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Novel Mutations in *FA2H*-Associated Neurodegeneration: An Underrecognized Condition?

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Abstract

Hereditary spastic paraplegias and related genetically heterogeneous disorders may be difficult to distinguish clinically. The *FA2H* gene has been associated with autosomal recessive neurodegenerative phenotypes encompassing spastic paraplegia with or without dystonia, and demyelinating leukodystrophy. To date, few individuals with mutations in the *FA2H* gene have been described. We report a 5-year-old girl of mixed Filipino and Vietnamese origin who presented with progressive lower limb spasticity and periventricular leukomalacia. The clinical diagnosis of *FA2H*-associated neurodegeneration was confirmed on the basis of 2 *novel* mutations in compound heterozygosity in the *FA2H* gene (p.S70L/p.P323L). This family highlights that *FA2H*-associated disorders may be underrecognized in children with neurodegeneration of many different ethnicities. Magnetic resonance imaging features play an important role as diagnostic clues in this and other hereditary spastic paraplegias. The consideration of this diagnosis is essential in providing families with important information on prognosis, as well as accurate genetic counseling.

Keywords

hereditary spastic paraplegia, SPG35, leukodystrophy, HSP, spastic paraparesis with dystonia, *FA2H*

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Childhood neurodegenerative disorders are difficult to diagnose.[1] Among these, hereditary spastic paraplegias are a large group of clinically and genetically heterogeneous neurodegenerative disorders. *FA2H*-associated neurodegeneration (OMIM 612319) is a rare autosomal recessive hereditary spastic paraplegia caused by biallelic mutations in *FA2H* (OMIM 611026), mapping to chromosome 16q21-q23.[2] This gene encodes fatty acid 2-hydroxylase, spanning 372 amino acids and 7 exons; it contains a cytochrome b5 heme-binding domain, and a fatty acid hydroxylase domain encoded by exons 5 through 7. *FA2H* is involved in the synthesis of 2-hydroxy fatty acid galactolipids, major components of the myelin sheath. The *fa2h*-deficient mouse model presents with progressive cerebellar motor incoordination and learning and memory deficits.[3] Distal axonal degeneration of motor and sensory neurons causes the characteristic progressive spasticity and weakness of the lower limbs.[4]

Case Report

A 5-year-old girl with a Filipino mother and Vietnamese father was born at term after an uncomplicated pregnancy. She has 5 healthy siblings. Her early milestones were reported as normal. She had onset of lower limb spasticity at 3 years and 2 months of age, first presenting with tip-toe walking, frequent falls, and mild loss of oromotor control. This was rapidly progressive so that by at age 4 she had loss of oromotor control and independent ambulation. Neurologic examination at age 4 years 1 month showed intact cranial nerves, appropriate upper limb and axial tone, spastic lower legs with ankle clonus, leg scissoring, and ankles in equinovarus position at rest. There was mild cognitive decline.

