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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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. 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 predicts that mutations in genes RPL10 (rs387906727), GABRA1 (rs121434579) and DRD2 (rs1801028) corresponding to ASD, Epilepsy and Schizophrenia respectively, are deleterious. Further, molecular docking shows that the mutated proteins resulting from these SNPs have altered protein-protein interaction with vital interacting partners, possibly impacting neuronal physiological pathways generally affected in the above-mentioned neurological disorders. Specifically, the abnormal interaction between D(2) dopamine receptor and neuronal calcium sensor 1, revealed through molecular docking, may hamper neurotransmitter regulation. Altogether, our study helps to further our understanding of synaptic signaling in the context of the Indian population with neurological disorders and potentiates the usage of a model and dynamic simulation systems to identify novel therapeutic targets aiding in the development of personalized therapy.

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. The human brain is an advanced network that also facilitates cognition, perception, judgement, memory, and retrieval (Greeno et al., 1996). Synaptic communication is critical for the 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 ultimately 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 etiology.

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), further classified as missense or nonsense (Edwards et al., 2007). nsSNPs substitute protein residues, thus 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 establishment of the likely biochemical pathway causing the disease and the creation of effective inhibitors to target the mutant proteins (Dong et al., 2021).

Our current study focuses on analyzing the existing gene mutations linked to ASD, Epilepsy and Schizophrenia with a special focus on the Indian population and their effect on protein structure, and function in mediating synaptic signaling. ASD and Schizophrenia are two different neurological conditions, with distinct phenotypic changes, yet display similarities in certain behaviors 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). By studying localized structural changes and its impact on the protein's orthosteric site and protein binding hot spots, we theorize on the mode of alteration of protein functions due to the SNPs.

MATERIALS AND METHODS:

Primary list of all mutated genes associated with neurological disorders such as ASD, Epilepsy and Schizophrenia in the Indian population was gathered from PubMed and Google Scholar (Supplementary Table 1). Using the SynGo database (Koopmans et al., 2019) the list of genes was segregated into synaptic and nonsynaptic genes, and only synaptic genes were considered for further analysis. The SNPs in synaptic genes were further sorted into exonic (synonymous and non-synonymous) and intronic based on position of occurrence. To sift out highly impactful mutations, Position-Specific Independent Counts Scores (PSIC) tools such as PolyPhen2 and SIFT were used (Adzhubei et al., 2013). 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. 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

86 different variants corresponding to a single synaptic gene, and further compared all the synaptic
87 genes to identify the most deleterious gene mutation per neurological condition. The nucleotide
88 sequence of the respective gene variants that are disease-causing were taken from NCBI the gene
89 code for *RPL10* is 6314, the gene code for *GABRA1* is 2554 and for *DRD2* the gene code is 1813.
90 and the mutations were induced by altering the nucleotide sequence (SNP) according to the
91 respective mutations. Amino acid sequences were identified for the wild type (wild type) and
92 mutated gene-encoded protein using Mutation Taster (Schwarz et al., 2014). The three-dimensional
93 protein structures of the wild-type and mutant variants were generated using SWISS-MODEL
94 (Waterhouse et al., 2018), and sequence identity was generated between wild-type and mutant
95 proteins. Molecular dynamics (MD) simulations were conducted using GROMACS version 2023.1
96 (Spoel et al., 2005) with ff19SB (Tian et al., 2020) Amber force field and TIP3P water model. The
97 proteins were centered in a 10nm simulation box, solvated, and electrically neutralized by 150mM
98 counter ions (K+/Cl-). Bond constraints were applied using the LINCS (Hess, 2008) algorithm.
99 First, proteins underwent minimization, followed by 2 staged equilibrations—NVT heating to
100 298K and NPT equilibration employing V-rescale (Bussi et al., 2007) thermostat and Parrinello-
101 Rahman (Martonák et al., 2003) pressure coupling algorithms. The final 20ns production was run
102 at physiological temperature(310K), from which frames at 0ns, 10ns, and 20ns were extracted for
103 subsequent analysis using GROMACS built-in tools. Furthermore, snapshots and interactions were
104 visualized and analyzed using Discovery Studio Visualizer (Biovia, 2021).

105 The list of interacting protein partners to the wild type protein were prepared using the STRING
106 (Search Tool for the Retrieval of Interacting Genes/Proteins) analysis tool (Szklarczyk et al., 2023),
107 with highest confidence (0.900) as minimum required interaction score. To check for the binding

108 differences between the wild type protein and mutated proteins with their interaction partners
109 ClusPro (Kozakov et al., 2017) was used.

