

## Letter to the Editor

The novel *de novo* mutation of *KIF1A* gene as the cause for Spastic paraplegia 30 in a Japanese case

## ARTICLE INFO

## Keywords:

KIF1A  
Spastic paraplegia 30: SPG30  
Moyamoya disease  
Gene  
HSAN2  
Mental retardation  
Autosomal dominant 9  
MRD9

## ABSTRACT

Spastic paraplegia 30 is a recently established autosomal recessive disease characterized by a complex form of spastic paraplegia associated with neuropathy. Homozygous mutations of *KIF1A* reportedly lead to hereditary spastic paraplegia or hereditary sensory and autonomic neuropathy type 2 (HSAN2), whereas heterozygous mutations can cause nonsyndromic and syndromic intellectual disability (MRD9). Here we report the case of a 37-year-old female who presented with gait disturbance complicated with moyamoya disease. **Results:** The patient exhibited hypotonia during infancy, after which intellectual disability, epileptic fits, spastic paraplegia, and cerebellar atrophy occurred. Genetic analysis revealed a novel *de novo* mutation (c.254C > A, p.A85D) in the motor domain of *KIF1A*.

## 1. Introduction

Hereditary spastic paraplegias (HSPs) are a group of clinically and genetically heterogeneous neurodegenerative diseases characterized by progressive spasticity and lower limb weakness. To date, as many as 79 loci [spastic paraplegia (SPG) 1–79] have been identified. In 2011, hereditary SPG 30 caused by a homozygous mutation in *KIF1A* (Kinesin family member1A) was identified in a Palestinian family presenting early childhood onset HSP [1]. Recessively inherited cases of the *KIF1A* mutations were further identified in several families [2,3]. *KIF1A* is located on the long arm of chromosome 2 (2q37.3) and is responsible for hereditary sensory and autonomic neuropathy type 2 (HSAN2) [4]. Furthermore, a heterozygous *KIF1A* mutation has been described to cause nonsyndromic intellectual disability (mental retardation, autosomal dominant 9: MRD9) [5]. Cases exhibiting a diverse range of symptoms caused by heterozygous mutations in *KIF1A*, such as nonsyndromic and syndromic intellectual disability, cerebellar atrophy, spastic paraplegia, and axonal neuropathy, have recently been reported [6,7].

Here we report the case of a 37-year-old woman who exhibited hypotonia during infancy, epileptic fits and intellectual disability during childhood, and late development of SPG and cerebellar atrophy during adulthood, in whom a novel heterozygous mutation of *KIF1A* (c.254C > A, p.A85D) was detected *via* exome analysis.

## 2. Methods

## 2.1. Patient

The proband and her parents (Japanese origin) were examined. This study was approved by the Kindai University genetics ethics committee and the institutional review board of the University of Tokyo. Written informed consent was obtained from the participant and her parents.

## 2.2. Whole exome sequence analysis and confirmation of the mutations

Genomic DNA was extracted from peripheral blood leukocytes

obtained from the proband and her parents, following standard procedures. Exome sequencing analysis was performed in the proband. In brief, exon sequences were enriched using the SureSelect Human All Exon v5 + UTRs kit (Agilent, Santa Clara, CA) and subjected to massively parallel sequence analysis employing an Illumina HiSeq2500 platform (Illumina, San Diego, CA), following manufacturer's instructions. Burrows Wheeler Aligner [8] and sequence alignment/map tools [9] were used with default settings for aligning raw reads and detecting variations. Variant confirmation was performed *via* direct nucleotide sequence analysis using the ABI 3730 Genetic Analyzer. The mutation in *KIF1A* and the variant in *RNF213* were analyzed using the primer pairs described in the studies conducted by Riviere et al. [6] and Liu et al. [10], respectively.

