



Original article

Clinical phenotype of hereditary spastic paraplegia due to *KIF1C* gene mutations across life span

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Abstract

Hereditary spastic paraplegias (HSPs) are a group of genetic disorders resulting in pyramidal tract impairment, predominantly in lower limbs. *KIF1C* gene has recently been identified as one of the genetic causes of HSP and associated with pure or complicated HSP. We present three patients with complicated HSP from two unrelated families, who had early onset progressive cerebellar signs and developed pyramidal tract signs during follow-up. Whole exome sequencing in these patients followed by segregation analysis identified novel truncating *KIF1C* mutations (c.463C> T; p.R155* and c.2478delA; p.Ala828Argfs*13). Neuroimaging findings showed cerebral and upper cervical spinal atrophy, bilateral symmetrical pyramidal tract involvement, and focal cerebral white matter lesions. Patients with *KIF1C* mutations may present with cerebellar signs and pyramidal findings may emerge later, therefore complicated HSP should be considered in the differential diagnosis of unidentified cases with cerebellar dysfunction.

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1. Introduction

Hereditary spastic paraplegia (HSP) is a group of inherited disorders characterized by weakness and spasticity of bilateral lower limbs. Genetic mutations cause neuropathy of the corticospinal tract axons resulting in progressive weakness and spasticity, extensor plantar responses and hyperactivity of deep tendon reflexes in

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lower extremities [1]. HSPs are clinically classified as uncomplicated and complicated forms based on neurological manifestations other than pyramidal signs. While pure HSPs are characterized by slowly progressive lower extremity spasticity and weakness, complicated HSP forms are characterized by the presence of additional neurological or systemic features including peripheral motor neuropathy and/or distal wasting, cognitive impairment, ataxia, loss of vision, epilepsy, abnormal neuroimaging findings or ichthyosis [2]. More than 60 genes have been associated with HSPs, most of which have roles in cellular trafficking. In addition, mutations of some genes involved in mitochondrial, cytochrome p450 and axonal transport pathways have also been identified in HSPs [3–5]. Mutations in *KIF1A* and *KIF5A* genes encoding kinesin family of proteins functioning as microtubule-based motor proteins were previously reported to cause different forms of HSP [6–9]. Similarly, mutations in *KIF1C* gene yet another kinesin family member have been associated with HSP [10–12].

Herein, we report clinical, neuroimaging, and genetic studies in three patients from two unrelated families with complicated HSP leading to identification of two novel *KIF1C* mutations.

2. Subjects

2.1. Patient 1 (Family A, II:1)

The first patient, a 25-year-old male, had a normal prenatal and birth history. Early developmental milestones were met until he started to have progressive tremor following inguinal hernia surgery at 3 years of age. Neurological evaluation at 10 years of age showed mild ataxia and wide based gait, borderline IQ. His gait deteriorated around 14 years of age, his speech became slower and dysarthric. He failed to respond to treatment with primidone and propranolol. After 15 years of age, he developed difficulty in walking due to increased tone and decreased strength in lower extremities. At 18 years, his weight was 68 kg (50–75p), his height was 185 cm (90p). He had severe dysarthria, ataxia and tremor with dystonia in upper extremities, bilateral spasticity, increased deep tendon reflexes and extensor flexor responses in lower extremities. Vitamin B12 treatment resulted in mild improvement (plasma B12 level: 188 pg/ml, N 160–900, homocysteine: 37.7 mmol/, N 5.5–17). He deteriorated over time with progression of pyramidal and cerebellar signs, he had swallowing difficulty, decreased joint position sense and was wheel chair bound at final examination at 24 years of age. He is on clonazepam and receives physical therapy.

Metabolic screening tests and biochemical tests during follow up including lysosomal enzymes, urine mucopolysaccharides and oligosaccharides, ceruloplasmin, copper in serum and 24 h collected urine, peroxi-

somal panel, biotinidase, urine and blood amino acids, urine organic acids, and carnitine-acylcarnitine profile were all normal. Homocysteine level was 37.7 μ mol/L (N 5.5–17). Neurophysiological studies at 10 years of age showed normal electroencephalography (EEG) and electromyography (EMG). He had bilateral prolonged central latencies in brainstem evoked potentials (BAEP), bilateral prolonged P1 latencies in visual evoked potentials (VEP). Muscle biopsy showed mild fiber size variability with scattered atrophic fibers. Oxidative enzyme stainings including cytochrome oxidase were not compatible with mitochondrial myopathy.

