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ORIGINAL ARTICLE

De novo KIF1A mutations cause intellectual deficit, cerebellar atrophy, lower limb spasticity and visual disturbance

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Recently, *de novo KIF1A* mutations were identified in patients with intellectual disability, spasticity and cerebellar atrophy and/or optic nerve atrophy. In this study, we analyzed a total of 62 families, including 68 patients with genetically unsolved childhood cerebellar atrophy, by whole-exome sequencing (WES). We identified five *de novo* missense *KIF1A* mutations, including only one previously reported mutation (p.Arg316Trp). All the mutations are located in the motor domain of KIF1A. In all patients, initial symptom onset was during the infantile period, and included developmental delay in three patients and gait disturbance in two. Thereafter, they showed gait disturbances, exaggerated deep tendon reflexes, cerebellar symptoms and cerebellar atrophy on brain magnetic resonance imaging. Four patients showed lower limb spasticity, upper limb clumsiness and visual disturbances. Nerve conduction study revealed peripheral neuropathy in three patients. This study further delineates clinical features of *de novo KIF1A* mutations. Genetic testing of *KIF1A* should be considered in children with developmental delay, cerebellar atrophy and pyramidal features.
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INTRODUCTION

Kinesin superfamily proteins (KIFs) are motor proteins that play important roles in transport of various cargos along microtubules.[1,2] KIFs comprise three major groups depending on the position of the motor domain: N-terminal motor domain KIFs, middle motor domain KIFs and C-terminal motor domain KIFs.[1] KIF1A belongs to the N-terminal motor domain KIFs and is composed of a motor domain, stark domain and tail region.[1] The motor domain binds to microtubules and moves along them by hydrolyzing adenosine triphosphate (ATP), whereas the tail region recognizes and binds to the cargo.[1]

Previous reports have identified *KIF1A* recessive mutations in patients with hereditary sensory and autonomic neuropathy type 2, and hereditary spastic paraplegias (SPG30).[3–5] Recently, *de novo KIF1A* mutations were identified in patients with intellectual disability, spasticity and cerebellar atrophy and/or optic nerve atrophy[6–8] that overlap with clinical features caused by recessive *KIF1A* mutations. Therefore, abnormal KIF1A function can affect both the central and peripheral nervous systems.

Here, we performed whole-exome sequencing (WES) of patients with childhood cerebellar atrophy. We identified *de novo KIF1A* mutations in five patients and analyzed their clinical phenotypes.

PATIENTS AND METHODS

Patients

We have previously described WES analysis of 25 patients with cerebellar atrophy.[9] In this study, a total of 62 families (including 68 patients with childhood cerebellar atrophy) were newly recruited as a second cohort and analyzed by WES. From the clinical point of view, it is difficult to make a definite genetic diagnosis in each patient. Both static and progressive cerebellar atrophy were included. Detailed clinical information was obtained from the clinicians.

Genetic analysis

Genomic DNA was obtained from peripheral blood leukocytes using Quick-Gene 610L (Wako, Osaka, Japan). Genomic DNA was captured using the SureSelect Human All Exon v4 or v5 Kit (51 Mb; Agilent Technologies, Santa Clara, CA, USA) and sequenced on a HiSeq2000 (Illumina, San Diego, CA, USA) with 101-bp paired-end reads. Exome data processing, variant calling and variant annotation were performed as described previously.[10] Rare

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nonsynonymous *KIF1A* variants, which were absent in dbSNP 137, the 6500 exomes of the National Heart, Lung and Blood Institute exome project, and our in-house 575 control exomes, were considered as candidate *KIF1A* mutations, and their segregation was examined by Sanger sequencing with trio samples (patients and their parents). In families showing *de novo* mutations, parentage was confirmed by microsatellite analysis, as previously described.[11] Pathogenicity of the mutations was predicted using Sorting Intolerant from Tolerant (SIFT; http://sift.jcvi.org/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://www.mutationtaster.org/). *KIF1A* mutations were annotated based on transcript variant 1 (NM_001244008.1). The *de novo KIF1A* mutations were deposited to a gene-specific database (http://databases.lovd.nl/shared/genes/KIF1A).

Standard protocol approvals and patient consents

Experimental protocols were approved by the institutional review board of Yokohama City University School of Medicine. Written informed consent was obtained from all individuals and/or their families in compliance with relevant Japanese regulations.