110 **Molecular Simulation Dynamics:**

111 Understanding the mechanisms by which mutations affect protein structure and stability is one of
112 the most important questions to be answered while studying the correlation between the occurrence
113 of a mutation and the manifestation of a disease. To validate the fidelity of the putative structure
114 and to study the structural perturbations in the secondary structure molecular dynamics simulations
115 followed by post-analysis using Ramachandran Plot analysis was done (Gromiha et al., 2002).

116 **RESULTS:**

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

118 Using the SynGO (cellular component) database, we narrowed down on synaptic genes from the
119 larger list of genes (Supplementary Table 1) whose mutations are implicated in the Indian
120 population with neurological disorders (Figure 1 and Supplementary Table 2). Indeed, except for
121 Epilepsy conditions, the synaptic genes are localised to both pre and post synaptic compartments
122 in ASD and Schizophrenia.

123 Intronic variants, despite not contributing to amino acid substitution or changes in the coding
124 sequence, have been implicated in various disorders (Baskin et al., 2011). Some of these variants
125 are present in linkage disequilibrium with other exonic variants, whereas other intronic mutations
126 cause mis-splicing and pseudo-exons (Vaz-Drago et al., 2017). Surprisingly, more than 50% of the
127 mutations implicated in Schizophrenia, both synaptic and non-synaptic genes are intronic variants,

128 whereas we did not observe this trend in case of ASD and Epilepsy (Supplementary Table 3 and
129 4).

130 **2. Severity of impact of mutations on protein structure**

131 Through the scores of both PolyPhen-2 and SIFT, we have concluded that the three genes *RPL10*,
132 *GABRA1*, *DRD2* and their mutations associated with ASD, Epilepsy and Schizophrenia,
133 respectively, are of highest impact potential (Table 1). All the three genes are predicted to be
134 damaging or deleterious based on the PolyPhen-2 and SIFT scores. Among the three genes (where
135 *RPL10* encodes for Large ribosomal subunit protein uL16, *GABRA1* encodes Gamma-
136 aminobutyric acid receptor subunit alpha-1, *DRD2* codes for and D(2) dopamine receptor).
137 *GABRA1* and *DRD2* encoded proteins are vital in neurotransmission related signal transduction
138 pathways and are highly impaired in the diseases they are associated with, whereas *RPL10* codes
139 for a ribosomal protein critical for the assembly of the polysomes (Noble, 2000; Maillard et al.,
140 2022; Klauck et al., 2006).

141 **3. Molecular Dynamics Simulation:**

142 First, we sought to visualize the structural differences in proteins coded by mutated genes in
143 comparison with wild type (Supplementary Figure 1). The sequence identity for the
144 aforementioned proteins are as follows: Large ribosomal subunit protein uL16 - 98.9%**,** Gamma-
145 aminobutyric acid receptor subunit alpha-1 - 99.07%, and D(2) dopamine receptor - 99.77%.

146 Further, we analyzed the φ-ψ angles using Discovery Studios to determine changes, if any, in the
147 secondary structure. Molecular dynamics simulation was done to study the behavior of a protein
148 molecule in solvent, to mimic its native state. The Ramachandran plot or φ-ψ plot uses torsion
149 angles to describe polypeptide and protein conformation and elucidates if a conformation is

allowed or not. The plot allows us to validate the fidelity of the wild type and mutated protein of interest and its associated protein obtained using structural biology techniques (Gromiha et al., 2002).

Root Mean Square Deviation (RMSD), which measures the stability of a protein by determining the protein backbone's deviation relative to its initial conformation during the course of the simulation. Smaller deviation implies more stability of the protein structure (Maruyama et al., 2023). Room Mean Square Fluctuation (RMSF) is used to determine the regional flexibility of the protein. The higher the RMSF, the higher is the flexibility.

The radius of gyration (Rg) is defined as the distribution of atoms of a protein around its axis (Lindahl, 2008). Rg is the length that represents the distance between the point when it is rotating and at the point where the transfer of energy has the maximum effect (Sneha et al., 2016). A relatively steady value of the Rg means stable folding of a protein and fluctuation of the Rg may imply structural destabilization (Liu et al, 2017). Solvent Accessible Surface (SASA) is commonly used to reflect the solvent-exposed surface area of proteins, and a variation of this parameter can reveal the dynamic nature of the protein structure. In general, the smaller SASA indicates that the protein molecule is in a more folded and compact structural state (Durham et al., 2009). Post analysis of our simulation studies revealed that the *DRD2* wild-type model, in the 0ns frame the Ser311 residue falls in the disallowed region but falls in the beta turn conformation in the 10ns and 20ns frame. In the mutant model, the Cys311 residue falls in the coil conformation region at 0ns but shifts to β- turn region in the 10ns and 20ns frame (Supplementary Figure 2a). But despite the secondary structure both being β-turn, the phi angle is observed to change significantly. This shows that there are significant structural constraints due to the amino acid substitution. In the root mean square deviation (RMSD) graph for DRD2 (Supplementary Figure 2b) after the 8ns of simulation,

