Am J Transl Res 2026;18(2):1195-1205
www.ajtr.org /ISSN:1943-8141/AJTR0166751

Case Report

Bioinformatics analysis of de novo missense variants in *Kinesin Family Member 1A (KIF1A)* in autism spectrum disorder: a case report and literature review

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Received June 12, 2025; Accepted December 24, 2025; Epub February 15, 2026; Published February 28, 2026

Abstract: Autism spectrum disorder (ASD) is a multifactorial neurodevelopmental disorder influenced by both rare and common genetic variants. Among the genes implicated, Kinesin Family Member 1A (KIF1A), which encodes a neuronal kinesin motor protein, has emerged as a potential contributor to ASD pathogenesis. In this case report and literature review, we describe a comorbidity-free ASD proband carrying a novel de novo KIF1A missense variant (c.A664C, p.N222H) and evaluate its pathogenic potential through comprehensive bioinformatics analyses. Whole-exome sequencing (WES) and Sanger sequencing were performed in a family trio, and gene expression profiling was conducted using the Human Brain Transcriptome (HBT) database to examine spatiotemporal KIF1A expression during neurodevelopment. The structural and functional impact of four KIF1A variants, including the novel one, was analyzed using PredictProtein, SWISS-MODEL, and PyMOL. KIF1A was found to be highly expressed during embryonic brain development and to stabilize postnatally, indicating an essential role in neuronal maturation. Structural modeling demonstrated that all four variants disrupted the secondary and tertiary conformations of the KIF1A protein, altering key hydrogen-bonding patterns and potentially impairing adenosine triphosphatase (ATPase)-driven vesicular transport along microtubules. The absence of comorbidities in the proband further supports a direct association between KIF1A dysfunction and ASD-related behavioral phenotypes. Although functional validation could not be performed, these bioinformatics findings highlight KIF1A as a plausible ASD risk gene and underscore the importance of integrating rare variant analysis with polygenic context in understanding ASD genetics.

Keywords: Kinesin Family Member 1A (KIF1A), autism spectrum disorder (ASD), missense variant, bioinformatics, pathogenicity, structural modelling

Introduction

Autism spectrum disorder (ASD) is an early-onset neurodevelopmental disorder with a high prevalence of approximately 1 in 54 individuals, characterized by deficits in social communication and restricted, repetitive patterns of behavior [1, 2]. In addition to these core clinical manifestations, the comorbidity rates of intellectual disability (ID) and epilepsy are approximately 31% and 20-25%, respectively [3-5]. Despite extensive research, no pharmacological therapy can cure the core symptoms of ASD, largely due to its complex and multifactorial etiology. Since 2011, whole-exome sequencing (WES) has been widely utilized in sporadic ASD family trios, leading to the identification of hundreds of candidate genes. It is currently estimated that 400-1,000 genes are associated with ASD susceptibility [6]. Studies have shown that many ASD-related genes, particularly those harboring rare de novo or novel variants, are functionally linked to synaptic growth, neural development, and neuronal survival as revealed by WES-based studies [7-9]. Previous studies have reported that individuals carrying de novo variants (DNVs) in KIF1A may present with a broad spectrum of phenotypes, including cognitive impairment, cerebellar atrophy, gait disturbances, exaggerated tendon reflexes, spastic paraplegia, optic nerve atrophy, peripheral neuropathy, ID, axial hypotonia, and epilepsy

https://doi.org/10.62347/EWYF1462

[10-14]. Three deleterious DNVs in KIF1A have been described in ASD probands to date - c.38G>A (p.R13H), c.746T>C (p.L249P), and c.37C>T (p.R13C) [11, 15, 16]. According to OMIM (Online Mendelian Inheritance in Man), Kinesin Family Member 1A (KIF1A) is primarily associated with NESCAV syndrome, neuropathy, and spastic paraplegia 30, and ASD has not been considered a primary phenotype of KIF1A-related disorders. Thus, direct evidence linking KIF1A variants to ASD onset remains limited. In the present study, we report a case of an ASD proband carrying a de novo KIF1A missense variant (c.A664C, p.N222H) who exhibited no additional neuropsychiatric or systemic comorbidities. Symptoms of autism typically manifest before the age of three, and the disorder is widely recognized as a congenital neurodevelopmental condition resulting from abnormal gene expression during embryonic brain development [17]. Therefore, preliminary evaluation of the pathogenic potential of KIF1A variants requires exploration of their expression during early neurodevelopmental stages.

To date, four de novo KIF1A variants, including the one reported herein, have been identified in ASD cases; however, no previous studies have systematically assessed the spatial and temporal expression of KIF1A in the human brain or examined how these variants might affect protein structure and function. In line with current models of ASD genetics, rare de novo mutations such as those in KIF1A may act in concert with a background of common polygenic risk, although this relationship could not be directly evaluated in the present study.

Given that the primary structure of a protein determines its spatial configuration and biological function, even a single missense variant can disrupt the secondary and tertiary structures of the encoded protein, thereby altering its biological activity [18]. Accordingly, this case report integrates genomic, bioinformatics, and structural modeling analyses to explore the potential impact of de novo KIF1A missense variants on ASD pathogenesis.

Case report

Case description

This case report describes a single male proband diagnosed with autism spectrum disorder (ASD) at the outpatient department. A psychiatrist initially applied the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) for a preliminary diagnosis of the child. Subsequently, two other psychiatrists independently used the Autism Diagnostic Observation Schedule (ADOS) and Autism Diagnostic Interview-Revised (ADI-R) to confirm the diagnosis in both the child and his parents. Additionally, the Kiddie Schedule for Affective Disorders and Schizophrenia - Present and Lifetime Version (K-SADS-PL) was employed to screen the child for other common psychiatric conditions. Prior to participation, the parents of the child provided written informed consent. The study protocol was reviewed and approved by the Ethics Committee of West China Second University Hospital, Sichuan University, and all procedures adhered to the ethical principles outlined in the Declaration of Helsinki.

