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KIF5A mutations cause an infantile onset phenotype including severe myoclonus with evidence of mitochondrial dysfunction

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Abstract

Missense mutations in *kinesin family member 5A* (*KIF5A*) cause spastic paraplegia 10. We report two patients with *de novo* stop-loss frameshift variants in *KIF5A* resulting in a novel phenotype that includes severe infantile onset myoclonus, hypotonia, optic nerve abnormalities, dysphagia, apnea, and early developmental arrest. We propose that alteration and elongation of the carboxy-terminus of the protein has a dominant-negative effect causing mitochondrial dysfunction in the setting of an abnormal kinesin “motor”. These results highlight the role of expanded testing and whole exome sequencing in critically ill infants and emphasize the importance of accurate test interpretation.

Introduction

Over the last 15 years, missense mutations in exon 8 of the *kinesin family member 5A* (*KIF5A*) have been linked to axonal degeneration of motor and sensory neurons and specifically hereditary spastic paraplegia 10 (SPG10)^{1,2,3} (OMIM # 604187).

Kinesin heavy chain isoform 5A is specific to neurons and is localized to the neuronal cytoplasm including the cell body, dendrites and axons. The kinesin heavy chain is essential

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Potential Conflicts of Interest: No conflicts to report in relation to this work.

Note in proof: During the review process a single published case report noted the same phenotype associated with a frameshift mutation in *KIF5A*. Rydzanicz M, Jagła M, Kosinska J, et al. *KIF5A* de novo mutation associated with myoclonic seizures and neonatal onset progressive leukoencephalopathy. Clin Genet. 2016 Jul 14. doi: 10.1111/cge.12831.

for mitochondrial transport.⁴ Impairment of this transport pathway results in dysregulation of the balance between healthy and damaged mitochondria contributing to the phenotype of patients with Charcot Marie Tooth and Parkinson diseases.

Animal studies have shown that *KIF5A* knockout mice are neonatal lethal.^{5,6} EEGs on these knockout mice display paroxysmal waveforms, consistent with neuronal hyperexcitability, but neither electrographic nor clinical seizures have been reported.⁷ GABA_A receptors (GABA_AR) are ligand-gated chloride channels that mediate inhibitory neurotransmission. A unique 73 amino acid region in the KIF5A C-terminus was noted to be sufficient for the binding of KIF5A to GABA_AR-associated protein (GABARAP).

Methods

The Institutional Review Boards (IRB) at Johns Hopkins University and Carolinas Health Care approved an exempt protocol for review of each case's medical records and use of clinical data for publication.

Clinical whole exome and mitochondrial sequencing were performed in case 1 at the Exome Laboratory at Baylor Miraca Genetic Laboratories, as previously described.⁸ A clinical Next Generation sequencing panel of 418 genes was utilized in case 2.⁹ The latter included all the nuclear genes encoding mitochondrial complexes, genes secondarily involved in mitochondrial function, and genes that phenotypically resemble mitochondrial disease. Targeted parental testing utilizing Sanger sequencing was utilized in both cases.

In case 2, muscle biopsy was performed by standard procedure. Biochemical testing in both cases included urine organic acids, plasma amino acids and plasma lactate.

Cases (TABLE 1)

Both cases presented shortly after birth with nearly continuous non-rhythmic, large-amplitude jerks occurring singly or in brief clusters of 2-3 jerks. Case 1 was intubated secondary to frequent episodes of apnea and associated bradycardia. He required gavage feeding due to an ineffective suck. Case 1 exhibited saccadic intrusions with marked post-saccadic drift toward midline. Case 2 has horizontal nystagmus. Both cases showed ptosis and optic nerve pallor. Neither patient fixed or followed a visual target. Motor exam was significant for axial and appendicular hypotonia in both patients.

Case 1 exhibited severe frequent myoclonus that was exacerbated by stimulation and persisted during sleep. Slower, athetoid limb movements were observed as well. Endoscopy revealed myoclonic movements of the pharyngeal muscles, likely accounting for apneic episodes. He remained intubated over the next 3 months without success at weaning. Myoclonus persisted with minimal developmental progress. After in-depth family discussions, the parents decided to withdraw support.

Case 2 required a prolonged stay in the Neonatal Intensive Care Unit due to a brief period of episodic hypoglycemia, persistently poor feeding requiring feeding tube placement, and intermittent apnea, although intubation was not necessary. She continues to have dysphagia,

gastrostomy tube-dependence and intermittent apnea requiring use of a home apnea monitor. At her most recent examination, the child was microcephalic and impaired globally with developmental arrest. She is now manifesting abnormal movements consisting of intermittent myoclonus and choreiform movements (including tongue).