173 there is a higher degree of fluctuation in the wild type of protein model than the mutated protein
174 model. This points in the direction that the mutant model shows more rigidity than the wild-type
175 model. In the root mean square fluctuation (RMSF) graph of DRD2 (Supplementary Figure 2c),
176 The Ser311 residue in the wild-type model falls in a higher peak region of the RMSF graph than
177 Cys311 in the mutant model, indicating relatively higher level fluctuation in its position in the
178 backbone in comparison with the mutant model, thus supporting the hypothesis that the mutant
179 model is relatively more stable than the wild type model. Such phenomena may be due to the
180 residues interacting spatially with free cysteines of non-membrane proteins revealed a predominant
181 association with hydrophobic residues and a lesser affinity for polar or charged residues
182 (PMC7102755) or less flexible/dynamic. The radius of gyration (Supplementary Figure 2d) and
183 solvent-accessible surface (Supplementary Figure 2e) for both wild type and mutant models is
184 similar within normal ranges of fluctuation. However, we did not find any significant alterations
185 in protein structure due to mutations in either RPL10 or GABRA1 (Supplementary Figure 3 and
186 4).

187 **4. Impact of mutation on protein-protein interaction:**

188 It is well understood that point mutations significantly contribute to stabilizing protein structures,
189 as demonstrated in studies on lysozyme (PMID: 3200317). Therefore, we anticipate that similar
190 mutational alterations in our protein may also impact its stability. So here in this study we have
191 identified the interaction partners of each of the impacted proteins in wild-type condition (Figure
192 2), using the STRING tool. From this list, we have assessed the most affected "interacting partner-
193 POI (protein of interest) interaction" (Table 2). In the case of ASD, the interaction between
194 RPL18A and the Large ribosomal subunit protein RPL10 is highly affected (difference in weighted
195 scores between wild-type and mutated conditions is -48.6), which might result in disruption of

196 polysome formation. For Epilepsy, the affected interaction is between GABBR2 and GABRA1
197 (difference in weighted scores is -375.7). We further identified that the strongly affected interaction
198 is between NCS1 and D(2) dopamine receptors, which is implicated in Schizophrenia (difference
199 in weighted scores is 890.1) (Table 2).

200 *FREQ* encodes Neuronal calcium sensor 1 protein, which is a highly conserved EF hand calcium
201 binding protein, a critical dopamine receptor interacting protein that has a variety of neuronal
202 functions like membrane traffic, cell survival, and ion channel and receptor signalling. Neuronal
203 calcium sensor 1 and D(2) dopamine receptors colocalize at sites of synaptic activity and this
204 interaction has shown to attenuate ligand-induced D(2) dopamine receptor internalisation and
205 receptor phosphorylation, enhancing D(2) dopamine receptor signalling (Cravchik et al., 1996;
206 Kabbani et al., 2002). Altogether our analysis and previous studies predict a strong influence of
207 genetic mutations on protein-protein interactions thus potentially affecting the neuronal functions.

208 **5. Molecular Docking:**

209 It is vital to analyze the change in the protein-protein interaction between the protein of interest
210 and its interacting partner to assess the gravity of the mutation. In RPL10, Leu206 participates in
211 the interaction between RPL10 and RPL18A. But post mutation, none of the initial residues,
212 including residue 206, interact with RPL18A. In terms of hydrogen bonding, the bonding
213 responsible for most directional interactions in the protein, there is an increase in number of
214 hydrogen bonds (20) from the initial number (5). There is also an overall increase in total number
215 of interacting residues post mutation, from 23 to 36. In wild type RPL10, RPL10Ala186 interacts
216 with both RPL18AArg161 and RPL18ALeu159 through alkyl bonding. This interaction is
217 completely absent in mutated RPL10. In addition, RPL10Trp209 interacts with RPL18ALeu154,

218 RPL18APhe152 and RPL18APro155, an interaction trio also absent in mutant RPL10- RPL18A
219 interaction (Supplementary Table 5a, 5b).