Brain magnetic resonance imaging (MRI) at 10 years of age showed T2A hyperintense symmetric lesions more prominent in the posterior regions involving bilateral cerebral hemispheres, periventricular white matter, and centrum semiovale. Bilateral pyramidal tracts showed hyperintensity, cerebellum and basal ganglia were spared. Repeat brain MRI at 24 years showed mild atrophy of the cerebral hemispheres especially perirolandic area brain stem, and upper cervical spinal cord (Fig. 1a). Very subtle volume loss in the upper vermis was present. Involvement of the splenium of the corpus callosum and pyramidal tracts up to the precentral gyri at the vertex were found as shown by increased T2 signal intensity (Fig. 1b–d). Spinal MRI was not obtained.

2.2. Patient 2 (Family A, II:2)

Second patient is a 19-year-old male, who is the brother of patient 1, he was also the product of an uncomplicated pregnancy and uneventful birth history. He was observed to be a less active infant. He was able to walk at 18 months. His gait had always been somewhat unsteady, and worsened after 4 years of age. He was observed to have difficulty in writing due to tremor of hands at primary school. On neurological examination at 11 years 5 months old, his weight was 56.4 kg (90p), his height was 157 cm (90–97p). He had a wide based ataxic gait and difficulty with tandem walking. He had titubation, upper-limb and lower-limb dysmetria more on the left side, and dysdiadochokinesia. He had mild muscle weakness in all extremities more prominent in lower extremities, and distally in upper extremities. His deep tendon reflexes were hyperactive in lower extremities and he had clonus and extensor plantar responses. After 12 years of age his instability increased and finally at the age of 14 he started homeschooling and using a wheel chair outside. At the age of 17, his titubation and tremors became more evident. He was able to stand with support. His speech was slower and dysarthric. Neuropsychological evaluation at 16 years old showed normal language and cognition level, and inability to take performance tests due to difficulty in visual-motor coordination.

