INTERNATIONAL JOURNAL OF APPLIED AND CLINICAL RESEARCH

IJACR

Computational Analysis of GRIN2A C1448R Variant in Epilepsy-Associated Intellectual Disability and their Structural Impact on NMDA Receptor Binding with Rapastinel and Dizocilpine

Sumaiya Yousaf[1], Rimsha Khan[1]*, Uzair Malik[1]

1 Center of Biotechnology and Microbiology, University of Peshawar, 25120, Khyber Pakhtunkhwa, Pakistan
* Correspondence: k.rimsha2002@gmail.com

Abstract: Epilepsy-associated intellectual disability (ID) encompasses a spectrum of neurodevelopmental disorders often caused by genetic mutations affecting synaptic transmission. This study employed a dry-lab, in silico approach to identify and characterize high-confidence candidate genes implicated in epilepsy-related ID, with a focus on *GRIN2A*, a subunit of the N-Methyl-D-Aspartate (NMDA) receptor complex. The pathogenic variant, c.4342T>C (p.Cys1448Arg), was subjected to computational analysis, including pathogenicity prediction, protein stability evaluation, and molecular docking. Two ligands, Rapastinel (GLYX-13) and Dizocilpine (MK-801) were selected for docking based on their clinical and pharmacological relevance to NMDA receptor modulation. Comparative docking revealed a reduction in binding affinity for Rapastinel (from −7.5 to −7.1 kcal/mol) and a slight increase for Dizocilpine (from −8.3 to −8.4 kcal/mol) in the mutant receptor. Protein–ligand interaction profiling showed that Rapastinel's hydrogen bonding network was disrupted, while Dizocilpine retained hydrophobic interactions. Normal mode analysis indicated increased structural flexibility, with lower eigenvalues and elevated B-factors in the mutant complex, suggesting destabilized receptor conformation. These findings provide molecular insight into how the C1448R mutation may contribute to synaptic dysfunction in epilepsy-associated intellectual disability. This study underscores the utility of computational biology in exploring genotype–phenotype relationships and may guide future drug development efforts targeting *GRIN2A* related epileptic disorders.

Keywords: Epilepsy GRIN2A, NMDA receptor, molecular docking, Rapastinel, Dizocilpine, C1448R mutation, ligand binding affinity, receptor conformation.

Introduction

Intellectual disability (ID) is a neurodevelopmental disorder characterized by impairments in intellectual functioning and adaptive behavior, affecting approximately 1–3% of the global population [1,2]. Epilepsy is characterized by abnormal neuronal activity, and frequently coexists with intellectual disability (ID), both of which often share overlapping etiologies and contribute to complex neurodevelopmental profiles [3]. Intellectual disability and epilepsy exhibit a shared pathophysiological basis involving aberrant regulation of activity-dependent protein translation. This mechanism is essential for synaptic plasticity, which underpins processes such as learning and memory. Disruptions in the synthesis of synaptic elements and ion channel proteins can contribute to both the cognitive impairments characteristic of intellectual disability and the neuronal hyper-excitability that manifests in epilepsy [4,5,6]. According to epidemiological studies approximately 20–30% of individuals with epilepsy also present with intellectual disability, highlighting a strong correlation between the two conditions. Both intellectual disability and epilepsy affect over 5% of the global population [7,8]. The *GRIN2A* gene encodes the GluN2A protein, which constitutes a subunit of the NMDA receptor (NMDAR). The neurotransmitters glutamate and glycine bind to the NMDAR, regulating the voltage-gated ion channel. However, the NMDA channel only opens following the ligand binding if the membrane is sufficiently depolarized. In an open channel, the cations (Na^+ and Ca^{2+}) flow into the cell, while K^+ flows out of the cell through the membrane pore in a voltage-dependent fashion [9,10]. Ca^{2+} flow through the NMDAR is crucial for synaptic plasticity, a cellular mechanism for learning and memory. Functional NMDA receptors are di or tri hetero-tetramers composed of two GluN1 subunits and two of four possible GluN2 (A-D) subunits or a combination of GluN2

and GluN3 (A and B) subunits [11,12]. The c.4342T>C mutation in the *GRIN2A* gene leading to the amino acid sequence change C1448R is predicted to destabilize the *GRIN2A* protein and may alter the conformational dynamics of the ligand binding domain. This destabilization potentially affects binding residues, making the mutant receptor a potent candidate for docking. Targeting such mutants can inform the development of therapeutics aimed at restoring receptor function or preventing neuronal excitations.