## 2.3. Clinical course

The patient's family history was unremarkable. She was delivered normally, and no abnormalities were noted at her 3-month checkup. At 5 months of age, she was examined at a local doctor's clinic owing to hypotonia; however, no clear developmental abnormality was identified. Because she exhibited short stature from 5 to 6 years of age, she was examined at another hospital at 11 years of age and subsequently received growth hormone therapy. Epileptic fits were noted at 12 years of age, and she underwent electroencephalography at a local clinic. Hence, administration of antiepileptic agents was initiated. At this time, no abnormal findings were noted on magnetic resonance imaging (MRI) or computed tomography scans. Muscle weakness gradually became apparent, and the patient was admitted to our hospital at 29 years of age for the evaluation of her gait disorder. Magnetic resonance angiography performed during hospitalization revealed moyamoya disease by chance; however, the disease was considered to have not contributed to her gait disturbance.

Her general physical examination was unremarkable upon admission. Neurological examination revealed mild proximal muscle weakness in the limbs, but tendon reflexes were normal. The Babinski sign was extensor on both sides. No abnormal limb coordination was noted.

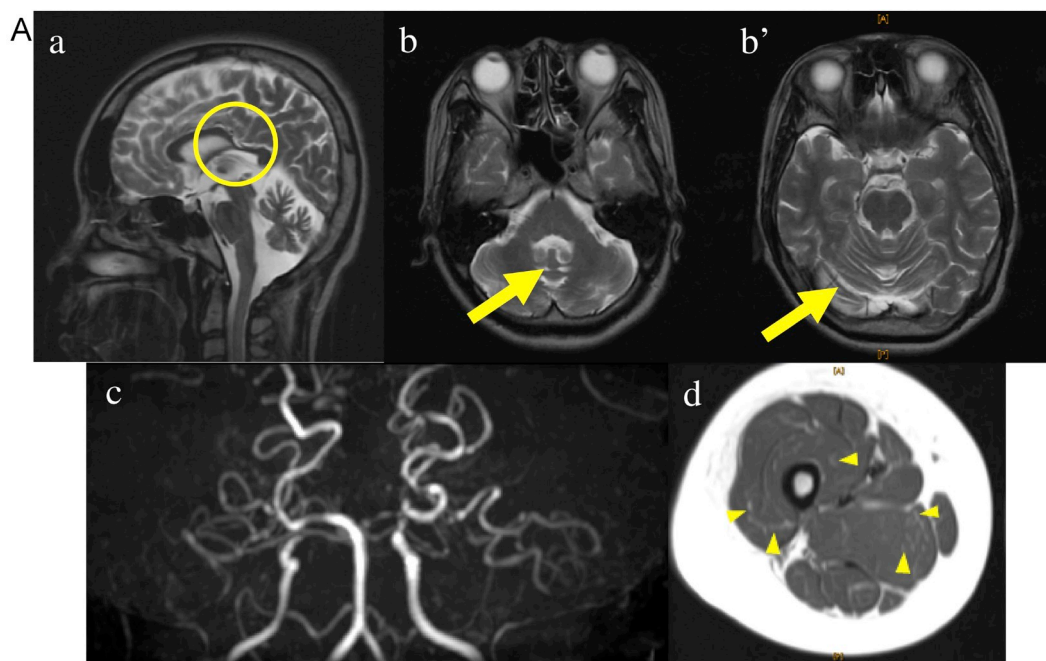
<https://doi.org/10.1016/j.ensci.2018.11.026>