RESULTS AND DISCUSSION

WES yielded an average of 87.1 million reads per sample (range 47.6–164.7 million reads per sample), resulting in an average read depth of 104.3 on the all RefSeq coding sequence (build 37/hg 19, range across all samples: 56.6–192.8). A total of five candidate missense *KIF1A* mutations were found in five patients, and all were confirmed as *de novo* events by Sanger sequencing using trio samples. In these five patients, no other candidate mutations, which were consistent with genetic model and segregation, were found in the other 180 genes previously reported in cerebellar atrophy.[9] All mutations were located in the motor domain (5/5, 100%) and substituted evolutionarily conserved amino acids (Figure 1). One mutation (p.Arg316Trp) had been previously reported.[7] SIFT, Polyphen2 and Mutation Taster predicted that all the mutations are highly damaging to the structure of *KIF1A* (Supplementary Table S1).

Clinical information on the patients with *KIF1A* mutations are summarized in Table 1, and magnetic resonance imaging findings are shown in Figure 2. Initial symptom onset was during the infantile period in all patients, with developmental delay in 3 patients (3/5, 60%) and gait disturbance in 2 patients (2/5, 40%). Subsequently, all patients showed gait disturbances, exaggerated deep tendon reflexes, cerebellar symptoms and cerebellar atrophy on brain magnetic resonance imaging (5/5, 100%). Four patients showed lower limb spasticity (4/5, 80%) and one patient had hypotonia (1/5, 20%). Three patients showed peripheral neuropathy that was demonstrated by abnormal nerve conduction studies (3/5, 60%). Three patients showed muscle weakness (3/5, 60%), and two had muscle hypertrophy (2/5, 40%). Upper limb clumsiness was observed in four patients (4/5, 80%). Three patients showed optic nerve atrophy (3/5, 60%), whereas one patient had hypermetropic astigmatism and light amblyopia (1/5, 20%). In patients 2 and 3, cerebellar atrophy was more severe in the vermis than the hemisphere. Periventricular white matter hyperintensities were observed on fluid-attenuated inversion recovery images in two patients (2/5, 40%). Dentate nucleus hyperintensity was observed on T2-weighted images in one patient (1/5, 20%). Case reports are available in Supplementary Information.

All the *de novo KIF1A* mutations we have identified here are located in the motor domain, and predicted to affect motor function based on structural models. Arg254, and Arg307 and Arg316 are located on loop L11 and the α5 helix of the switch II cluster, respectively, that associates γ-phosphate release during ATP hydrolysis and KIF1A binding to microtubules.[7,12,13] The p.Glu253Lys mutation adjacent to Arg254 was previously predicted to change the charge of salt bridge-forming residues, resulting in suppression of γ-phosphate release.[7] In addition, the p.Arg316Trp mutation was predicted to disrupt stabilization of loop L8 that binds to microtubules.[7,13] Thus, it is likely that the three mutations we have identified disrupt function of the switch II cluster. Located on loop L7, Glu148 is a Mg-stabilizer along with Arg203 and Asp248.[14] Therefore, the Glu148 mutation may affect Mg stability that is crucial for kinesin regulation and mortality.[14]