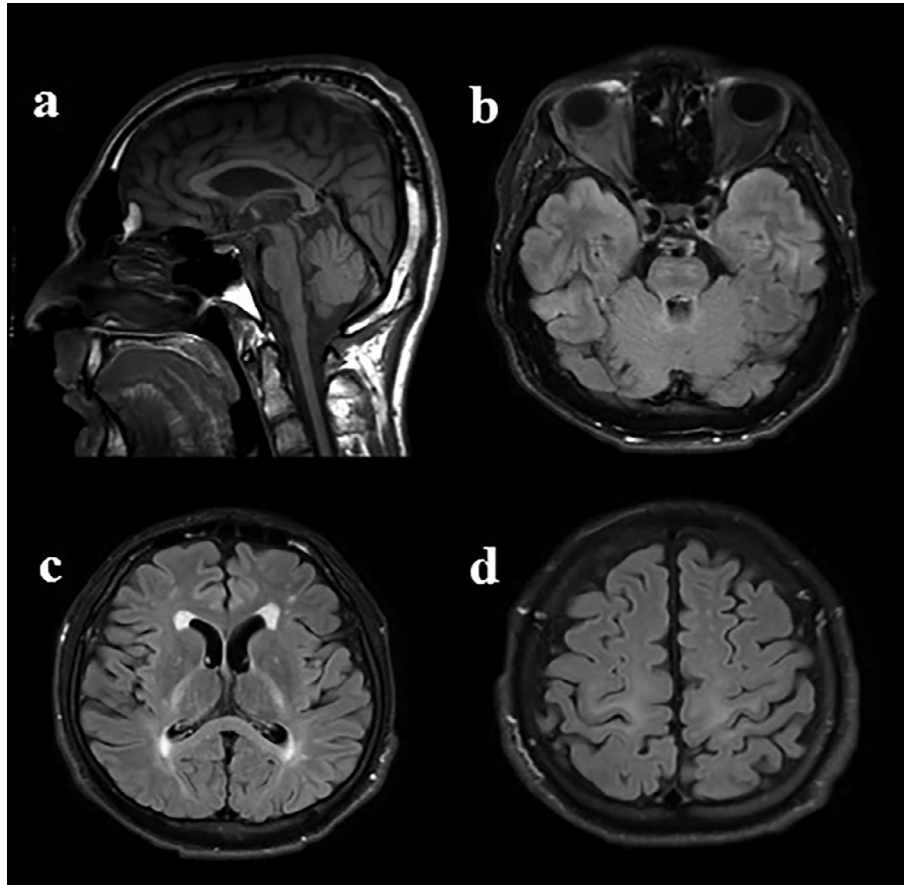


Fig. 1. Brain MRI of the patient 1, elder brother, at the age of 24 years. Sagittal T1 weighted (W) (TR/TE; 550/15 ms) image (a) shows mild atrophy of the brain stem, upper vermis and upper cervical spinal cord. Axial fluid-attenuated inversion-recovery (FLAIR) (TR/TE/TI; 9000/100/2000 ms) images (b–d) show hyperintensity in the brain stem (b), internal capsule (c) and perirolandic area (d) along the pyramidal tracts, in the splenium of the corpus callosum (c) as well as minute hyperintense foci in the cerebral white matter (c, d). Please note more prominent atrophy in the perirolandic area (d).

Initial metabolic screening tests were normal except homocysteine level at 46 $\mu\text{mol/L}$ (N: 5.5–17 $\mu\text{mol/L}$), vitamin B12 126.4 pg/mL (N: 200–860 pg/mL). Based on biochemical results, he was treated for vitamin B12 deficiency. Brain MRI at 17 years old revealed a pattern of involvement very similar to his elder brother. However, atrophy of the cerebrum, marked at the perirolandic area at the vertex and upper cervical spinal cord were more severe than his elder brother (Fig. 2a–d).

2.3. Patient 3 (Family B, II:1)

Third patient, who is now a 27-year-old woman, is from a different family. Her first symptom was tremor, which started at the age of 3. She had a mild motor developmental delay (walked at the age of 2). She also complained from difficulty in speech and mild balance problems leading to infrequent falls. These symptoms progressed very mildly in the course of the disease.

Neurological examination at the age of 22 showed mild ataxia, titubation of the head, and significant postural and kinetic tremor in the upper extremities along with dysarthria and mild cerebellar signs. Her deep tendon reflexes were brisk but no Babinski sign was observed. Vibration sense was found to be diminished in the lower and upper extremities. Her gait was slightly impaired due to the ataxia but she was able to walk independently. Her mental status was intact. Cerebrospinal fluid and EMG analyses were normal. Brain MRI revealed very similar findings.

Clinical, laboratory and neuroimaging findings of the patients are summarized in Table 1.

Institutional approval from the Ethics Review Board for this research project was obtained (Hacettepe University, TBK11/03-37 and İstanbul University 2012/907-1091). Written informed consent for participation and publication of clinical data was obtained from the patients and family members.

