De Novo Mutations in the Motor Domain of KIF1A Cause Cognitive Impairment, Spastic Paraparesis, Axonal Neuropathy, and Cerebellar Atrophy


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ABSTRACT: KIF1A is a neuron-specific motor protein that plays important roles in cargo transport along neurites. Recessive mutations in KIF1A were previously described in families with spastic paraparesis or sensory and autonomic neuropathy type-2. Here, we report 11 heterozygous de novo missense mutations (p.S58L, p.T99M, p.E253K, and p.R316W) in KIF1A that are found in unrelated cases. All these de novo mutations affect the transport of the mitochondria (MD) along the MD. Transfection studies suggested that at least five of these mutations affect the transport of the MD along axons. Individuals with de novo mutations in KIF1A display a phenotype characterized by cognitive impairment and variable presence of cerebellar atrophy, spastic paraparesis, optic nerve atrophy, peripheral neuropathy, and epilepsy. Our findings thus indicate that de novo missense mutations in the MD of KIF1A cause a phenotype that overlaps with, while being more severe, than that associated with recessive mutations in the same gene.


KEY WORDS: KIF1A; intellectual disability; spastic paraparesis; axonal neuropathy; de novo mutations

Introduction

The Kinesin family proteins (KIFs) are microtubule-dependent molecular motors that participate in the transport of membrane vesicles/organelles, protein complexes, and mRNAs along neurites,
thus playing important roles in neuronal function [Hirokawa et al., 2009]. KIF1A is a neuron-specific motor protein composed of an N-terminal motor domain (MD) followed by a neck coil, a C1C1-FHA-CC2-CC3, a liprin-α-binding and PH domains [Shin et al., 2003]. KIF1A is responsible for fast anterograde transport of synaptic vesicles (SV) precursors along axons [Okada et al., 1995; Lee et al., 2003]. Through its interaction with the scaffolding protein liprin-α, KIF1A also transports cargo vesicles containing postsynaptic proteins, such as GRIP, GIT1, and AMPA receptors, which play an important role in synaptic plasticity and transmission as well as learning and memory [Ko et al., 2003; Shin et al., 2003; Huganir and Nicoll, 2013].

Recessive mutations in KIF1A (MIM# 601253; NM_001244008.1) have been described in cases with neurodegenerative disorders. Homozygous and compound heterozygous truncating mutations (p.L947fs+4 and p.S1758fs+7), located downstream of the KIF1A MD, were first reported in individuals with peripheral nerve degeneration causing a condition known as hereditary sensory and autonomic neuropathy type (HSAN2) [Riviere et al., 2001]. Two additional homozygous missense mutations (p.A255V and p.R350G), affecting the distal part of the KIF1A MD, were later described in three consanguineous families with autosomal recessive hereditary spastic paraparesis (HSP; Spastic Paraplegia-30, SPG30) [Erlich et al., 2011; Klebe et al., 2012].

In parallel, we previously reported a de novo missense mutation (p.T99M), affecting the ATP-binding site of the MD of KIF1A, in a child with developmental delay and cerebellar atrophy [Hamdan et al., 2011]. Although we found that this mutation affected the transport of KIF1A MD along axons, its pathogenicity remained uncertain. Here, we identified, by exome sequencing and targeted gene sequencing, 13 additional patients with de novo missense mutations in the KIF1A MD. These patients display a complex neurologic phenotype characterized by moderate to severe developmental delay and/or intellectual disability (ID), variable cerebellar atrophy, visual loss, spastic paraparesis, peripheral neuropathy, and epilepsy. Our findings thus provide further evidence that de novo missense mutations in the MD of KIF1A cause a phenotype that overlaps with, while being more severe, than that associated with recessive mutations in the same gene.

