteins like ion channels and GPCRs can be majorly affected by mutations, and their interactions with other proteins present in their vicinity within the cell or matrix are also impacted (Figure 3 and Table 2). Studying the physiological functions and mechanisms facilitated by the interactions between the unmutated proteins, and the impact of mutations on these protein interactions aid us in determining the mechanism of disorder manifestation (Rao et al., 2014; Bugge et al., 2020).

ASD is used to describe individuals with a specific pattern of social impairment paired with repetitive behaviours, often with niche interests and/or sensory behaviours with an early onset. Subtle anatomical and functional deviations have been observed in the brain through postmortem studies despite not characterising the disorder by gross brain pathology (Lord et al., 2020). Large ribosomal subunit protein uL16 is pivotal for joining the 60S and 40S subunits in a late step of translation initiation, and structural data suggests that it is present in the interface of the 60s subunit in a cleft between the central protuberance and the L7/L12 stalk in the vicinity of the A and P sites and the catalytic trans-peptidyl transferase centre (Spahn et al., 2001). Studies showing the two missense mutations *RPL10* [L206M] and *RPL10* [H213Q] in ASD candidates suggest an impairment of a complete translating ribosome complement, that is, the polysome population rather than basic translation, per se (Klauck et al., 2006).

Juvenile myoclonic epilepsy, or Janz syndrome, is a prevalent form of epilepsy constituting up to 10% of all epilepsy and 18% of idiopathic generalised epilepsy (IGE) (Camfield et al., 2013). The *GABRA1* gene codes for the α1 subunit of heteropentameric gamma-aminobutyric acid A receptors (GABAARs), which functions in mediating fast primary inhibition in the brain and is critical for brain development. Impairment of GABAergic transmission by genetic mutations has been shown

to induce epileptic seizures (Sperk et al., 2004). Ala322Asp mutant GABAA receptors show reduced amplitude of GABA activated currents *in vitro*, alluding that seizures may be a consequence of loss of function of GABA gated channels (Cossette et al., 2002). Application of GABA to HEK cells expressing receptor complexes that included the mutant subunit showed a reduced chloride flux compared to receptors formed with wild type subunits (Noebels et al., 2002). *GABRA1*[A332V] should be viewed as a "molecular change of function" variant which induces an increased sensitivity to low GABA concentrations along with accelerated desensitisation and some impact on agonist efficacy (Steudle et al., 2020).

Schizophrenia is a syndrome hallmarked by evident signs of psychosis. Schizophrenia presents with paranoid delusions and auditory hallucinations late in adolescence or in early adulthood. Manifestations of the illness are commonly divided into positive (delusions, hallucinations, thought disorganisation, paranoia), negative (lack of drive and motivation, alogia, social withdrawal), and cognitive symptoms (poor performance on cognitive tasks involving attention and working memory). Hyperactivity of dopamine in the mesolimbic pathway in the subcortical region augments D(2) dopamine receptor activation and mediates positive psychotic symptoms. The negative and cognitive symptoms are thought to arise from reduced D(1) dopamine receptor stimulation (Shen et al., 2012; Brisch et al., 2014). D(2) dopamine receptor regulates the release of dopamine through a negative feedback loop when localised in the presynaptic compartment. It plays an integral role in maintaining dopamine concentrations in the synapse, and any dysfunction can lead to hyperdopaminergia, which is extensively discussed in the Schizophrenia hypothesis (Howes et al., 2009).

We further observed that there is a significant percentage of intronic variants in the list of implicated mutations in the synaptic genes (Supplementary Figure 1). The intron–exon structure

of eukaryotic genes has played a major role in the creation of new genes through exon shuffling (Berk., 2016), and the ability to alternatively select different combinations of exons has been critical for generating gene expression diversity in more complex organisms (Keren et al., 2010). Alterations in pre-mRNA splicing are increasingly recognized as responsible for monogenic disorders, with about 15% to 50% affecting pre-mRNA splicing (Vaz-Drago et al., 2017). Mutations affecting intronic splicing enhancers or silencers have been shown to result in mis-regulated splicing and then in most cases rapidly degrade (Sharp et al., 1987). Some genes are purely snoRNA or microRNA generating, with no protein-coding capacity (Bortolin and Kiss., 1998; Flynt et al., 2010; Tycowski et al., 1996). Recent transcriptomic analysis revealed that many introns are actively retained in polyadenylated transcripts and contribute to down regulating gene expression (Wong et al., 2013; Yap et al., 2012).