Whole-exome sequencing and trio analysis

A 5 mL peripheral blood sample was collected from the proband and both parents using EDTA anticoagulation tubes. Genomic DNA extraction was performed using the phenol extraction method, and DNA concentration and purity were quantified using the Qubit dsDNA HS Assay Kit on a Qubit® 3.0 fluorometer (Life Technologies, CA, USA). Exon capture and library construction were carried out using the Agilent SureSelect liquid capture system. Low-quality sequences and duplicates were removed, and high-quality reads were aligned to the human reference genome (GRCh37/hg19) using the Burrows-Wheeler Aligner (BWA).

Sequencing data were processed using SAMtools and Picard for sorting, duplicate marking, and BAM file generation. Duplicate reads were excluded from downstream analyses. Variants, including single-nucleotide variants (SNVs) and insertions/deletions (indels), were identified using SAMtools mpileup and bcftools. The resulting data were submitted to the China National GeneBank (CNGB) database (https://db.cngb.org/, Reference ID: sub040372).

Variant annotation and filtering

Variant annotation was conducted using ANNOVAR, following the methodologies described by Huganir and Nicoll (2013). Filtering criteria were applied to focus on rare exonic and splice-

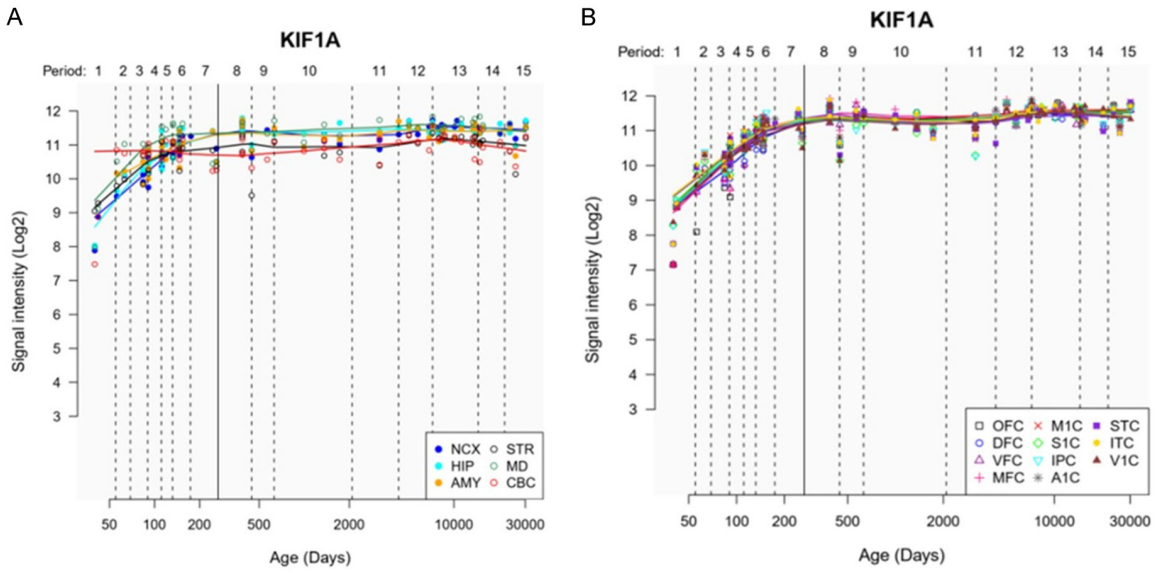

Figure 1. Expression profile of Kinesin Family Member 1A (KIF1A) in different brain regions and developmental stages. A. *KIF1A* expression levels in six brain regions: neocortex (NCX), striatum (STR), hippocampus (HIP), mediodorsal nucleus of the thalamus (MD), amygdala (AMY), and cerebellar cortex (CBC). B. *KIF1A* expression levels in eleven neocortical areas, including the orbital (OFC), dorsolateral (DFC), ventrolateral (VFC), medial (MFC), and primary motor (M1C) cortices of the frontal lobe; the primary somatosensory (S1C) and posterior inferior parietal (IPC) cortices; the primary auditory (A1C), posterior superior temporal (STC), and anterior inferior temporal (ITC) cortices; and the primary visual (V1C) cortex of the occipital lobe. The solid line represents birth, and the dotted lines indicate different developmental stages. Signal intensity (log_2) denotes gene expression level, with higher values indicating stronger expression.

site variants with a minor allele frequency (MAF) <1% across population databases, including the 1000 Genomes Project, ESP6500, and gnomAD (ALL and East Asian subsets). Variants were cross-referenced against dbSNP, CADD, and HGMD for annotation of mutation type, genomic position, and conservation scores. Deleteriousness prediction was performed using SIFT (http://sift.jcvi.org/www/SIFT), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (http://www.mutationtaster.org/), and CADD. Variants predicted to be damaging by at least two tools were validated through PCR amplification and Sanger sequencing. Classification of pathogenicity followed the American College of Medical Genetics and Genomics (ACMG) guidelines.

Literature selection for comparative analysis

To identify previously reported KIF1A variants associated with ASD, a comprehensive literature search was performed across MEDLINE, EMBASE, PsycINFO, and CNKI databases. The search terms included “autism”, “autism spectrum disorder”, “ASD”, “Asperger syndrome”, and “KIF1A”. We included peer-reviewed studies published in English or Chinese up to November 2024. Based on the inclusion criteria, three relevant articles published between 2017 and 2019 were identified [11, 15, 16]. These publications described three deleterious de novo KIF1A variants (p.R13H, p.L249P, p.R13C) in ASD probands from different populations. These variants were used for comparative bioinformatics and structural analyses with our case.

