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Computational Analysis of Epilepsy Mutations in the Kinesin Motor Domain of *KIF1A*

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ABSTRACT

Background: The Kinesin Family Member-1A (*KIF1A*) gene encodes a motor protein crucial for axonal transport and synaptic vesicle transport in neurons. *KIF1A* mutations are associated with neurological disorders, including Hereditary Spastic Paraplegia, Intellectual Disability, and Autism. Mutations in *KIF1A* have been found to increase the hyperactivity of the motor protein, altering the axonal transport of synaptic vesicles precursors. Mutant *KIF1A* proteins with hyperactive motor functions have been shown to increase excitatory synaptic transmission, suggesting a potential role in the development of epileptic seizures. **Aim:** This study emphasizes on the computational analysis of previous reported missense mutations of *KIF1A* causing epilepsy. **Methodology:** Comparative protein models of normal and all selected mutations (R13C and A85D were identified in Japanese patient, T99M, T258M and R316W were found in Korean patients) was prepared. Pathogenic prediction was performed using Meta-SNP and PREDICT SNP, Phylogenetic analysis was performed using MEGA software and molecular docking by using previously discovered drugs was performed using PyRx tool. **Results:** All mutations were predicted highly pathogenic and reported to cause epilepsy with other associated symptoms. Compounds with highest binding affinity were selected for docking which binds with the receptor making it a successful docking. Phylogenetic analysis revealed the changes in their conserved regions making these mutations pathogenic. **Conclusion:** This study gives a time and cost-effective analysis of *KIF1A* gene causing epilepsy and genetic mutations that play a key role in development and cause of disease.

Keywords: KIF1A, Epilepsy, Computational analysis, molecular docking, Protein modelling.

INTRODUCTION

The Kinesin Family Member 1A (*KIF1A*) gene is an encoding motor protein necessary in the effective translocation of essential cellular freight in neurons. This protein is a member of the kinesin-3 subfamily, which is a cellular delivery system that helps to transport dense core vesicles and synaptic vesicle precursors. These transport systems have important constituents such as the neuro transmitters which help communication between neurons and mitochondria, the energy producers of the cell. The

accurate and sufficient delivery of these materials by the KIF1A protein is an essential factor in the health and performance of the neurons [1].

Recent literature has closely associated mutations in KIF1A gene with development of epilepsy. Although the processes are yet to be studied, it is evident that the dysfunction of KIF1A protein has a great influence on the communication of neurons and the general functionality of the brain. Vitet H. et al., (2023) have a study of the knockout mic model of KIF1A. Phenotype of KIF1A knockout mice contains the decrease in the population of synaptic vesicles in nerve terminals, motor and sensory defects, and the presence of clear vesicles in nerve cell bodies. KIF1A knock-out mice demonstrated motor and sensory disturbances, diminished vesicles density in the synapses and distorted vesicle collection in the nerve cells. These mice reportedly had poor synaptic vesicle transport and premature death, which testifies to the significance of KIF1A in the survival and functioning of neurons [2].

KIF1A plays a critical role in synapse development and maturation because it helps in the transport of neurotransmitter-filled synaptic vesicles. This is necessary in the normal operation and excitability of neural networks [3]. The sensitive balance of brain functioning may be disrupted by the *KIF1A* gene mutations resulting into the abnormal levels of neurotransmitters, malfunctioning of communication between neurons and development of seizures. Such mutations may also disrupt the formation of synapses between neurons, which exposes them to seizures [4].

Since epilepsy is a complicated disease with many factors behind it, and *KIF1A* mutations have also been linked to some of the epilepsy types, it is only one of the puzzle pieces. More studies are required to unveil the complex correlation between *KIF1A* and epilepsy, and the end result is the creation of specific medications that could be used in patients with this condition.

Therefore, this study focuses on protein structure modeling of genes *KIF1A* gene and their mutated forms R13C [5], A85D [6], T99M [7], T258M [8] and R316W [7] which also involves wild and mutant type comparison, protein interaction studies, docking and phylogenetic history based on representative ortholog species.