220 In GABRA1, some interactions are partially conserved pre and post-mutation i.e., the amino acid
221 in GABBR2 involved in the interaction with GABRA1 is the same, but the residue number
222 changes. Nonetheless, the residue interacting post-mutation is less than 5 residues away from the
223 initial interacting amino acid. But there is an increase in Pi-Alkyl interactions between GABRA1
224 and GABBR2, from 6 to 20 bonds, highlighting the importance of hydrophobic interactions
225 between two proteins. There is a reduction in number of interacting residues from 29 to 22. There
226 is a Phe323-Leu666-Ala322 inteteraction present in wild type GABRA1-GABBR2 interaction,
227 which is absent in mutant protein where Ala322 is replaced with Asp322. (Supplementary Table
228 6a, 6b).

229 In the case of DRD2, Ser311 is not involved in the interaction of the protein with NCS1. The
230 mutation causes structural changes dire enough that none of the residues in DRD2 wild type model
231 that interact with NCS1, participate in the interaction pair after mutation (Supplementary Table 7a,
232 7b). Interestingly, there is a formation of salt bridges observed in mutant DRD2 and NCS1, whereas
233 salt bridges are not observed in binding between WT DRD2 and NCS1. The introduction of salt
234 bridges into the binding site may have a destabilising effect on the bonding (Hendsch & Tidor,
235 1994). Thus, despite not being directly involved in the interaction between DRD2 and NCS1, the
236 Ser311Cys mutation in DRD2 shows a substantiated effect on the interaction between these
237 proteins. This is highlighted by the stark difference in the interacting residues of DRD2 in wild
238 type model and mutant model.

239 **DISCUSSION:**

240 Our current study elucidates the impact of existing single nucleotide polymorphisms on the overall
241 structure and function of proteins coded by genes whose mutations are implicated in ethnically
242 Indian populations having neurological disorders. Our *in silico* analysis demonstrates that known
243 mutations in genes *RPL10*, *GABRA1* and *DRD2*, associated with ASD, Epilepsy and Schizophrenia
244 respectively, are the most potentially damaging ones with respect to the protein structure and
245 subsequent protein functions (Figure 2). Interestingly, all the three candidates - Large ribosomal
246 subunit protein uL16 (coded by *RPL10*), Gamma-Aminobutyric Acid Receptor Subunit alpha-1
247 (coded by *GABRA1*) and D(2) dopamine receptor (coded by *DRD2*), - are essential for optimum
248 neurological function in the disorders that their mutations have been correlated in.

249 Synaptic proteins regulate ion channel localisation, synaptic vesicle cycle, positional priming and
250 other critical functions (Gundelfinger et al., 2012). Dysfunction in synaptic genes leads to synaptic
251 protein dysregulation and function. Indeed, except for Epilepsy conditions, the synaptic genes are
252 localised to both pre and post synaptic compartments in ASD and Schizophrenia. Thus, by studying
253 the mutations in these genes, we can understand the mechanism by which these mutations may
254 contribute to the aetiology of synaptopathies mentioned above.

255 Intronic variants, despite not contributing to amino acid substitution or changes in the coding
256 sequence, have been implicated in various disorders (Baskin et al., 2011). Some of these variants
257 are present in linkage disequilibrium with other exonic variants, whereas other intronic mutations
258 cause mis-splicing and pseudo-exons (Vaz-Drago et al., 2017). Thus, it is vital to study intronic
259 variations parallel to exonic variants when establishing a roster of susceptibility genes for any
260 disorder. Surprisingly, more than 50% of the mutations implicated in Schizophrenia, both synaptic
261 and non-synaptic genes are intronic variants, whereas we did not observe this trend in case of ASD
262 and Epilepsy (Supplementary table 3 and 4).

263 ASD is used to describe individuals with a specific pattern of social impairment paired with
264 repetitive behaviours, often with niche interests and/or sensory behaviours with an early onset.
265 Subtle anatomical and functional deviations have been observed in the brain through post mortem
266 studies despite not characterising the disorder by gross brain pathology (Lord et al., 2020). Large
267 ribosomal subunit protein uL16 is pivotal for joining the 60S and 40S subunits in a late step of
268 translation initiation, and structural data suggests that it is present in the interface of the 60s subunit
269 in a cleft between the central protuberance and the L7/L12 stalk in the vicinity of the A and P sites
270 and the catalytic trans-peptidyl transferase centre (Spahn et al., 2001). Studies showing the
271 missense mutation *RPL10* Leu206Met in ASD candidates suggest an impairment of a complete
272 translating ribosome complement, that is, the polysome population rather than basic translation,
273 per se (Klauck et al., 2006).