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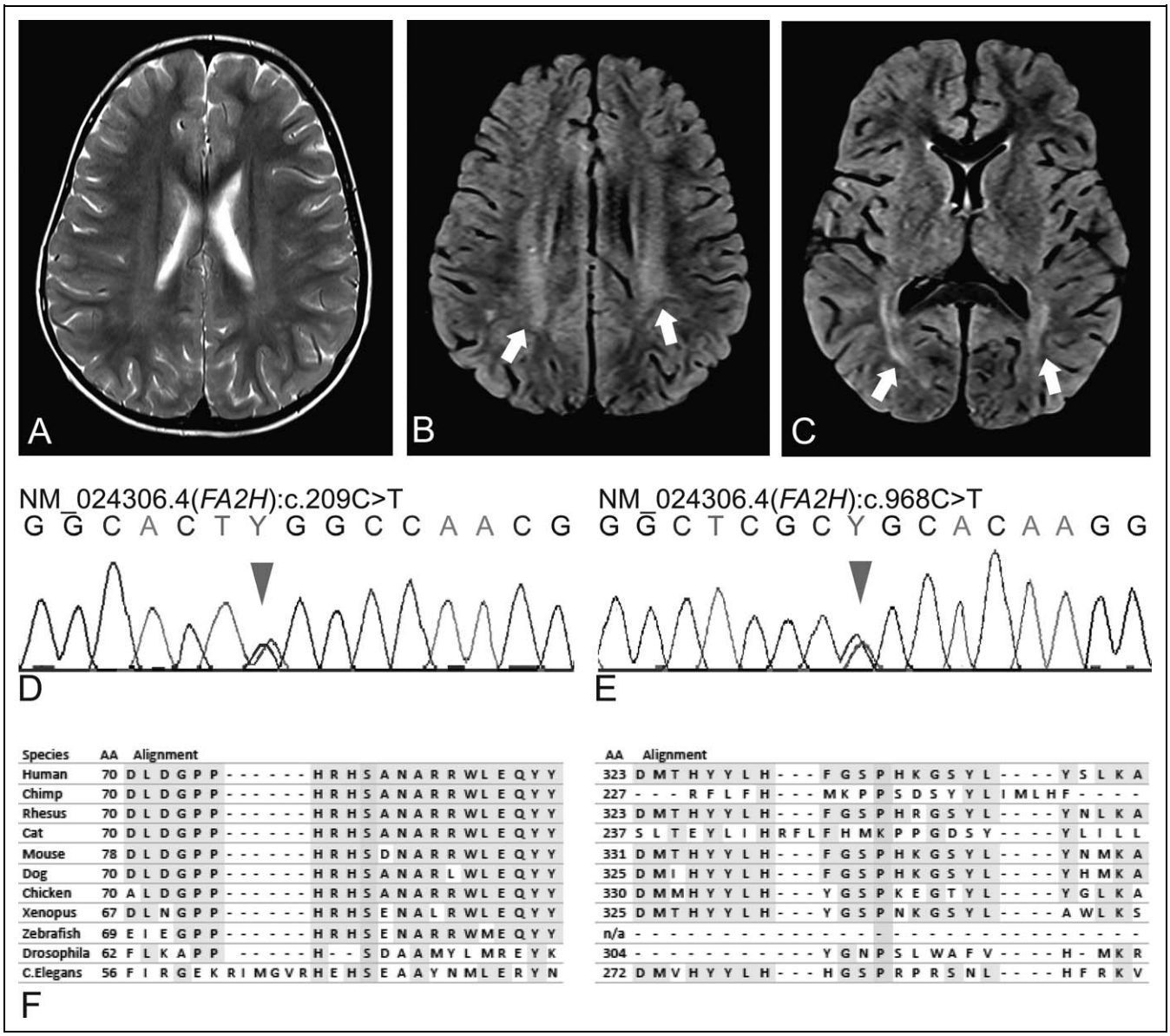

Figure 1. Diagnostic studies in the proband with progressive spastic paraparesis. The brain magnetic resonance imaging showed normal grey/white matter differentiation, and abnormal high-signal-intensity areas in the periventricular white matter with a posterior predominance (white arrows) seen on both axial T2 (A) and axial fluid-attenuated inversion recovery (B, C) sequences. Panels D and E show the partial chromatograms of Sanger sequencing for exons 1 (D) and 6 (E) of the *FA2H* gene, identifying 2 substitutions confirmed to be in trans by parental segregation (data not shown): c.209C>T in exon 1 (maternal), and c.968C>T in exon 6 (paternal), leading to the substitution of highly conserved amino acids as shown by the orthologous sequences (F): p.S70L (left) and p.P323L (right), respectively. AA indicates amino acid position.