METHODOLOGY

2.1 Genetic variants retrieval and protein modelling

The focus on *KIF1A* missense variants that (i) were previously reported in patients with epilepsy or epilepsy-associated phenotypes, ensuring clinical relevance [5–8]; (ii) localize to the N-terminal motor domain (~aa 1–350) responsible for ATP hydrolysis and microtubule binding, where substitutions are most likely to perturb kinesin mechanochemistry; (iii) are single-amino-acid substitutions (no truncations/frameshifts), enabling like-for-like structural modeling and stability prediction across variants; (iv) have sufficient primary literature detail (phenotype description, zygosity/de novo status) to support interpretation; and (v) show high cross-species conservation at or near the altered residue, increasing prior probability of functional impact. Variants meeting all criteria at the time of study were R13C and A85D (reported in Japanese patients) and T99M, T258M, and R316W (reported in Korean cohorts).

Protein structure homology modeling of wild-type and mutant KIF1A (R13C, A85D, T99M, T258M, and R316W) was conducted on SWISS-MODEL (https://swissmodel.expasy.org) using the template PDB ID: 2HXF, selected based on highest sequence identity (≥70%) and GMQE/QMEAN scores. Energy minimization was performed automatically by the SWISS-MODEL pipeline.

2.2 Validation of structures

Model validation employed SAVES v6.0 (https://saves.mbi.ucla.edu), including PROCHECK for Ramachandran plots, ERRAT, and Verify3D for structure quality assessment.

2.3 Pathogenicity prediction

Meta-SNP (https://snps.biofold.org/meta-snp/) and PREDICT SNP (https://loschmidt.chemi.muni.cz/predictsnp/) were used to analyze the impact of a mutation on protein stability. These tools assess how likely the mutation is to disrupt the protein's structure and function, potentially leading to abnormal protein activity.

2.4 Phylogenetic relationship

MEGA software was used to trace the evolutionary history of a protein called *KIF1A* and its related proteins in other species. Protein sequences of various organisms were retrieved from UniProt (https://www.uniprot.org) database. Muscle Alignment of these sequences was performed using MEGA software. This aligned the sequences both individually and in pairs, calculating how similar they are to each other. A family tree was created, also known as a bootstrap consensus tree, to illustrate the evolutionary history of the analyzed proteins. This tree was generated by running the analysis 100 times. Collapsed branches in the tree indicate groups of proteins that did not consistently cluster together in at least half of the analyses.

2.5 Protein-protein interaction

STRING (https://string-db.org) was used to investigate potential interactions between the *KIF1A* gene and its neighboring genes on the chromosome.

2.6 Molecular docking interaction

For molecular docking, ligand structures were retrieved from PubChem in SDF format and converted to PDBQT using Open Babel. Docking was carried out in AutoDock Vina (via PyRx v0.8) using a grid box of size 40 × 40 × 40 Å centered on the predicted active site residues (identified from template-based structural alignment). Default exhaustiveness was set to 8, with 10 binding poses generated per ligand. The receptor was prepared by removing water molecules and adding polar hydrogens and Kollman charges. The ligands were chosen based on their therapeutic relevance in epilepsy (e.g., Carbamazepine, Diazepam, Selurampanel, and barbituric acid derivatives) and binding site complementarity as determined by preliminary blind docking. Binding affinities were recorded in kcal/mol, and interaction visualization was done using Discovery Studio Visualizer.

RESULTS

3.1 Protein Modelling and validation

3.1.1 Structure modeling

Swiss-Model generated 3D models of *KIF1A* protein with specific mutations (R13C, A85D, T99M, T258M, and R316W). The mutated models were compared to the normal *KIF1A* structure to see how the mutations affect the protein's structure. Evaluation of the models focused on the Ramachandran plot, which indicates the most favorable (sterically allowed) positions for amino acids in a protein. A high percentage of amino acids in the modeled mutants fell within these favored regions, suggesting the models are reliable [Added in Supplementary file]. These results not only confirm the reliability of the 3D models but also provides understandings of how these mutations alter the normal structure of the *KIF1A* protein.