Methodology

Gene Identification and Selection for Epilepsy

The genes associated with Epilepsy were identified and selected using various databases and tools. Epilepsy related genes were retrieved using the DisGeNET and MalaCards databases, which provide human gene-disease associations. The genes obtained from both databases were compared for similarity by Venn diagram analysis, using the Venny 2.1 tool [13,14,15]. The results showed *GRIN2A* and *GRIN2B* genes as the potential targets responsible for causing epilepsy. To explore the functional context of the genes and their related epilepsy associated genes, KEGG pathway enrichment analysis was performed using ShinyGO. These pathways underscore the involvement of *GRIN2A* and *GRIN2B* in synaptic plasticity, neurotransmitter regulation, and neurological excitability, all of which are central to the pathophysiology of epilepsy. Additionally, the OMIM database was consulted to investigate the functional roles and disease associations of the identified genes. Based on their relevance to the disease, *GRIN2A* was prioritized for further evaluation [16, 17].

Variant Selection

To identify relevant genetic variations in the *GRIN2A* gene, population-specific variant data were retrieved from the gnomAD database, with a particular focus on the South Asian cohort. Among the reported variants, the missense mutation c.4342T>C, with an amino acid substitution p.Cys1448Arg, was selected due to its potential link to epilepsy. This mutation was introduced into the wild-type nucleotide sequence using MutationTaster, a tool used to predict the disease-causing potential of genetic alterations [18,19].

Pathogenicity Prediction of Selected Variant

To evaluate the potential pathogenicity of the selected *GRIN2A* variant, various in silico tools were utilized to predict its impact on protein function and structural stability. SIFT was employed to determine whether the amino acid substitution would affect protein function based on sequence homology and evolutionary conservation. PolyPhen-2 further assessed the likely structural and functional consequences of the variant by analyzing physical properties of amino acid changes. In parallel, FATHMM, which uses hidden Markov models and conservation metrics, was applied to predict the likelihood of functional disruption [20,21,22].

Structure Prediction of Wild-Type and Mutant Proteins

To enable structural analysis and molecular docking, three-dimensional models of both the wild-type and mutant forms of the *GRIN2A* protein were generated. Both sequences were submitted to AlphaFold, an advanced deep learning-based tool for protein structure prediction, which produced high confidence PDB models [23]. The resulting structural files were preserved for downstream stability analysis and molecular docking, allowing visualization of potential conformational changes caused by the mutation

Prediction of Protein Stability

To assess whether the selected mutation could alter the structural stability of the *GRIN2A* protein, a set of computational tools was employed. I-Mutant 3.0 was used to estimate changes in protein stability based on the protein sequence and mutation site. MuPro and INPS-MD were used to evaluate potential shifts in protein folding and stability using a machine learning-based approach [24,25,26]. These predictions offered a preliminary understanding of how the mutation might compromise protein integrity and function. This evaluation was essential for establishing whether the altered structure could contribute to functional disruption of the NMDA receptor complex, thereby supporting its potential role in epilepsy.

Protein-Protein Interaction (PPI) Network Analysis

To investigate the broader functional context of *GRIN2A* within cellular pathways, a Protein–Protein Interaction (PPI) network was constructed using the STRING database [27]. This tool integrates known and predicted interactions based on experimental data, co-expression patterns, and computational predictions. A network of interacting proteins was visualized, highlighting role in synaptic transmission, ion channel regulation, and neurodevelopmental processes. Functional analysis of

these interacting proteins revealed several components linked to neuronal excitability and neurotransmitter signaling, suggesting that disruptions in *GRIN2A* may have cascading effects on multiple pathways essential for brain function.

Ligand Selection

To identify suitable ligands for docking analysis, molecules associated with the function of *GRIN2A* were analyzed. Two NMDA receptor targeting ligands Rapastinel and Dizocilpine (MK-801) were selected based on their relevance to glutamatergic signaling. Rapastinel is a positive allosteric modulator and enhances synaptic plasticity without inducing the dissociative side effects typical of NMDA antagonists, while Dizocilpine is a potent non-competitive antagonist of NMDA receptors and has been widely used in experimental epilepsy models for its ability to block excessive excitatory neurotransmission. Both ligands directly interact with NMDA receptor subunits, including *GRIN2A*, making them suitable candidates. Their selection was further supported by literature linking these compounds to therapeutic modulation in neurodevelopmental and seizure-related disorders. The three-dimensional structure of ligands was retrieved from the PubChem database for molecular docking simulations [28].