The proband's magnetic resonance imaging (MRI) of the brain done at ages 3 years 6 months and 4 years showed mild periventricular leukodystrophy (Figure 1A-C). No iron accumulation was seen. Electromyography and motor and sensory nerve conduction studies were normal; specifically, right sural nerve response amplitude and conduction velocity was within the normal range (31.7 μV and 59 m/s). The proband had a normal echocardiogram and ophthalmologic examination including flash visual evoked response. Metabolic investigations were negative for vitamin responsive, peroxisomal, neurotransmitter, lysosomal storage and nucleic acid metabolism disorders. Infection screen was negative. A clinical diagnosis of hereditary spastic paraplegia type 35 was made based on examination and imaging findings. Molecular testing was initiated for the *FA2H* gene, simultaneously with *C1P7BI*, *KIAA184* and *SPG7* to rule out potential differential diagnoses (Centogene, Rostock, Germany).

Genomic DNA from peripheral blood was extracted using standard techniques. The *FA2H* gene was analyzed by polymerase chain reaction and sequencing of both DNA strands of the entire coding region and the highly conserved exon-intron splice junctions. Two heterozygous mutations in the

FA2H gene, which encodes a fatty acid hydroxylase involved in the production of ceramide species necessary for the synthesis of essential lipid components of normal myelin[5,6] were detected: c.209C>T in exon 1, leading to a p.S70L substitution; and c.968C>T in exon 6, leading to a p.P323L substitution of the primary transcript (Figure 1D, E). Segregation of the mutations from the parents was confirmed by Sanger sequencing. The 2 variants have not been reported in the Single Nucleotide Polymorphism database (dbSNP132), and are not observed in 1094 samples of the 1000Genomes project (release 2010/11/23), the National Heart, Lung, and Blood Institute Exome Sequencing Project,[7] or the HAPmap project (The International HapMap Project, available at http://hapmap.ncbi.nlm.nih.gov/). Both these amino acids are highly conserved (Figure 1F). The p.S570L mutation is predicted to alter a cytochrome b5 heme-binding site. Analysis with programs Polyphen 2 (Polymorphism Phenotyping v2, Harvard Medical School, Boston, MA),[8] SIFT (J. Craig Venter Institute; available at http://sift.jcvi.org), Align GVGD (International Agency for Research on Cancer, available at http://agvgd.iarc.fr), and Mutation Taster (available at http://www.mutationtaster.org), which predict the possible impact of sequence alterations, indicates the p.S70L substitution as highly damaging. The p.P323L mutation is within the fatty acid hydroxylase domain and is predicted to be disease causing by Polyphen 2 and Mutation Taster.

On subsequent clinical follow-up, at 6 years of age, she was ambulant with a walker with assistance. She had limited speech, but could use 3 words at a time, with preserved receptive language. There was hypophonia and bradylalia as well as mild dysphagia. She had vertical nystagmus with upward gaze. She had mild dysmetria. She had lower limb spasticity, with sustained ankle clonus and upgoing plantars; upper limbs were only mildly involved.

Discussion

In this clinical report, we describe a 5-year-old girl who presented with progressive lower limb spasticity and periventricular leukomalacia and was diagnosed clinically with dysmyelinating leukodystrophy and spastic paraparesis with or without dystonia /autosomal recessive spastic paraplegia 35 (SPG35; OMIM 612319). She was compound heterozygous for 2 *novel* mutations in the *FA2H* gene.

Biallelic mutations in *FA2H* have been associated with a broad spectrum of neurodegeneration, which includes spastic paraparesis (spastic paraplegia 35) with or without dystonia,[2,5,9,10] demyelinating leukodystrophy,[2] and Neurodegeneration with Brain Iron Accumulation (NBIA).[6] A total of 28 patients with *FA2H*-associated neurodegeneration have previously been reported: from 5 Middle Eastern, South Asian, and southern European consanguineous families, and 2 families with no reported consanguinity (Table 1).