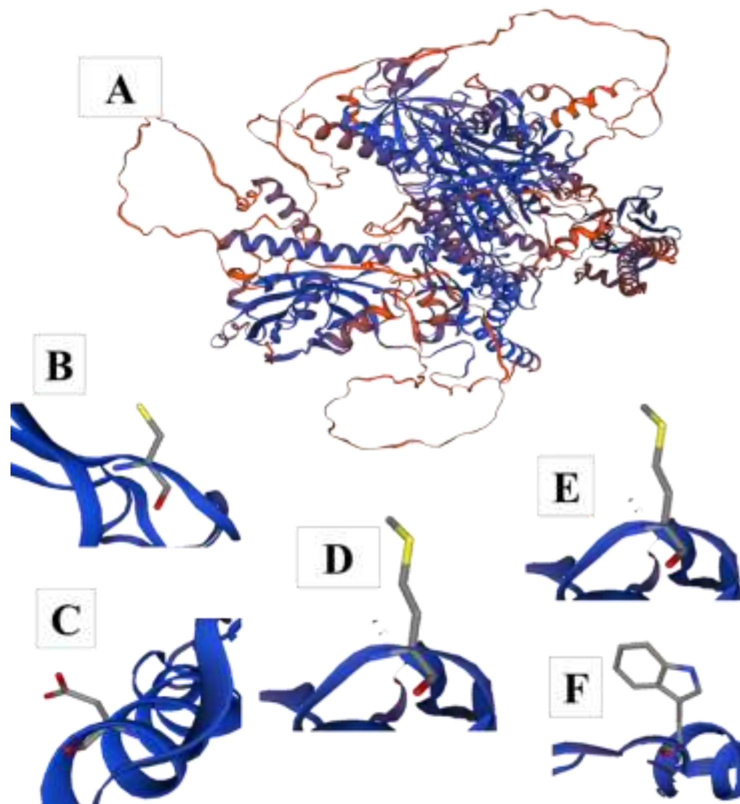

Figure 1. A) Full view model of KIF1A protein. B) R13C mutated KIF1A protein model. C) A58D mutated KIF1A protein model. D) T99M mutated KIF1A protein model. E) T258M mutated KIF1A protein model. F)R316W mutated KIF1A protein model.

3.1.2 Structure evaluation

Mutation p. R13C in *KIF1A* represents a change in amino acid at location 13 from Arginine (R) to Cysteine (C). This causes a slight loss in overall molecular weight and hydrophilic to slight hydrophobic change of protein (shown in fig. 2).

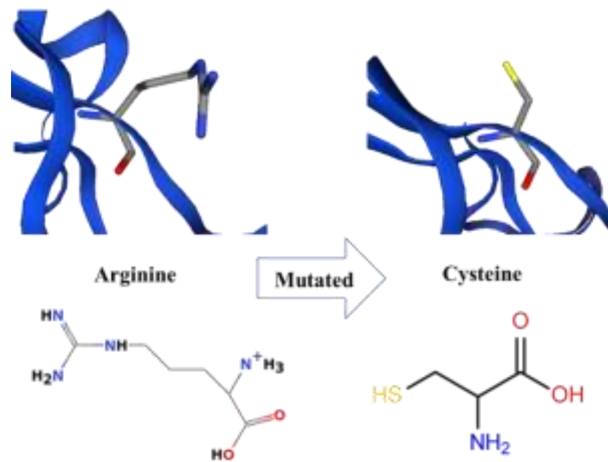

Figure 2. Effect of mutation R13C on KIF1A protein

Mutation p. A85D in *KIF1A* represents a change in amino acid at location 85 from Alanine (A) to Aspartic Acid (D). This causes a slight gain in overall molecular weight and hydrophobic to slight hydrophilic change in nature of protein (shown in fig. 3).

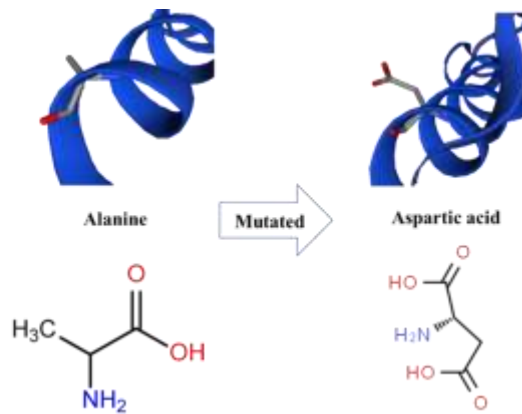

Figure 3. Effect of mutation A85D on KIF1A protein

Mutation p. T99M in *KIF1A* represents a change in amino acid at location 99 from Threonine (T) to Methionine (M). This causes a slight gain in overall molecular weight and hydrophilic to slight hydrophobic change in nature of protein (shown in fig. 4).

