American Journal of Medical Genetics Part A

WILEY

AMERICAN JOURNAL OF medical genetics PART A

ORIGINAL ARTICLE OPEN ACCESS

Expanding the Molecular and Clinical Phenotype of Patients With *De Novo* Variants in *KIF5C*: A Six Patient Case Series

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Received: 11 August 2024 | **Revised:** 1 October 2024 | **Accepted:** 21 October 2024

Funding: This work was supported by Research Council of Finland.

Keywords: developmental delay | epilepsy | failure to thrive | intellectual disability | *KIF5C*

ABSTRACT

Heterozygous *de novo* loss of function variants in the motor domain of *KIF5C* are associated with a neurodevelopmental disorder characterized by infantile-onset epilepsy, frontal cortical dysplasia, and developmental delays including motor and speech impairments. Previously, only three missense variants in *KIF5C* were known to be pathogenic. We identified an additional six patients with significant developmental delays with heterozygous *de novo* variants in the *KIF5C* gene (Glu237Val, Thr93Ile, Thr93Asn, Ser90del, Lys92Arg, and Glu237Lys), of which four variants have not been reported before. Functional assessment was performed on fluorescently-tagged KIF5C variants expressed in isolated hippocampal neurons. The pathogenic *de novo* variants displayed significantly reduced motor function compared to the wild-type KIF5C. We conclude that the pathogenic *de novo* variants presented have decreased motor domain activity and that is likely to be the etiology of the patients' symptoms given the gene's constraint in the population. By adding these patients to the seven patients previously reported, we are able to expand the phenotypic spectrum associated with pathogenic *KIF5C* variants. Evaluation of the neurodevelopmental phenotype of additional individuals with loss of function variants in *KIF5C* is indicated to further characterize the spectrum of associated phenotypes.

1 | Introduction

The kinesin superfamily of proteins (KIF) act as ATP-dependent molecular motors to facilitate intracellular transport along microtubules (Hirokawa et al. 2009), a process that is critical in brain development, functioning, plasticity, and survival (Hirokawa, Niwa, and Tanaka 2010). The KIF proteins regulate transport of cargo such as vesicles, organelles, protein complexes, mRNAs, and chromosomes along microtubules within axons, dendrites, and synapses (Willemsen et al. 2014). KIF proteins are regulated through phosphorylation. For example, c-Jun NH_2-terminal kinase 3 (JNK3) phosphorylates the

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motor domain of KIF5C (one of three variants also known as kinesin-1 (Miki et al. 2001)), preventing KIF5C from binding to microtubules (Morfini et al. 2009). Disruption in the normal process of phosphorylation of KIF5C proteins has been suspected as a pathophysiologic mechanism for neurodegenerative conditions. The abnormally long polyglutamine repeat in the pathogenic form of huntingtin enhances JNK3 activity causing hyperphosphorylation of KIF5C suggesting that pathology of Huntington disease may in part result from the decreased transportation of cargo along microtubules by KIF5 proteins (Morfini et al. 2009).

Similarly, genetic variants that directly disrupt the functioning of the motor domain also produce neurological phenotypes (Duquesne et al. 2020; Naim et al. 2022; Banerjee et al. 2024; Becker et al. 2024). The *KIF5C* gene has been established as a genetic cause for a neurodevelopmental disorder characterized by infantile-onset epilepsy, frontal cortical dysplasia, and developmental delays including motor and speech impairments with seven patients reported in the literature to date (Willemsen et al. 2014; Duquesne et al. 2020; Jamuar et al. 2014; Poirier et al. 2013; Cavallin et al. 2016; Michels et al. 2017). The neurodevelopmental disorder is the result of *KIF5C*-encoded kinesin proteins harboring a non-functional motor domain and losing the ability to bind hydrolyzed ATP (Padzik et al. 2016) which impairs or inhibits KIF5C-mediated transport of cargo along microtubules. Interestingly, five of the seven patients reported have had variants involving amino acid Glu237, with four of these individuals sharing the same missense variant, p.Glu237Lys, suggesting a hot spot for variants and potential genotype–phenotype correlations (Michels et al. 2017).

To date only three missense variants in *KIF5C* have been reported (Willemsen et al. 2014; Jamuar et al. 2014; Poirier et al. 2013; Cavallin et al. 2016; Michels et al. 2017). KIF5C gene was first identified to cause of malformation of cortical development (Poirier et al. 2013). Affected individuals typically present with neurodevelopmental delay, infantile-onset epilepsy, intellectual disability and psychomotor retardation and behavioral issues (Duquesne et al. 2020; Banerjee et al. 2024; Michels et al. 2017). The malformation in cortical development can include polymicrogyria and pachgyria as well as brain atrophy (Duquesne et al. 2020; Banerjee et al. 2024; Michels et al. 2017). However, clinical features such as epilepsy, developmental delays and intellectual disabilities are common clinical presentations for a wide variety of genetic and multifactorial conditions and therefore not immediately suggestive of an underlying *KIF5C* etiology. The increasing use of Next Generation Sequencing (NGS) technologies as part of the diagnostic evaluation for these children leads to the discovery of variants of uncertain significance in *KIF5C*. Here we report six additional children with *de novo* variants in *KIF5C*, four of which have not been reported before. By adding these patients to the seven patients previously reported, the phenotypic spectrum associated with pathogenic *KIF5C* variants is expanded.

2 | Methods

2.1 | Ethical Considerations

This case series was prepared based on Colorado Multiple Institutional Review Board (COMIRB) protocol #19–0751.