Methods

Subjects and Mutation Analysis

The affected individuals with de novo mutations in KIF1A were recruited from different countries, including Canada (patients 1, 6, 11, 12), USA (patients 2, 7, 8), the Netherlands (patients 4, 5, 9, 10, 13, 14), and Finland (patient 3). Informed consent was obtained from each participant or legal guardian and the study was approved by the ethics committee of the CHU Sainte-Justine Hospital (Montreal, Quebec, Canada). Exome capture and sequencing was done on a clinical basis at three different facilities: Whole Genome Laboratory and Medical Genetics Laboratory at Baylor College of Medicine (BCM) (patients 6–8; Roche Nimblegen [Madison, WI, USA] VCRome v2.1 exome capture and HiSeq2000 sequencing), GeneDx (patient 2; Agilent SureSelect XT2 v4 [Santa Clara, CA, USA] capture and HiSeq2000 sequencing), and Radboud university medical center (Radboudumc) (Agilent SureSelect XT 50Mb capture and HiSeq2000 [patients 4, 5, 10, 13, 14] or SOLiD 5500xl [patient 9] sequencing). Read processing, mapping to human genome reference hg19, variant calling, annotations, and filtering for rare variants (minor allele frequency ≤1%–5%) affecting the coding sequence and/or consensus splice sites were performed as previously described [Neveling et al., 2013; Yang et al., 2013; Dhamija et al., 2014]. Briefly, variants affecting coding and splice sites that were present at minor allele frequencies ≤1%–5% in public databases (e.g., 1000 Genomes, NHLBI Exome Sequencing Project [ESP] Exome Variant Server [EVS]) and in in-house control datasets were selected. Among these variants, only those present in known disease genes (OMIM, HGMD) or in specific sets of disease genes relevant to the phenotype of interest (patients 4, 9, 10 filtered against a panel of 200 genes known disease genes causing movement disorders: http://www.radboudumc.nl/Informatievoorverwijzers/Genoomdiagnostiek/Users/folders/ngs-movement_disorders_panel_181213.pdf) were further considered. For patients 2, 5, 10, exome sequencing was also performed in their unaffected parents, facilitating the identification of de novo mutations [de Ligt et al., 2012]. The exome of patient 12 was captured (Agilent SureSelect 50 Mb) and sequenced (HiSeq2000) as part of the Care4Rare Canada research project at the McGill University and Genome Quebec Innovation Centre (Montreal, Canada). Sequence analysis and filtering for rare variants in known disease OMIM genes previously associated with movement disorders and ID were done as previously described [Sourr et al., 2012].

Only genes associated with hereditary spastic paraplegia genes, including KIF1A, were sequenced in patient 3, as previously described [Ylikallio et al., 2014]. Target enrichment was done with the HaloPlex system (Agilent) and sequencing was performed with the MiSeq system (Illumina). The data were processed as previously described [Ylikallio et al., 2014].

Nucleotide numbering of the mutations herein reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the NCBI reference sequence NM_001244008.1, while the amino positions are based on the corresponding NCBI reference sequence NP_001230937.1. All de novo KIF1A mutations identified herein have been submitted to the ClinVar database: http://www.ncbi.nlm.nih.gov/clinvar/?term=KIF1A. Predictions of the effects of the de novo missense KIF1A mutations on the protein function were done using PolyPhen-2 [Adzhubei et al., 2010] (version 2.2.2r398; http://genetics.bwh.harvard.edu/pph2/) and SIFT [Kumar et al., 2009] (version1.03, SIFT/PROVEAN Human SNPs; http://sift.jcvi.org/), respectively.

Structural Modeling of the KIF1A MD

The structural modeling was based on the crystal structure of the MD of KIF1A [PDB ID: 1VFV; http://www.rcsb.org/pdb/home/home.do]. All structural images were generated using the PyMOL (version 1.3; http://www.pymol.org/) molecular visualization software [Schrödinger, 2010]. The mutations of interest were introduced in the models by using the mutagenesis option of PyMOL with backbone-dependent rotamer parameters. Energy minimization and loop flexible modeling were performed by using the loop modeling function in the Molder software and default parameters (version 9.13; https://salilab.org/modeller/about_modeller.html) [Fiser et al., 2000].