Results

EEG in case 1 showed a continuous slow background with scattered sharp waves without a consistent focus. EEG in case 2 showed diffuse slowing. There were no epileptiform discharges during periods of myoclonus in either case (no ictal correlation).

Routine laboratory studies were within normal limits. Biochemical testing including urine organic acids (UOA), plasma amino acids (PAA), free and total carnitine, and lactate was normal.

Monoamine neurotransmitter disorders were considered in each case. In case 1, pyridoxine was initiated while awaiting results of neurotransmitter studies and did not have any clinical benefit.

Sequencing revealed *de novo* variants in *KIF5A*, specifically c.2854delC and c.2934delG in case 1 and case 2, respectively. Based on predictive models, the mRNA transcripts are not subjected to nonsense-mediated decay.¹⁰ These mutations disrupt the C-terminal domain of the protein at a region unique to *KIF5A* (FIGURE 1). The laboratories reported these variants as likely pathogenic in case 1 and a variant of uncertain significance in case 2 based on the ACMG guidelines for variant classification.¹¹

In both cases, brain MRIs showed no abnormalities in the infant period. Patient 2 developed increased T2 signal in the brainstem and pons noted at age 2 years (FIGURE 2).

Muscle biopsy was performed in case 2 and showed nonspecific myopathic features. There was increased variability of fiber diameters, and atrophy that appeared to involve type 1 fibers somewhat more than type 2 fibers. Electron transport studies revealed a borderline complex IV deficiency (TABLE 2). Complex I testing was inconclusive.

Discussion

We report two cases characterized by myoclonus, eye movement abnormalities, ptosis, optic nerve abnormalities, dysphagia, apnea, hypotonia and developmental arrest associated with stop-loss frameshift mutations in *KIF5A* causing elongation of the protein and altered C-terminus of *KIF5A*. The C-terminus of *KIF5A* has been identified as an area of binding of GABARAP (FIGURE 1).⁶ GABARAP clusters neurotransmitter receptors by mediating interaction with microtubules. These data suggest that the myoclonus in these patients may be caused by increased neuronal excitation due to aberrant GABA signaling.^{6,12,13} Negative EEG findings suggest that the myoclonus may be of spinal cord origin, which is supported by expression of *KIF5A* in the peripheral nervous system and swelling of cell bodies of lower motor neurons in the spinal cord of *KIF5A* knockout mice.¹⁴ Levetiracetam trials had no benefit in treating the myoclonus in either case.

We hypothesize that the elongated protein has a dominant-negative effect on the kinesin-1 complex, thus disrupting the transport of organelles, protein complexes and mRNAs.¹⁵ The stop-loss frameshift mutations cause elongation of the amino acid sequence and result in a gain of new and abnormal protein function affecting the C-terminal docking site and altering the tertiary structure of the protein. KIFs appear to be involved in higher brain functions and developmental patterning, providing a possible explanation for the severe developmental delays.

The remarkable similarity of the phenotype in these patients suggests that the *KIF5A* mutation is pathogenic in both cases. The presentations reflect findings of diseased mitochondria including myopathy, myoclonus, developmental delay and ocular abnormalities. Further evidence includes the atrophy of type I muscle fibers in case 2, which supports the role of *KIF5A* in the trafficking of mitochondria¹⁶ and maintenance of a functional mitochondrial population to meet local energy demands and calcium buffering requirements within the neuron.¹⁷ Case 2 was diagnosed with complex IV deficiency based on a high suspicion of mitochondrial disease given the clinical presentation and borderline findings on electron transport chain studies. These functional data in case 2 more likely reflect an inability for the mitochondria to be transported to their proper intracellular locations based on the role of *KIF5A* in mitochondrial trafficking.¹⁸

The availability of rapid sequencing will change the diagnostic and potentially therapeutic approaches to severe neurological phenotypes. Extensive genetic counseling and clear communication among the medical team and with the family are paramount. Rapid whole exome sequencing in case 1 provided specific diagnostic information utilized in conjunction with the infant's clinical course to inform prognosis and aid the family's decision to withdraw supportive care. In case 2, the variant was listed with a number of variants of uncertain significance. The use of molecular modeling, mutational databases and available functional data including mouse and *Drosophila* models are essential in determining pathogenesis. As sequencing becomes more cost-effective, the neurologist, geneticist and genetic counselor will play an increasingly pivotal role in providing phenotypic information to the testing laboratory and in interpreting results for families.