2.2 | Molecular Testing

Using genomic DNA from the proband and parents, the exonic regions and flanking splice junctions of the genome were captured using the IDT xGen Exome Research Panel v1.0. Massively parallel (NextGen) sequencing was done on an Illumina system with 100 bp or greater paired-end reads. Reads were aligned to human genome build GRCh37/UCSC hg19 and analyzed for sequence variants using a custom-developed analysis tool. Additional sequencing technology and variant interpretation protocol has been previously described (Retterer et al. 2016). The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (https://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/).

2.3 | Plasmids

KIF5C(1-560)-3xmCit variants were cloned into the P3 mCit-C1 vector; K92R, T93I, T93N, E237V, E237K, and A268S were prepared by insertional overlapping PCR using mutagenic and flanking primers as described previously (Padzik et al. 2016).

2.4 | Cell Culture and Transfection

Primary hippocampal neurons were prepared from newborn rats and maintained as before (Deshpande et al. 2020; Hollos et al. 2020). Neurons at 4 days in vitro were transfected with KIF5C(1–560)-3xmCit variants as indicated, ECFP-C1 and pCMV in the ratio of 4:3:3 using Lipofectamine 2000 (ThermoFisher Scientific) according to the manufacturer's protocol. The constructs were expressed for 24 h and the samples were fixed with 4% paraformaldehyde at 4°C.

2.5 | Distribution Analysis

Images were acquired with 20× objective using a Nikon Eclipse Ti2 microscope equipped with a Hamamatsu sCMOS Orca Flash4.0 camera. The cytosolic space was visualized by the CFP expression and the distribution of KIF5C(1–560)-3xmCit variants to soma, neurites and tips was visually assessed. For every neuron, neurite distance from soma to the most distal site of visible KIF5C-3xmCit was measured. The experimenter was blinded to the treatment groups during the image acquisition and analysis.

2.6 | KIF5C Structure

For analysis of KIF5C microtubule co-structure, Mol* 3D Viewer (https://www.rcsb.org/3d-view) was used (Morikawa et al. 2015).

2.7 | Motor Tracking Analysis

For kymograph analysis of the 93I variant, hippocampal neurons were cultured in glass-bottomed dishes (Matek) and transfected with Lipofectamine 2000 (Invitrogen) reagent at 4–5 days in vitro. Imaging was performed 4–6 h after transfection to minimize clustering of KIF5C molecules. Neurons were imaged

with a Zeiss LSM880 microscope. 1024×1024 images were acquired using a 63×oil-immersion objective. Laser line 514 nm was used at 2% excitation and emission was captured with a PTM detector in emission range 520–600 nm. Images were acquired at 600 ms intervals for over 2 min. To compensate for XY drift in the dish, time-series were stack registered using the StackReg plugin from Image J and median filtered with a 1 pixel radius to enhance the signal to noise ratio. Particle tracking was performed using TrackMate v2.5.4 from ImageJ as previously described (Padzik et al. 2016). Particles that could not be tracked frame by frame, but otherwise fitted the diameter criteria of 0.20 ± 0.02 μm and had a minimum intensity that was 15 units above background were scored as "diffuse."

3 | Results

3.1 | Patient Clinical History

3.1.1 | Patient 1

Patient 1 presented for genetics evaluation at 21 months of age with developmental delays, hypotonia, and failure to thrive. She was the product of an uncomplicated pregnancy and born at 39 weeks 4 days gestation to a G2P1–2 mother. She had normal growth parameters and no delivery or neonatal complications were reported. Her history of developmental concerns began at 5 months of age when she was failing to achieve developmental milestones. She was able to sit independently at 1 year of age but could not crawl or walk at 21 months of age. Evaluations determined her motor skill development to be at an 8–10 month level. She had only 3 words at 21 months of age. Her speech delays were partly attributed to low tone in her jaw muscles for which she received speech and occupational therapy. At 21 months of age she was in the eighteenth percentile for height and twenty-seventh percentile for head circumference but had fallen to below the second percentile for weight.

Chromosomal microarray, 5-cell karyotype, and serum amino acids were normal. Initial urine organic acids were concerning for possible HSD10 disease, an X-linked disorder, however repeat analyses were within normal limits. The Autism/ID Xpanded Panel (GeneDx) identified a *de novo* variant of uncertain significance in *KIF5C* (p.T93I; c.278C>T).

The patient has made some developmental gains. She began to walk at 2 years 3 months of age and utilizes an assistive device for verbal communication. There has been no regression of skills nor seizures and therefore she has not had neurological imaging. She consumes a pureed diet.

3.1.2 | Patient 2

Patient 2 is a 6-year-old female, the product of a twin pregnancy conceived with in vitro fertilization. Mother was age 29 years and father age 30 years at time of delivery. Pregnancy was complicated by subchorionic hematoma and chronic abruption of co-twin placenta. She was born at 35+ weeks gestation, appropriate for gestational age for weight and length, head circumference unknown. Neonatal course was uncomplicated. Hypotonia and developmental delay were evaluated at 14 months with microcephaly, axial hypotonia, and appendicular hypertonia documented. Brain MRI at age 14 months demonstrated decreased white matter volume and thin corpus callosum. Mild spastic diplegia was diagnosed at age 17 months. She began walking independent steps at age 20 months. Language delay is significant, she used two words by age 18 months, 5 words by age 3 years. Oral motor hypotonia with excessive drooling, oral phase dysphagia, and dysarthria have persisted. At age 6 she remains globally delayed, uses two-word sentences with echolalia and is not toilet trained. Behavioral concerns have also become increasingly apparent over time. Neuropsychological testing at age 6 years confirmed average functioning at 2-year-old level with the inability to complete formal neurocognitive testing secondary to her severe symptoms of ADHD, autism spectrum disorder, and language delays. She is generally healthy with slow growth (height and weight −2.0–2.5 standard deviations below mean for age) with microcephaly (OFC −2.5 to −3 standard deviations below mean for age). She wears glasses for hyperopia and amblyopia. Genetic evaluation detected no significant minor anomalies. Family history is negative for similar developmental concerns including her co-twin. Chromosomal microarray was normal. Exome sequencing performed as trio (GeneDx, Gaithersburg, MD) detected a *de novo* pathogenic variant in *KIF5C* (p.T93I, c.278C>T).