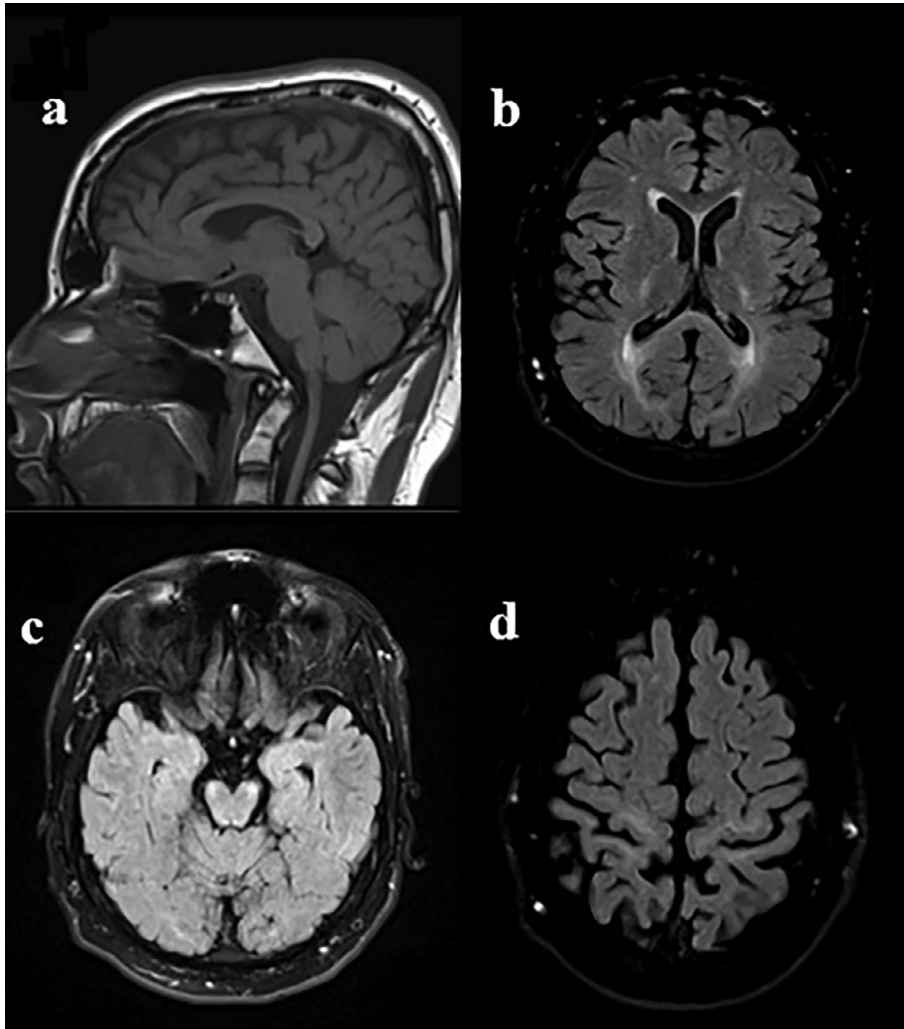


Fig. 2. Brain MRI of the patient 2, younger brother of patient 1, at the age of 17 years shows much similar findings with involvement of both pyramidal tracts, splenium of the corpus callosum, hyperintense cerebral white matter foci (b–d). However in this patient, atrophy of the upper cervical spinal cord (a) and perirolandic atrophy (d) are more severe.

3. Methods and results

3.1. Whole exome sequencing

Whole exome sequencing was performed in the DNA samples of patients and their healthy family members using deep sequencing core facility of The Scientific and Technical Research Council Marmara Research Center (TÜBİTAK-MAM, İGBAM, Turkey). Illumina TruSeq Sample Preparation Kit (San Diego, CA) was used for sample preparation and exonic DNA was captured using Illumina TruSeq Exome Enrichment Kit. Captured DNA fragments were sequenced on an Illumina HiSeq 2000 system (Illumina, San Diego, California, USA).

Variants were filtered according to their GMAF and ESP6500 MAF scores being lower than 0.01 in dbSNP (build 138) and in our in-house database ($n = 1003$).

The nucleotide variations were filtered according to a segregation pattern in family members for autosomal recessive mode of inheritance. The final remaining nucleotide changes $c.463C > T$; $p.R155^*$ and $c.2478delA$; $p.Ala828Argfs^*13$ in *KIF1C* gene (NM_006612.5) were visually inspected by using Integrative Genomics Viewer (IGV). In silico analyses for the novel mutations detected in these patients were performed by Mutation Taster. The Mutation Taster analyses showed that the mutations are assigned as disease causing pathogenic variations. None of the nucleotide changes were found in The Exome Aggregation Consortium (ExAC) and 1000 Genomes.

Detected mutations by exome sequencing analyses in *KIF1C* gene were confirmed by direct DNA sequencing.