Our *in silico* analysis in the current study demonstrates the effect of the mutations in different genes related to neurological conditions on their encoded protein structures and predicted the possible alteration in synaptic signaling, specifically the *DRD2*. In addition to its schizophrenia association, *DRD2* is one of the strongest candidates linked to ASD, and targeting its activity might be a potential therapeutic strategy (Annamneedi et al., 2023). Further studies are required to address the altered interaction between Neuronal calcium sensor 1 and D(2) dopamine receptor, due to the mutation and its relevance to both schizophrenia and ASD and in developing the common therapeutic strategies.

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Conflict of Interest:

Authors declare no conflict of interest.

Author's contributions:

P.K.D., N.M., S.M., S.K wrote the initial manuscript. P.K.D., N.M., S.M., S.K contributed to the data mining and formal analysis. P.K.D and N.M contributed to bioinformatic and simulation analysis and image processing. A.A designed and supervised the study. All the authors contributed to the final version of the manuscript.

References:

1. Adzhubei, Ivan et al. “Predicting functional effect of human missense mutations using PolyPhen-2.” Current protocols in human genetics vol. Chapter 7 (2013): Unit 7.20.
2. Annamneedi, Anil, et al. "Ablation of the presynaptic organizer Bassoon in excitatory neurons retards dentate gyrus maturation and enhances learning performance." *Brain Structure and Function* 223 (2018): 3423-3445.
3. Ashton-Beaucage, Dariel, et al. "The exon junction complex controls the splicing of MAPK and other long intron-containing transcripts in Drosophila." Cell 143.2 (2010): 251-262.
4. Baskin, Berivan et al. “Duchenne muscular dystrophy caused by a complex rearrangement between intron 43 of the DMD gene and chromosome 4.” *Neuromuscular disorders : NMD* vol. 21,3 (2011): 178-82.
5. Berk, Arnold J. “Discovery of RNA splicing and genes in pieces.” Proceedings of the National Academy of Sciences of the United States of America vol. 113,4 (2016): 801-5.
6. Bortolin, M L, and T Kiss. “Human U19 intron-encoded snoRNA is processed from a long primary transcript that possesses little potential for protein coding.” *RNA (New York, N.Y.)* vol. 4,4 (1998): 445-54.
7. Brisch, Ralf, et al. "The role of dopamine in schizophrenia from a neurobiological and evolutionary perspective: old fashioned, but still in vogue." Frontiers in psychiatry 5 (2014): 47.
8. Bugge, Katrine, et al. "Interactions by disorder–a matter of context." Frontiers in Molecular Biosciences 7 (2020): 110.
9. Camfield, Carol S et al. “Epidemiology of juvenile myoclonic epilepsy.” *Epilepsy & behavior : E&B* vol. 28 Suppl 1 (2013): S15-7.
10. Cascella, Nicola G et al. “Schizophrenia and epilepsy: is there a shared susceptibility?.” *Neuroscience research* vol. 63,4 (2009): 227-35.
11. Canitano, Roberto, and Mauro Pallagrosi. “Autism Spectrum Disorders and Schizophrenia Spectrum Disorders: Excitation/Inhibition Imbalance and Developmental Trajectories.” *Frontiers in psychiatry* vol. 8 69. 1 May. 2017
12. Cossette, Patrick, et al. "Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy." Nature genetics 31.2 (2002): 184-189.
13. Cravchik, Anibal, David R. Sibley, and Pablo V. Gejman. "Functional analysis of the human D2 dopamine receptor missense variants." Journal of Biological Chemistry 271.42 (1996): 26013-26017.
14. Edwards, D., Forster, J.W., Chagné, D., Batley, J. (2007). What Are SNPs?. In: Oraguzie, N.C., Rikkerink, E.H.A., Gardiner, S.E., De Silva, H.N. (eds) Association Mapping in Plants. Springer, New York, NY.
15. Flynt, Alex S., and Eric C. Lai. "Biological principles of microRNA-mediated regulation: shared themes amid diversity." Nature Reviews Genetics 9.11 (2008): 831-842.