3.1.3 | Patient 3

Patient 3 is a female whose pregnancy history was remarkable for echogenic foci in several organs including the brain, heart, liver, and kidneys that reportedly cleared and persistent echogenic foci in the stomach. No follow-up imaging was done postnatally due to insurance issues. She was large for gestational age weighing 4139 g and 55.25 cm long. She was doing well until 2 months of age when she developed difficulties swallowing. She eventually required G-tube feedings followed by a GJ tube. She continues to have failure to thrive with weight and length less than the third percentile.

Around the second month of life there was also report of developmental delays. She has continued to have delays and has had only mild improvements through therapy. There is no history of developmental regression. She also has a history of seizures that started at 6 months of age that have never been completely controlled. She follows with neurology with a diagnosis of partial symptomatic epilepsy with complex partial seizures. She has had a brain MRI that showed partial agenesis of the corpus callosum. Additional findings include proteinuria that was later believed to be secondary to valproic intake. She also has hip dysplasia and scoliosis. She has chronic lung disease felt to be secondary to hypotonia and chronic aspiration. Family history is significant for a maternal half-sister who passed away from seizures at 6 years of age.

Genetic testing using the Autism/ID Xpanded Panel (GeneDx, Gaithersburg, MD) identified a heterozygous *de novo* pathogenic variant in *KIF5C* (p.T93N; c.278C>A).

3.1.4 | Patient 4

Patient 4 presented to genetics clinic at 12 months of age with significant hypotonia, ptosis, congenital Horner syndrome, microcephaly, developmental delay, lack of weight gain, and MRI findings of hypomyelination. She was the product of an uncomplicated pregnancy and delivery. On exam, she is eumorphic. Initial microarray was normal. Biochemical testing showed evidence of possible mitochondrial disease. Whole exome sequencing identified a *de novo* pathogenic variant in *KIF5C* (p.S90del; c.268_270delTCA).

At 3 years of age, the patient is growing well, but remains G-tube dependent. She is making progress in development with most skills in the 5–7 months range. She has self-injurious behaviors. Subsequent MRIs show delayed myelination and pachygyria.

3.1.5 | Patient 5

Patient 5 was first referred to genetics at 3 years of age due to hypotonia, developmental delay, intractable epilepsy, Dandy-Walker malformation, and bilateral optic nerve hypoplasia. He was noted shortly after birth to have hypotonia. At 8 months of age he developed seizures which were found to be infantile spasms. At three years of age, he remained non-verbal and was unable to sit without support. He could lift briefly to his elbows when prone but did not use his hands purposefully. He could bear weight only briefly on his legs with assistance. He consumed a pureed diet. Growth parameters at 3 years of age included weight at the 18th percentile, length at the 29th percentile, and head circumference at the 24th percentile. He was noted to have bilateral Darwinian tubercles and prominent ear lobes, without significant other dysmorphic features.

A chromosomal microarray was normal. Whole exome sequencing revealed a pathogenic variant in *KIF5C* (p.E237K, c.709G>A). The variant was not found in his mother; paternal sample was not available for testing.

At 5 years of age, he was noted to have continued medication-resistant epilepsy, as well as decreased sleep.

3.1.6 | Patient 6

Patient 6 was born at term. She was found to have ventricular enlargement based on obstetrical ultrasound. She was delivered by caesarean section because of failure of labor to progress, but was a healthy newborn and no acute problems were encountered.

She experienced infantile myoclonic spasms beginning at 2–3 months of age, associated with a hypsarrhythmic EEG. Brain MRI confirmed the in utero ventricular enlargement that was likely due to reduced central white matter. This finding has been static. She has never achieved seizure control despite treatment with various antiepileptic drugs used alone or in combination. A ketogenic diet has also not improved seizure control.

Development has been severely delayed, globally. She has never sat or walked independently, nor has she ever spoken.

She had early genetic testing for conditions such as Rett syndrome with negative results. Whole exome sequence analysis revealed a pathogenic *de novo* variant in *KIF5C* (p.K92R, c.275A>G).

3.2 | *De Novo* Variants in *KIF5C* Motor Domain Disrupt Its Motility in Axons

To evaluate the functionality of *de novo* variants in *KIF5C*, we engineered single site variants (Lys92Arg, Thr93Ile, Thr93Asn, Glu237Lys, Glu237Val, Ala268Ser) into KIF5C motor domain (amino acids 1–560). We incorporated a triple mCitrine (3xmCit) fluorescent tag at the C-terminal to facilitate visualization of the motor without interfering with motility. The KIF5C(1–560) motor domain lacks the stalk and cargo binding autoinhibitory tail (Coy et al. 1999), and is used for studies of motor domain function in cells (Craig, Jareb, and Banker 1992). As KIF5C is a neuron-specific kinesin (Miki et al. 2001), we expressed the pathological variants in neurons isolated from rat hippocampus, and examined their motility using the neurite tip accumulation assay. This relies on the directional motility of KIF5C towards the plus-ends of microtubules (Case et al. 1997), which are oriented towards the axon tip (Rao and Baas 2018). Thus, transiently expressed KIF5C variants move from the soma where they are synthesized, to tips of axons (Padzik et al. 2016).