Nucleotide change $c.463C > T$; $p.R155^*$ in exon 7 of *KIF1C* gene was homozygous in the affected children (Family A, II:1 and II:2) and heterozygous in the

Table 1
Clinical and neuroimaging findings of patients with *KIF1C* mutations.

	Family A, II:1	Family A, II:2	Family B, II:1
Genotype	c.463C> T (p.R155*)	c.463C> T (p.R155*)	c.2478delA (p.Ala828Argfs*13)
Origin	Turkey	Turkey	Turkey
Sex	Male	Male	Female
Age at final examination (yrs)	23	17	22
Age at onset	3	4	3
First symptom	Tremor	Unsteady gait, tremor	tremor
Increased DTR (lower extremity)	+	+	+
Spasticity (lower extremity)	+	+	–
Babinski reflex	+	+	–
Vibration defect	?	?	+
Trunkal ataxia	+	+	+
Nystagmus	+	+	+
Dysarthria	+	+	+
Dysmetria	+	+	+
Titubation	+	+	+
Extrapyramidal signs	Dystonia	–	–
Sphincter disturbance	–	–	–
Cognitive decline	Borderline IQ, no decline	–	–
MRI	Cerebral and upper cervical spinal atrophy, bilateral symmetrical pyramidal tract involvement, focal cerebral white matter lesions	Cerebral, craniocervical junction and upper cervical spinal atrophy, pyramidal tract involvement, parietal posterior periventricular and occipital subcortical involvement	White matter abnormalities of periventricular white matter, capsula interna, brainstem and cerebellum

healthy brother and parents (I:1, I:2, II:3) (Fig. 3A). c.2478delA; p.Ala828Argfs*13 mutation in exon 22 of *KIF1C* gene was homozygous in the single affected child (Family B, II:1) and heterozygous in parents and healthy brother (I:1, I:2, II:2) (Fig. 3B). These mutations were absent in public databases.

4. Discussion

We present three new patients from two unrelated families with complicated form HSP due to *KIF1C* mutations (Table 1). Our patients were diagnosed with complicated HSP, based on prominent cerebellar signs. Early features of the disease were cerebellar, such as tremor and gait disturbance, manifested at 3–4 years of age. Of note, two patients had delayed gross motor development and walked at 18 months (patient 2) and 2 years (patient 3). Pyramidal signs, the hallmark of HSP, became manifest during the course of the disease in the second decade. Common MRI findings were bilateral and symmetrical involvement of the pyramidal tracts, T2 hyperintense foci in the cerebral white matter, brain stem and cervical spinal atrophy (Figs. 1 and 2).

Twenty-one patients from 7 families with *KIF1C* mutations have been identified in literature and were summarized in Supplement Table 1 along with our patients [10–13]. Majority of the patients had early onset cerebellar findings similar to our patients. Overall twelve patients had brain MRI studies, ranging from normal findings to white matter abnormalities and/or cerebellar atrophy. Very subtle upper vermian atrophy was just found in one of our patients (Fig. 1) in MRI investigations. Similarly, Dor et al. reported an 18 years old patient with cerebellar findings without apparent cerebellar atrophy on MRI; the authors suggested that cerebellar signs in HSP patients with *KIF1C* mutations result from lesions of the efferent cerebellar pathways or their connections and are not due to primary involvement of the cerebellum [11]. Patients with HSP may sometimes develop cerebellar ataxia; also mild cerebellar symptoms may be the presenting features of HSP patients as in *KIF1A* and *KIF5A* mutations that belong to the kinesin family. Patients with *KIF1C* mutations reported in literature clinically differ from other HSP patients as majority of them have early onset prominent cerebellar signs and symptoms, and pyramidal signs become evident later in life. Therefore, we suggest that