16. Gao, R, and P Penzes. “Common mechanisms of excitatory and inhibitory imbalance in schizophrenia and autism spectrum disorders.” Current molecular medicine vol. 15,2 (2015): 146-67.
17. Greeno, James G., Allan M. Collins, and Lauren B. Resnick. "Cognition and learning." Handbook of educational psychology 77 (1996): 15-46.
18. Grellscheid, Sushma-Nagaraja, and Christopher WJ Smith. "An apparent pseudo-exon acts both as an alternative exon that leads to nonsense-mediated decay and as a zero-length exon." Molecular and Cellular Biology 26.6 (2006): 2237-2246.
19. Gundelfinger, Eckart D., and Anna Fejtova. "Molecular organization and plasticity of the cytomatrix at the active zone." Current opinion in neurobiology 22.3 (2012): 423-430.
20. Howes, Oliver D., and Shitij Kapur. "The dopamine hypothesis of schizophrenia: version III—the final common pathway." Schizophrenia bulletin 35.3 (2009): 549-562.
21. Jutla, Amandeep et al. “Autism spectrum disorder and schizophrenia: An updated conceptual review.” *Autism research : official journal of the International Society for Autism Research* vol. 15,3 (2022): 384-412.
22. Kabbani, Nadine, et al. "Interaction with neuronal calcium sensor NCS-1 mediates desensitization of the D2 dopamine receptor." Journal of Neuroscience 22.19 (2002): 8476-8486.
23. Kelemen, Olga, et al. "Function of alternative splicing." Gene 514.1 (2013): 1-30.
24. Keller, Roberto et al. “Autism, epilepsy, and synaptopathies: a not rare association.” *Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology* vol. 38,8 (2017): 1353-1361.
25. Keren, Hadas, Galit Lev-Maor, and Gil Ast. "Alternative splicing and evolution: diversification, exon definition and function." Nature Reviews Genetics 11.5 (2010): 345-355.
26. Klauck, S. M., et al. "Mutations in the ribosomal protein gene RPL10 suggest a novel modulating disease mechanism for autism." Molecular psychiatry 11.12 (2006): 1073-1084.
27. Koopmans, Frank et al. “SynGO: An Evidence-Based, Expert-Curated Knowledge Base for the Synapse.” Neuron vol. 103,2 (2019): 217-234.e4.
28. Kozakov, Dima et al. “The ClusPro web server for protein-protein docking.” Nature protocols vol. 12,2 (2017): 255-278.
29. Lepeta, Katarzyna, et al. "Synaptopathies: synaptic dysfunction in neurological disorders–A review from students to students." Journal of neurochemistry 138.6 (2016): 785-805.
30. Lord, Catherine, et al. "Autism spectrum disorder." Nature reviews Disease primers 6.1 (2020): 1-23.
31. Maillard, Pierre-yves, et al. "Molecular and clinical descriptions of patients with GABAA receptor gene variants (GABRA1, GABRB2, GABRB3, GABRG2): A cohort study, review of literature, and genotype–phenotype correlation." Epilepsia 63.10 (2022): 2519-2533.