274 Juvenile myoclonic epilepsy, or Janz syndrome, is a prevalent form of epilepsy constituting up to
275 10% of all epilepsy and 18% of idiopathic generalised epilepsy (IGE) (Camfield et al., 2013). The
276 *GABRA1* gene codes for the α1 subunit of heteropentameric gamma-aminobutyric acid A receptors
277 (GABAARs), which functions in mediating fast primary inhibition in the brain and is critical for
278 brain development. Impairment of GABAergic transmission by genetic mutations has been shown
279 to induce epileptic seizures (Sperk et al., 2004). Ala322Asp mutant GABAA receptors show
280 reduced amplitude of GABA activated currents *in vitro*, alluding that seizures may be a
281 consequence of loss of function of GABA gated channels (Cossette et al., 2002). Application of
282 GABA to HEK cells expressing receptor complexes that included the mutant subunit showed a
283 reduced chloride flux compared to receptors formed with wild type subunits (Noebels et al., 2002).

284 Hyperactivity of dopamine in the mesolimbic pathway in the subcortical region augments D(2)
285 dopamine receptor activation and mediates positive psychotic symptoms of Schizophrenia, where

286 the manifestation of the illness is divided into positive (delusions, hallucinations, thought
287 disorganisation, paranoia) or negative (lack of drive and motivation, alogia, social withdrawal)
288 symptoms, with cognitive impairments. The negative symptoms and cognitive decline are thought
289 to arise from reduced D(1) dopamine receptor stimulation (Shen et al., 2012; Brisch et al., 2014).
290 D(2) dopamine receptor regulates the release of dopamine through a negative feedback loop when
291 localised in the presynaptic compartment. It plays an integral role in maintaining dopamine
292 concentrations in the synapse, and any dysfunction can lead to hyperdopaminergia, which is
293 extensively discussed in the Schizophrenia hypothesis (Howes et al., 2009; Kesby et al., 2018).

294 We further observed that there is a significant percentage of intronic variants in the list of
295 implicated mutations in the synaptic genes. The intron–exon structure of eukaryotic genes has
296 played a major role in the creation of new genes through exon shuffling (Berk., 2016), and the
297 ability to alternatively select different combinations of exons has been critical for generating gene
298 expression diversity in more complex organisms (Keren et al., 2010). Alterations in pre-mRNA
299 splicing are increasingly recognized as responsible for monogenic disorders, with about 15% to
300 50% affecting pre-mRNA splicing (Vaz-Drago et al., 2017). Mutations affecting intronic splicing
301 enhancers or silencers have been shown to result in mis-regulated splicing and then in most cases
302 rapidly degrade (Sharp et al., 1987). Some genes are purely snoRNA or microRNA generating,
303 with no protein-coding capacity (Bortolin and Kiss., 1998; Flynt et al., 2010; Tycowski et al.,
304 1996). Recent transcriptomic analysis revealed that many introns are actively retained in
305 polyadenylated transcripts and contribute to down regulating gene expression (Wong et al., 2013;
306 Yap et al., 2012).

307 Molecular simulation dynamics predicts the movement of each and every atom present in a protein
308 in a specified timeframe, using a general model of physics governing interatomic interactions as

309 the template (Karplus and Mc Cammon, 2002). These simulations can replicate many vital
310 biomolecular processes including but not limited to conformational change, ligand binding, and
311 protein folding, determining the positions of the atoms at femtosecond temporal resolution. Most
312 importantly, MSD simulates how proteins respond at atomic levels to mutations. These simulations
313 are used hand in hand with experimental structural biology techniques. We also observed how the
314 mutation affects interacting amino acid residues of RPL10, GABRA1 AND DRD2 with the protein
315 of interest, there are no similar amino residues interacting in the mutated type and wild type RPL10
316 with its interacting partner RPL18A. In case of GABRA1 also there no common interacting
317 residues

318 In the case of RPL10 and GABRA1, using MSD we can conclude that the mutation does not
319 drastically impact the structure of the protein, and that the fluctuations are well within normal range
320 (Supplementary Figure 3a, 5a). This hints at the fact that despite being in the coding region and a
321 missense SNP, the mutation contributes to the disorder without destabilising the structure. In the
322 case of DRD2, it can be observed that surprisingly, the mutant variant is more rigid than the wild
323 type (Supplementary Figure 2d).

324 Protein-protein interactions are vital for cellular functions, and disruptions in these interactions can
325 lead to various disorders. For example, in ASD, PTEN-AKAP8L interaction is said to affect
326 neuronal growth, and both proteins are POI for ASD aetiology (Pintacuda et al., 2023). By
327 investigating these interactions, we gain insights into disease causes and identify potential drug
328 targets, offering hope for more effective treatments. Our results reveal, proteins like ion channels
329 and GPCRs can be majorly affected by mutations, and their interactions with other proteins present
330 in their vicinity within the cell or matrix are also impacted (Figure 2 and Table 2). Studying the
331 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).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., N.M contributed to the data mining and formal analysis. P.K.D, N.M., N.M and B.G.A contributed to bioinformatics, simulation analysis and image processing. A.A designed and supervised the study. All the authors contributed to the final version of the manuscript.

