

Hereditary Spastic Paraplegia Associated With Axonal Neuropathy: A Novel Mutation of SPG3A in a Large Family

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Abstract

Spastic paraplegia Type 3A is an autosomal-dominant pure or uncomplicated hereditary spastic paraplegia. It is caused by mutations in *SPG3A*, the only gene associated with this condition. We identified a novel mutation, c.1040T>C (p. M347T), in a family with axonal neuropathy in addition to spastic paraplegia. This expands the spectrum of neurologic complications associated with *SPG3A* and highlights the importance of long-term follow-up and neurological surveillance in this patient population.

Key Words: HSP, atlastin, hereditary spastic, paraplegia, *SPG3A*, axonal neuropathy

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INTRODUCTION

Hereditary spastic paraplegias (HSP) are heterogeneous clinical and genetic disorders characterized by progressive spasticity, lower limb weakness, and extensor plantar response.¹ They are classified as pure if the paraplegia occurs in isolation or complicated if it occurs with other features, including amyotrophy, cardiac defects, cerebellar signs, deafness, dementia, epilepsy, extrapyramidal signs, sensory neuropathy, retinal changes, or white matter abnormalities.² They are also classified according to the mode of inheritance or the locus if known.³ To date, 41 spastic paraplegia gene loci have been mapped and 17 genes identified.¹ Autosomal-dominant hereditary spastic paraplegia caused by *SPG3A* mutations is the most common cause of HSP with onset before age 10 years.⁴ The *SPG3A* gene encodes atlastin-1, a member of the dynamin family of large guanosine triphosphatases.⁵

PATIENTS AND METHODS

We evaluated six members of a family, four of whom were affected with spastic gait disturbance (Fig 1). A detailed clinical history, standardized neurological examination, imaging of the brain and spine, and electrophysiological studies were carried out for all patients.

Genomic DNA was isolated from white blood cells (QIAamp Tissue Kit; Qiagen, Hilden, Germany). All exon structures of the gene were amplified from 50 ng (2.5 μ L from 20 g/ μ L) of purified DNA using the following thermoprofile: 15 minutes initial denaturation at 95°C; 13 cycles at 94°C for 30 seconds, 66°C for 30 seconds, 72°C for 20 seconds; followed by eight cycles at 94°C for 30 seconds, 46.5°C for 30 seconds, 72°C for 20 seconds, and an additional 16 cycles at 94°C for 30 seconds, 54.5°C for 30 seconds, 72°C for 20 seconds, and final elongation for 5 minutes at 72°C. The polymerase chain reactions were carried out in a 22- μ L reaction volume containing 0.5 μ L (10 μ M) of each primer 6.26 μ L HotStarTaq (Qiagen), 2.25 μ L RNase-free Aqua bidest, 0.5 μ L DMSO.

For the sequencing reaction, the polymerase chain reaction fragments were purified Exosap. Direct sequence analysis of both strands of the polymerase chain reaction fragments was performed using a nonradioactive, fluorescent-labeled primer dideoxynucleotide chain termination protocol, Taq cycle-sequencing, and the sequencing machine from ABI (Foster City, CA).

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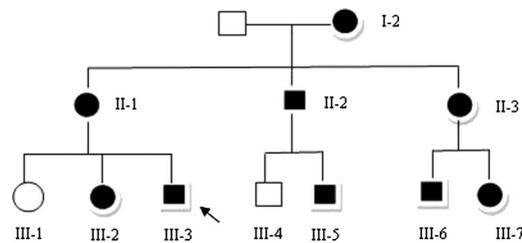


FIGURE 1. Family pedigree with clinically affected individuals indicated as darkened symbols.

RESULTS

The proband (III-3) was a 10-year-old boy who developed an abnormal gait and spasticity of the lower limbs at the age of 2 years. He had normal cognitive development, has had no speech or swallowing difficulties, and no bladder disturbance. Clinical examination at age 10 years revealed bilateral weakness of the lower extremities (Medical Research Council Grade 4/5), hyperactive reflexes at the knees and ankles, and bilateral extensor plantar response. He had a spastic gait with marked circumabduction. Cranial nerve, sensory, and coordination testing were normal. Examination of muscle tone and strength of the upper extremities was normal. Standard metabolic tests (plasma amino acids, urine organic acids, ammonia, lipid profile, vitamin B12 and E levels, very long chain fatty acid levels, beta-hexosaminidase analysis) and brain magnetic resonance imaging were normal.