Prediction of Active Binding Sites

To identify potential binding regions on the *GRIN2A* protein, annotated structural data from the UniProt database was consulted [29]. This resource provided information on functionally important residues based on experimental evidence and domain analysis. The amino acid positions 511, 513, 518, 689, and 731 were identified as potential candidates involved in ligand interaction. These residues were considered during molecular docking to ensure targeted interaction analysis at biologically relevant sites.

Molecular Docking with Ligand

To investigate the interaction between the mutant *GRIN2A* protein and the ligands, molecular docking was performed using AutoDock Vina [30]. The three-dimensional structure of the mutated *GRIN2A* protein, obtained using AlphaFold3 was loaded into AutoDock Vina, where preliminary preparations were carried out. Water molecules were removed, and polar hydrogens along with appropriate charges were added to the protein. The processed structure was then saved in PDBQT format to prepare it for docking. A grid box was defined around the predicted active site residues (positions 511, 513, 518, 689, and 731) to restrict the docking region and focus on relevant binding interactions. The docking simulation was executed via Command Prompt, and output files containing binding poses and binding affinity values were generated. These files were further analyzed in PyMOL, where both the protein and docked complex were visualized [31]. To analyze he interactions, the online tool PLIP was used [32]. This analysis provided a breakdown of hydrogen bonds and hydrophobic contacts which were visualized and tabulated for reporting purposes.

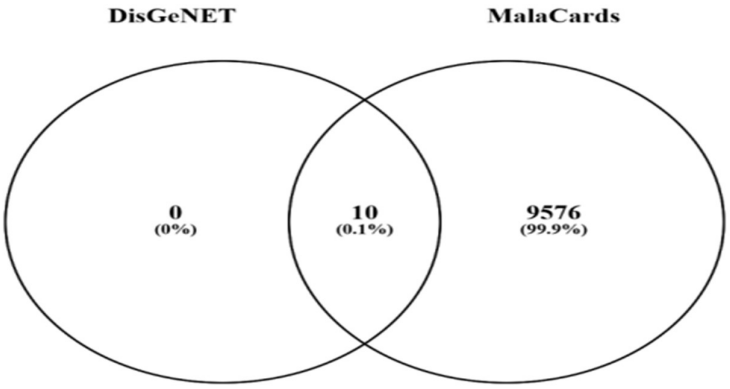
Molecular Dynamics Simulation

For preliminary insight into the dynamic behavior of the docked complex, a basic molecular dynamics simulation was performed using iMODS, a web-based tool for normal mode analysis [33]. These analyses clarify whether the ligand binding induces significant conformational changes, offering additional support for the predicted interaction's biological relevance.

Results

Gene Selection for Epilepsy Associated Intellectual Disability

A list of 10 epilepsy associated genes was retrieved from DisGeNET and 9576 genes from MalaCards. By comparing these datasets using the Venny 2.1 tool, a total of 10 genes were found to be common between both databases, suggesting a higher level of confidence in their relevance to epilepsy (Figure 1): *SCN1A, SCN2A, KCNQ2, SCN8A, ABCB1, GRIN2A, MECP2, GRIN2B, POLG*, and *ATP1A3*.



To further select genes relevant to epilepsy with intellectual disability, functional pathway analysis was performed using ShinyGO. Among the 10 shortlisted genes, *GRIN2A* and *GRIN2B* were found to be involved in the glutamatergic synapse pathway which is an essential component of brain signaling pathways, including excitatory neurotransmission, synaptic plasticity, and cognitive processes. These two genes encode subunits of the N-Methyl-D-Aspartate (NMDA) receptor complex, which is crucial for calcium signaling and neuronal development. Dysfunctions in NMDA receptor signaling are widely implicated in epileptic encephalopathies and neurodevelopmental disorders, including intellectual disability. While both *GRIN2A* and *GRIN2B* share functional roles, *GRIN2A* was selected for downstream structural and functional analysis due to its higher frequency of pathogenic mutations reported in literature specific to epilepsy and intellectual disability, and its role in glutamate-mediated neurotransmission.