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506 **Figure legends:**

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

510 **Figure 2:** Protein-protein interactions in wild type condition. Protein-protein interactions form the
511 foundation of complex biological functions. Thus, we compile the interacting partners of our POI
512 using STRING and assessed the change in binding strength between the interacting pairs. The most
513 affected protein-POI is encircled in a box for each potential protein.

514 **Figure 3:** Molecular Docking of protein-protein interaction. (a) Protein-protein interaction
515 molecular docking of (i) wild type RPL10 and RPL18A and (ii) mutant RPL10 and RPL18A
516 (Supplementary Table 5). (b) Protein-protein interaction molecular docking of (i) wild type
517 GABRA1 and GABBR2 and (ii) mutant GABRA1 and GABBR2 (Supplementary Table 6). (c)
518 Protein-protein interaction molecular docking of (i) wild type DRD2 and NCS1 and (ii) mutant
519 DRD2 and NCS1 (Supplementary Table 7).

520 **Table descriptions:**

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

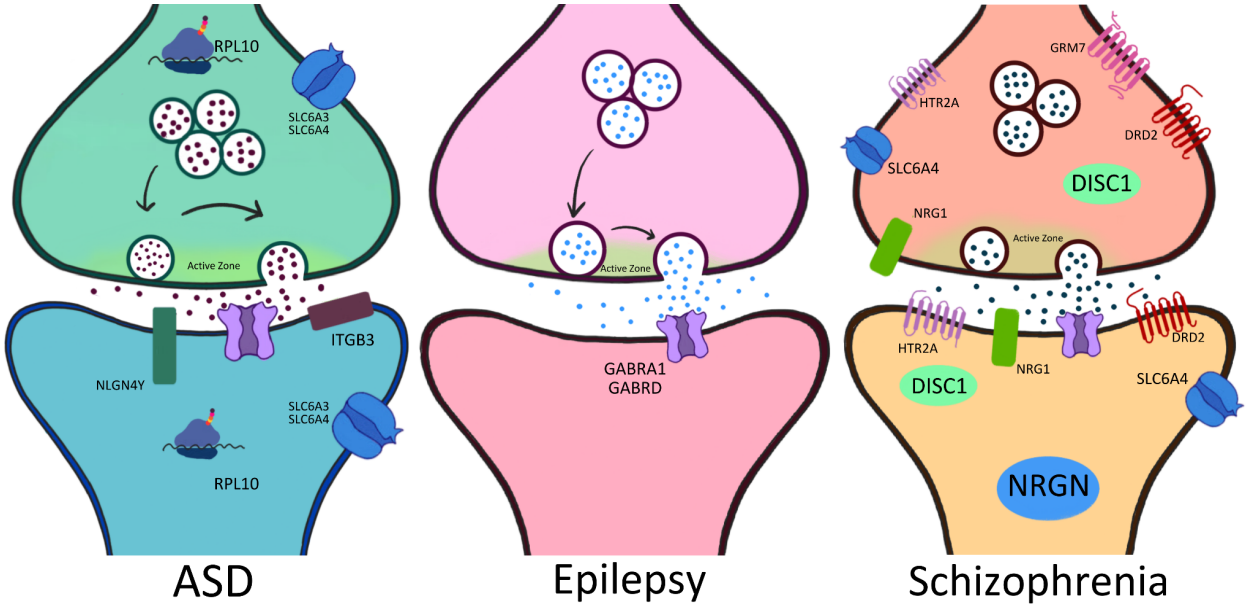
524 **Table 2:** Protein-protein interaction scores (Lowest Energy). To understand the impact of mutation
525 on protein-protein interaction, we use ClusPro and observe the change in lowest energy scores.