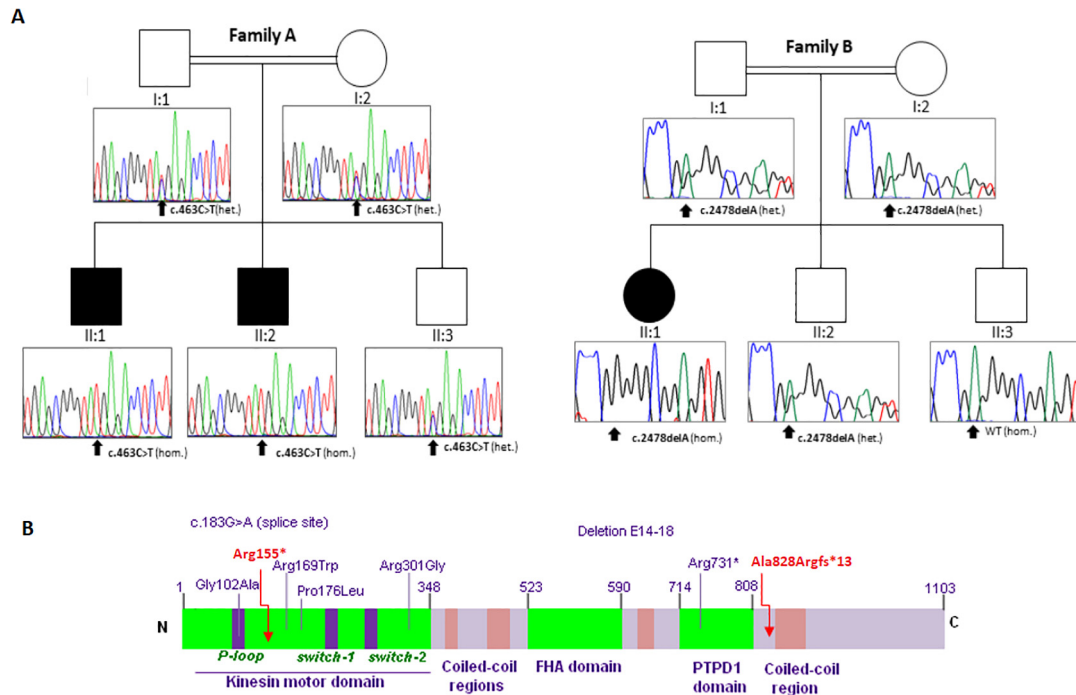


Fig. 3. A Pedigrees of two different Turkish families (Family A and B) with consanguineous marriages. Electropherograms indicating the position and type of the *KIF1C* mutations are shown on the right. B Domain structure and localisation of reported *KIF1C* mutations. Novel mutations detected in this study are shown by red and previously reported mutations by dark blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

KIF1C mutations may be considered in the differential diagnosis of patients who present with early cerebellar findings without prominent structural cerebellar abnormalities on MRI.

So far 9 different *KIF1C* mutations including missense, nonsense, splicing or frame shift types were described in HSP patients (SPG58) (Fig. 3.B) [10–12]. *KIF1C* mutations described in patients with SPG58 cause dysfunction of microtubule-based motor protein by disrupting adenosine triphosphate hydrolysis and microtubule binding. The novel mutations (NM_006612.5: c.463C> T; p.R155* and c.2478delA; p.Ala828Argfs*13) either cause formation of truncated protein products or absence of any protein product due to a nonsense mediated RNA decay mechanism, especially for the former mutation. In the truncation scenario, c.463C> T; p.R155* affects all critical protein domains particularly the conserved kinesin motor domain. c.2478delA; p.Ala828Argfs*13 on the other hand, is located in the 22th exon of *KIF1C* gene, which is composed totally of 23 exons. Nevertheless, it still can cause truncation of almost 250 amino acids in the C terminal of the mature protein.

According to the available data in literature, all patients with *KIF1C* mutations have complicated HSP form and have homozygous state for the mutations. However, in the series of Oteyza et al., the parents (THI26001-1 and THI26001-2) carrying a heterozygous

mutation have subclinical phenotype characterized by mild signs of pyramidal involvement and demyelinating sensory motor neuropathy each in one patient. On the other hand, the sibling (IHG25125-3) with a heterozygous mutation presented with complicated HSP [10]. Oteyza et al. proposed that HSP due to *KIF1C* mutations have a mild dominant and more severe recessive disease phenotype. Therefore, we evaluated the parents and the brother with heterozygous c.463C> T mutation for carrier phenotype. While the mother had normal neurological examination, the father and 23-year-old brother had hypoactive deep tendon reflexes in the upper extremities compared to lower extremities as well as pes cavus deformity. Their neurological examinations were otherwise unremarkable. Electroneuromyography of the father revealed normal nerve conduction velocities as well as normal needle electromyography. Family members of patient 3 were not available for further evaluation.