32. Noble, Ernest P. "The DRD2 gene in psychiatric and neurological disorders and its phenotypes." Pharmacogenomics 1.3 (2000): 309-333.
33. Noebels, Jeffrey L.. “Human Epilepsy Can Be Linked to a Defective Calcium Channel.”*Epilepsy currents* vol. 2,3 (2002): 95
34. Pintacuda, Greta, et al. "Protein interaction studies in human induced neurons indicate convergent biology underlying autism spectrum disorders." Cell Genomics 3.3 (2023).
35. Rao, V. Srinivasa, et al. "Protein-protein interaction detection: methods and analysis." International journal of proteomics 2014 (2014).
36. Rong, Yu, et al. "DDRS: Detection of drug response SNPs specifically in patients receiving drug treatment." Computational and Structural Biotechnology Journal 19 (2021): 3650-3657.
37. Schwarz, Jana Marie et al. “MutationTaster2: mutation prediction for the deep-sequencing age.” Nature methods vol. 11,4 (2014): 361-2.
38. Sharp, P M, and W H Li. “The codon Adaptation Index--a measure of directional synonymous codon usage bias, and its potential applications.” *Nucleic acids research* vol. 15,3 (1987): 1281-95.
39. Shen, Hai-Ying, et al. "Adenosine augmentation ameliorates psychotic and cognitive endophenotypes of schizophrenia." The Journal of clinical investigation 122.7 (2012): 2567-2577.
40. Spahn, Christian MT, et al. "Structure of the 80S ribosome from Saccharomyces cerevisiae—tRNA-ribosome and subunit-subunit interactions." Cell 107.3 (2001): 373-386.
41. Sperk, Günther, et al. "GABA and its receptors in epilepsy." Recent advances in epilepsy research (2004): 92-103.
42. Steudle, Friederike, et al. "A novel de novo variant of GABRA1 causes increased sensitivity for GABA in vitro." Scientific Reports 10.1 (2020): 2379.
43. Szklarczyk, Damian et al. “The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest.” Nucleic acids research vol. 51,D1 (2023): D638-D646.
44. Tycowski, Kazimierz T., Mei-Di Shu, and Joan A. Steitz. "A mammalian gene with introns instead of exons generating stable RNA products." Nature 379.6564 (1996): 464-466.
45. Vaz-Drago, Rita, Noélia Custódio, and Maria Carmo-Fonseca. "Deep intronic mutations and human disease." Human genetics 136 (2017): 1093-1111.
46. Waterhouse, Andrew et al. “SWISS-MODEL: homology modelling of protein structures and complexes.” Nucleic acids research vol. 46,W1 (2018): W296-W303.
47. Wong, Justin J-L., et al. "Orchestrated intron retention regulates normal granulocyte differentiation." Cell 154.3 (2013): 583-595.
48. Yang, Yang et al. “AWESOME: a database of SNPs that affect protein post-translational modifications.” *Nucleic acids research* vol. 47,D1 (2019): D874-D880.

49. Yap, Karen, et al. "Coordinated regulation of neuronal mRNA steady-state levels through developmentally controlled intron retention." Genes & development 26.11 (2012): 1209-1223.

Figure legends:

Figure 1: Localization of synaptic genes. The list of genes identified from various studies conducted on the Indian population with respect to neurological conditions, are stratified into synaptic genes, both pre and post synapse using SynGO-cellular component.

Figure 2: Structural comparison of WT and mutated proteins. To further study the impact of the mutations on protein structure, we visualize the WT and mutant proteins. In RPL10, Leu206 is replaced with Met206. In GABA receptor subunit alpha-1, Ala322 is replaced by Asp. In dopamine receptor D2, Ser311 is substituted by Cys311.

Figure 3: Protein-protein interactions in WT condition. Protein-protein interactions form the foundation of complex biological functions. Thus, we compile the interacting partners of our POI using STRING and assessed the change in binding strength between the interacting pairs. Most affected protein-POI is encircled in a box for each potential protein.

Table descriptions:

Table 1: Position-Specific Independent Counts Scores. To determine if the mutation is potentially damaging, and to assess the degree of impact on the structure and function of the protein, we use PSIC softwares Polyphen-2 and SIFT.

Table 2: Protein-protein interaction scores (Lowest Energy). To understand the impact of mutation on protein-protein interaction, we use ClusPro and observe the change in lowest energy scores.