Transfection Studies

Mutations in KIF1A were introduced by site-directed mutagenesis into a mouse cDNA expression construct encoding KIF1A MD (aa 1–365) (KIF1A-MD-EGFP) [Lee et al., 2004]. Primary hippocampal...
De Novo Mutations in KIF1A

We previously reported the identification of a de novo missense mutation in KIF1A (c.296C>T [p.T99M]; NM_001244008.1) in the context of a project that aimed to sequence candidate synaptic genes in individuals with ID (patient 1 in [Hamdan et al., 2011]). Here, we report the identification of de novo missense mutations in KIF1A in 13 additional patients, including 2 monozygotic twins, with developmental delay or ID. These mutations were identified by exome sequencing (12 families) or targeted next-generation sequencing (1 family). Sanger sequencing confirmed that these mutations were present in the blood DNA of the probands but not in their parents. These heterozygous KIF1A mutations included: c.173C>T (p.S58L), c.296C>T (p.T99M), c.305G>A (p.L249Q), c.430G>T (p.V144F), c.499C>T (p.R167C), c.604G>T (p.T99M), c.643A>G (p.R216P), c.746C>T (p.V144F), c.757G>A (p.E253K), and c.946C>T (p.S316F) (positions according to NCBI RefSeq NM_001244008.1) (Fig. 1A). Interestingly, both c.296C>T (p.T99M) and c.757G>A (p.E253K), which affect CpG dinucleotides, were recurrent, each being identified in 2 unrelated patients (Table 1).

All of these mutations are predicted to be damaging according to SIFT (damaging, score: 1.00) and Polyphen-2 (probably damaging; HumDiv score 1.00; sensitivity: 0.00, specificity: 1.00) and affect well-conserved residues (Fig. 1B). All these mutations were absent from public SNP databases (dbSNP138, 1000 Genomes and EVS) and from >3500 exomes (in-house and Radboudumc databases).

Structural Impact of De Novo Missense Mutations in the MD of KIF1A

All of the 11 de novo KIF1A mutations identified here affect residues located in the MD of KIF1A (Fig. 1). The crystal structures of the MD of KIF1A in complex with diverse ATP/ADP analogues such as AMP-PCP, ADP, AMP-PNP, ADP-vanadate, and ADP-AlFx have been determined [Kikkawa et al., 2001; Nitta et al., 2004]. These studies have elucidated the molecular mechanisms underlying ATP hydrolysis, microtubule binding, and movement of KIF1A. ATP hydrolysis by KIF1A induces conformational changes in two important structures of the MD, termed the switch I region (helix α3, loop L9, and β sheet β12) and the switch II cluster.
<table>
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<th>Gender</th>
<th>Age (years)</th>
<th>GDD/ID</th>
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<th>Language development</th>
<th>Optic nerve atrophy</th>
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Note: 
- Gender: F, female; M, male.
- Age: years or months.
- GDD/ID: Global Developmental Delay/Intellectual Disability.
- Ambulation: Walks independently (indep.), Walks with aid.
- Language development: Delayed sentences, Normal.
- Optic nerve atrophy: Yes, No.
- Microcephaly: Yes, No.
- Epilepsy: Yes, No.
- Ataxia: Yes, No.
- Spasticity: Spastic parapar., Spastic.
- Neuropathy: Yes, No.
- MRI findings: -Cerebellar atrophy, -Cerebral atrophy, -Optic nerve atrophy.

* Nerve conduction studies/electromyography performed.
* No investigations performed.
* Muscle biopsy.
Figure 2. Functional influences of KIF1A mutations based on structural modeling. A: Overall structure of the motor domain of KIF1A [PDB ID: 1VFV]. Three missing loops, L9, L11, and L12, are indicated by dashed lines. The switch I, switch II, P-loop, and neck linker are indicated in blue, magenta, green, and yellow, respectively.

B: Locations of KIF1A mutations in the motor domain. The mutation-related amino acid (aa) residues from this study and a previous study on recessive HSP (A255 and R350) are represented by a stick model with red and black label, respectively. The non-hydrolysable ATP analogue AMP-PNP (adenylyl imidodiphosphate) in the structure is represented by a stick model.