When we compared the relative accumulation of KIF5C variants at axonal tips after 24 h of expression, both the wild-type KIF5C(1-560)WT and KIF5C(1–560)268S variant were visible at the neurite tips and were clearly depleted at the soma (Figure 1A–D). In contrast, KIF5C(1–560)92R, KIF5C(1–560)93I, KIF5C(1–560)93 N, KIF5C(1–560)237 K, and KIF5C(1–560)237 V variants remained at the soma after 24 h of expression, indicating defective motor function (Figure 1A–D). Notably, though the 268S variant was closer to the wild-type in terms of tip accumulation, it appeared to lose axon specificity, and accumulated at the tips of both axons and dendrites (Figure 1B). In summary, with the exception of the 268S variant, all pathological variants displayed significantly compromised anterograde motility in neurons (Figure 1D). KIF5C(1–560)268S on the other hand, displayed some motility, but it was no longer restricted to axons.

We next investigated the positions of *KIF5C* variants on the crystal structure of KIF5C motor domain protein complexed with ADP (PDB ID 3X2T) (Morikawa et al. 2015) (Figure 1E, left panel). This shows that the 92R, 93I, and 93 N variants are located in the nucleotide-binding region close to the ADP binding site. The defective motility most likely results from defective ATP hydrolysis. Consistent with this, kymograph analysis of 93I demonstrated a severe motility loss (Figure S1A–F). The 237 K, 237 V, and 268S variants are shown on the cryo EM-generated structure of KIF5C motor complexed to tubulin (PDB ID 3J6H) (Figure 1E, right panel). Ala268 and E237 are very close to the microtubule interface.

In conclusion, our data on KIF5C motor function in neurons indicates that the 92R, 93 N 93I, and 237 K or V variants are most disruptive in terms of motility, showing grossly inhibited accumulation at axonal tips. The 268S variant however, which