Figures:

1. **Synaptic gene mutations implicated in the Indian population**

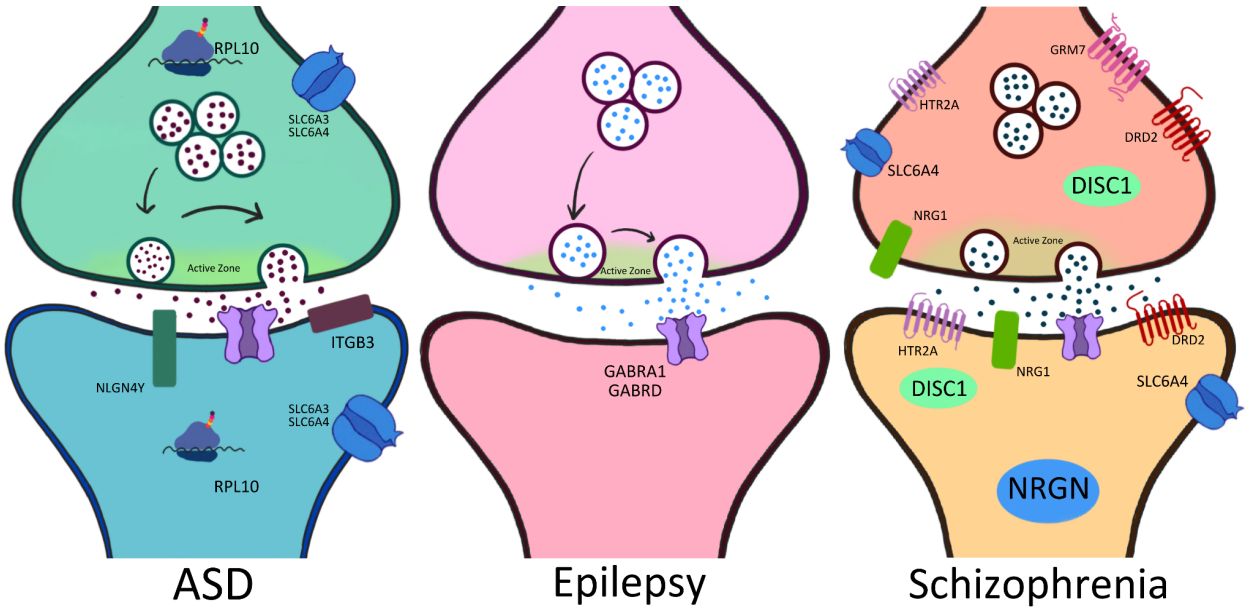
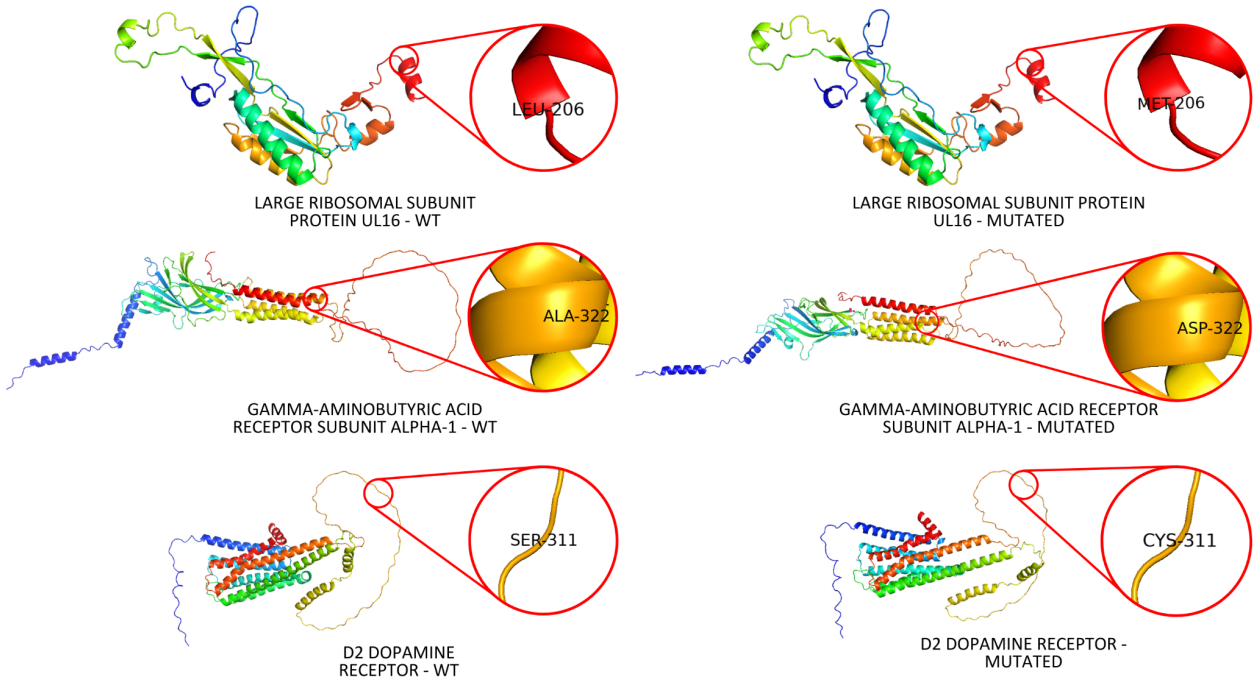