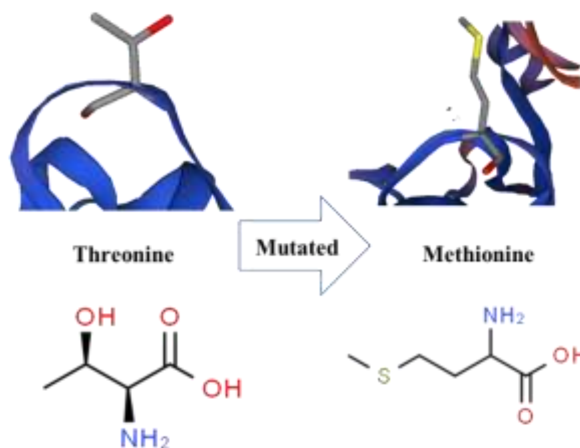

Figure 4. Effect of mutation T99M on KIF1A protein

Mutation p. T258M in *KIF1A* represents a change in amino acid at location 258 from Threonine (T) to Methionine (M). This causes a slight gain in overall molecular weight and hydrophilic to slight hydrophobic change in nature of protein (shown in fig. 5).

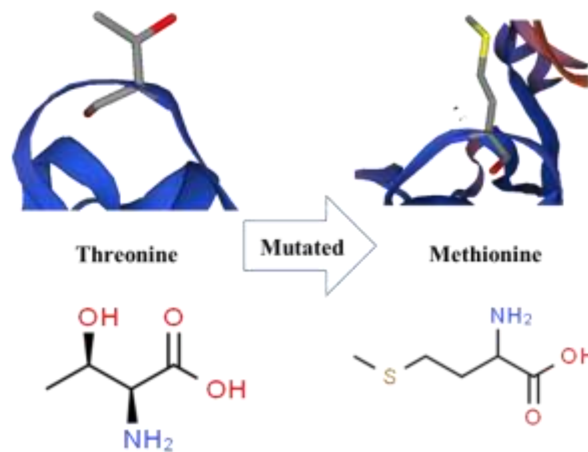

Figure 5. Effect of mutation T258M on KIF1A protein

Mutation p. R316W in *KIF1A* represents a change in amino acid at location 316 from Arginine (R) to Tryptophan (W). This causes a slight gain in overall molecular weight and hydrophilic to slight hydrophobic change in nature of protein (shown in fig. 6).

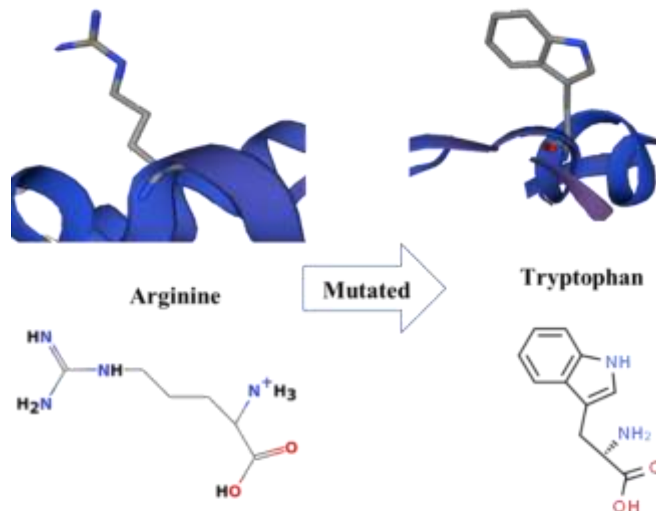

Figure 6. Effect of mutation R316M on KIF1A protein

3.2 Protein pathogenicity prediction

This study employed two prediction tools, PREDICT SNP and Meta-SNP, to analyze how mutations affect proteins (shown in Figure 7). Mutations predicted as deleterious by PREDICT SNP often correspond to natural variations known to cause clinical issues. In many cases, these deleterious mutations also coincide with decreased protein stability.

Meta-SNP utilizes several different predictors (including PATHER, PhD-SNP, SIFT, and SNAP) to assess the impact of mutations on normal protein function. The specific values reported for each prediction tool are shown in Figure 8. All the mutations are pathogenic predicted by both tools.