Temporal and spatial expression of KIF1A in the human brain

To explore the spatiotemporal expression profile of KIF1A during brain development, expression data were extracted from the Human Brain Transcriptome (HBT) database (http://www.hbatlas.org/). Gene expression across multiple regions - including the cerebellar cortex, mediodorsal nucleus of the thalamus, striatum, amygdala, hippocampus, and 11 neocortical regions - was analyzed (**Figure 1A**, **1B**). This publicly available database provides transcriptomic data for both developing and adult human brains, allowing evaluation of KIF1A expression trends during neurodevelopmental stages. We

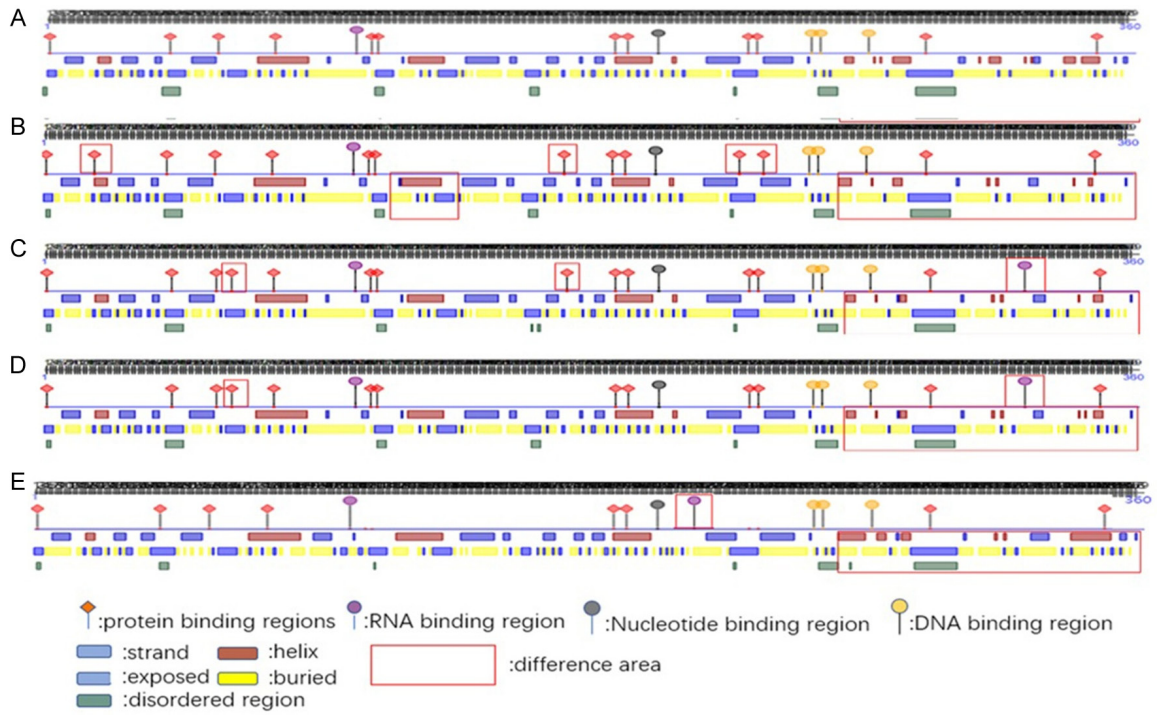

Figure 2. Secondary structure predictions of the *Kinesin Family Member 1A (KIF1A)* protein. A. Wild-type *KIF1A*. B. Mutant *KIF1A* (p.N222H). C. Mutant *KIF1A* (p.R13H). D. Mutant *KIF1A* (p.L249P). E. Mutant *KIF1A* (p.R13C).

specifically focused on embryonic and postnatal periods to assess whether KIF1A expression coincides with critical windows of synaptic formation relevant to ASD pathogenesis.

Prediction and analysis of secondary and tertiary structures of KIF1A protein

To evaluate the potential structural impact of KIF1A missense variants, we used PredictProtein (https://www.predictprotein.org/) to analyze secondary structures of the wild-type and mutant KIF1A proteins (**Figure 2A-E**). As no experimentally solved structure of the mutant KIF1A protein was available, homology modeling was performed using SWISS-MODEL (https://www.swissmodel.expasy.org/). The human KIF1A protein sequence was input, and the most homologous reference template was automatically selected. The accuracy of the generated models was evaluated using Global Model Quality Estimation (GMQE) and QMEAN Z-scores, where values closer to 1.0 and 0, respectively, indicate better model quality. To further assess model reliability, the PROCHECK tool in SAVES v5.0 (https://servicesn.mbi.ucla.edu/PROCHECK/) was used to generate Ramachandran plots, with >90% residues in favored regions indicating acceptable model geometry. Alterations in hydrogen-bonding patterns between wild-type and mutant proteins were visualized using PyMOL v2.4 (https://pymol.org/) to examine potential structural disruptions at the atomic level. All computational predictions were performed using publicly available bioinformatics tools, and no wet-lab functional experiments were conducted; this limitation is acknowledged and discussed in the manuscript.

Results

Case description and identification of de novo variants

Whole-exome sequencing (WES) and Sanger validation identified a de novo missense variant in *KIF1A* (c.A664C, p.N222H) and a novel variant in *Crumbs homolog 1, Drosophila (CRB1)* (c.C1982A, p.A661E) in a male proband diagnosed with autism spectrum disorder (ASD). Both variants were absent in the proband's parents and the gnomAD database. Conservation prediction and multiple in silico algorithms suggested both variants to be deleterious. Following ACMG criteria, the *KIF1A* vari-

Table 1. Scores on Autism Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic Observation Schedule (ADOS) scales for the proband

Scale	ADI-R	ADOS
Subscale	Abnormal quality of social interaction	Abnormal quality of verbal communication
Score	10 (=10)	14 (=8)

Values in parentheses indicate the boundary scores of the diagnostic algorithm.