526

527 **Figures:**

528

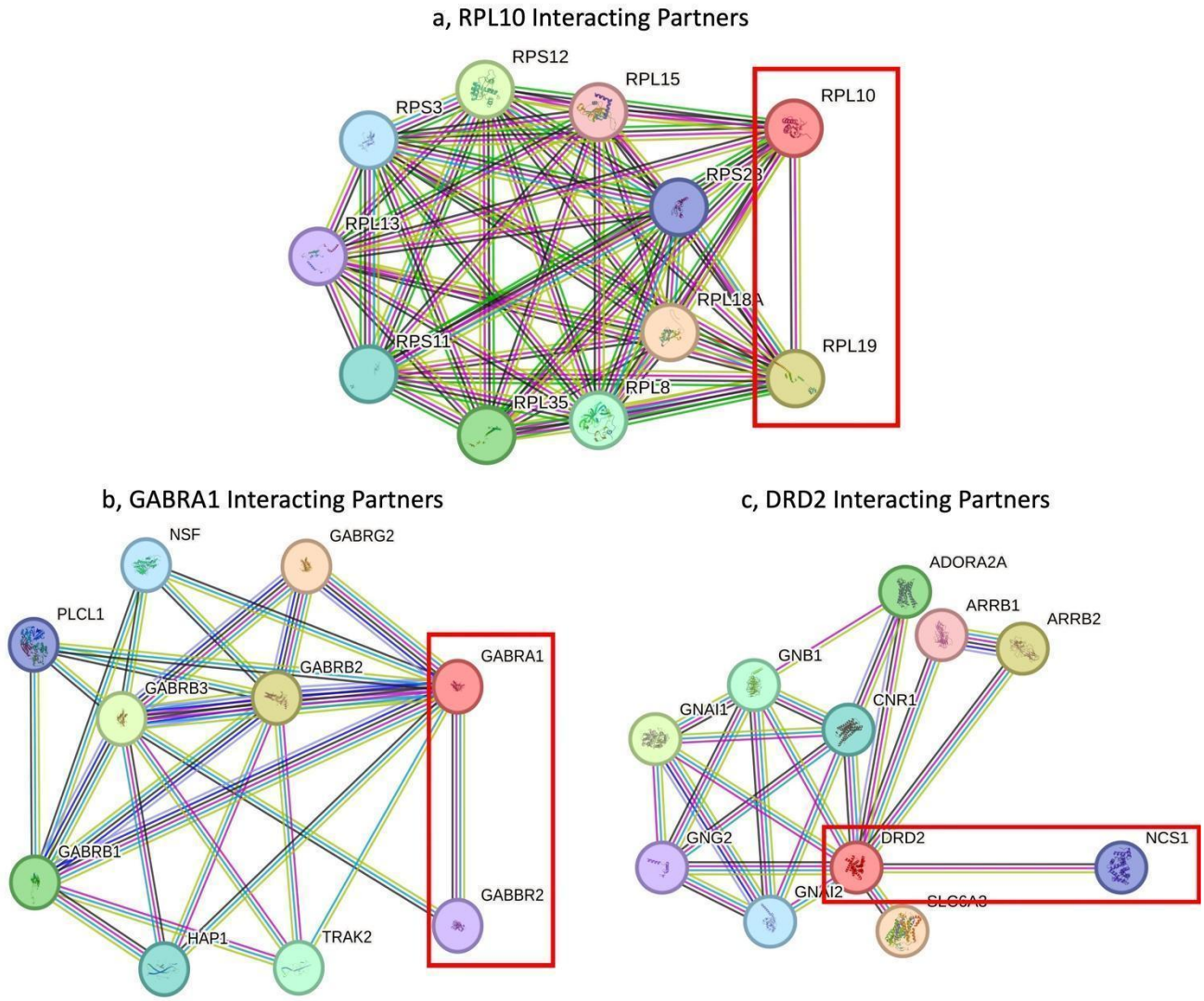
529 **1. Synaptic gene mutations implicated in the Indian population**



530

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

534 ## 2. Impact of Mutation on Protein-Protein Interaction



535

536 ***Figure 2: Protein-protein interactions in wild type condition.*** *Protein-protein interactions*
537 *form the foundation of complex biological functions. Thus, we compile the interacting*
538 *partners of our POI using STRING and assessed the change in binding strength between*
539 *the interacting pairs. The most affected protein-POI is encircled in a box for each potential*
540 *protein.*