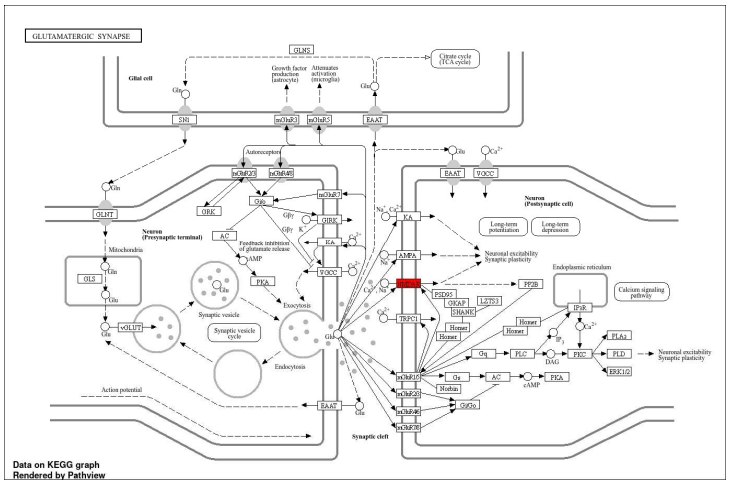


Figure 2. KEGG pathway analysis illustrates the involvement of GRIN2A in the glutamatergic synapse pathway. GRIN2A is mapped to the NMDA receptor complex, a critical component in excitatory neurotransmission and synaptic plasticity.

Mutation Pathogenicity Analysis

In silico analysis of the pathogenic potential of the GRIN2A c.4342T>C (p.C1448R) mutation revealed a damaging effect on protein function.

Tool	Prediction	Score
SIFT	Not Tolerated	0.05
PolyPhen-2	Probably Damaging	0.975
MutationTaster	Disease Causing	High Confidence
FATHMM	Tolerated	2.81

Table 1. Computational prediction of the pathogenicity of the c.4342T>C (p.C1448R) mutation in the GRIN2A gene

Protein Stability Assessment

Evaluation of the impact of the mutation on protein stability predicted a destabilizing effect, indicating that this mutation could affect the conformational flexibility and proper functioning of the GluN2A protein, potentially leading to altered NMDA receptor signaling.

Tool	ΔΔG (kcal/mol)	Prediction
Mupro	–1.385	Decreased Stability
INPS-MD	–1.49761	Decreased Stability
I-Mutant2.0	+0.48	Increased Stability

Table 2. Prediction of protein stability changes due to mutation

Molecular Docking Analysis

To assess the potential impact of the mutation on ligand binding, molecular docking was performed using Rapastinel and Dizocilpine against both wild-type and mutant structures of the GluN2A protein. The binding affinity of Rapastinel decreased slightly in the mutant (–7.1 kcal/mol) compared to the wild-type (–7.5 kcal/mol), indicating a reduction in binding strength. Conversely, Dizocilpine exhibited a marginally stronger affinity for the mutant protein (–8.4 kcal/mol) than for the wild-type (–8.3 kcal/mol).

Ligand	Protein Type	Best Binding Affinity (kcal/mol)
Rapastinel	Wild-type	–7.5
Rapastinel	Mutant	–7.1
Dizocilpine	Wild-type	–8.3
Dizocilpine	Mutant	–8.4

Table 3. Binding affinities (kcal/mol) of Rapastinel and Dizocilpine with wild-type and mutant GRIN2A proteins as determined through molecular docking using AutoDock Vina

While both ligands demonstrated favorable binding to the receptor in all conditions, subtle shifts in binding affinity were observed between wild-type and mutant complexes.

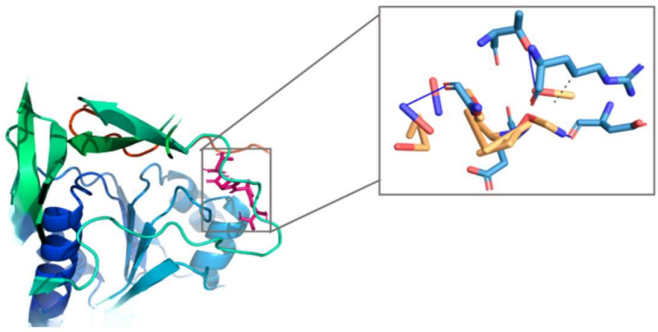


Figure 3. Close-up view of the Rapastinel-bound GRIN2A C1448R mutant complex

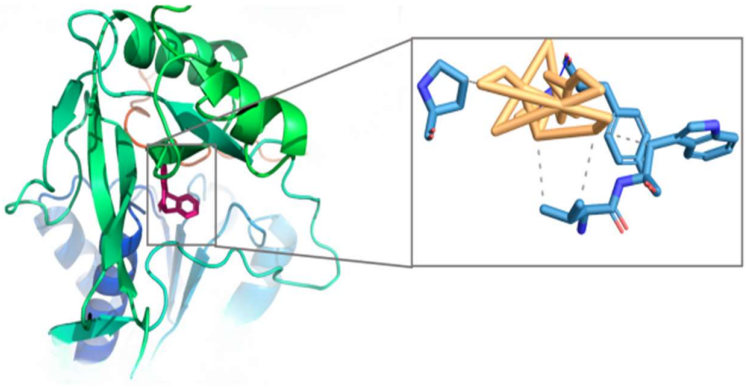


Figure 4. Zoomed in visualization of the Dizocilpine-bound GRIN2A C1448R mutant complex