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Mutation	PANTHER	PhD-SNP	SIFT	SNAP	Meta-SNP	RI
R13C	Disease 0.988	Disease 0.860	Disease 0.000	Disease 0.795	Disease 0.921	8
A85D	Disease 0.614	Disease 0.856	Disease 0.000	Disease 0.650	Disease 0.773	5
T99M	Disease 0.985	Disease 0.866	Disease 0.000	Disease 0.790	Disease 0.901	8
T258M	Disease 0.782	Disease 0.679	Disease 0.000	Disease 0.655	Disease 0.667	3
R316W	Disease 0.889	Disease 0.766	Disease 0.000	Disease 0.530	Disease 0.767	5

Figure 7. Pathogenic prediction from PREDICT SNP

Mutation	PredictSNP	MAPP	PhD-SNP	PolyPhen-1	PolyPhen-2	SIFT	SNAP
R13C	87 %	63 %	88 %	74 %	81 %	79 %	87 %
A85D	87 %	91 %	88 %	74 %	81 %	79 %	72 %
T99M	87 %	63 %	88 %	74 %	81 %	79 %	72 %
T258M	76 %	63 %	88 %	74 %	65 %	79 %	50 %
R316W	87 %	56 %	73 %	74 %	81 %	79 %	72 %

Figure 8. Pathogenic prediction from META-SNP

3.3. Phylogenetic Analysis

The phylogenetic study compared *KIF1A* sequences from various representative species to create a phylogenetic tree (shown in Fig. 9). The analysis revealed a close evolutionary relationship between the studied *KIF1A* gene and those found in Chimpanzees, Bovine, Rat, Mouse, Zebra fish, Fruit fly, Social amoeba, Yellow fever mosquito and African malaria mosquito. Additionally, the study examined the evolutionary rates of the *KIF1A* coding sequence across different species for comparison. According to *KIF1A* tree, human is making cluster with Chimpanzee with bootstrap value of 100. Other clusters with highest bootstrap value i.e., 100 include cluster of Rat and Mouse with Zebra fish, Fruit fly, Social amoeba, Yellow fever mosquito and African malaria mosquito. High bootstrap values of the clusters correspond to highest reliability. Tree is reconciling species divergence time. Evolutionary time for the tree is 0.01.

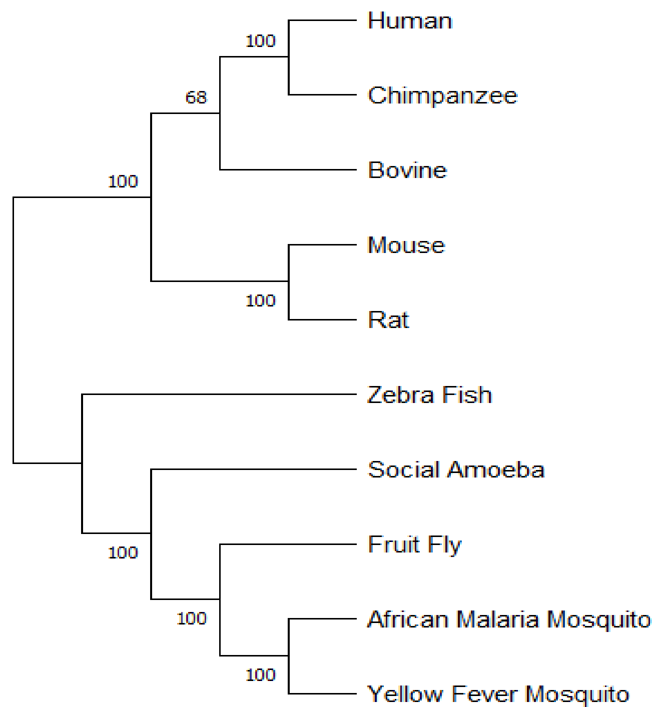

Figure 9. Evolutionary relationships through Neighbor Joining Method of *KIF1A* gene

3.4. Interaction Studies

3.4.1. Protein-Protein Interaction

KIF1A kinesin-like protein showed the interaction with *TUBA1A, TUBB2B, KLC2* and *KIF1B* with confidence score of 0.960, 0.939, 0.857 and 0.850 respectively. TUBA1A and TUBB2B belong to tubulin family, are the major constituents of microtubules. These proteins bind with two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain. As *KIF1A* is a transporter protein and tubulin proteins allow the exchangeable sites therefore *TUBA1A* and *TUBB2B* is interacting with the highest score (Shown in Fig. 10).