His sister (III-2) presented with a spastic gait at the age of 11 years. She did not have any urinary symptoms, and her cognitive function was normal. On examination, she had bilateral weakness (Medical Research Council Grade 4/5) and spasticity of the lower extremities and extensor plantar response with normal cranial nerve, sensory, and cerebellar examinations.

The mother (II-1) developed an abnormal gait with lower extremity spasticity and weakness at age 11 years. Her condition was slowly progressive and at age 47 she is still ambulatory but requires the use of a brace on her right ankle. She developed axonal neuropathy in her early 20s, which was confirmed by nerve conduction studies.

Her brother and sister (II-2 and II-3) have a similar history as does her mother (I-2). All developed spasticity and lower extremity weakness during childhood, which were slowly progressive. None had upper limb involvement or urinary or bowel dysfunction. All developed axonal, predominantly motor neuropathy in their early 20s. The daughter of Patient II-3 (Patient III-7) had an extremely mild presentation with no spasticity or weakness, although she did have hyperreflexia on clinical examination (Table 1).

Molecular genetic testing of the *SPG3A* gene revealed a novel mutation, c.1040T>C (p. M347T) in exon 10. The mutation segregated with the affected phenotype in this family (II-1, III-2, III-3, III-7, and II-3) and was absent in unaffected family members (III-1), who had normal neurologic examinations. We analyzed this variant using Biointeractive software Alamut (Rouen, France) and it predicted the variant to be pathogenic. It affected a highly conserved nucleotide (score: 1.0 [0–1]). The corresponding amino acid is highly conserved up to *C. elegans* among 11 species. There was a moderate physicochemical difference between Met and Thr with a Grantham distance of 81 [0–215]). This variation is in the following protein domain: guanylate-binding protein-like, N-terminal amino acid. The mutation was not detected in 224 control chromosomes.

DISCUSSION

We report a family with autosomal-dominant hereditary spastic paraplegia caused by a novel mutation, c.1040T>C (p. M347T), in exon 10 of the *Alta* gene. The patients presented with slowly progressive spastic gait disturbance at an average age of onset of 8 years. The adult members of the family all developed axonal motor neuropathy in their 20s, which was confirmed by nerve conduction studies, and in some cases led to an initial diagnosis of Charcot-Marie-Tooth disease.

Autosomal-dominant HSP represents approximately 70% of all HSP.¹ Of the

autosomal-dominant HSP, approximately 40% of cases are caused by mutations in the *SPG4* gene (which encodes the spastin protein), whereas *SPG3A* accounts for approximately 10% of cases.⁶ Mutations of *SPG3A* are the most common cause of HSP with onset before age 10 years, and the average age of onset of symptoms is 6 years (range, 2–50 years).⁴

SPG3A is typically considered a pure or uncomplicated form of HSP, but advances in genetic testing have revealed this diagnosis in patients with additional features such as neuropathy.

In a large cohort of 182 families studied by Ivanova et al,⁷ there were six patients with axonal motor neuropathy associated with R495W, F151S, M408T, G469A, and Q191R mutations in *SPG3A*. The R495W mutation had previously been reported to be associated with motor neuropathy by Scarano et al⁸ in a family with three affected members. Meijer et al⁹ described two families with a p.del436N mutation in *SPG3A*, which was associated with later-onset neuropathy in two of nine affected individuals. Recently Fusco et al¹⁰ described a patient with infantile-onset HSP also associated with axonal neuropathy, who carried a novel S398F mutation in exon 12 of *SPG3A*.

Precise genotype-phenotype correlations are currently being studied, and at present there are more than 30 reported mutations of *SPG3A*. The mutations are predominantly of the missense type, which suggests a gain-of-function pathogenic mechanism.^{11,12} Most of the mutations described to cause polyneuropathy in combination with *SPG3* are localized in the area of the amino acids 398 to 495, including our new mutation describing the area of the N-terminal cytoplasmic domain.