Protein-Ligand Interaction Analysis

The impact of mutation on ligand binding was evaluated by performing interaction profiling for Rapastinel and Dizocilpine. In the wild-type GRIN2A structure, Rapastinel formed four hydrogen bonds with VAL430, THR433, and ASP476, while the mutant structure also maintained four hydrogen bonds but involved GLY247, THR249, ASP398, and GLU400. Notably, the mutant showed additional hydrophobic interactions with ARG244 and GLU400, indicating a shift in the binding environment. Dizocilpine retained its interaction pattern across both structures. While the wild-type showed six hydrophobic contacts and one hydrogen bond with GLU803, the mutant retained similar hydrophobic interactions with altered residues (VAL389, TRP390, and PRO435) and a relocated hydrogen bond with TYR165. These changes suggest a reshaping of the binding pocket in the mutant, potentially affecting ligand affinity and receptor responsiveness.

Ligand	Protein Type	H-Bonds	Key Residues (H-Bonds)	Hydrophobic Contacts
Rapastinel	Wild-Type	4	VAL430, THR433, ASP476	None
Rapastinel	Mutant	4	GLY247, THR249, ASP398, GLU400	ARG244, GLU400
Dizocilpine	Wild-Type	1	GLU803	PHE658, ALA739, VAL748
Dizocilpine	Mutant	1	TYR165	VAL389, TRP390, PRO435

Table 4. Comparison of hydrogen bonding and hydrophobic interactions between Rapastinel and Dizocilpine with wild-type and C1448R mutant GRIN2A proteins, predicted by PLIP

Normal Mode and Flexibility Analysis

For the Rapastinel–GluN2A complex, Normal Mode Analysis revealed differences in structural flexibility between the wild-type and mutant forms. The mutant complex showed higher flexibility compared to the wild-type, particularly in the C-terminal region. This was evident by elevated peaks in deformability and B-factor plots. This increased motion near the binding site suggests a less stable ligand interaction environment which may reduce the stability of the ligand–receptor interaction observed during docking.

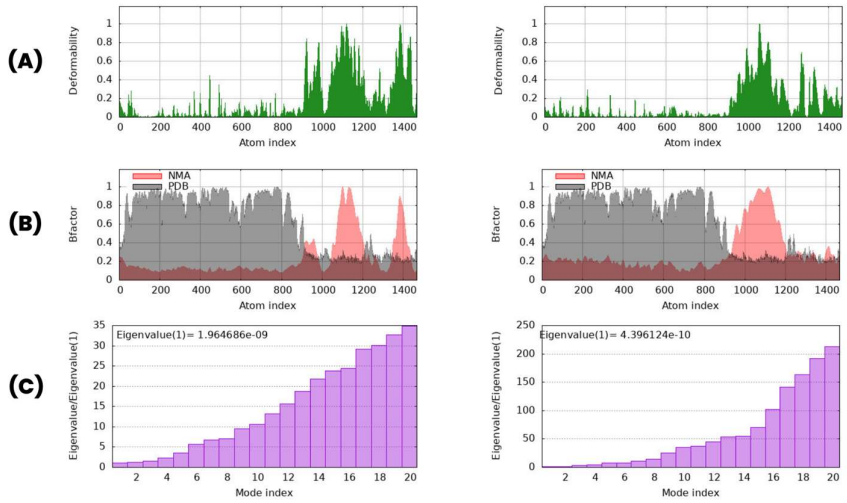


Figure 5. Comparative Normal Mode Analysis (NMA) of wild-type and C1448R mutant GluN2A-Rapastinel complexes

Similarly, the Dizocilpine-bound mutant complex exhibited more flexibility than the wild-type. While the overall binding region remained intact, deformability and B-factor plots revealed increased fluctuations in the mutant. These findings suggest that the C1448R mutation introduces structural changes that may influence ligand binding efficiency and receptor stability, supporting the docking and interaction analysis results, providing structural insight into potential functional consequences of the C1448R substitution.