Table 2. Clinical phenotypes of Autism spectrum disorder (ASD) probands with de novo missense variants in the motor domain of *Kinesin Family Member 1A (KIF1A)*

	Demographic characteristics	Mutations related	Hazardous prediction	Concomitant chromosomal abnormality	Clinical features	Comorbidities
	Sex	Age (y)	Nation	Protein change	Nucleotide change	Position (hg 19)
Our proband	Male	15	China	p.N222H	c.A664C	241724462
1	Male	20	France	p.R13H	c.38G>A	N
2	Female	N	UK	p.L249P	c.746T>C	241723208
3	Male	18	Japan	p.R13C	c.37C>T	001244008

ant was classified as "likely pathogenic" (PM6, PM2, PP2, PP3), whereas the *CRB1* variant was designated a variant of uncertain significance (VUS) (PM6, PM2, PP2). No chromosomal abnormalities were detected. The sequence data have been deposited in the China National GeneBank Database (CNGBdb, accession no. sub040372). The patient was a 15-year-old boy with a full-scale IQ of 122 and no comorbid psychiatric or neurological disorders. He was born to non-consanguineous, healthy parents via natural delivery after an uneventful, full-term pregnancy. Family history spanning three generations revealed no neuropsychiatric illness.

Since infancy, the proband exhibited reduced eye contact and delayed language development until two years of age. In school, he demonstrated impaired social interaction, lack of peer relationships, and restricted interests, often engaging in repetitive behaviors such as rotating fans and wheels. Financial limitations delayed medical evaluation. Clinical assessment with ADOS and ADI-R confirmed the diagnosis of ASD, and the proband presented no other systemic, somatic, or chronic medical conditions (**Table 1**). Comparative phenotypic data for the present proband and three previously reported ASD probands carrying deleterious *KIF1A* de novo variants are summarized in **Table 2**. Importantly, although a variant was also identified in *CRB1*, a gene primarily associated with inherited retinal dystrophies (e.g., RP12 and LCA8), neither the proband nor his family members exhibited any visual impairment or retinal abnormalities. Moreover, large-scale ASD genetic studies have not implicated *CRB1* as an autism risk gene, supporting its classification as a non-causal VUS. Thus, the *KIF1A* p.N222H variant is more consistent with the proband's neurodevelopmental phenotype.

Spatiotemporal expression profile of KIF1A in the human brain

To explore the potential developmental relevance of *KIF1A* to ASD, expression patterns were analyzed using the Human Brain Transcriptome (HBT) database. The analysis demonstrated broad expression of *KIF1A* across multiple brain regions, including the cerebellar cortex, thalamus, striatum, amygdala, hippocampus, and neocortex (**Figure 1A**, **1B**). Expression of *KIF1A* increased progressively from the early embryonic stage, peaking around 150 postconceptional days (period 6), corresponding to a key stage of neuronal differentiation and synaptogenesis. Thereafter, expression remained stable and elevated into adulthood. This developmental pattern suggests that KIF1A may play an essential role during early brain wiring and synaptic maturation - critical processes implicated in ASD pathogenesis.

Predicted structural consequences of KIF1A missense variants

Because the *KIF1A* variant p.N222H is located within the motor domain (first 360 amino acids), we restricted our analysis to this region.