C: A model for KIF1A binding to tubulin. The crystal structure of KIF1A [PDB ID: 1VFV] was superimposed to KIF5B in complex with tubulin [PDB ID: 4HNA]. The microtubule binding mode of KIF1A was modelled by energy minimization using the Modeller software. The α- and β-tubulin are labeled. R167 in the loop L8 (shown in red) is represented by a stick model.

D: Location of R316 in the KIF1A motor domain. R316 and E170, which interacts with R316 by hydrogen bonds, are represented by a stick model.

E: Mutation-related KIF1A residues around the nucleotide-binding pocket of KIF1A and their roles in ATP binding (S58, T99, G102, and S215) and salt bridge formation for the back door (R216 and E253). The AMP-PNP and aa residues in the structure are represented by a stick model. The αP, βP, and γ-phosphate of AMP-PNP. The residues involved in ATP binding but not mutated are represented by a stick model and labeled with black color.

F–P: Structural influences of KIF1A mutations. Conformational changes in the loop structures (H, I, L, M, N, P), modelled using the Modeller software, are indicated by arrows. All structural images were generated using PyMOL.
suggests that the p.T99M and p.S215R mutations would abolish ATP binding of the MD. Therefore, the mutations affecting these residues (p.S58L, p.R316W) are expected to disrupt ATP binding of the MD. Specifically, inspection of the structural models suggests that the p.T99M and p.S215R mutations would abolish the interactions of KIF1A with the γ-phosphate of ATP (Fig. 2F and G). The p.G102D mutation, which lies in the conserved P-loop (GX,G[TH/S]) [Nider and Houry, 2008] is predicted to induce a significant conformational change in the loop structure (Fig. 2H), also disrupting the interaction with the phosphate region of ATP. The p.S58L mutation is likely to disrupt the conformation of L2 containing the Y67 residue, which contributes to ATP binding by interacting with the adenine group of ATP molecule (Fig. 2I).

After ATP hydrolysis, the γ-phosphate is released from the nucleotide-binding pocket through a specialized structure termed the back door, which represents the salt bridge formed by the ion interaction between conserved R216 (loop L9) and E253 (loop L11) [Nitta et al., 2004] (Fig. 2B and E). The p.R216P and p.E253K mutations in KIF1A, which directly change the structure and charge of the salt bridge-forming residues, respectively, are predicted to disrupt the structure of the back door and thus suppress γ-phosphate release, additional ATP binding, and conformational changes in switch I and II (Fig. 2J and K). Indeed, substitutions of these salt bridge-forming residues in the back door by alanine in the related motor protein Kar3 (p.R598A and p.E631A) abolish ATP hydrolysis [Yun et al., 2001]. The p.L249Q mutation, which is located on L11 in close proximity to the salt bridge-forming residue E253, would alter the conformation of L11, likely affecting the structure of the back door and γ-phosphate release (Fig. 2L). In addition, the A202 residue is located in the switch I loop I loop L9, in proximity to the salt bridge-forming residue R216. L9 is known to undergo beta-to-alpha conformational transition during γ-phosphate release [Nitta et al., 2004]. The p.A202P mutation is predicted to induce a significant change in the conformation of this loop, likely disrupting the structure of the back door or the efficient γ-phosphate release and additional ATP binding (Fig. 2M). The p.V144F mutation located on the β-strand (β7) near the α3 helix in the switch I region is likely to disrupt the helical structure of α3 and convert it into a loop structure (Fig. 2N). This might destabilize the whole switch I region, including L9, and thus affect the ATP binding or γ-phosphate release of the MD.