Received 28 October 2018; Accepted 17 November 2018

Available online 22 November 2018

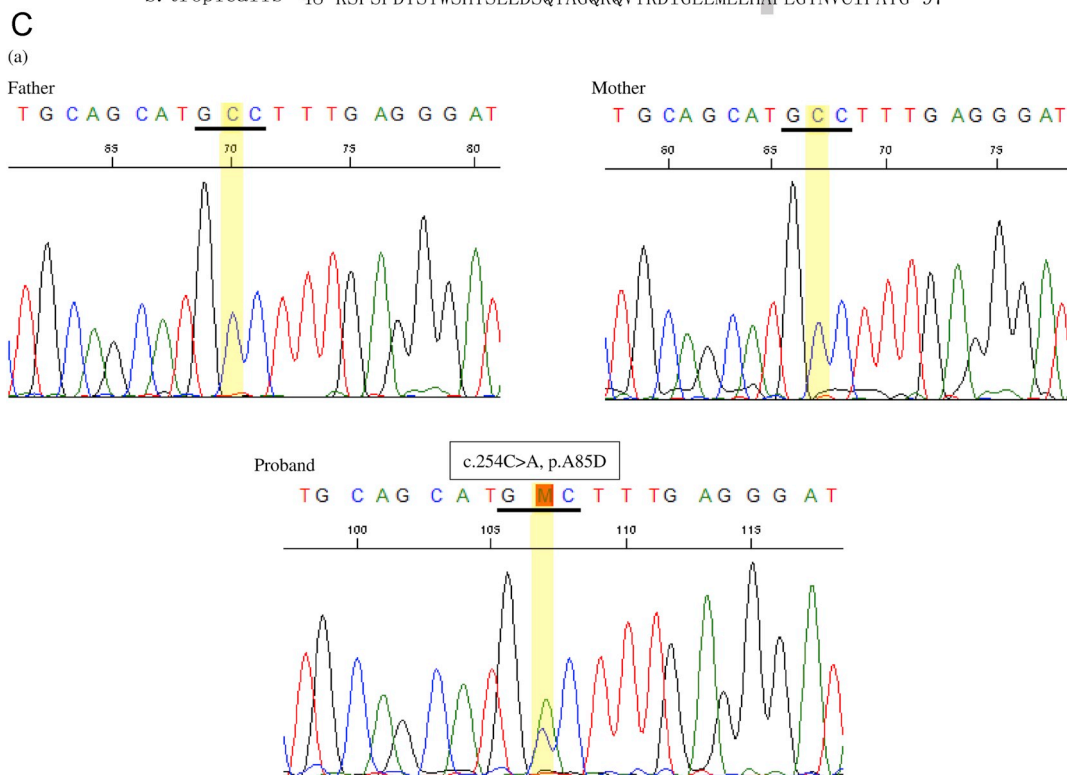
2405-6502/© 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).