All affected patients, except for 1 had homozygous mutations identified in *FA2H*; recently, Pierson et al[10] reported on a young boy with compound heterozygous mutations in the *FA2H* gene detected using single nucleotide polymorphism analysis and exome sequencing. A progressive gait disorder, spasticity, and weakness of the lower limbs were seen in all reported patients, with upper limbs rarely affected. Variably seen features include later-onset dystonia, cognitive decline, seizures, optic atrophy, and leukodystrophy. This proband and at least 8 other reported cases[2,5,6,9] in whom electromyography and sensory nerve conduction studies were performed (and reported) had normal results. Only 1 case was reported to have an axonal sensory neuropathy, with a previous normal sural nerve biopsy.[10] It is unclear whether this may be a low penetrance or later onset feature of *FA2H*-associated neurodegeneration, and/or the effect of patient-specific genetic background. *fa2h*-deficient mice show late-onset axon and myelin sheath degeneration.[13] Nerve conduction velocities of adult *fa2h* null mice do not differ from wild type in adult mice; however, data for aged mice has not been reported. Further investigations and long-term follow-up of patients may provide additional information.

Pierson et al[10] proposed a correlation of mutation type and disease severity; however, the age of onset, presence of dystonia, seizures, dysarthria/dysphagia, and cognitive decline in the reported cases[2,5,6,9,11] (Table 1) do not correlate with the type of mutation or the involvement of the fatty acid hydroxylase domain of *FA2H* in the mutation as demonstrated by the present report, and the Italian patients reported by Kruer et al.[6] No clear genotype-phenotype correlation can be made at this time. We admit this may be due to the few cases reported.

To date, 54 clinical forms of hereditary spastic paraplegia have been described. Mutations in 25 different genes are found as an underlying cause in more than half; the underlying gene is unknown in the remainder. The various types of hereditary spastic paraplegias are often difficult to distinguish clinically so that classification based on the underlying genotype has replaced that based on phenotype.[14] The European Federation of Neurological Societies has recently made recommendations for sequential testing for the most common forms of spastic paraplegias (see reference).[15] We add that spastic paraplegia 35 with mutation testing for the *FA2H* gene should be considered in childhood onset of progressive pyramidal tract dysfunction. Periventricular leukomalacia, though not invariably present, is an important diagnostic handle (Table 1). Given that patients may not present with a characteristic phenotype, and that the relatively recent description of this condition may underestimate phenotypic heterogeneity, the advent of next generation sequencing technologies is appealing for the clinical diagnosis of clinically heterogeneous disorders, such as hereditary spastic paraplegias.[16] Pippucci et al[17] used a whole exome sequencing and homozygosity mapping to demonstrate the utility of the approach in a previously reported family.[5] However, it was Pierson et al[10] that provided the first report of a compound heterozygous patient diagnosed using whole exome sequencing. Thus far, only 2 instances of compound heterozygosity have been reported including the present report. We propose that *FA2H*-associated neurodegeneration is an underdiagnosed condition, and is an important diagnostic consideration in individuals with spastic paraparesis of all ethnicities,