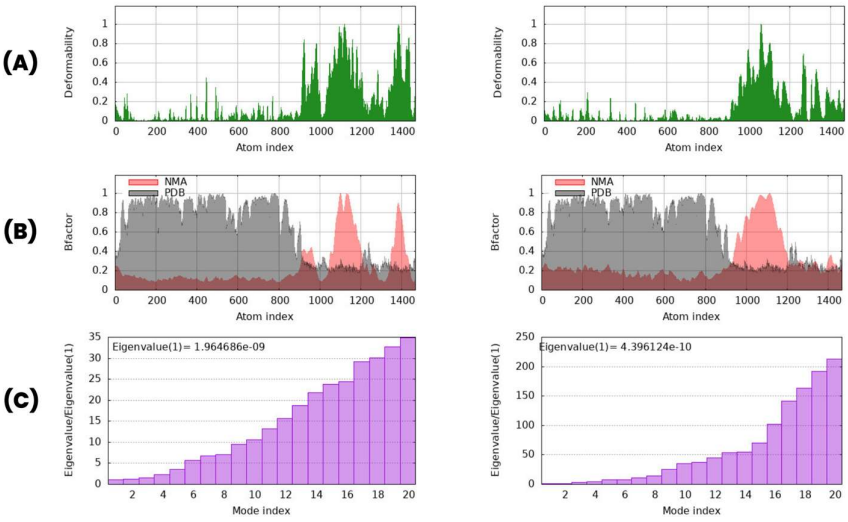


Figure 6. Comparative Normal Mode Analysis (NMA) of wild-type and C1448R mutant GluN2A-Dizocilpine complexes

Discussion

This study investigated the potential impact of the Cys1448Arg missense mutation in the GRIN2A gene on NMDA receptor function, with a specific focus on how this mutation alters receptor dynamics and interactions with two known NMDA receptor modulators: Rapastinel (GLYX-13) and Dizocilpine (MK-801). By combining molecular docking and normal mode analysis, we aimed to characterize the structural consequences of this mutation and explore its relevance in the context of epilepsy associated intellectual disability (ID). Our results revealed that this mutation compromises receptor stability and modifies ligand-binding behavior. Rapastinel showed reduced binding affinity in the mutant receptor, while Dizocilpine slightly enhanced its interaction strength. This suggests that the mutation affects the binding pocket in a ligand specific manner, depending on their binding mechanisms and structural compatibility. This outcome aligns with recent studies which have reported that GRIN2A mutations yield diverse functional phenotypes ranging from gain-of-function to loss-of-function, depending on variant type and position [34]. Normal Mode Analysis further revealed that mutant receptor complexes had increased flexibility, higher B-factors, and lower eigenvalues, which are hallmarks of structural destabilization. Such instability may impair channel gating and excitatory signaling, contributing to epileptogenesis and cognitive deficits. Recent multi-omics studies support these findings, showing that *GRIN2A* mutations in mice lead to widespread transcriptomic disruptions across neuronal and glial cells, along with reduced synaptic glutamatergic proteins. Clinically, *GRIN2A* variants are associated with diverse responses to anti-epileptics, underscoring the need for mutation specific therapeutic strategies [35,36]. Our findings align with Ogden et al. (2017), who showed that mutations in the NMDA receptor's pre-M1 region impair gating, yet MK-801 retains efficacy [37]. Similarly, we observed that Dizocilpine maintained stable binding in the C1448R mutant, suggesting its potential as a mutation-tolerant therapeutic. With the identification of C1448R as a mutation that significantly alters NMDA receptor conformation and ligand dynamics, our study emphasizes the role of computational modeling in identifying how specific mutations affect ligand-receptor interactions. Our findings suggest that structure-based optimization of modulators like Rapastinel, or the selection of mutation tolerant compounds like Dizocilpine may guide future personalized interventions for GRIN2A related neurodevelopmental disorders. While this study offers valuable insights into the structural and functional implications of the GRIN2A Cys1448Arg mutation, future work could be expanded to include experimental validation of the predicted effects through electrophysiological assays or protein expression studies. Screening a broader library of therapeutic compounds may also help identify novel modulators capable of compensating for the altered binding behavior observed in the mutant receptor. Rational drug design could focus on enhancing ligand adaptability or improving receptor rigidity through allosteric modulators. Integrating patient specific genetic data can pave the way for personalized in silico drug testing pipelines for epilepsy and intellectual disability syndromes. This study underscores the significance of integrative in-silico pipelines in early-stage therapeutic evaluation, providing valuable molecular insights that can direct laboratory research and clinical decision-making in a cost effective and time efficient manner.