Patients with HSP have a wide spectrum of clinical phenotype along with genetic and allelic heterogeneity. Patients presented here are remarkable for early onset of cerebellar findings long before pyramidal signs become evident, which is a similar course seen in several patients with *KIF1C* mutations reported in the literature. In summary, we propose that *KIF1C* mutations might be considered in the differential diagnosis of patients who present with cerebellar findings and

without remarkable structural cerebellar abnormalities on MRI. In addition, carriers for *KIF1C* mutations may become symptomatic, even later in life. We conclude that all carriers including asymptomatic individuals should be considered for long-term follow up.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.braindev.2018.02.013>.

References

- [1] Lo Giudice T, Lombardi F, Santorelli FM, Kawarai T, Orlacchio A. Hereditary spastic paraplegia: clinical-genetic characteristics and evolving molecular mechanisms. *Exp Neurol* 2014;26:518–39.
- [2] Fink JK. Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. *Acta Neuropathol* 2013;126:307–28.
- [3] de Souza PV, de Rezende Pinto WB, de Rezende Batistella GN, Bortholin T, Oliveira AS. Hereditary spastic paraplegia: clinical and genetic hallmarks. *Cerebellum* 2017;16:525–51.
- [4] Schüle R, Schols L. Genetics of hereditary spastic paraplegias. *Semin Neurol* 2011;31:484–93.
- [5] Dion PA, Daoud H, Rouleau GA. Genetics of motor neuron disorders: new insights into pathogenic mechanisms. *Nat Rev Genet* 2009;10:769–82.
- [6] Klebe S, Lossos A, Azzedine H, Mundwiler E, Sheffer R, Gaussen M, et al. KIF1A missense mutations in SPG30, an autosomal recessive spastic paraplegia: distinct phenotypes according to the nature of the mutations. *Eur J Hum Genetics* 2012;20:645–9.
- [7] Reid E, Kloos M, Ashley-Koch A, Hughes L, Bevan S, Svenson IK, et al. A kinesin heavy chain (KIF5A) mutation in hereditary spastic paraplegia (SPG10). *Am J Hum Genet* 2002;71:1189–94.
- [8] Crimella C, Baschirotto C, Arnoldi A, Tonelli A, Tenderini E, Airoidi G, et al. Mutations in the motor and stalk domains of KIF5A in spastic paraplegia type 10 and in axonal Charcot-Marie-Tooth type 2. *Clin Genet* 2012;82:157–64.
- [9] Erlich Y, Edvardson S, Hodges E, Zenvirt S, Thekkat P, Shaag A, et al. Exome sequencing and disease-network analysis of a single family implicate a mutation in KIF1A in hereditary spastic paraparesis. *Genome Res* 2011;2:658–64.
- [10] Oteyza CA, Battaloğlu E, Ocek L, Lindig T, Reichbauer J, Rebelo AP et al. Motor protein mutations cause a new form of hereditary spastic paraplegia. *Neurology* 2014;3(82):2007–16.
- [11] Dor T, Cinnamon Y, Raymond L, Shaag A, Bouslam N, Bouhouche A et al KIF1C mutations in two families with hereditary spastic paraparesis and cerebellar dysfunction. *J Med Genet* 2014;51:137–42.
- [12] Novarino G, Fenstermaker AG, Zaki MS, Hofree M, Silhavy JL, Heiberg AD, et al. Exome sequencing links corticospinal motor neuron disease to common neurodegenerative disorders. *Science* 2014;343:506–11.
- [13] Bouslam N, Bouhouche A, Benomar A, Hanein S, Klebe S, Azzedine H, et al. A novel locus for autosomal recessive spastic ataxia on chromosome 17p. *Hum Genet* 2007;121:413–20.