Binding of KIF1A to microtubules is critical for its movement along the neurites. The R167 residue is located in the loop L8 (Fig. 2A and B), which binds the microtubules in an ATP hydrolysis-independent manner [Kikkawa et al., 2001; Nitta et al., 2004]. The p.R167C mutation may thus weaken the binding of L8 to microtubules by suppressing the interaction between R167 and a negatively charged region in tubulin (Fig. 2C and O). In addition, R316 on the helix α5 of the switch I cluster is thought to stabilize L8 by forming two hydrogen bonds with E170 on L8 (Fig. 2D). The p.R316W mutation is predicted to disrupt this interaction and L8-dependent microtubule binding (Fig. 2P).

Two homozygous missense mutations in KIF1A, p.A255V and p.R350G, have been shown to cause recessive HSP (SPG30) [Klebe et al., 2012]. As these mutations also affect the MD of KIF1A, we also looked at their potential impact on its structure. The A255 residue is located in loop L11 of the switch II cluster whose detailed structure is currently unknown. However, A255 is situated very close to E253 (two residues away), which directly participates in the formation of the back-door salt bridge (Fig. 2B). Therefore, the A255V mutation is likely to affect KIF1A function by altering the structure of L11 (in switch II) or the back door. Although the modelled structure does not predict any interaction between R350 and microtubules or other parts of the MD, this residue is in close proximity to the neck domain, which links the MD with the cargo-binding regions (Fig. 2B). The p.R350G mutation might thus affect KIF1A function by altering the structure of the neck linker.

Functional Impact of KIF1A MD Missense Mutations

We previously showed that the expression of EGFP tagged KIF1A MD (aa 1–365) (KIF1A-MD-EGFP) in rat hippocampal neurons results in its dramatic accumulation in distal regions of the neurites in a large proportion of the transfected cells [Lee et al., 2004]. In contrast, KIF1A-MD-EGFP with a point mutation (p.T312M) that impairs the activity of the MD showed a greatly reduced P.A. [Lee et al., 2003; Lee et al., 2004]. We previously used this functional assay to test the impact of p.T99M on the MD of KIF1A and found that EGFP-KIF1A-MD-T99M also showed greatly reduced distal localization in neurites [Hamdan et al., 2011]. We employed here the same strategy and expressed the wild-type (WT) or selected mutant KIF1A-MD-EGFP constructs in hippocampal neurons and monitored their localization in neurites (Fig. 3A). WT KIF1A-MD showed extensive P.A. (93.2%, 8 trials), but each of the five mutants that were tested showed greatly reduced distal localization compared with WT: KIF1A-MD-T99M (15.9%, 5 trials), KIF1A-MD-A202P (9.9%, 7 trials), KIF1A-MD-S215 (13.5%, 7 trials), KIF1A-MD-R216P (13.4%, 7 trials), KIF1A-MD-E253K (19.3%, 7 trials) (Fig. 3B). Transfection of these constructs in HEK293 cells followed by Western blotting indicated that the mutant and WT KIF1A-MD proteins were expressed at similar levels (Supp. Fig. S1).

We also assessed the impact of point mutations (p.A255V and p.R350G) identified in cases with HSP on the localization profile of KIF1A-MD (Fig. 4A). KIF1A-MD-A255V showed mildly reduced distal localization (80.8%, 5 trials) when compared with the WT MD (Fig. 4B). In contrast, the distal localization of KIF1A-MD-R350G was comparable to that associated with T99M (20.7%, 5 trials) (Fig. 4B). The proximal distribution of KIF1A-MD-A255V and KIF1A-MD-R350G was increased when compared with the WT MD but decreased when compared with KIF1A-MD-T99M (Fig. 4C). Overall, these results suggest that the motor activity of KIF1A-MD-A255V and KIF1A-MD-R350G is decreased but not as much as in KIF1A-MD-T99M.