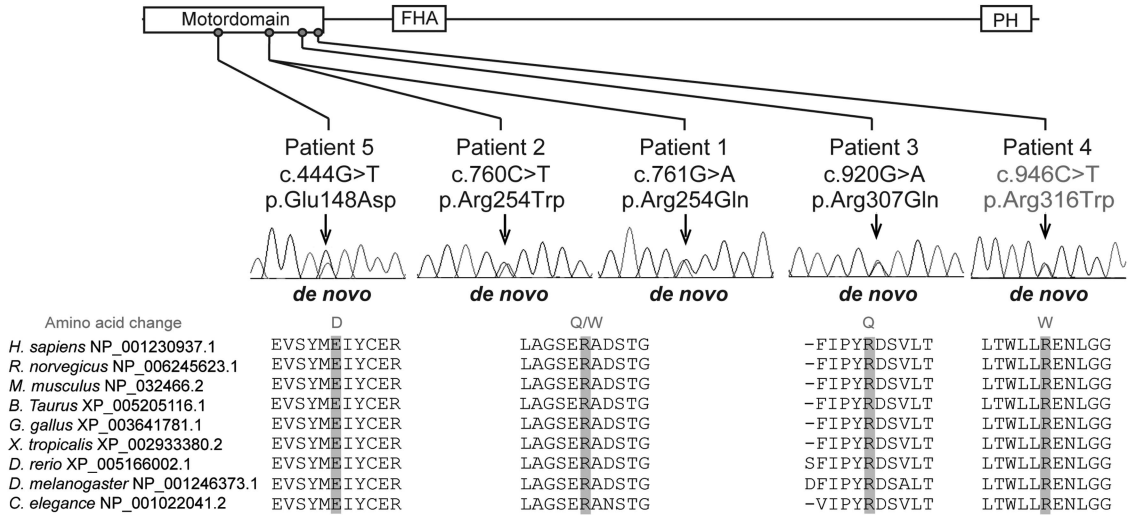

Figure 1 Schematic presentation of KIF1A and evolutionary conservation of substituted amino acids by *KIF1A* mutations. The KIF1A subunit contains three domains: motor domain, forkhead-associated domain (FHA) and pleckstrin homology domain (PH).[7] Mutations are annotated according to NM_001244008.1. The p.Arg316Trp mutation was reported previously (red characters).[7] All the *KIF1A* mutations occur at evolutionarily conserved amino acids. Orthologous sequences were aligned using CLUSTALW (http://www.genome.jp/tools/clustalw/). A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 1 Clinical features of patients with *KIF1A* mutations

Patient ID	*1*	*2*	*3*	*4*	*5*
Age, sex	8 yr, male	27 yr, female	6 yr, female	33 yr, male	8 yr, female
Initial diagnosis	Cerebellar ataxia	Cerebral palsy (spastic diplegia)	Spinocerebellar degeneration	Cerebellar ataxia	Cerebellar ataxia
Mutation	c.761G>A (p.Arg254Gln)	c.760C>T (p.Arg254Trp)	c.920G>A (p.Arg307Gln)	c.946C>T (p.Arg316Trp)	c.444G>T (p.Glu148Asp)
Inheritance	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*
Initial symptom	Equinus gait	Unsteady gait	Developmental delay	Developmental delay	Developmental delay
Age at onset	5 yr	1 yr 9 mo	8 mo	7 mo	10 mo
Deep tendon reflexes	Exaggerated	Exaggerated, ankle clonus at 10 yr; exaggerated (patellar only) (20 yr)	Exaggerated (patellar)	Exaggerated (patellar), absent (Achilles)	Exaggerated (patellar)
Babinski reflex	−	−	+	−	−
Muscle tone	Spasticity (lower limbs)	Spasticity	Spasticity	Spasticity	Hypotonia
Muscle hypertrophy	−	+ (arm and calf muscles)	−	+ (biceps muscle)	−
Muscle weakness[a]	+ (lower extremities, 4/5)	+ (lower extremities, 3/5)	−	+ (lower extremities, 3/5)	−
Peripheral neuropathy	−	+ (sensory and motor)	+ (sensory)	+ (sensory and motor)	−
Sensory deficit	−	Deep sense and light touch	+	Sense of pain	−
Nerve conduction study	Not performed	Mild decrease in MCV of posterior tibial nerves ($32-37\ m\ s^{-1}$), SCV of lower extremities was undetectable	Mild slowing in SCV and reduction in SNAP amplitude, normal MCV	Decrease in MCV of posterior tibial nerves ($24\ m\ s^{-1}$)	Normal
Visual disturbance	−	Bilateral optic atrophy and severe visual field narrowing	Bilateral optic atrophy	Bilateral optic atrophy (18 yr)	Hypermetropic astigmatism and light amblyopia
Ataxia	+	+ (truncal, ataxic gait)	−	+ (truncal and limbs)	+ (ataxic gait)
Dysmetria	−	−	−	+	−
Ocular pursuit	+ (saccadic)	+ (saccadic)	+ (saccadic)	+ (saccadic)	+ (saccadic)
Speech	Fluent	Fluent	Fluent	Slurred speech	Fluent
Hand clumsiness	+	+	−	+	+
Other neurological features	Oculomotor apraxia	Slight terminal dysmetria, Urinary urgency	Nystagmus, intention tremor	Nystagmus, intension tremor, epilepsy	Nystagmus
Walking	Ataxic and equinus gait	Planovalgus and crutch gait (10 yr); ataxic and equinus gait (20 yr)	Walking with support	Crutch gait (until 18 yr)	Ataxic and equinus gait
Current state	Walks unaided and attends a normal class	Crutch gait	Walks with support	Crawls	Walks unaided
Intellectual disability	Mild (DQ ~70)	Mild (DQ ~70)	Severe (DQ 25)	Severe	Mild (DQ 50–60)
Brain MRI	Cerebellar atrophy (superior)	Cerebellar atrophy (vermis>hemisphere) (11 yr); cerebellar atrophy without progression (as compared with the earlier study) (26 yr)	Progressive cerebellar atrophy (vermis>hemisphere), periventricular hyperintensities around the posterior horns on T2WI and FLAIR images (6 yr), optic nerve atrophy	Cerebellar atrophy (severe), cerebral atrophy (mild), slightly high intensity areas surrounding the lateral ventricles bilaterally on T1WI and FLAIR images	Cerebellar atrophy, T2 dentate nucleus hyperintensity