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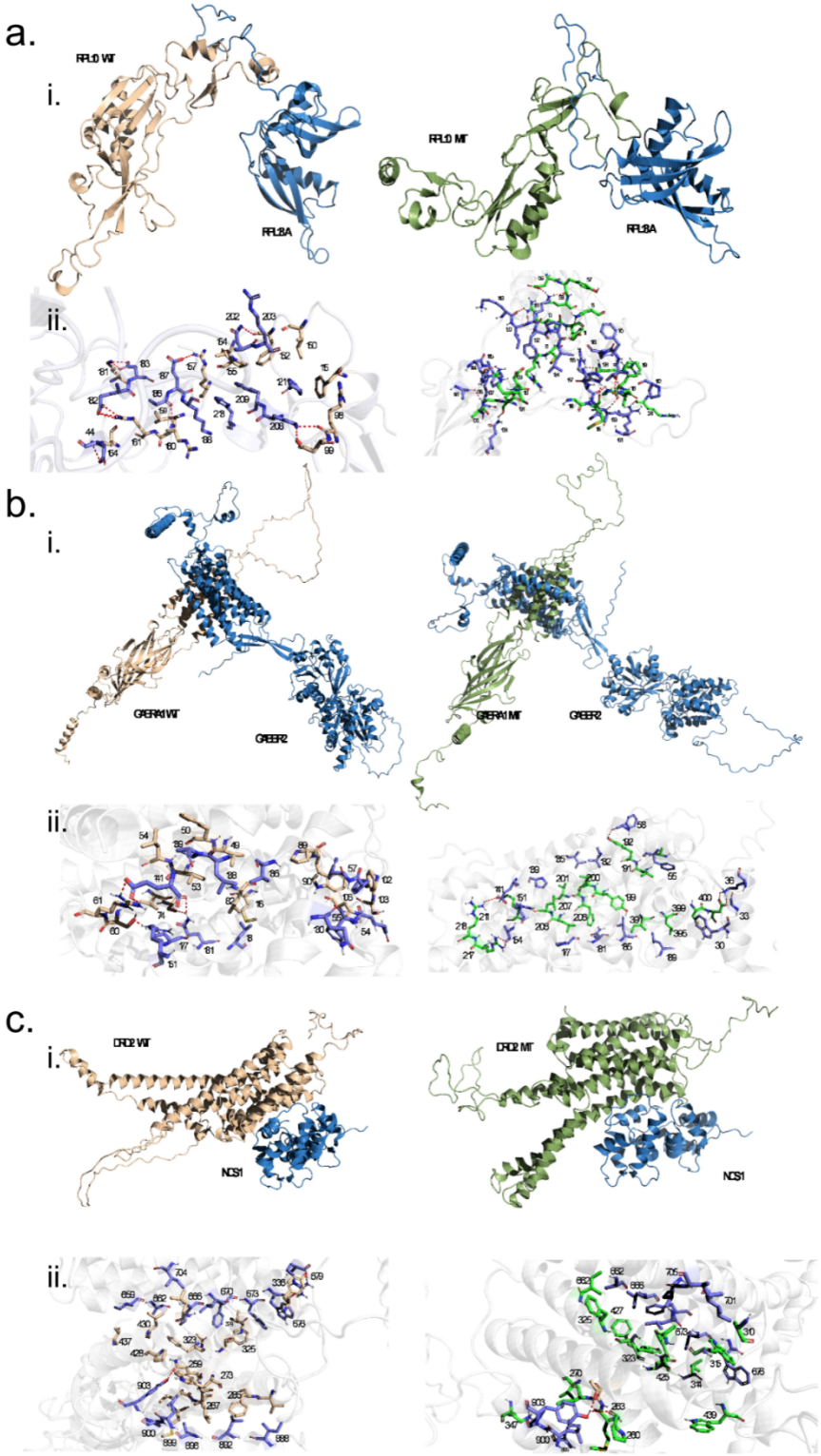

Figure 3: Molecular Docking of protein-protein interaction. *(a) Protein-protein interaction molecular docking of (i) wild type RPL10 and RPL18A and (ii) mutant RPL10 and RPL18A (Supplementary Table 5). (b) Protein-protein interaction molecular docking*

570 *of (i) wild type GABRA1 and GABBR2 and (ii) mutant GABRA1 and GABBR2*
571 *(Supplementary Table 6). (c) Protein-protein interaction molecular docking of (i) wild type*
572 *DRD2 and NCS1 and (ii) mutant DRD2 and NCS1 (Supplementary Table 7).*

573 **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	*GABRA1*	rs121434579	965C>A Ala322Asp	0.992	Probably Damaging	0.035	Deleterious
Schizophrenia	*DRD2*	rs1801028	932C>G Ser311Cys	0.999	Probably Damaging	0.027	Deleterious

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

577

578 **1. Impact of Mutation on Protein-Protein Interaction**

Disease	Protein of Concern	Interacting Protein (Gene)	Weighted Score- Wild Type Protein	Weighted Score - Mutated Protein	Difference
ASD	Large ribosomal subunit protein uL16 (rs782521991)	*RPL13*	-887.8	-888.4	0.6
		RPL15	-765.6	-765.9	0.3
		RPL18A	-844.7	-789.3	-55.4
		RPL19	-727.2	-680.1	-47.1
		RPL35	-680.4	-654	-26.4
		RPL8	-904.2	-856.5	-47.7
		RPS11	-741.4	-741	-0.4
		RPS3	-1049.8	-1050.3	0.5
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

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