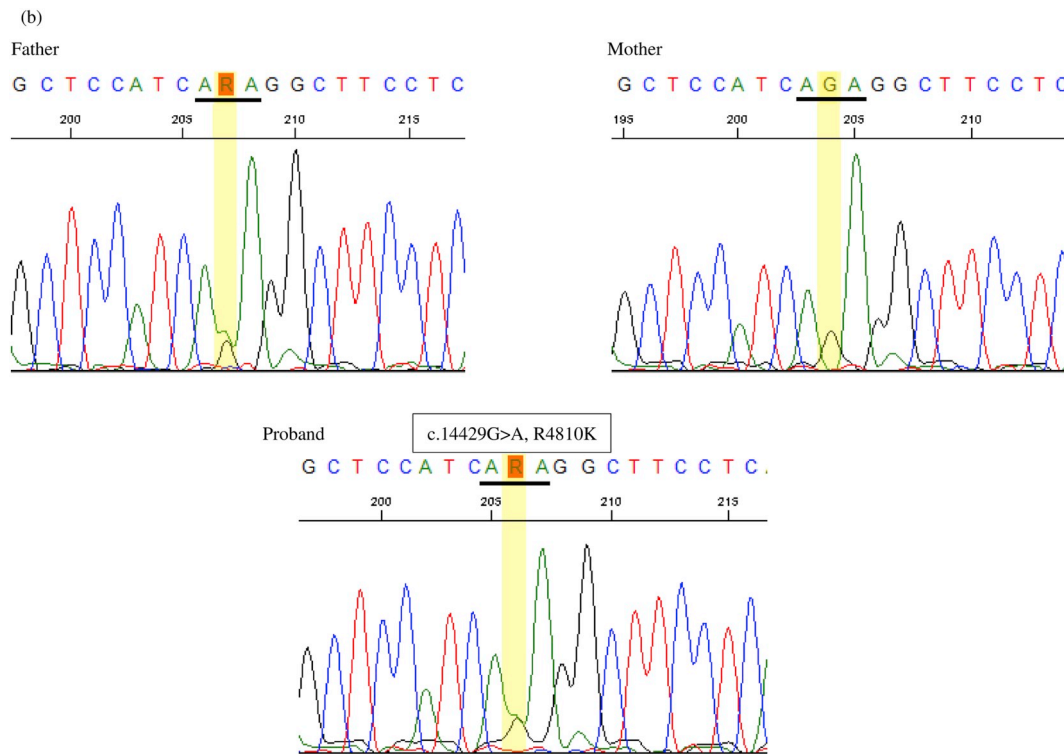
**B**

		A85D
H. sapiens	48	KSFSFDYSYWSHTSPEDINYASQKQVYRDIGEEMLQHAFEGYNVCIFAYG 97
C. lupus	48	KSFSFDYSYWSHTSPEDINYASQKQVYRDIGEEMLQHAFEGYNVCIFAYG 97
B. taurus	48	KSFSFDYSYWSHTSPEDINYASQKQVYRDIGEEMLQHAFEGYNVCIFAYG 97
M. musculus	48	KSFSFDYSYWSHTSPEDINYASQKQVYRDIGEEMLQHAFEGYNVCIFAYG 97
R. norvegicus	48	KSFSFDYSYWSHTSPEDINYASQKQVYRDIGEEMLQHAFEGYNVCIFAYG 97
G. gallus	48	KSFSFDYSYWSHTTPADINYASQKQVYRDIGEEMLQHAFEGYNVCIFAYG 97
D. rerio	47	KSFNFDYSYWSHTSPEDINYASQQVYRDIGEEMLLHAFEGYNVCIFAYG 96
D. rerio	47	KSFNFDYSYWSHTTPEDVNYACQKQVYRDIGEEMLLHAFEGYNVCIFAYG 96
C. elegans	45	FSFNFDHSYWSF-ARNDPHFITQKQVYEELGVEMLEHAFEGYNVCIFAYG 93
S. tropicalis	48	KSFSFDYSYWSHTSEEDSQYAGQRQVYRDIGEEMLLHAFEGYNVCIFAYG 97



(caption on next page)

**Fig. 1.** A. Brain and femoral magnetic resonance imaging data of the proband (a) The sagittal T2-weighted image shows thinning of corpus callosum (indicated by circle). (b, b') T2-weighted images show atrophy of cerebellar vermis and hemispheres (indicated by arrow). (c) Occlusions from the origin of the middle cerebral arteries on both sides are shown in the magnetic resonance angiography. Perforating branch and collateral pathway can be observed. (d) Diffuse atrophy of muscle tissues (particularly observed in the quadriceps femoris muscle), mesh-patterned high-signal intensity (arrow head) are shown in the T2-weighted image. B. Amino acid sequence alignment of KIF1A. Positions 48–97 of KIF1A are highly conserved among species. C. Results of the genetic analysis. (a) Electropherography revealed a missense mutation (c.254C > A, p.A85D) in exon 3 of KIF1A and (b) a high-risk variant (c.14429G > A, p.R4810K) in the RNF213 gene.



**Fig. 1.** (continued)

In the sensory system, vibration sensation was mildly diminished in both legs, and spastic gait was noted. The Hasegawa's dementia scale-revised (HDS-R) score was 18/30. Blood examination revealed no electrolyte, thyroid function, or adrenal gland function abnormalities. Cerebrospinal fluid examination revealed normal results. Head MRI scan indicated atrophic cerebellar vermis and mild cerebral atrophy. MR angiography revealed occlusion from the origin of the middle cerebral arteries on both sides accompanied by rich perforating branches and collateral pathways on the parietal side, consistent with the diagnosis of moyamoya disease. Leg MRI scans revealed diffuse atrophy of the muscle tissue, with prominent atrophy of the quadriceps femoris muscle. A mesh-patterned, high-signal intensity was noted, indicating fatty degeneration (Fig. 1A). Nerve conduction studies revealed decreased sensory nerve conduction velocity of the ulnar nerve and motor nerve conduction velocity of the posterior tibial nerve, indicating a mildly demyelinating pattern. Needle electromyography revealed low amplitude potential in the left biceps brachii, and myopathy was suspected. Muscle biopsy of the vastus muscle on the outer side of the left femur revealed differences in the muscle fiber sizes, with the same extent of atrophy of type I & type II fibers. Abnormal intermyofibrillar networks were observed within muscle fibers, with two to three muscle fibers with cytoplasmic bodies observed in the entire region. However, there were few specific findings, and only mild myopathy-like changes observed. Neurogenic changes were not apparent. Electroencephalography revealed the widespread appearance of a 1–2 Hz

spike-and-slow-wave complex.