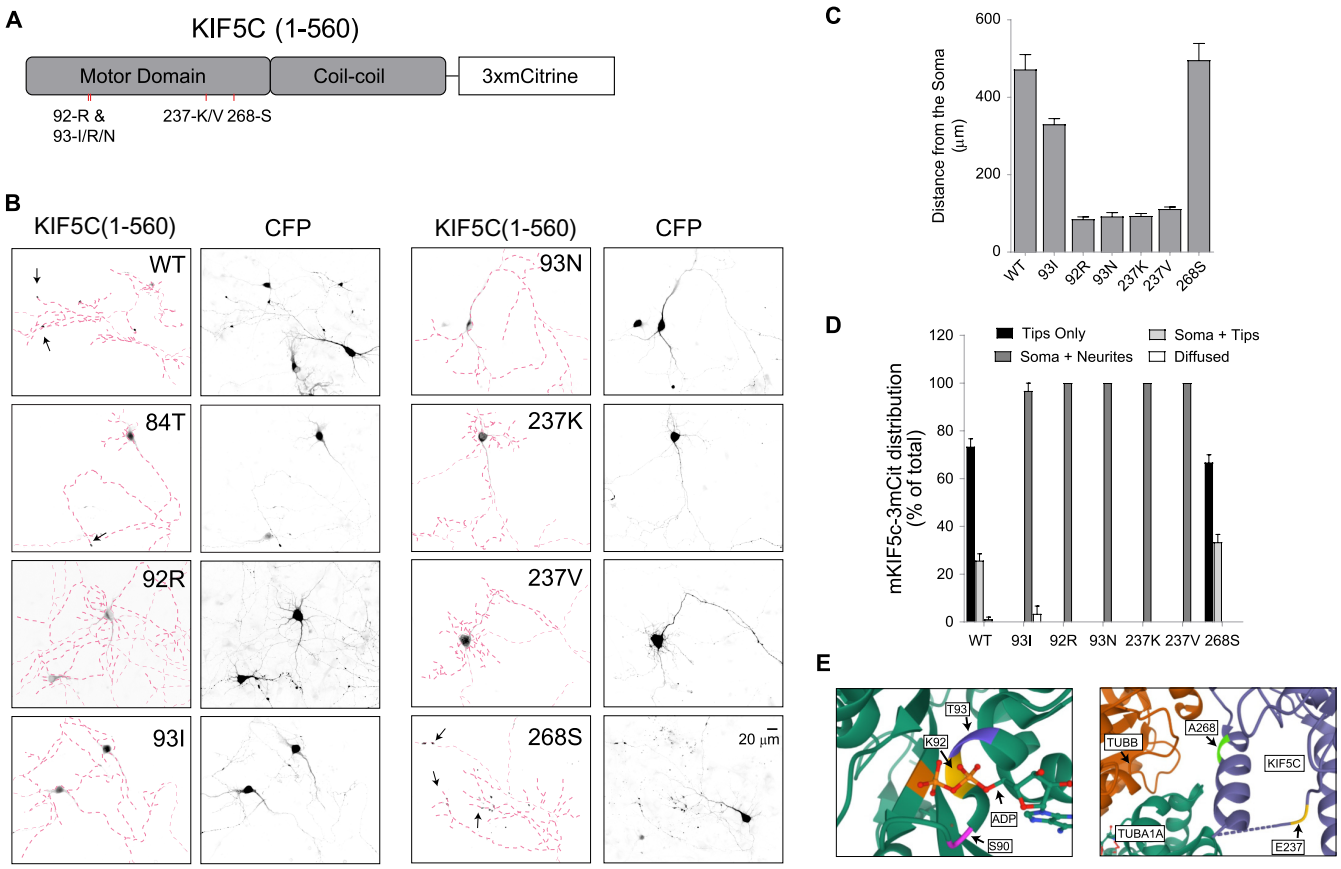

FIGURE 1 | Characterization of *KIF5C* variant motor function. (A) A domain map of KIF5C(1–560)-3mCit depicting patient-specific variants on KIF5C protein. (B) Representative images of hippocampal neurons at 4 days post plating. Cells express citrine-tagged *KIF5C* variants (left hand side; KIF5C) and co-transfected cyan fluorescent protein (CFP; a space filler to visualize neuronal architecture). Neuronal processes from the CFP image are superimposed on the KIF5C images and shown as red dotted lines. Arrowheads point to examples of enriched motors. (C) The distance of KIF5C(1–560)-3xmCit variants from the soma is shown. Mean values ± S.E.M. from 5 to 14 neurons. (D) Relative distribution of KIF5C(1–560) pathological variant accumulation in the soma, neurite and neurite tips. (E) Structures of KIF5C motor domain complexed with ADP (PDB ID 3X2T, left hand side), and the nucleotide-free KIF5C motor cryo EM structure complexed with GMPCPP-microtubule (PDB ID 3J6H, right hand side). Patient-specific variants in the KIF5C motors are indicated.

is at the tubulin interface, displays and intermediate phenotype which indicates that axon specificity is lost.

4 | Discussion

Previously, the key features of *KIF5C*-related disorders have been reported to include cortical dysplasia with simplification of the gyral pattern (100% of patients), variable callosal, and cerebellar abnormalities (83% of patients), early infantile-onset epilepsy (67% of patients), severe intellectual disability including absent speech and language (67% of patients), and abnormal motor development and neurobehavioral issues including self-injurious behaviors (50% of patients) (Michels et al. 2017).

Here we report an additional six patients with pathogenic variants in *KIF5C*. Patient 4 was found to have the Glu237Lys variant that is the single most common *KIF5C* variant reported to date, now documented in 6 of 11 individuals reported with *KIF5C* variants (Duquesne et al. 2020; Naim et al. 2022). This individual similarly shared the phenotype that has been consistently reported with this variant including infantile-onset seizures that are resistant to antiepileptic medications, lack of purposeful hand movements, and severe delays including being both non-verbal and non-ambulatory (Willemsen et al. 2014; Cavallin et al. 2016; Michels et al. 2017). This individual provides further evidence for a genotype–phenotype correlation for the Glu237Lys missense variant (Table 1).

The additional five individuals newly reported in this case series and the four individuals previously reported (Banerjee et al. 2024; Jamuar et al. 2014; Poirier et al. 2013; Cavallin et al. 2016), all have unique *de novo* variants in *KIF5C*. The reported clinical features suggest phenotypic variability, broadening the spectrum of both occurrence and severity of symptoms (Table 2). The new variants that we report, p.(The93Ile), p.(The93Asn), and p.(Lys92Arg) are absent from the Genome Aggregation Database (gnomAD) (Karczewski et al. 2019). In addition, we report p.(Ser90del), for which only one other case is reported (Banerjee et al. 2024). There are 45 KIF genes in mammals including humans and mice (Hirokawa, Niwa, and Tanaka 2010). The motor domain has 30%–60% sequence homology, retaining the highest homology of the amino acid sequence compared to other domains. *KIF5C* is an extremely constrained gene with an observed/expected ratio of 0.04 for loss of function variants, which

TABLE 1 | Phenotypes of individuals with *KIF5C* variants p.Glu237Lys, c.709G>A (Total $N = 7$; $N = 6$ previously reported, $N = 1$ reported in this study-patient 5).

	Willemsen et al. 2014	Cavallin et al. 2016	Michels et al. 2017	Michels et al. 2017	Duquesne et al. 2020	Naim et al. 2022	Patient 5 (this study)
Gender	Female	Male	Male	Female	Female	Male	Male
Age last assessed when reported	15 years	7 years	13 years	11 years	16 years	5 years	5 years
Seizures	+	−	+	+	+	+	+
Seizure onset	6 months	NA	1 month	3 months	12 months	1 day	8 months
Seizure types	ND	NA	Generalized epilepsy	Febrile, GTC	Febrile, GTC, absence	GTC	Infantile spasms
Tone abnormalities	Mild hypotonia	ND	Spastic quadriplegia	Hypotonia	Mild hypotonia	Appendicular hypotonia	Hypotonia
Behavioral problems	Stereotypic hand movements	Stereotypic hand movements, inappropriate laughter	Stereotypic hand movements	Self-injurious behavior, aggression, impulsivity	Stereotypic hand movements	Stereotypic hand movements, mild autism	Non-purposeful use of hands
Intellectual disability	Severe ID, non-verbal, walked with support at age 9–10 years	Severe delays, non-verbal	Non-verbal, non-ambulatory	Non-verbal, non-ambulatory	Severe delay, non-verbal	Severe, non-ambulatory, non-verbal	Non-verbal, non-ambulatory
Dysmorphic facies	Apparent microcephaly	ND	Synophrys, laterally prominent ears, prominent nasal bridge, relatively large mouth	Low sloping forehead, apparent prognathism, relatively large mouth with downturned corners, mildly arched eyebrows, mildly high-arched palate	Large mouth, big eyes	Prominent forehead, smooth philtrum and thin upper vermilion lip	Bilateral Darwinian tubercles, prominent earlobes
Other notable medical problems	Severe auto mutilation	Auto mutilation	Static encephalopathy, bilateral hip dysplasia, superior mesenteric artery syndrome	Pica, nocturnal enuresis	Sleeping troubles	Failure to thrive	Decreased sleep
Cortical abnormalities	Pachygyria	Pachygyria	Mild pachygyria	Mild pachygyria	Mild pachygyria	Pachygyria	ND