Clinical Phenotype of Patients with De Novo Missense Mutations in KIF1A

A summary of the clinical features of the affected individuals is presented in Table 1. A more detailed description of the characteristics and evolution of each patient is available in the Supporting Information section. The main clinical features of the patients with de novo mutations in the MD in KIF1A were: (1) moderate to severe developmental delay or ID (all cases), (2) cerebellar atrophy on MRI (9/14 cases) with variable cerebral atrophy (see Fig. 5 for
Figure 3. Functional impact of KIF1A motor domain de novo missense mutations. A: EGFP-tagged motor domain (MD) constructs of KIF1A were expressed in cultured hippocampal neurons and visualized by immunofluorescence staining with anti-EGFP antibody. The MD of wild-type (WT) KIF1A (KIF1A-MD) accumulated in distal regions of the axons (arrow heads), but the KIF1A-MD of the tested mutants showed a greatly reduced peripheral accumulation (P.A.). The neuronal cell bodies (arrow) and dendrites were visualized by immunofluorescence using anti-MAP2 antibody. B: Quantitative analysis of the P.A. of KIF1A-MD (mean±s.e.m.). The distal distribution of KIF1A-MD was analyzed as described in the methods section. Significant decreases (*, compared with WT) are indicated.
Figure 4. Functional impact of KIF1A motor domain recessive missense mutations. A: EGFP-tagged motor domain (MD) constructs of KIF1A were expressed in cultured hippocampal neurons and visualized by immunofluorescence staining with anti-EGFP antibody. The MD of WT KIF1A (WT KIF1A-MD) accumulated primarily in distal regions of the axons (arrow heads), but the KIF1A-MD-T99M showed a greatly reduced peripheral accumulation (P.A.). KIF1A-MD-A255V and R350G showed distinguishable phenotypes from WT or T99M mutant. The neuronal cell bodies (arrow) and dendrites were visualized by immunofluorescence using anti-MAP2 antibody.

B: Quantitative analysis of the P.A. of KIF1A-MD (means ± s.e.m.). The distal distribution of KIF1A-MD was analyzed as described in the methods section. Significant decreases (∗) are indicated.

C: Quantitative analysis of the proximal distribution of KIF1A-MD (means ± s.e.m.). The proximal distribution of KIF1A-MD was analyzed by measuring the brightness of the proximal neurites (blue asterisk) and the soma (red asterisk) as described in the methods section. Significant increases (+) are indicated.

mutations are pathogenic. First of all, these mutations appear to disrupt KIF1A function. They affect evolutionary conserved residues located in the MD of the protein and are predicted to be highly damaging by several in silico algorithms and structural modelling. Indeed, functional studies of 5 of these mutations suggested that they all affect the ability of the MD to localize to distal aspects of neurites. In addition, all the patients with de novo mutations in KIF1A share a similar phenotype. They presented with moderate to severe developmental delay or ID and the majority showed cerebellar atrophy, optic nerve atrophy, axonal sensorimotor neuropathy, and spastic paraparesis. Importantly, some aspects of the phenotype appear to be progressive as shown by the course of spastic paraparesis, neuropathy and cerebellar atrophy in these patients. Finally, sequencing the exome or relevant gene panels in these patients
did not reveal any other rare predicted-damaging variants in genes known to be associated with ID, neurodegenerative, or movement disorders.

Interestingly, the most severely affected individuals carry the same p.E253K mutation. These two individuals presented at birth with severe hypotonia, respiratory insufficiency, and axonal sensorimotor neuropathy of the lower limbs. They had severe developmental delay, were non-verbal, non-ambulatory, with facial diplegia, ptosis, and swallowing dysfunction resulting in recurrent aspiration pneumonias. These patients were noted to be spastic in the upper limbs but not in the lower limbs, as the important peripheral neuropathy was likely masking the upper-motor neuron signs. Their phenotype reflects significant involvement of both the central nervous system—at the level of the brain and spinal cord—and peripheral nervous system. Both patients died at an early age from complications of respiratory tract infections.