#### 2.4. Genetic analysis

Because the patient exhibited decreased muscle strength, spastic gait, intellectual disability, and short stature, diseases including mitochondrial disease, chromosomal disorders, and hereditary spastic paraplegia of complex form were suspected for differential diagnosis. No mutations were identified at m.3243 or m.8344 in the mitochondrial DNA using Sanger sequencing. Furthermore, no chromosomal abnormalities were noted using G-banding.

Exome analysis revealed a novel heterozygous mutation (c.254C > A, p.A85D) in *KIF1A*, which is absent in the in-house database of 1261 control subjects or the public gnomAD database (<http://gnomad.broadinstitute.org/>). No other potential causative variants were detected in the genes known to cause HSP. The mutation was located in the motor domain of *KIF1A*, with A85 evolutionally conserved among species (Fig. 1B). Sanger sequencing revealed that this mutation was absent in the parents; therefore, the mutation in *KIF1A* was considered a *de novo* mutation. Sanger sequencing of seven rare variants located in different chromosomes identified *via* exome analysis of the proband were present in either of the biological parents. Moreover, a high-risk variant (rs112735431) for moyamoya disease in *RNF213* was detected and found to be transmitted from the father (Fig. 1C).

### 3. Discussion

While homozygous mutations in *KIF1A* cause hereditary sensory and autonomic neuropathy type 2C and HSP type 30, heterozygous mutations in the motor domain of *KIF1A* reportedly cause pure SPG, nonsyndromic intellectual disability, or syndromic intellectual disability with SPG. KIFs comprise a 40-kDa molecular weight spherical domain called the motor domain (MD). *KIF1A* comprises 1791 amino acids, and the MD is made up of amino acids 1–361 at the amino-terminus. Previously reported heterozygous *KIF1A* mutations are located in the MD [ref]. The mutation identified in our study (c.254C > A, p.A85D) affects a highly conserved amino acid in the MD. The mutation was absent in the in-house database of 1261 control subjects as well as in the public Genome Aggregation Database (gnomAD). *De novo* occurrence of the mutation was confirmed based on the trio analysis. These findings strongly support the pathogenicity of the novel mutation identified in the patient. Moreover, the results of the pathogenicity prediction database (PROVEAN, Polyphen2) indicate strong pathogenicity due to protein structural changes. Because intellectual disability, cerebellar atrophy, epileptic fits, and axonal neuropathy are the common clinical characteristics of patients with heterozygous *KIF1A* mutations, we conclude that these findings in the proband are attributable to the *KIF1A* mutation.

Although the patient had not experienced any clinical symptoms attributable to cerebral ischemia, magnetic resonance angiography performed at admission revealed cerebral vessel abnormalities consistent with that observed in moyamoya disease. Because moyamoya disease has been found to be highly associated with a SNP (rs112735431) of *RNF213* in Asian populations [10,11], we examined it when performing exome sequencing. The data showed that the patient was a heterozygous carrier of the high-risk variant rs112735431. Posey et al. reported that the frequency of patients with two or more disease loci was approximately 4.9% (101 of 2076) and that among those patients, 97 received dual molecular diagnoses. Dual molecular diagnosis could be divided into two categories based on the exhibited features: distinct clinical features or similar phenotypic features [12]. Our case diagnosis can be categorized as that with distinct clinical features. However, it is difficult to clarify the complexity of the blending phenotype in a single patient with two molecular variants. Because this case did not present transient motor disturbances as referred to in the OMIM database, it seems to have a distinct phenotype. Regarding the modes of inheritance, variants in autosomal dominant disease genes were the most common [12], in line with the inheritance pattern of both variants in *KIF1A* and *RNF213* noted here. Going forward, with the accumulation of genetic testing, many genetic variants will be discovered and detailed data from more cases will be investigated, eventually leading to easier and faster interpretation of cases with dual molecular diagnosis.

### Acknowledgments

This study was supported in part by Ministry of Health, Labor, and

Welfare of Japan and the Ministry of Education, Culture, Sports, Science, and Technology of Japan. This study was also supported by grants (Nos. 16kk0205001h0001 and 17kk0205001h0002) from the Japan Agency for Medical Research and Development (AMED) to S.T.