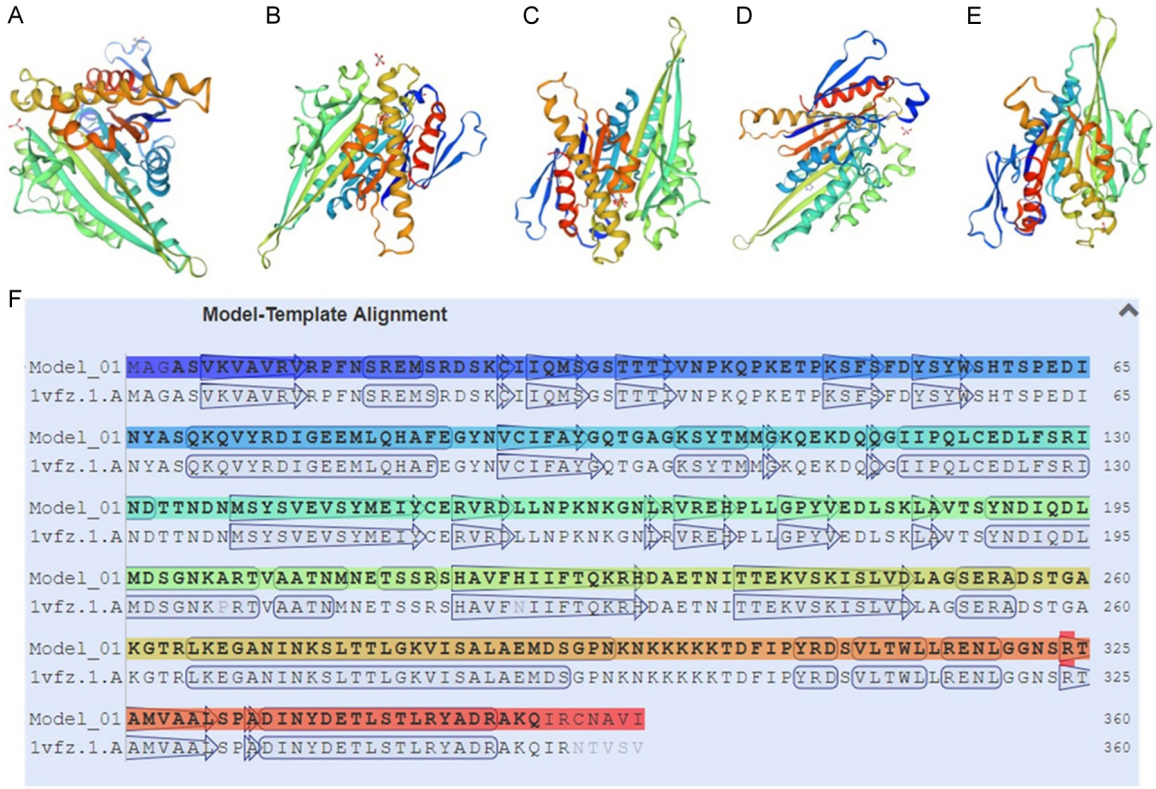

Figure 3. Homology-modeled 3D structures and model-template alignment of wild-type and mutant *KIF1A* proteins. A. Model of wild-type Kinesin Family Member 1A (KIF1A) protein. B. Mutant model p.L249P (c.746T>C). C. Mutant model p.N222H (c.A664C). D. Mutant model p.R13C (c.37C>T). E. Mutant model p.R13H (c.38G>A). F. Model-template alignment of wild-type protein. PDB ID 1VFZ.1.A was used as the template for *KIF1A* protein modeling.

Table 3. Reliability analysis of wild-type and mutant models of *Kinesin Family Member 1A (KIF1A)* protein

Model	SWISS-MODEL	PROCHECK
	Template	Sequence identity (%)
Wild-type	1VFZ.1.A	98.33
p.N222H (c.A664C)		98.06
p.L249P (c.746T>C)		98.06
p.R13C (c.37C>T)		98.06
p.R13H (c.38G>A)		98.06

PredictProtein analysis revealed that the wild-type KIF1A motor domain contained 15 α-helices and 19 β-strands, representing 19.17% and 19.12% of residues, respectively, with 61.11% forming loop regions (**Figure 3A**). Comparative analysis of the mutant versus wild-type protein revealed multiple secondary structure alterations (Tables S1, S2, S3, S4, S5). Specifically, the p.N222H variant introduced an additional β-strand (residues 105-107) and α-helix (residues 208-209), and created new potential protein-, RNA-, and DNA-binding regions (residues 104-217). Conversely, some secondary elements present in the wild type - such as strand 281-283 and helix 310-were lost in the mutant form (**Figure 3B**). Similar secondary-structure perturbations were observed for three previously reported ASD-related *KIF1A* variants (p.R13H, p.L249P, and p.R13C). Each mutation produced distinct combinations of gained or lost α-helices, β-strands, and binding regions, indicating a convergent pattern of structural destabilization within the motor domain (**Figure 3C-E**). Homology modeling via SWISS-MODEL yielded highly reliable tertiary structures, with sequence identities exceeding 98%, GMQE values >0.75, and QMEAN Z-scores close to 0, confirming high model accuracy (**Table 3**; **Figure 3F**). Ramachandran plot assessment using PROCHECK revealed >90% of residues in favored regions, consistent with acceptable stereochemical quality. Comparative visualization using PyMOL demonstrated specific disruptions in hydrogen-bonding networks following