Limitations

This study was conducted through computational methods, which lack the in vivo biological complexity and variability. The structural predictions relied on modeled proteins rather than experimentally resolved crystal structures, which may limit the precision of docking and interaction analyses. Additionally, only two ligands were studied for therapeutic interactions, which may not represent the full spectrum of potential pharmacological responses. Moreover, the analysis focused on a single nucleotide variation in *GRIN2A*, without exploring the broader spectrum of mutations or gene to gene interactions that contribute to epilepsy and intellectual disability.

References

1. Schalock, R. L. (2014). Intellectual disability. In Encyclopedia of Quality of Life and Well-Being Research (pp. 3292–3295). Springer, Dordrecht.
2. Maulik, P. K., Mascarenhas, M. N., Mathers, C. D., Dua, T., & Saxena, S. (2011). Prevalence of intellectual disability: A meta-analysis of population-based studies. *Research in Developmental Disabilities, 32*(2), 419–436.
3. Brizard, B. A., Limbu, B., Baeza-Velasco, C., & Deb, S. (2021). Association between epilepsy and psychiatric disorders in adults with intellectual disabilities: Systematic review and meta-analysis. BJPsych Open, 7(3), e95.
4. Malone, T. J., & Kaczmarek, L. K. (2022). The role of altered translation in intellectual disability and epilepsy. Progress in Neurobiology, 213, 102267.

5. Wang, T., Kloth, A. D., & Badura, A. (2013). SynGAP regulates synaptic strength and plasticity through modulation of the ERK/RHEB/mTOR pathway. Journal of Neuroscience, 33(46), 18443–18454.
6. Gamache, T. R., Araki, Y., & Huganir, R. L. (2020). SynGAP1-related intellectual disability. Nature Reviews Neurology, 16(6), 347–358.
7. Hsieh, K., Hsieh, L., & Hsieh, P. (2015). Prevalence of epilepsy among people with intellectual disabilities: A systematic review. Seizure: European Journal of Epilepsy, 33, 56–63.
8. Olusanya, B. O., Wright, S. M., Nair, M. K. C., Boo, N. Y., Halpern, R., Kuper, H., ... & Global Research on Developmental Disabilities Collaborators (GRDDC). (2020). Global burden of childhood epilepsy, intellectual disability, and sensory impairments. Pediatrics, 146(1), e20192623.
9. Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. Pharmacological Reviews, 51(1), 7–61.
10. Paoletti, P., & Neyton, J. (2007). NMDA receptor subunits: Function and pharmacology. Current Opinion in Pharmacology, 7(1), 39–47.
11. Li, H., & Tsien, J. Z. (2009). Memory and the NMDA receptors. Science, 323(5919), 1227–1228.
12. Furukawa, H., & Gouaux, E. (2003). Mechanisms of activation and antagonism in ionotropic glutamate receptors. Annual Review of Biophysics and Biomolecular Structure, 32, 195–225.
13. Piñero, J., Bravo, Á., Queralt-Rosinach, N., Gutierrez-Sacristán, A., Deu-Pons, J., Centeno, E., ... & Furlong, L. I. (2017). DisGeNET: A comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Research, 45(D1), D833–D839. https://www.disgenet.org
14. Rappaport, N., Twik, M., Nativ, N., Stelzer, G., Bahir, I., Stein, T. I., ... & Lancet, D. (2017). MalaCards: An integrated compendium for diseases and their annotation. https://www.malacards.org
15. Oliveros, J. C. (2007). Venny: An interactive tool for comparing lists with Venn diagrams (Version 2.1). https://bioinfogp.cnb.csic.es/tools/venny/
16. Ge, S. X., Jung, D., & Yao, R. (2020). ShinyGO: A graphical gene-set enrichment tool for animals and plants. Bioinformatics, 36(8), 2628–2629. https://bioinformatics.sdstate.edu/go/
17. Amberger, J. S., Bocchini, C. A., Scott, A. F., & Hamosh, A. (2015). OMIM.org: Online Mendelian Inheritance in Man (OMIM®), an online catalog of human genes and genetic disorders. Nucleic Acids Research, 43(D1), D789–D798. https://www.omim.org
18. Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., ... & MacArthur, D. G. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. Nature, 581(7809), 434–443. https://gnomad.broadinstitute.org
19. Schwarz, J. M., Cooper, D. N., Schuelke, M., & Seelow, D. (2014). MutationTaster2: Mutation prediction for the deep-sequencing age. Nature Methods, 11(4), 361–362. https://www.mutationtaster.org/
20. Ng, P. C., & Henikoff, S. (2003). SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Research, 31(13), 3812–3814. https://sift.bii.a-star.edu.sg/
21. Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P., ... & Sunyaev, S. R. (2010). A method and server for predicting damaging missense mutations. Nature Methods, 7(4), 248–249. https://genetics.bwh.harvard.edu/pph2/
22. Shihab, H. A., Gough, J., Cooper, D. N., Stenson, P. D., Barker, G. L. A., Edwards, K. J., ... & Gaunt, T. R. (2013). Predicting the functional, molecular, and phenotypic consequences of amino acid substitutions using hidden Markov models. Human Mutation, 34(1), 57–65. http://fathmm.biocompute.org.uk/
23. Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., ... & Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. Nature, 596(7873), 583–589. https://www.deepmind.com/research/highlighted-research/alphafold
24. Capriotti, E., Fariselli, P., & Casadio, R. (2005). I-Mutant2.0: Predicting stability changes upon protein mutation from the sequence or structure. Nucleic Acids Research, 33(Web Server issue), W306–W310. https://folding.biofold.org/i-mutant/i-mutant2.0.html
25. Cheng, J., Randall, A., & Baldi, P. (2006). Prediction of protein stability changes for single-site mutations using support vector machines. Proteins: Structure, Function, and Bioinformatics, 62(4), 1125–1132. http://mupro.proteomics.ics.uci.edu/