The missense mutation we report, M347T, is located at the predicted N-terminal cytoplasmic domain of the atlastin-1 protein.¹³ Atlastin-1 is a member of the dynamin family of large guanosine triphosphatases and a Golgi body transmembrane protein, usually assembled as a homotetramer. It is expressed in the endoplasmic reticulum and cis-Golgi in

TABLE 1. Clinical Features of Affected Patients*

Patient	Age at Onset (years)	Age at Examination n (years)	Lower Limb Hyperreflexia	Spasticity	Weakness	Sensory Impairment	Extensor		Upper Extremity		Bladder Disturbance	Disability†	Axonal Neuropathy on Nerve Conduction Velocity
							Plantar Response	Involvement	Involvement	Disturbance			
III1	11	47	+	+	+	+	+	+	+	+	+	2	+
III3	11	53	+	+	+	+	+	+	+	+	+	1	+
III2	11	12	+	+	+	+	+	+	+	+	+	1	-
III5	2	10	+	+	+	+	+	+	+	+	+	3	-
III7	25	25	+	+	+	+	+	+	+	+	+	1	N/A

*III-1 had normal neurologic examinations (not shown).

†Disability graded according to the following scale: 1, normal gait or very slight stiffness in the legs; 2, moderate gait stiffness; 3, unable to run but able to walk alone; 4, able to walk with help; and 5, wheelchair-bound.

N/A, not available.

brain tissue. The function of atlastin-1 is not known, but it is suggested that abnormal atlastin-1 impairs normal formation of axons and dendrites.^{14,15} Mammalian atlastins interact with proteins necessary for tubule formation in the endoplasmic reticulum, which appears to have a direct impact on axonal development.¹⁶

We present a family with a novel mutation of SPG3A associated with adult-onset axonal neuropathy in addition to the typical features of spastic paraplegia. This expands the phenotype associated with SPG3A mutations and highlights the importance of long-term follow-up and surveillance for neurologic complications in these patients.

REFERENCES

- Salinas S, Proukakis C, Crosby A, et al. Hereditary spastic paraplegia: clinical features and pathogenetic mechanisms. *Lancet Neurol.* 2008;7:1127-1138.
- McDermott CJ, Shaw PJ. Hereditary spastic paraplegia. *Int Rev Neurobiol.* 2002;53.
- Fink JK. Hereditary spastic paraplegia. *Curr Neurol Neurosci Rep.* 2006;6:65-76.
- Namekawa M, Ribai P, Nelson I, et al. SPG3A is the most frequent cause of hereditary spastic paraplegia with onset before age 10 years. *Neurology.* 2006;66:112-114.
- Zhao X, Alvarado D, Rainier S, et al. Mutations in a newly identified GTPase gene cause autosomal dominant hereditary spastic paraplegia. *Nat Genet.* 2001;29:326-331.
- Fink JK. The hereditary spastic paraplegias: nine genes and counting. *Arch Neurol.* 2003;60:1045-1049.
- Ivanova N, Claeys KG, Deconinck T, et al. Hereditary spastic paraplegia 3A associated with axonal neuropathy. *Arch Neurol.* 2007;64:706-713.
- Scarano V, Mancini P, Criscuolo C, et al. The R495W mutation in SPG3A causes spastic paraplegia associated with axonal neuropathy. *J Neurol.* 2005;252:901-903.
- Meijer IA, Dion P, Laurent S, et al. Characterization of a novel SPG3A deletion in a French-Canadian family. *Ann Neurol.* 2007;61:599-603.
- Fusco C, Frattini D, Farnetti E, et al. Hereditary spastic paraplegia and axonal motor neuropathy caused by a novel SPG3A de novo mutation. *Brain Dev.* 2010;32:592-594.
- Depienne C, Stevanin G, Brice A, et al. Hereditary spastic paraplegias: an update. *Curr Opin Neurol.* 2007;20:674-680.
- Rismanchi N, Soderblom C, Stadler J, et al. Atlastin GTPases are required for Golgi apparatus and ER morphogenesis. *Hum Mol Genet.* 2008;17:1591-1604.
- Zhu PP, Patterson A, Lavoie B, et al. Cellular localization, oligomerization, and membrane association of the hereditary spastic paraplegia 3A (SPG3A) protein atlastin. *J Biol Chem.* 2003;278:49063-49071.
- Muriel MP, Dauphin A, Namekawa M, et al. Atlastin-1, the dynamin-like GTPase responsible for spastic paraplegia SPG3A, remodels lipid membranes and may form tubules and vesicles in the endoplasmic reticulum. *J Neurochem.* 2009;110:1607-1616.
- Zhu PP, Soderblom C, Tao-Cheng JH, et al. SPG3A protein atlastin-1 is enriched in growth cones and promotes axon elongation during neuronal development. *Hum Mol Genet.* 2006;15:1343-1353.
- Hu J, Shibata Y, Zhu PP, Voss C, et al. A class of dynamin-like GTPases involved in the generation of the tubular ER network. *Cell.* 2009;138:549-561.