Abbreviations: DQ, developmental quotient; FLAIR, fluid-attenuated inversion recovery; MCV, motor nerve conduction velocity; mo, month; MRI, magnetic resonance imaging; SCV, sensory nerve conduction velocity; SNAP, sensory nerve action potential; T1WI, T1-weighted; yr, year.
[a]Value of manual muscle testing (an estimated value in patient 4) is shown.

The clinical features of the five patients reported here are consistent with previous reports in which patients with *de novo KIF1A* mutations show intellectual disability, peripheral neuropathy, cerebellar atrophy, optic nerve atrophy and lower limb spasticity.[6–8] It is interesting to note that two of our patients showed periventricular white matter hyperintensities on fluid-attenuated inversion recovery images, and one patient showed dentate nucleus hyperintensity on T2-weighted images.

In conclusion, we have identified five *de novo KIF1A* mutations in patients with childhood cerebellar atrophy. Our data shed light on understanding the phenotypic spectrum of *de novo KIF1A* mutations.

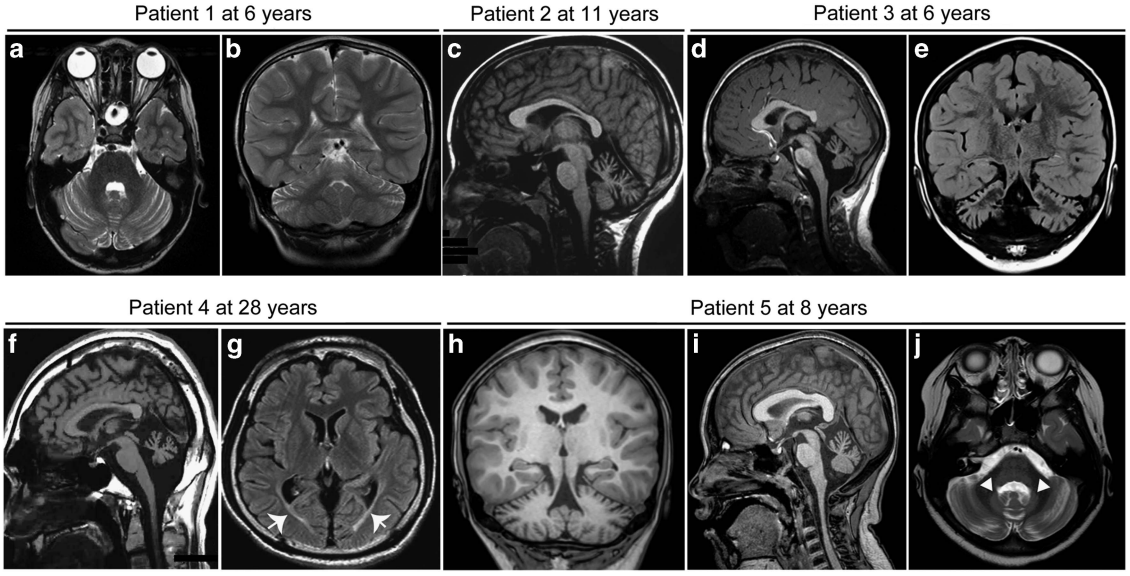

Figure 2 Brain magnetic resonance imaging (MRI) of patients. (**a**, **j**) T2-weighted axial images, (**b**) T2-weighted coronal image, (**c**, **d**, **f**, **i**) T1-weighted sagittal images, (**e**) fluid-attenuated inversion recovery (FLAIR) coronal and (**g**) axial images and (**h**) T1-weighted coronal image. (**a**, **b**) Patient 1 at 6 years of age, (**c**) patient 2 at 11 years, (**d**, **e**) patient 3 at 6 years, (**f**, **g**) patient 4 at 28 years and (**h**, **i**, **j**) patient 5 at 8 years. Cerebellar atrophy was observed in all patients (**a**–**f**, **h**, **i**). In patient 4, a slightly high intensity area surrounding the lateral ventricles bilaterally was observed on FLAIR images (arrows). In patient 5, a hyperintense dentate nucleus was observed on T2-weighted images (arrowheads).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)