Table 1. Reported Cases With *FA2H*-Associated Neurodegeneration

Individual		Ethnicity	Consanguinity	DNA change	Protein change	Age of onset (y)	FAH domain intact	Dystonia	Cognitive Decline	Leukodystrophy	Spasticity	Seizures	Dysarthria
Edvardson et al[9]	1-122	Israeli Arab	Yes	c.[786+1G>A]; [786+1G>A]	? exon 4-5 skipping	4.5	–	+	+	+	+	+	+
	1- 21	Israeli Arab	Yes	Idem	Idem	6	–	+	+	+	+	–	+
	1-1211	Israeli Arab	Yes	Idem	Idem	5	–	+	+	+	+	–	+
	2-2761	Israeli Arab	Yes	c.[786+1G>A]; [786+1G>A]	? exon 4-5 skipping	4.5	–	+	+	+	+	+	+
	2-2758	Israeli Arab	Yes	Idem	Idem	4.5	–	+	+	+	+	–	+
	2-2762	Israeli Arab	Yes	Idem	Idem	5	–	+	+	+	+	–	+
	2-2769	Israeli Arab	Yes	Idem	Idem	4	–	+	+	+	+	–	+
	3-2327	Israeli Arab	Yes	c.[103G>T]; [103G>T]	p.[D35Y]; [D35Y]	4	+	–	–	nt	+	–	–
	3-2328	Israeli Arab	Yes	Idem	Idem	6	+	–	–	–	+	–	–
Dick et al[2,11]	VII-2	Omani	Yes	c.[703C>T]; [703C>T]	p.[R235C]; [R235C]	6	–	?	+	+	+	–	+
	VII-6	Omani	Yes	Idem	Idem	6	–	?	–	nr	+	+	+
	VII-12	Omani	Yes	Idem	Idem	10	–	?	+	nr	+	–	+
	VIII-3	Omani	Yes	Idem	Idem	11	–	?	+	nr	+	–	+
	VIII-5	Omani	Yes	Idem	Idem	11	–	?	+	nr	+	–	+
	VIII-6	Omani	Yes	Idem	Idem	9	–	?	–	nr	+	+	+
	VIII-7	Omani	Yes	Idem	Idem	9	–	?	+	nr	+	–	nt
	IV-1	Pakistani	Yes	nt[b]		nr		+[c]	+[c]	nr	+[c]	+[c]	nr
	IV-2	Pakistani	Yes	nt[b]		nr		+[c]	+[c]	nr	+[c]	+[c]	nr
	IV-3	Pakistani	Yes	c.[157-174del]; [157-174del]	p.[R53_I58del]; [R53_I58del]	nr	+	+	+	nr	+	+	nr
	IV-8	Pakistani	Yes	Idem	Idem	4	+	+	+	+	+	+	+
Garone et al[5]	IV-2	Italian	Yes	c.[270+3A>T]; [270+3A>T]	? loss of exons 2-7	7	–	–	+	+	+	+	+
	IV-4	Italian	Yes	Idem	Idem	4	–	–	+	+	+	–	nr
Kruer et al[6]	II-1	Italian	Yes	c.[460C>T]; [460C>T]	p.[R154C]; [R154C]	4-5	+	–	–	+	+	1 of 3	+
	II-3	Italian	Yes	Idem	Idem	4-5	+	–	–	+	+	1 of 3	+
	II-4	Italian	Yes	Idem	Idem	4-5	+	–	–	+	+	1 of 3	+
	I	Albanian	None known[a]	c.[509_510delAC]; [509_510delAC]	p.[Y170X]; [Y170X]	4	–	+	+	+	+	+	+
	2	Albanian	None known[a]	Idem	Idem	3	–	+	+	+	+	–	+
Pierson et al[12]	II-2	nr	No	c.[707T>C]; c.[507-?_1119+?del]	p[F263S]; ? loss of exons 3-7	3		+	+	+	+	–	+
Current case		Filipino/ Vietnamese	No	c.[209C>T]; [968C>T]	p.[S70L]; [P323L]	3	+/–	–	+	+	+	–	+

Abbreviations: idem, the same as previous; NCV, nerve conduction velocity studies; nr, not reported; nt, not tested; ?, "possible" dystonia reported.
[a]From same small village.
[b]Same genotype assumed.
[c]"Similar phenotype" reported.

whether from consanguineous families or not, particularly in children with periventricular leukomalacia.

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Author Contributions

RR wrote the first draft and subsequent revisions of the manuscript under the guidance of CD. JH, SM, MB, and AR reviewed the manuscript and provided thoughtful insights. CD, JH, and SM clinically assessed the patient. AR performed the genetic testing. MB and RR provided family history and genetic background information. The final draft was approved by all authors. SM is currently affiliated with the Department of Pediatrics, University of Toronto and The Hospital for Sick Children, Toronto.

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Ethical Approval

Individual case reports are considered to be a medical/educational activity by the University of British Columbia's research ethics boards (REBs) and may be published without ethical review when patients are made aware of the publication. The authors have complied with these requirements.

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