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Figure 1. Sequence chromatogram and altered protein sequence

(A). Sequence chromatogram showing *de novo* frameshift mutation c.2854delC in case 1. (B): Comparison of wildtype, c.2854delC (case 1) and c.2934delG (case 2) sequences with altered protein sequence underlined. Frameshift mutation results in a stop-loss with read through the normal termination codon to create an elongated protein in case 1. Black bar is the GABARAP interacting tail. Italics indicates unique sequence between case 1 and both WT and case 2. Note the altered protein at the location of the unique 73 amino acid region in the KIF5A C-terminus sufficient for binding the GABA_AR-associated protein (GABARAP).

Figure 2. Brain MRI scan of case 2 illustrating T2 hyperintensities and delayed myelination at 2 years of age

Table 1
Clinical characteristics of case 1 and case 2

Characteristic	Patient 1	Patient 2
Sex/age	Male/deceased at 3 months when respiratory support withdrawn	Female/5 years
KIF5A mutation	c.2854delC	c.2934delG
Pregnancy	term, unremarkable	36 weeks, unremarkable
Presentation	At birth: continuous myoclonus; apnea associated with episodes of bradycardia; ineffective suck	At birth: myoclonus; poor feeding; hypoglycemia
Dysmorphic features	None	None
Development	Global delay	Global delay
Tone	Hypotonia	Hypotonia
GI	Dysphagia; feeding tube dependent	Dysphagia; mild nasal pharyngeal penetration; poor oral bolus formation; feeding tube dependent
Respiratory	Apnea, ventilation required to sustain life; endoscopy revealed myoclonic movements of the pharyngeal muscles	Apnea; home apnea monitor
Ophthalmologic findings	Oculogyric crises; optic nerve atrophy	Bilateral ptosis; optic nerve pallor; cortical vision impairment
Biochemical testing	Plasma ammonia, carnitine profile, plasma amino acids, and urine organic acids were normal	Plasma amino acids, urine organic acids, urine amino acids and lactate were all normal; mild elevation in ammonia
CSF findings	5-HIAA, HVA and 3-O-methyldopa within the normal range	5-H1AA, HVA and 3-O-methyldopa within the normal range
EEG	Occasional posterior sharp and polysharp waves, more prominent on the right side, without correlation with the observed movements; no electrographic seizures were noted	No electrographic seizures were noted
NCS	Not performed	Normal
Muscle biopsy	Not performed	Increased variability of fiber diameters, atrophy type 1 fibers > than type 2 fibers
Electron transport chain studies	Not performed	29% activity of cytochrome C oxidase (Table 2)
Brain MRI	Normal	Normal in the newborn period; increased T2 signal in the brainstem and pons at 2 years
Additional Testing	Epilepsy panel negative; mitochondrial sequencing, VUS on SNP array; urine catecholamines, abdominal imaging negative	Karyotype, microarray, SMA testing, methylation studies for Prader-Willi, and transferrin isoelectric focusing were all normal; echocardiogram normal
Differential Considered	opsoclonus myoclonus; mitochondrial disorder; AADC, hereditary hyperekplexia	AADC; mitochondrial depletion syndrome, hereditary hyperekplexia

* AADC: Aromatic L-amino acid decarboxylase deficiency; 5-HIAA: 5-hydroxyindoleacetic acid; HVA: homovanillic acid; 3-O-methyldopa: 3-O-methyl-Dopa; VUS: variant of uncertain significance; SNP: single nucleotide polymorphism

Electron transport chain data for case 2 showing borderline activity of cytochrome C oxidase

Table 2

ETC Complexes		Patient	Prior Controls (Mean $\pm$ SD) N=49	Range	% Patient/Mean
I, III	NADH-cyt. c reductase (rotenone sensitive)	0.2	1.2 $\pm$ 1.1	0.2 – 4.7	16%
I,"	NADH-ferricyanide reductase	34.2	29.9 $\pm$ 12.9	11.5-60.1	114%
II,III	Succinate –cyt. c reductase (antimycin sensitive)	1.3	2.1 $\pm$ 1.2	0.5-4.9	81%
"II"	Succinate dehydrogenase	0.9	0.8 $\pm$ 0.4	0.1-2.0	114%
III	Decylubiquinol-cyt. c reductase	17.4	15.2 $\pm$ 6.8	6.8-35.2	114%
IV	Cytochrome c oxidase	43.9	148.9 $\pm$ 67.2	57.3-373.0	29%
	Citrate synthase	11.7	18.8 $\pm$ 4.7	9.4-30.0	63%
	Lactate dehydrogenase	175.0	$\pm$	-	
	Non-collagen protein (mg/g ww)	75.8	$\pm$	-	