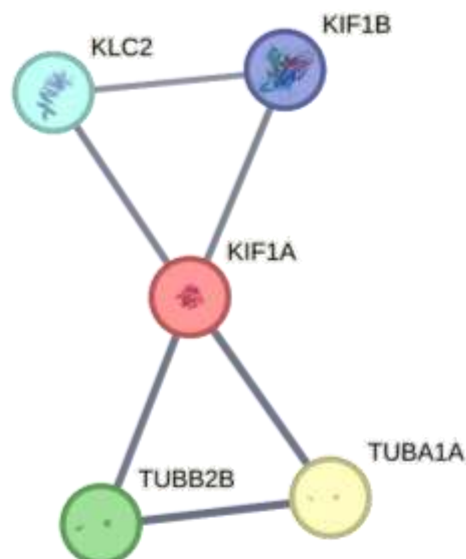

Figure 10. Interaction of *KIF1A* with other proteins with highest confidence score

3.4.2. Docking Interaction

All 07 compounds were docked but the compounds with the highest binding energies were selected. Carbamazepine, Diazepam, Selurampanel and 1,3-Dimethoxymethyl-5,5-diphenylbarbituric acid were selected because these ligands showed highest binding affinity -8.3, -8.3, -8.2 and -8.1 respectively. All the selected ligands were docked and hydrogen bonds are

shown fig. 11-14. Summary of binding affinity and hydrogen bonds is shown in Table 1 [Supplementary file]. All the ligands showed same type of interactions with all mutated protein so only one figure is added (Fig. 11.

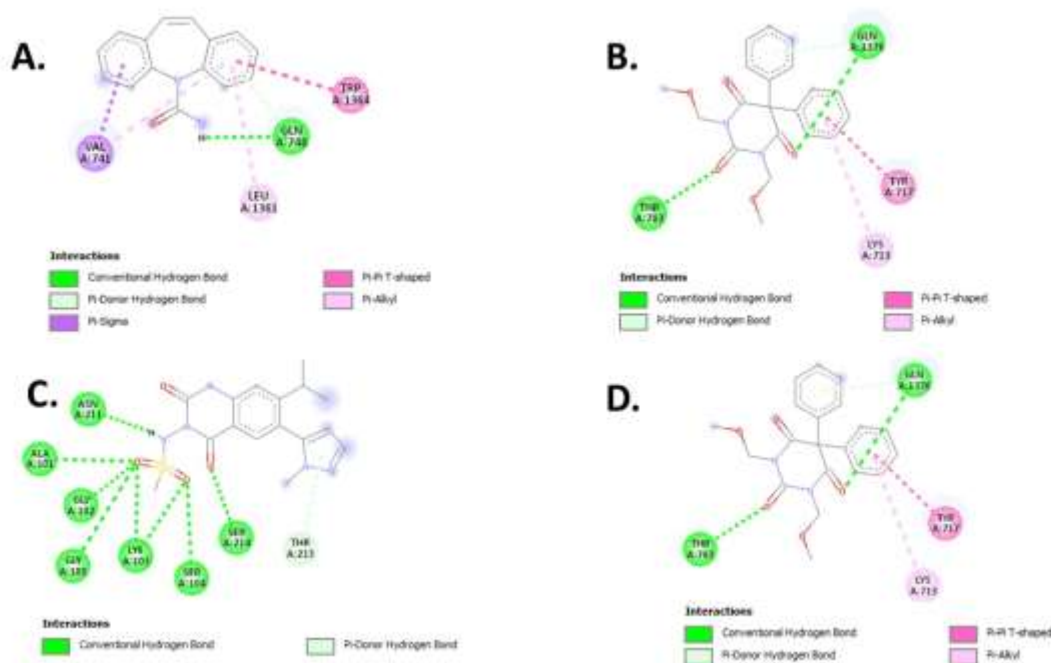

Figure 11. A. Docking interaction of Carbamazepine with *KIF1A:* **B.** Docking interaction of Diazepam with *KIF1A*: **C.** Docking interaction of Selurampanel with *KIF1A*; **D.** Docking interaction of 1,3-Dimethoxymethyl-5,5-diphenylbarbituric acid with *KIF1A*

DISCUSSION

The *KIF1A* gene encodes a neuron-specific kinesin motor protein essential for anterograde transport of synaptic vesicle precursors and dense-core vesicles along microtubules [1]. The analysis targeted motor-domain missense variants with documented epilepsy presentations, a strategy that balances biological evidence with modeling. By restricting to the ATPase/microtubule-interacting motor core, the chance was maximized that observed physicochemical changes (charge, polarity, side-chain volume) map onto transport defects relevant to neuronal hyperexcitability. Limiting to missense events avoided confounding by nonsense-mediated decay and allowed direct structure–function comparisons across variants.