There is overlap in the clinical features of our patients and those previously reported carrying recessive mutations in KIF1A resulting in HSAN2 or SPG30. Two mutations (p.L947Rfs*4 and p.S1758Qfs*7) were described in individuals with HSAN2, both of which are truncating and situated downstream of the MD (Fig. 1). The p.L947Rfs*4, found homozygous in three families and in trans (compound heterozygous) with the distal p.S1758Qfs*7 mutation in 1 family, affects an alternatively spliced exon of KIF1A that is present in a subset of the KIF1A transcripts [Riviere et al., 2011]. The HSAN2 patients who were homozygous for the p.L947Rfs*4 mutation in the alternate transcript had a pure peripheral phenotype with severe axonal predominantly sensory neuropathy, and absence of central nervous system abnormalities. On the other hand, the individual who was compound heterozygous for the p.L947Rfs*4 mutation and p.S1758Qfs*7 only displayed axonal sensory neuropathy but also language delay and borderline intelligence (IQ 80) with normal brain imaging, suggesting central nervous system involvement as seen in our patients with de novo mutations in the MD of KIF1A.

Two homozygous missense mutations (p.A255V and p.R350G) were identified in three consanguineous families with HSP [Erlich et al., 2011; Klebe et al., 2012]. Although these mutations also target the KIF1A MD, the patients were less severely affected than the ones with the de novo mutations. In addition to HSP, a subset

Figure 5. Brain MRI of individuals with de novo mutations in KIF1A. A–J: Sagittal T1 images. A′–H′: Axial T2 images. I′: Axial T1 image. MRI was performed in patient 1 at age 2 years (A, A′), in patient 2 at age 8 months (B, B′), in patient 3 at age 22 years (C, C′), in patient 4 at age 11 years (D, D′), in patient 5 at age 9 months (E, E′), in patient 6 at age 14 months (F, F′), in patient 8 at age 5 days (G, G′) and at 15 months of age (H, H′), in patient 9 at 3 months of age (I, I′), and in patient 10 at age 2 years (J, J′). Note cerebellar hypoplasia (arrow), thinning of corpus callosum (arrow head) and cerebral atrophy with widening of the sulci, ventricular dilatation and decreased posterior white matter volume. Dashed arrow indicates a hyperintense lesion in the left thalamus of patient 4.
of affected individuals (64%) also had a concomitant peripheral sensorimotor neuropathy and signs of cerebellar impairment (21%). With the exception of one patient who had mild cerebellar atrophy, they did not show cerebellar abnormalities on the brain MRI. None of the patients had developmental delay or cognitive impairment, and the parents carrying single heterozygous mutations were reportedly asymptomatic. These patients have primarily spinal cord involvement with the majority also exhibiting peripheral nerve dysfunction, but mainly sparing the brain. It is possible that the observed milder phenotypes associated with these HSP mutations are due to the fact that they may be less detrimental to KIF1A function. Indeed, our structural modelling (Fig. 2) and functional testing (Fig. 4) suggest that both HSP mutations maybe less deleterious to the KIF1A MD function than the de novo ones.

KIF1A was initially considered as a globular monomer because it lacks the long stalk region and has the positively charged K loop. However, it was also shown that KIF1A could dimerize through the coiled coil formation in the neck region, and be converted into a functional motor after dimerization in vivo [Tomishige et al., 2002]. The interaction between the FHA and CC2 domains was shown to regulate the dimerization and activity of the KIF1A motor [Lee et al., 2004]. Moreover, it was shown that only KIF1A dimeric motors, not monomeric motors, undergo ATP-dependent progressive motility although monomeric motor could undergo one-dimensional diffusion [Hammond et al., 2009]. Recently, evidence from protein crystallography showed that dimerization of the CCI-FHA tandem could promote KIF1A dimer formation and motor activity [Huo et al., 2012; Yue et al., 2013]. Collectively, these findings indicate that KIF1A functions as an active dimer motor along microtubules in living cells, raising the possibility that the deleterious de novo mutations in KIF1A may be exerting a dominant negative effect, potentially explaining why the associated phenotype is more severe than that observed in patients with recessive mutations.

In conclusion, we have shown that damaging de novo missense mutations in the MD of KIF1A cause an earlier onset and a more severe neurological presentation than the previously published recessive mutations involved in HSP and HSAN2, thus further expanding the phenotypic spectrum associated with mutations in this gene. More work is needed to elucidate the mechanisms by which these de novo mutations disrupt brain function and development.

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