(Continues)

TABLE 1 | (Continued)

	Willemsen et al. 2014	Cavallin et al. 2016	Michels et al. 2017	Michels et al. 2017	Duquesne et al. 2020	Naim et al. 2022	Patient 5 (this study)
Ventriculomegaly	ND	+	+	+	+	+	Dandy-Walker malformation
Callosal abnormalities	ND	Thin, dysplastic	Thin	Thin	ND	Thin	ND
Testing method	Trio-based WES	Trio-based WES	Targeted NGS panel	Targeted NGS panel	Targeted NGS panel	Targeted NGS panel	Duo-based WES
Inheritance	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*

Abbreviations: NA, not applicable; ND, not documented; NGS, next generation sequencing; WES, whole exome sequencing.

implies that heterozygous loss of function is likely to be clinically significant. This is not surprising given the abundance of this motor and its enrichment in the nervous system. The scaled Combined Annotation Dependent Depletion (CADD) scores are 21.5 for p.(Ser90del), 25.9 p.(Thr93Asn), and 26.6 for p.(Thr93Ile) which are also suggestive of pathogenicity (Rentzsch et al. 2019). A CADD score could not be generated for p.(Lys92Arg).

Functional analyses of the *KIF5C* variants provides insights into the mechanism of disease. Assays revealed significant decreases in KIF5C movement along microtubules as demonstrated by the high levels in the soma and absence of accumulation in the axonal tips. This is in stark contrast to the wild-type KIF5C motor which trafficks to the end of the polarized microtubule tracks and accumulates at tips (Figure 1). This implies a loss of function of the motor domain. Specifically, the most severe variants in terms of motility readout were the T92R, T93N that form the nucleotide-binding pocket, and E237K and E237V variants. KIF5C-E237 is believed to form a salt bridge with R204 when the motor domain is in the ATP-bound state (Cavallin et al. 2016). The replacement of the negatively charged glutamate with positively charged lysine or neutral valine is expected to disrupt ATP hydolysis. This was previously been shown to be the case for the p.Ser90del *de novo* pathogenic variant, which is also in the nucleotide-binding site (Banerjee et al. 2024). Together our functional analysis demonstrates abnormal KIF5C movement along microtubules also in the newly reported *de novo* variants. The phenotypic spectrum of neurodevelopmental anomalies are caused by a loss of function effect in the motor domain of KIF5C. The variants displaying most functional disruption were also those that induced earlier neurodevelopmental symptoms (Tables 1 and 2).

We also investigated the motor activity of the 268S variant which was previously reported in a patient (Jamuar et al. 2014). This variant showed an intermediate motility deficiency that was less severe than the 93I/N and the 237K variants. However, among all variants tested, only KIF5C-268S variant was detected in dendrites (Figure 1B). This was unexpected given the characteristic preference of the KIF5C motor for transport in axons (Nakata and Hirokawa 2003). Kinesin-1 motors are known to move towards the microtubule plus-ends (Hirokawa, Niwa, and Tanaka 2010; Craig, Jareb, and Banker 1992), and differential posttranslational modifications of tubulin, such as acetylation, polyglutamylation and tyrosination, alter KIF5 binding, processivity, and specificity for axons (Konishi and Setou 2009; Hammond et al. 2010; Sirajuddin, Rice, and Vale 2014; Janke and Magiera 2020). Our data suggests that the c.805G>T p.Ala268Ser variant which is proximal to the motor:microtubule interface may interfere with KIF5C recognition of the tubulin "code" that normally enriches it in axons.

In summary, we report an additional six patients with *KIF5C* variants in the motor domain, including four entirely new variants, nearly doubling the number of known cases. We demonstrate that all variants are functionally defective in neurons and are therefore likely to play a causal role in the pathology. Our data thus supports the general phenotype of *KIF5C* related disorders as a spectrum of cortical dysplasia, infantile-onset epilepsy,

TABLE 2 | Phenotypes of individuals with *KIF5C* variants not reported in other individuals (Total $N = 7$; $N = 2$ previously reported, $N = 5$ reported in this study).

	Poirier et al. 2013	Jamuar et al. 2014	Banerjee et al. 2024	Patient 1	Patient 2	Patient 3	Patient 4	Patient 6
Gender	Male	Female	Male	Female	Female	Female	Female	Female
Age last assessed when reported	1 month	ND	6 years	3 years	6 years	ND	3 years	ND
Seizures	+	ND	+	−	−	+	−	+
Seizure onset	1 month	ND	6 months	NA	NA	6 months	NA	2–3 months
Seizure types	Clonic	ND	Tonic and absence seizures	NA	NA	Symptomatic epilepsy with complex partial seizures	NA	Infantile myoclonic spasms
Tone abnormalities	Spastic quadriplegia	ND	Tone abnormalities	Hypotonia	Axial hypotonia with spastic diplegia	Hypotonia	Hypotonia	ND
Behavioral problems	ND	ND	Psychomotor retardation involuntary limb movement lacked social responses	−	ADHD, autism spectrum disorder	ND	Self-injurious behaviors	ND
Intellectual disability	ND	ND	Severe developmental delay	Walked at 2 years 3 months, severe speech delays	Suspected	Developmental delays	Severe developmental delays	Non-verbal, non-ambulatory
Dysmorphic facies	ND	ND	ND	Telecanthus	Non-dysmorphic	ND	Ptosis, congenital Horner syndrome	ND
Other notable medical problems	IUGR, severe arthrogryposis	ND	Requires liquid diet	Requires puree diet	Oral phase dysphagia, slow growth	GJ tube fed, hip dysplasia, scoliosis, chronic lung disease	G-tube dependent	ND
Cortical abnormalities	Polymicrogyria, pachygyria	Subcortical pachygyria	Cerebral atrophy	NE	Mildly reduced white matter volume loss	ND	Pachygyria, hypomyelination	Reduced central white matter