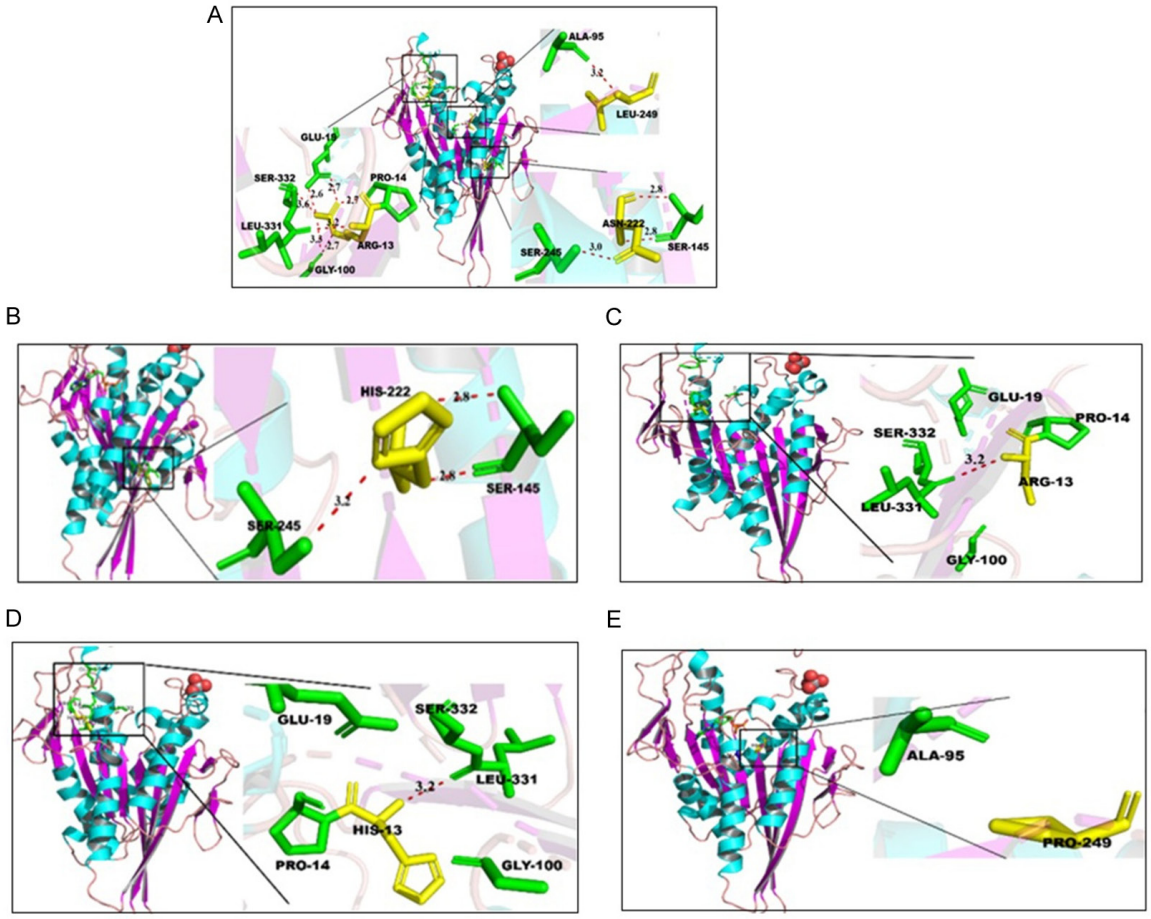

Figure 4. Hydrogen-bonding patterns in wild-type and mutant *Kinesin Family Member 1A (KIF1A)* proteins. A. Hydrogen bonds formed by residues 222, 13, and 249 in the wild-type protein. B-E. Hydrogen bonds involving residues 222, 13, and 249 in mutant proteins: p.N222H (c.A664C), p.R13H (c.38G>A), p.R13C (c.37C>T), and p.L249P (c.746T>C), respectively.

mutation (**Figure 4**). In the wild-type KIF1A protein, residue Asn222 formed three hydrogen bonds with residues 145 and 245, whereas in the mutant protein, the hydrogen bond involving residue 245 exhibited a noticeable shortening, indicating altered local interactions (**Figure 4A**, **4B**). Similarly, the R13H variant showed a marked reduction in the number of hydrogen bonds - from seven in the wild type to only one in the mutant - resulting in significant changes in local folding (**Figure 4C**, **4D**). In contrast, for the L249P variant, the hydrogen bond between residues 249 and 95 present in the wild-type structure was completely absent following mutation (**Figure 4E**). Collectively, these observations indicate that missense mutations within the KIF1A motor domain disrupt local hydrogen-bonding interactions, leading to secondary structure instability and potentially impairing ATPase-driven vesicular transport along neuronal microtubules.

Discussion

In our investigation, we conducted whole-exome sequencing (WES) on an autism spectrum disorder (ASD) family trio, revealing that the proband harbored a de novo missense variant in Kinesin Family Member 1A (KIF1A) (c.A664C, p.N222H) and another variant in *Crumbs homolog 1, Drosophila* (*CRB*) (c.C1982A, p.A661E). Both were confirmed by Sanger sequencing. Additionally, we identified three previously reported de novo *KIF1A* variants from ASD studies. Comprehensive bioinformatics analysis demonstrated that all four *KIF1A* variants induced significant structural changes in the protein, supporting a functional

link between *KIF1A* and ASD. To our knowledge, this study provides the first integrated bioinformatics-based exploration of *KIF1A* pathogenicity in ASD.

KIF1A, a member of the kinesin family, encodes a motor protein essential for anterograde transport of synaptic vesicle precursors along axons [19, 20]. This protein is critically involved in synaptic transmission, neuronal connectivity, learning, and memory [10, 21]. Knockout studies in mice have shown that loss of *KIF1A* function results in early postnatal death due to axonal degeneration in the central nervous system, underscoring its indispensable role in neuronal maintenance and synaptic plasticity [22]. Given that ASD is fundamentally a neurodevelopmental disorder, disruption of such key neuronal transport mechanisms could plausibly contribute to its etiology [23]. To date, four de novo likely pathogenic *KIF1A* variants have been identified, three of which were associated with additional comorbidities such as attention-deficit/hyperactivity disorder (ADHD), intellectual disability (ID), or seizures. In contrast, our proband exhibited the *KIF1A* variant without any accompanying neurological or psychiatric comorbidities, suggesting that this mutation may specifically underlie ASD-related behavioral phenotypes in the absence of global developmental impairment. This strengthens the argument for a direct link between *KIF1A* dysfunction and ASD pathogenesis. Previous studies have demonstrated that cortical synaptogenesis begins as early as 18 gestational weeks and peaks around 15 months postnatally [24-26]. Aberrant synaptic pruning and dysregulated early neurodevelopment have been repeatedly implicated in ASD [27, 28]. Consistent with these findings, our analysis revealed widespread and progressively increasing expression of *KIF1A* during embryonic development, stabilizing at a high level after birth (**Table 2**). This expression pattern aligns with critical periods of neuronal differentiation and synaptic formation, suggesting that *KIF1A* plays an essential role in the maturation of neuronal circuits relevant to ASD.

De novo *KIF1A* variants have previously been associated with neurodevelopmental disorders such as developmental delay, cerebellar and optic nerve atrophy, progressive spasticity, and peripheral neuropathy [10, 12-14]. These conditions, like ASD, involve abnormal neuronal development and axonal dysfunction, highlighting a shared mechanistic pathway. The four *KIF1A* variants identified in this and prior studies are localized within the motor domain (amino acids 1-361), a region responsible for microtubule binding and ATP-dependent cargo transport [29-31]. Our secondary and tertiary structure analyses confirmed that these variants significantly alter the distribution of α-helices, β-strands, and nucleic acid-binding regions, potentially impairing motor domain stability and vesicular trafficking efficiency. Altered hydrogen-bonding patterns and local structural distortions further suggest impaired conformational dynamics of the motor protein. These findings are consistent with earlier reports that missense variants can alter not only the primary but also the secondary and tertiary structures of proteins, thereby disrupting biological function [17]. Collectively, these data imply that *KIF1A* missense variants may contribute to ASD by perturbing axonal transport and synaptic connectivity during early neurodevelopment. In our case, the proband also carried a rare variant in *CRB1* (c.C1982A, p.A661E). *CRB1* encodes a human homolog of the *Drosophila* gene involved in maintaining apical-basal polarity during retinal development. Mutations in *CRB1* are well established as causative in severe retinal dystrophies, including retinitis pigmentosa 12 (RP12) and Leber congenital amaurosis 8 (LCA8) [32, 33]. However, our proband and his family members exhibited no visual impairment or retinal abnormalities, and *CRB1* has not been reported as an ASD-associated gene in large-scale genome-wide studies. Therefore, the *CRB1* variant was classified as a variant of uncertain significance (VUS) and is unlikely to explain the proband's ASD phenotype.