26. Savojardo, C., Fariselli, P., Martelli, P. L., & Casadio, R. (2016). INPS-MD: A web server to predict stability of protein variants from sequence and structure. Bioinformatics, 32(16), 2542–2544. http://inpsmd.biocomp.unibo.it/
27. Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., ... & Bork, P. (2019). STRING v11: Protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Research, 47(D1), D607–D613. https://string-db.org/
28. (2019). PubChem 2019 update: Improved access to chemical data. Nucleic Acids Research, 47(D1), D1102–D1109. https://pubchem.ncbi.nlm.nih.gov
29. The UniProt Consortium. (2023). UniProt: The universal protein knowledgebase in 2023. Nucleic Acids Research, 51(D1), D523–D531. https://www.uniprot.org
30. Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry, 31(2), 455–461. http://vina.scripps.edu/
31. DeLano, W. L. (2002). The PyMOL Molecular Graphics System (Version 1.2r3pre). http://www.pymol.org
32. Salentin, S., Schreiber, S., Haupt, V. J., Adasme, M. F., & Schroeder, M. (2015). PLIP: Fully automated protein–ligand interaction profiler (Version Webserver W1) [Computer software]. Nucleic Acids Research, 43(W1), W443–W447. https://projects.biotec.tu-dresden.de/plip-web
33. López-Blanco, J. R., Aliaga, J. I., Quintana-Ortí, E. S., & Chacón, P. (2014). iMODS: Internal coordinates normal mode analysis server. Nucleic Acids Research, 42(Web Server issue), W271–W276. http://imods.chaconlab.org
34. Shepard, N., Farsi, Z., Aryal, S., et al. (2024). Differential functional consequences of GRIN2A missense variants: Implications for receptor modulation and neurodevelopmental pathophysiology. bioRxiv. https://doi.org/10.1101/2023.10.29.565332
35. Farsi, Z., Nicolella, A., Simmons, S. K., Aryal, S., Shepard, N., Brenner, K., & Dulac, C. (2023). Brain-region-specific changes in neurons and glia and dysregulation of dopamine signaling in Grin2a-deficient mice. Neuron, 111(21), https://doi.org/10.1016/j.neuron.2023.08.004
36. Venkateswaran, S., McCarthy, M., Chaturvedi, A., & Hogan, R. E. (2014).
37. A novel GRIN2A mutation in intractable epilepsy with developmental delay. Neurology, 82(24), 1954–1958.
38. Ogden, K. K., Chen, W., Swanger, S. A., McDaniel, M. J., Fan, L. Z., Hu, C., ... & Traynelis, S. F. (2017). Molecular mechanism of disease-associated mutations in the pre-M1 helix of NMDA receptors and potential rescue pharmacology. Epilepsia, 58(8), 1499–1508.