(Continues)

TABLE 2 | (Continued)

	Poirier et al. 2013	Jamuar et al. 2014	Banerjee et al. 2024	Patient 1	Patient 2	Patient 3	Patient 4	Patient 6
Ventriculomegaly	+	+	+	NE	Ex-vacuo from volume loss	ND	–	+
Callosal abnormalities	Thin	Thin, dysplastic	Thin corpus callosum	NE	Thin corpus callosum	Partial agenesis of the corpus callosum	ND	ND
Testing method	ND	Targeted NGS panel	Whole exome sequencing	Targeted NGS panel	Trio-based WES	Targeted NGS panel	Trio-based WES	ND
cDNA	c.710A>T	c.805G>T	c.265_267delTCA	c.278C>T	c.278C>T	c.278C>A	c.268_270delTCA	c.275A>G
Amino acid	p.Glu237Val	p.Ala268Ser	p.Ser90del	p.Thr93Ile	p.Thr93Ile	p.Thr93Asn	p.Ser90del	p.Lys92Arg
Inheritance	Maternal germline mosaicism	ND	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*

Abbreviations: NA, not applicable; ND, not documented; NE, not evaluated; NGS, next generation sequencing; WES, whole exome sequencing.

and severe developmental delays in the absence of significant dysmorphic features.

Author Contributions

S.G. compiled the clinical data and wrote the first draft of the manuscript. P.D. and P.H. cloned the KIF5C variants and carried out the cellular experiments. A.L., K.D.D., R.J.F., D.M.M., A.B.P., J.A.S.S., M.R.B., L.H.S., and K.B. A.L. collected and shared patient data. E.C., K.D.D., R.J.F., D.M.M., A.B.P., J.A.S.S., M.R.B., L.H.S., K.B., P.D., and P.H. contributed to the editing of the final manuscript.

Acknowledgments

We thank the patients and their families for their contributions. The work was funded by a Research Council of Finland grant #310583 to E.C.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

Banerjee, S., Q. Zhao, B. Wang, et al. 2024. "A Novel in-Frame Deletion in KIF5C Gene Causes Infantile Onset Epilepsy and Psychomotor Retardation." *MedComm* 5, no. 4: e469. https://doi.org/10.1002/mco2.469.

Becker, L. L., K. L. Makridis, A. T. Abad-Perez, et al. 2024. "The Importance of Routine Genetic Testing in Pediatric Epilepsy Surgery." *Epilepsia Open* 9: 800–807. https://doi.org/10.1002/epi4.12916.

Case, R. B., D. W. Pierce, N. Hom-Booher, C. L. Hart, and R. D. Vale. 1997. "The Directional Preference of Kinesin Motors Is Specified by an Element Outside of the Motor Catalytic Domain." *Cell* 90: 959–966. https://doi.org/10.1016/s0092-8674(00)80360-8.

Cavallin, M., L. Hubert, V. Cantagrel, et al. 2016. "Recurrent KIF5C Mutation Leading to Frontal Pachygyria Without Microcephaly." *Neurogenetics* 17: 79–82. https://doi.org/10.1007/s10048-015-0459-8.

Coy, D. L., W. O. Hancock, M. Wagenbach, and J. Howard. 1999. "Kinesin's Tail Domain Is an Inhibitory Regulator of the Motor Domain." *Nature Cell Biology* 1: 288–292. https://doi.org/10.1038/13001.

Craig, A. M., M. Jareb, and G. Banker. 1992. "Neuronal Polarity." *Current Opinion in Neurobiology* 2: 602–606. https://doi.org/10.1016/0959-4388(92)90025-g.

Deshpande, P., D. Flinkman, Y. Hong, et al. 2020. "Protein Synthesis Is Suppressed in Sporadic and Familial Parkinson's Disease by LRRK2." *FASEB Journal* 34: 14217–14233. https://doi.org/10.1096/fj.202001046R.

Duquesne, S., M. C. Nassogne, P. Clapuyt, K. Stouffs, and Y. Sznajer. 2020. "Phenotype Description in KIF5C Gene Hot-Spot Mutations Responsible for Malformations of Cortical Development (MCD)." *European Journal of Medical Genetics* 63: 103991. https://doi.org/10.1016/j.ejmg.2020.103991.

Hammond, J. W., C. F. Huang, S. Kaech, C. Jacobson, G. Banker, and K. J. Verhey. 2010. "Posttranslational Modifications of Tubulin and the Polarized Transport of Kinesin-1 in Neurons." *Molecular Biology of the Cell* 21: 572–583. https://doi.org/10.1091/mbc.e09-01-0044.