Importantly, the role of rare de novo variants such as *KIF1A* p.N222H should be viewed within the broader context of ASD's polygenic architecture. Although we did not perform polygenic risk score (PRS) analysis due to sample limitations, existing evidence suggests that rare, high-impact mutations often interact with background polygenic risk to modulate ASD severity and expressivity. Thus, our findings may represent a scenario in which a rare variant in a critical neuronal transport gene acts synergistically with a higher polygenic liability to produce ASD symptoms. This study marks the first identifica-

tion of a de novo pathogenic *KIF1A* variant (c.A664C, p.N222H) in an ASD proband from China. Our results provide bioinformatic evidence supporting *KIF1A* as a plausible contributor to ASD pathogenesis through its essential role in axonal transport and synaptic maintenance.

This study has several limitations. First, it is based on a single-family trio, and although we verified the *KIF1A* variant's pathogenic potential through bioinformatic analyses, the sample size limits generalizability and causal inference. Second, no in vitro or in vivo functional experiments were conducted to confirm the impact of the variant on neuronal structure or function. Third, genome-wide data for polygenic risk score (PRS) analysis were unavailable, precluding the evaluation of common-variant background effects. Future work should include functional validation using cellular and animal models and integrate PRS analysis across larger ASD cohorts to clarify how rare *KIF1A* mutations interact with polygenic susceptibility in ASD pathogenesis.

Conclusions

In summary, this study provides new evidence supporting the potential involvement of *KIF1A* variants in the pathogenesis of autism spectrum disorder (ASD). The identification of a comorbidity-free ASD proband carrying a novel de novo *KIF1A* missense variant (c.A664C, p.N222H) strengthens the hypothesis that disruption of neuronal motor protein function may contribute directly to ASD phenotypes.

Comprehensive bioinformatics and structural modeling analyses demonstrated that the mutation induces significant alterations in the secondary and tertiary structures of the *KIF1A* protein, likely affecting its axonal transport and synaptic regulatory functions. These findings extend current understanding of the molecular heterogeneity of ASD and emphasize the role of microtubule-based transport mechanisms in neurodevelopmental disorders.

Future research should include larger cohorts and functional validation studies to clarify the precise cellular effects of *KIF1A* variants. Moreover, integrating rare variant analysis with polygenic risk assessment may help elucidate how *KIF1A*-related disruptions interact with broader genetic susceptibility to influence ASD expression.

Acknowledgements

This work was supported by the Key Research Project from the Ministry of Science and Technology, China (Grant No. 2016YFC1306104).

Disclosure of conflict of interest

None.

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Table S1. Comparison of STRAND and amino acid number change of KIF1A before and after mutation

Wild-type	6-12 (7)	25-30 (6)	35-39 (5)	47-55 (9)	91-96 (6)	*	118 (1)	139-150 (12)	154-156 (3)	166-170 (5)	182-186 (5)	219-229 (11)	237-248 (12)	253 (1)	281-283 (3)	303-304 (2)	325-331 (7)	354-356 (3)	359 (1)
c.A664C, p.N222H	6-12 (7)	25-30 (6)	36-38 (3)▼	48-56 (9)△	93-94 (2)▼	105-107 (3)▲	118 (1)	139-150 (12)	154-156 (3)	166-170 (5)	182-185 (4)▼	219-230 (12)▲	238-248 (11)▼	253 (1)	*,▼	303-304 (2)	327-330 (4)▼	*,▼	359 (1)
c.38G>A, p.R13H	6-12 (7)	25-30 (6)	36-38 (3)▼	48-56 (9)△	93-94 (2)▼	105-107 (3)▲	118 (1)	139-150 (12)	154-156 (3)	166-170 (5)	182-185 (4)▼	219-230 (12)▲	238-248 (11)▼	253 (1)	282 (1)▼	303-304 (2)	327-331 (5)▼	*,▼	359 (1)
c.746T>C, p.L249P	6-12 (7)	25-30 (6)	36-38 (3)▼	48-56 (9)△	93-94 (2)▼	105-107 (3)▲	118 (1)	139-150 (12)	154-156 (3)	166-170 (5)	182-185 (4)▼	219-230 (12)▲	238-248 (11)▼	253 (1)	282 (1)▼	303-304 (2)	327-330 (4)▼	*,▼	359 (1)
c.37C>T, p.R13C	6-12 (7)	25-30 (6)	35-39 (5)	48-55 (8)▼	91-97 (7)▲	104▲	*,▼	139-150 (12)	153-155 (3)	166-170 (5)	182-186 (5)	219-229 (11)	237-248 (12)	253 (1)	281-283 (2)	303-304 (2)	325-331 (7)	354-356 (3)	359 (1)

The change of secondary structure in KIF1A mutant protein. The "▼" corresponds to the area where the amino acid is reduced. The "▲" corresponds to the area where the amino acid is increased. The "△" corresponds to the area where the amino acid is reduced by one as well as increased by one thus the total number of amino acid is unaltered. "*" represents no amino acid at this site.