The modeled structural deviations in mutant KIF1A proteins suggest that even subtle amino acid substitutions can have large-scale effects on motor function. For example, arginine-to-cysteine substitution (R13C) introduces sulfhydryl reactivity that can alter inter- or intramolecular bonding within the motor domain, potentially destabilizing ATP-binding or microtubule interaction surfaces. Equally, A85D and T99M replacements occur to change polarity and hydrophobicity, which is likely to change the local folding environment required in kinesin conformational cycling. These physicochemical imbalances can destabilize the processive migration of *KIF1A* along axons, causing synaptic vesicle retention and the release of neurotransmitters abnormally, which is a pathological process that is consistent with hyperexcitability and epileptic phenotypes of patients.

All five mutations were consistently found to be deleterious by the pathogenicity analyses of PREDICT-SNP and Meta-SNP which showed that they were likely to contribute to the disease progression through protein destabilization. This is consistent with the previous functional data that motor hyperactivity or hypofunction of *KIF1A* may equally cause disrupted synaptic vesicle dynamics and disruptive excitatory-inhibitory balance in cortical circuits. Therefore, disrupted neuronal signaling and seizure vulnerability could be caused by the destabilization of mutant proteins, which has been predicted.

The phylogenetic analysis of *KIF1A* showed that it is highly conserved among vertebrates with the important bootstrap support

joining the human and chimpanzee orthologs at the same position. The fact that the key residues are conserved in the motor domain underlines the fact that it is only minor differences in the sequence that are tolerated even through the evolutionary process, which makes the studied substitutions all the more pathogenic. The evolutionary conservation also suggests that human *KIF1A* dysfunction is likely to recapitulate model organism mechanistic deficits because *KIF1A* loss-of-function or gain-of-function mutations cause synaptic depletion and locomotor impairments in model organisms.

The analysis of protein-protein interactions showed that *KIF1A* is strongly correlated with tubulin isoforms (*TUBA1A* and *TUBB2B*) and other kinesin representatives (*KLC2*, *KIF1B*). This network points out to the fact that it is integrated into the microtubule motor machinery. Any disturbance of these interactions would disrupt the efficiency of axonal transport and the stability of the microtubules, which would add to the harmful impact of KIF1A mutations on neuron connectivity and neurotransmission.

Clinically relevant compounds that included carbamazepine and diazepam were studied using docking techniques and found to have preferred binding affinities to mutant KIF1A proteins. Even though these compounds have been historically characterized based on their antiepileptic and GABAergic modulatory effect, their possible interaction with the motor domain of *KIF1A* suggest interesting possibilities of off-target axonal transport modulation. Nevertheless, the findings are tentative and indicate that they should be experimentalized to find out whether it is possible to stabilize or control the activity of mutant *KIF1A* using small molecules as the means of therapeutic treatment.

CONCLUSION

Epilepsy, a complex neurological disorder with unclear causes, serves as a good example. This study has provided a cost-effective approach to examine the *KIF1A* gene which has a potential role in epilepsy. The normal and mutated variant of the KIF1A protein was studied by modeling and analysis and provided a deeper insight into how the mutations may have led to the onset and progression of the disease. In addition, the research investigated the evolutionary connection of the various variations of the *KIF1A* gene. This was done through the construction of phylogenetic trees, which illustrate the evolutionary record of the gene and its variants in other organisms. Through categorizing these related genes (orthologs), ancestral and descendant associations can be defined, which gives more context in regard to the role of *KIF1A* gene in epilepsy.

DECLARATIONS

Ethical approval: Not applicable

Consent to participate: Not applicable

Availability of data and materials: The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: Neither of the authors has any conflict of interest to disclose

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