Figure 1: Localization of synaptic genes*. The list of genes identified from various studies conducted on the Indian population with respect to neurological conditions, are stratified into synaptic genes, both pre and post synapse using SynGO-cellular component.*

2. Structural visualization of putative structures of WT and Mutated proteins



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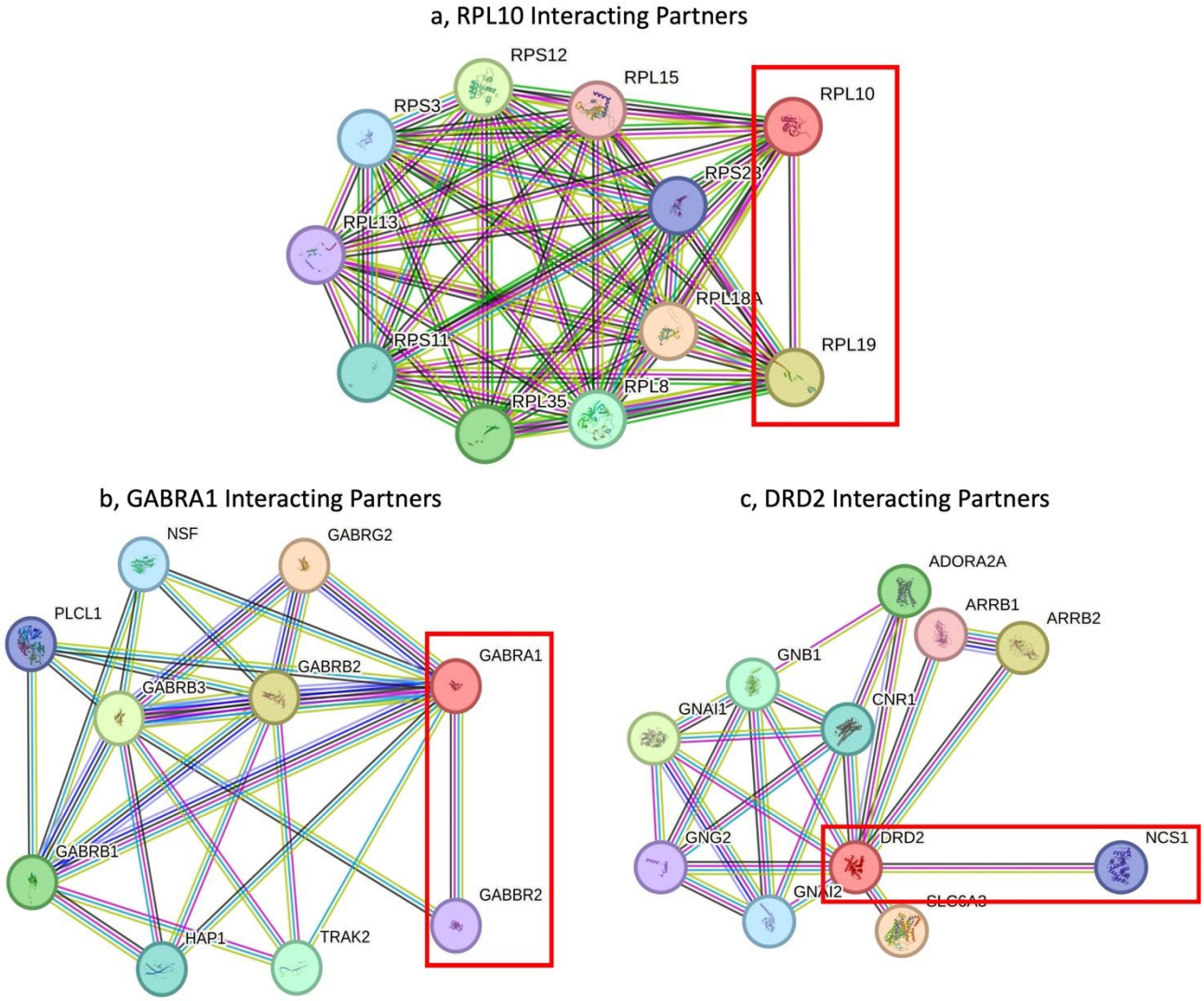
3. Impact of Mutation on Protein-Protein Interaction