Table S2. Comparison of HELIX and amino acid number change of KIF1A before and after mutation

Wild-type	17-19 (3)	70-87 (18)	119-132 (14)	189-201 (13)	*	263-271 (9)	275-277 (3)	284-285 (2)	310 (1)	313-314 (2)	316-317 (2)	338-342 (5)	344-351 (8)
c.A664C, p.N222H	17-21 (5)▲	70-87 (18)	119-132 (14)	189-201 (13)	208-209 (2)▲	264-268 (5)▼	275 (1)▼	282-285 (4)▲	*,▼	313-314 (2)	316-317 (2)	342 (1)▼	347-348 (2)▼
c.38G>A, p.R13H	17-21 (5)▲	70-87 (18)	120-132 (13)▼	189-201 (13)	208-209 (2)▲	265-268 (4)▼	275 (1)▼	283-285 (3)▲	*,▼	313-314 (2)	316-317 (2)	342 (1)▼	347-350 (4)▼
c.746T>C, p.L249P	17-21 (5)▲	70-87 (18)	120-132 (13)▼	189-201 (13)	208-209 (2)▲	265-268 (4)▼	275 (1)▼	283-285 (3)▲	*,▼	313-314 (2)	316-317 (2)	342 (1)▼	344 (1) 347-350 (4)▼
c.37C>T, p.R13C	17-20 (4)▲	70-87 (18)	118-133 (16)▲	189-201 (13)	*	262-271 (10)▲	275-278 (4)▲	283-286 (4)▲	*,▼	313-314 (2)	316-317 (2)	338-351 (14)▲	*,▼

The change of secondary structure in KIF1A mutant protein. The "▼" corresponds to the area where the amino acid is reduced. The "▲" corresponds to the area where the amino acid is increased. The "△" corresponds to the area where the amino acid is reduced by one as well as increased by one thus the total number of amino acid is unaltered. "*" represents no amino acid at this site.

Table S3. Comparison of protein binding region and amino acid number change of KIF1A before and after mutation

Wild-type	*	*	*	149-154 (6)	*	278 (1)	305-318 (14)	337-338 (2)	344(1)
c.A664C, p.N222H	104-106 (3)▲	116-120 (5)▲	122-123 (2)▲	148-153 (6)△	216-217 (2)▲	278 (1)	306-318 (13)▼	336-347 (12)▲	*,▼
c.38G>A, p.R13H	104-106 (3)▲	115-120 (6)▲	122 (1)▲	147-153 (7)▲	216-217 (2)▲	278 (1)	306-318 (13)▼	336-347 (12)▲	*,▼
c.746T>C, p.L249P	104-106 (3)▲	115-120 (6)▲	122 (1)▲	148-153 (6)△	216-217 (2)▲	278 (1)	306-318 (13)▼	336-347 (12)▲	*,▼
c.37C>T, p.R13C	*	*	*	*▼	*	278 (1)	305-318 (14)	337-340 (4)▲	*,▼

The change of secondary structure in KIF1A mutant protein. The "▼" corresponds to the area where the amino acid is reduced. The "▲" corresponds to the area where the amino acid is increased. The "△" corresponds to the area where the amino acid is reduced by one as well as increased by one thus the total number of amino acid is unaltered. "*" represents no amino acid at this site.

Table S4. Comparison of RNA-binding region and amino acid number change of KIF1A before and after mutation

Wild-type	7-15 (9)	*	*	203-220 (18)	*	*	261-276 (16)	296-313 (18)	344-359 (16)
c.A664C, p.N222H	9-14 (6)▼	51-52 (2)▲	99-106 (8)▲	201-206 (6)▼	214-222 (9)▲	*	262-279 (18)▲	296-315 (20)▲	344-357 (14)▼
c.38G>A, p.R13H	9-14 (6)▼	50-52 (3)▲	99-106 (8)▲	201-206 (6)▼	214-222 (9)▲	*	261-278 (18)▲	296-314 (19)▲	345-357 (13)▼
c.746T>C, p.L249P	9-14 (6)▼	*	99-106 (8)▲	201-206 (6)▼	214-222 (9)▲	247 (1)▲	261-278 (18)▲	297-314 (18)△	344-357 (14)▼
c.37C>T, p.R13C	10-13 (4)▼	*	*	202-220 (19)▲	*	*	261-276 (16)	297-314 (18)△	343-358 (16)△

The change of secondary structure in KIF1A mutant protein. The "▼" corresponds to the area where the amino acid is reduced. The "▲" corresponds to the area where the amino acid is increased. The "△" corresponds to the area where the amino acid is reduced by one as well as increased by one thus the total number of amino acid is unaltered. "*" represents no amino acid at this site.

Table S5. Comparison of DNA-binding region and amino acid number change of KIF1A before and after mutation

Wild-type	7-17 (11)	43-48 (6)	*	*	204-219 (16)	241-279 (39)	295-313 (19)	*	320-326 (7)	344-355 (12)
c.A664C, p.N222H	8-17 (10)▼	46-53 (8)▲	93-109 (17)▲	165 (1), 167 (1), 169 (1)▲	199-220 (22)▲	244-282 (39)	295-326 (32)▲	*	*,▼	341-355 (15)▲
c.38G>A, p.R13H	8-16 (9)▼	46-53 (8)▲	93-109 (17)▲	*	199-220 (22)▲	244-282 (39)	294-315 (22)▲	317-326 (10)▲	*,▼	342-355 (14)▲
c.746T>C, p.L249P	8-17 (10)▼	46-53 (8)▲	93-109 (17)▲	163-171 (9)▲	199-220 (22)▲	243-283 (41)▲	294-315 (22)▲	317 (1)▲	319-326 (8)▲	342-35 (15)▲
c.37C>T, p.R13C	9-15 (7)▼	44-48 (5)▼	*	*	203-219 (17)▲	241-279 (39)	295-313 (19)	*	320-325 (6)▼	344-355 (12)

The change of secondary structure in KIF1A mutant protein. The "▼" corresponds to the area where the amino acid is reduced. The "▲" corresponds to the area where the amino acid is increased. The "△" corresponds to the area where the amino acid is reduced by one as well as increased by one thus the total number of amino acid is unaltered. "*" represents no amino acid at this site.