Hirokawa, N., S. Niwa, and Y. Tanaka. 2010. "Molecular Motors in Neurons: Transport Mechanisms and Roles in Brain Function, Development, and Disease." *Neuron* 68: 610–638. https://doi.org/10.1016/j.neuron.2010.09.039.

Hirokawa, N., Y. Noda, Y. Tanaka, and S. Niwa. 2009. "Kinesin Superfamily Motor Proteins and Intracellular Transport." *Nature Reviews. Molecular Cell Biology* 10: 682–696. https://doi.org/10.1038/nrm2774.

Hollos, P., J. M. John, J. V. Lehtonen, and E. T. Coffey. 2020. "Optogenetic Control of Spine-Head JNK Reveals a Role in Dendritic Spine Regression." *eNeuro* 7. https://doi.org/10.1523/ENEURO.0303-19.2019.

Jamuar, S. S., A. T. Lam, M. Kircher, et al. 2014. "Somatic Mutations in Cerebral Cortical Malformations." *New England Journal of Medicine* 371: 733–743. https://doi.org/10.1056/NEJMoa1314432.

Janke, C., and M. M. Magiera. 2020. "The Tubulin Code and Its Role in Controlling Microtubule Properties and Functions." *Nature Reviews. Molecular Cell Biology* 21: 307–326. https://doi.org/10.1038/s41580-020-0214-3.

Karczewski, K. J., L. C. Francioli, G. Tiao, et al. 2019. "Variation Across 141,456 Human Exomes and Genomes Reveals the Spectrum of Loss-of-Function Intolerance Across Human Protein-Coding Genes." *Biorxiv*. https://doi.org/10.1101/531210.

Konishi, Y., and M. Setou. 2009. "Tubulin Tyrosination Navigates the Kinesin-1 Motor Domain to Axons." *Nature Neuroscience* 12: 559–567. https://doi.org/10.1038/nn.2314.

Michels, S., K. Foss, K. Park, et al. 2017. "Mutations of KIF5C Cause a Neurodevelopmental Disorder of Infantile-Onset Epilepsy, Absent Language, and Distinctive Malformations of Cortical Development." *American Journal of Medical Genetics. Part A* 173: 3127–3131. https://doi.org/10.1002/ajmg.a.38496.

Miki, H., M. Setou, K. Kaneshiro, and N. Hirokawa. 2001. "All Kinesin Superfamily Protein, KIF, Genes in Mouse and Human." *Proceedings of the National Academy of Sciences of the United States of America* 98: 7004–7011. https://doi.org/10.1073/pnas.111145398.

Morfini, G. A., Y. M. You, S. L. Pollema, et al. 2009. "Pathogenic Huntingtin Inhibits Fast Axonal Transport by Activating JNK3 and Phosphorylating Kinesin." *Nature Neuroscience* 12: 864–871. https://doi.org/10.1038/nn.2346.

Morikawa, M., H. Yajima, R. Nitta, et al. 2015. "X-Ray and Cryo-EM Structures Reveal Mutual Conformational Changes of Kinesin and GTP-State Microtubules Upon Binding." *EMBO Journal* 34: 1270–1286. https://doi.org/10.15252/embj.201490588.

Naim, A., A. Accogli, E. Amadori, et al. 2022. "Abnormal Course of the Corticospinal Tracts in KIF5C-Related Encephalopathy." *European Journal of Medical Genetics* 65: 104622. https://doi.org/10.1016/j.ejmg.2022.104622.

Nakata, T., and N. Hirokawa. 2003. "Microtubules Provide Directional Cues for Polarized Axonal Transport Through Interaction With Kinesin Motor Head." *Journal of Cell Biology* 162: 1045–1055. https://doi.org/10.1083/jcb.200302175.

Padzik, A., P. Deshpande, P. Hollos, et al. 2016. "KIF5C S176 Phosphorylation Regulates Microtubule Binding and Transport Efficiency in Mammalian Neurons." *Frontiers in Cellular Neuroscience* 10: 57. https://doi.org/10.3389/fncel.2016.00057.

Poirier, K., N. Lebrun, L. Broix, et al. 2013. "Mutations in TUBG1, DYNC1H1, KIF5C and KIF2A Cause Malformations of Cortical Development and Microcephaly." *Nature Genetics* 45: 639–647. https://doi.org/10.1038/ng.2613.

Rao, A. N., and P. W. Baas. 2018. "Polarity Sorting of Microtubules in the Axon." *Trends in Neurosciences* 41: 77–88. https://doi.org/10.1016/j.tins.2017.11.002.

Rentzsch, P., D. Witten, G. M. Cooper, J. Shendure, and M. Kircher. 2019. "CADD: Predicting the Deleteriousness of Variants Throughout the Human Genome." *Nucleic Acids Research* 47: D886–D894. https://doi.org/10.1093/nar/gky1016.

Retterer, K., J. Juusola, M. T. Cho, et al. 2016. "Clinical Application of Whole-Exome Sequencing Across Clinical Indications." *Genetics in Medicine* 18: 696–704. https://doi.org/10.1038/gim.2015.148.

Sirajuddin, M., L. M. Rice, and R. D. Vale. 2014. "Regulation of Microtubule Motors by Tubulin Isotypes and Post-Translational Modifications." *Nature Cell Biology* 16: 335–344. https://doi.org/10.1038/ncb2920.

Willemsen, M. H., W. Ba, W. M. Wissink-Lindhout, et al. 2014. "Involvement of the Kinesin Family Members KIF4A and KIF5C in Intellectual Disability and Synaptic Function." *Journal of Medical Genetics* 51: 487–494. https://doi.org/10.1136/jmedgenet-2013-102182.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.