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1. **Severity of impact of mutations on protein structure**

DISORDER	GENE	RSID	MUTATION	POLYPHEN-2 SCORE	POLYPHEN-2 PREDICTION	SIFT SCORE	SIFT PREDICTION
ASD	*RPL10*	rs387906727	616C>A L206M	0.999	Probably Damaging	0.008	Deleterious
Epilepsy	*GABRA 1*	rs121434579	965C>A Ala322Asp	0.992	Probably Damaging	0.035	Deleterious
Schizophrenia	*DRD2*	rs1801028	932C>G Ser311Cys	0.999	Probably Damaging	0.027	Deleterious

Table 1: Position-Specific Independent Counts Scores. *To determine if the mutation is potentially damaging, and to assess the degree of impact on the structure and function of the protein, we use PSIC softwares Polyphen-2 and SIFT.*

2. Impact of Mutation on Protein-Protein Interaction

Disease	Protein of Concern	Interacting Protein (Gene)	Weighted Score-WT Protein	Weighted Score - Mutated Protein	Difference
ASD	Large ribosomal subunit protein uL16 (rs782521991)	*RPL18A*	-844.7	-828	-16.7
		RPL19	-727.2	-678.6	-48.6
		RPL35	-680.4	-675.4	-5
		RPL8	-904.2	-881.2	-23
		RPS11	-741.4	-740.8	-0.6
		RPS12	-814.8	-805.8	-9
		RPS23	-630.6	-582.9	-47.7
		RPS3	-1049.8	-1049.9	0.1
Epilepsy	Gamma-aminobutyric acid receptor subunit alpha-1 (rs121434579)	*GABBR2*	-1704.2	-1328.5	-375.7
		GABRB2	-1733.3	-1639	-94.3
		GABRB3	-1919.4	-1879.9	-39.5
		GABRG2	-1699.8	-1707.6	7.8
		HAP1	-1967.2	-1630.7	-336.5
		PLCL1	-1481.2	-1476.3	-4.9
Schizophrenia	D(2) dopamine receptor (rs1801028)	*ADORA2A*	-2139.6	-2140.8	1.2
		ARRB1	-1280.2	-1285.2	5
		ARRB2	-1828	-1828.1	0.1
		GNAI1	-1301.7	-1301.1	-0.6
		GNB1	-1157.1	-1157	-0.1
		NCS1	-1333.4	-2223.5	890.1

Table 2: Protein-protein interaction scores (Lowest Energy). *To understand the impact of mutation on protein-protein interaction, we use ClusPro and observe the change in lowest energy scores. Highlighted are the most significantly affected interaction partners due to mutation.*