### Declaration of interest

None.

### References

- [1] Y. Erlich, S. Edvardson, E. Hodges, et al., Exome sequencing and disease-network analysis of a single family implicate a mutation in *KIF1A* in hereditary spastic paraparesis, *Genome Res.* 21 (2011) 658–664.
- [2] S. Klebe, H. Azzedine, A. Durr, et al., Autosomal recessive spastic paraplegia (SPG30) with mild ataxia and sensory neuropathy maps to chromosome 2q37.3, *Brain* 129 (2006) 1456–1462.
- [3] S. Klebe, A. Lossos, H. Azzedine, et al., *KIF1A* missense mutations in SPG30, an autosomal recessive spastic paraplegia: distinct phenotypes according to the nature of the mutations, *Eur. J. Hum. Genet.* 20 (2012) 645–649.
- [4] J.B. Riviere, S. Ramalingam, V. Lavastre, et al., *KIF1A*, an axonal transporter of synaptic vesicles, is mutated in hereditary sensory and autonomic neuropathy type 2, *Am. J. Hum. Genet.* 89 (2011) 219–230.
- [5] F.F. Hamdan, J. Gauthier, Y. Araki, et al., Excess of *de novo* deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability, *Am. J. Hum. Genet.* 88 (2011) 306–316.
- [6] J.R. Lee, M. Srour, D. Kim, et al., *De novo* mutations in the motor domain of *KIF1A* cause cognitive impairment, spastic paraparesis, axonal neuropathy, and cerebellar atrophy, *Hum. Mutat.* 36 (2015) 69–78.
- [7] C. Ohba, K. Haginoya, H. Osaka, et al., *De novo* *KIF1A* mutations cause intellectual deficit, cerebellar atrophy, lower limb spasticity and visual disturbance, *J. Hum. Genet.* 60 (2015) 739–742.
- [8] H. Li, R. Durbin, Fast and accurate short read alignment with Burrows-Wheeler transform, *Bioinformatics* 25 (2009) 1754–1760.
- [9] H. Li, B. Handsaker, A. Wysoker, et al., The sequence alignment/map format and SAMtools, *Bioinformatics* 25 (2009) 2078–2079.
- [10] W. Liu, D. Morito, S. Takashima, et al., Identification of *RNF213* as a susceptibility gene for moyamoya disease and its possible role in vascular development, *PLoS ONE* 6 (2011) e22542.
- [11] F. Kamada, Y. Aoki, A. Narisawa, et al., A genome-wide association study identifies *RNF213* as the first Moyamoya disease gene, *J. Hum. Genet.* 56 (2011) 34–40.
- [12] J.E. Posey, T. Harel, P. Liu, et al., Resolution of disease phenotypes resulting from multilocus genomic variation, *N. Engl. J. Med.* 376 (2017) 21–31.

Keisuke Yoshikawa<sup>a</sup>, Motoi Kuwahara<sup>a</sup>, Kazumasa Saigoh<sup>a,b,\*</sup>,  
Hiroyuki Ishiura<sup>c</sup>, Yuko Yamagishi<sup>a</sup>, Yuta Hamano<sup>b</sup>,  
Makoto Samukawa<sup>a</sup>, Hidekazu Suzuki<sup>a</sup>, Makito Hirano<sup>a</sup>,  
Yoshiyuki Mitsui<sup>a</sup>, Shoji Tsuji<sup>d,e</sup>, Susumu Kusunoki<sup>a</sup>

<sup>a</sup> Department of Neurology, Faculty of Medicine, Kindai University, Japan

<sup>b</sup> Department of Life Science, Faculty of Science and Engineering, Kindai University, Japan

<sup>c</sup> Department of Neurology, Faculty of Medicine, The University of Tokyo, Japan

<sup>d</sup> Department of Molecular Neurology, Graduate School of Medicine, The University of Tokyo, Japan

<sup>e</sup> Institute of Medical Genomics, International University of Health and Welfare, Japan

E-mail address: saigoh@k.email.ne.jp (K. Saigoh).

\* Corresponding author at: Department of Life Science, Faculty of Science and Engineering, Kindai University, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan.