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***SPG11* mutations are common in familial cases of complicated hereditary spastic paraplegia (HSP)**

Coro Paisan-Ruiz, PhD^{1,CA}, Okan Dogu, MD², Arda Yilmaz, MD², Henry Houlden, MD³, and Andrew Singleton, PhD¹

¹Molecular Genetics Unit, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892

²Department of Neurology, Faculty of Medicine, Mersin University, 33070 Mersin, Turkey

³Department of Molecular Neuroscience, Institute of Neurology, Queen Square, London, WC1N 3BG, England, United Kingdom

Abstract

Objective—Autosomal recessive hereditary spastic paraplegia (ARHSP) with thin corpus callosum (TCC) is a common form of complex HSP. The genetic lesion underlying ARHSP-TCC was localized to chromosome 15q13-q15 and given the designation *SPG11*. Recently the gene encoding spatacsin (*KIAA1840*), has been shown to contain mutations that underlie the majority of ARHSP-TCC cases.

Methods—Here we present a complete analysis of the 40 coding exons of this gene in patients with sporadic (n = 25) or familial (20 probands) complex hereditary spastic paraplegia with and without thinning of the corpus callosum.

Results—We identified seven mutations, including deletions, insertions and nonsense mutations, which were all predicted to lead to premature truncation of the protein.

Conclusion—We conclude that mutations on *KIAA1840* are frequent in complex ARHSP but an infrequent cause of sporadic complex HSP.

INTRODUCTION

Hereditary spastic paraplegias (HSPs) are a large group of inherited neurological disorders characterized by slowly progressive weakness and spasticity affecting primarily lower extremities. They are classified genetically by autosomal dominant, autosomal recessive and X-linked HSP; and clinically as pure HSP when the spastic paraplegia is the only clinical manifestation, and as complicated HSP when it is accompanied by additional neurological or non-neurological features, such as mental retardation, ataxia, peripheral neuropathy, muscle atrophy, dementia and dysarthria¹. To date 33 loci associated with HSPs have been described and 15 genes have been identified.

Autosomal recessive hereditary spastic paraplegia with thin corpus callosum (ARHSPTCC, MIM 604360) is a common type of complex ARHSP and is characterized by mental retardation, slowly progressive spastic paraparesis, peripheral neuropathy and the presence of thin corpus callosum (TCC). The ARHSP-TCC was first linked to the *SPG11* locus on chromosome 15q13-q15 in 7 spastic paraplegia families from North America and Europe, whose members showed pure and complicated HSP², other HSP-TCC families from different ethnicities were also

CAruizc@mail.nih.gov.

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linked to the *SPG11* locus (²⁻⁵). Refinement of the *SPG11* locus to a region of 6 cM, which contained at least 70 genes, was described in 20 Mediterranean *SPG11* families ³ and in a recent study of 20 Turkish families the *SPG11* locus was narrowed to 2.93cM ⁴.

Recently 10 different mutations in *KIAA1840* were described in 11 ARHSP-TCC families, in which the *SPG11* locus was first restricted to an interval of 2.53Mb (D15S778-D15S659) containing ~40 genes. *KIAA1840* has a 7515 bp/7332 bp open reading frame encoding a 2443 amino acid protein of unknown function, named spatacsin. Spatacsin is predicted to contain a Glycosyl hydroxylase F1 signature (482-490 aa), a Leucine zipper (611-632 aa), a short coiled coil domain (1556-1590 aa), a Myb domain (1766-1774 aa), and 4 transmembrane domains (TM) located between amino acid residues 163-194, 200-240, 1239-1267 and 1471-1493. Spatacsin is expressed ubiquitously in the nervous system but most prominently in the cerebellum, cerebral cortex, hippocampus and pineal gland ⁵.

We identified 22 families and 25 apparently sporadic cases of complex HSP from Turkey, the UK and Asia. We defined apparently sporadic as disease occurring in the absence of a positive family history. We had previously shown that three of the families were linked to the chromosome 15q13-q15 locus. To ascertain the frequency of mutations, genetic heterogeneity and clinical phenotype of our group of patients we sequenced the coding region and flanking introns of *KIAA1840* in our group of complex HSP patients.

METHODS

Patients

We have studied 44 subjects from 5 unrelated consanguineous HSP-TCC families in addition to 17 families and 25 apparently sporadic cases with complex HSP, of which 14 showed known parental consanguinity. Informed consent was obtained from all participants in this study.

Spastic paraplegia was present in all cases along with the addition of at least one of the following features: cognitive decline, sensory or motor neuropathy, seizures, ataxia, speech and swallowing problems and brainstem signs ⁶. The majority (28) of the cases analysed had at least three additional neurological manifestations typical of the phenotype of *SPG11*. The patients were fully investigated for acquired causes of SPG such as multiple sclerosis, adrenoleukodystrophy and mitochondrial disease, all underwent imaging of brain and spinal cord, CSF and very long chain fatty acid examination. In a large proportion of cases a muscle biopsy was carried out for histopathology and biochemistry.

Molecular Analysis

Genomic DNA from peripheral blood was extracted by using the Promega Wizard Genomic DNA Isolation Kit (www.promega.com).

To analyze all 40 exons of the *KIAA1840* gene and at least 50bp of flanking intronic sequence, PCR analysis was performed using 10pmol of both forward and reverse genomic primers (table E-1) previously designed by ExonPrimer (<http://ihg.gsf.de/ihg/ExonPrimer.html>) and FastStart Taq DNA polymerase (www.roche-applied-science.com). Then each purified product was sequenced using forward or reverse primers with Applied Biosystems BigDye terminator v3.1 sequencing chemistry as per the manufacturers instructions; the resulting reactions were resolved either on an ABI3730 or an ABI3100 genetic analyzer (Applied Biosystems, Foster city, CA) and analyzed with Sequencher software (Genecodes, VA/(Gene Codes Corporation, Ann Arbor, MI)). Sequencing was performed in a proband from each ARHSP family and in all apparently sporadic HSP cases. All mutations were verified in both directions and segregation of mutations within families was tested by sequencing of specific exons in each available family member.

None of the cases was screened for any of the known genes associated with autosomal recessive forms of SPG, except for a negative *SPG11* familial case (22445) that was also screened for the *Spartin* gene; no mutation was found. To examine the entire coding region of the *Spartin* gene, we used the same procedure that we used to analyze the *KIAA1840* gene

RESULTS

We identified seven novel *SPG11* variants, including a nonsense mutation, 3 small deletions, 2 small insertions and an insertion/deletion (Indels), in five of the 20 ARHSP families and one of 25 sporadic complex HSP cases, Sp1 (figure E-1). All mutations were located on coding regions of the *SPG11* gene and all were homozygous except for a non consanguineous family (Family 4) where affected members carried a compound heterozygous mutation (table 1).

Familial cases

Family F1—Three affected subjects, who were born to healthy first-degree cousins, showed a homozygous CTGT deletion (c.5985delCTGT), which creates a frameshift change at 1997 amino acid position and causes a stop codon at 2056 amino acid position (p.L1997MfsX2056), located in exon 31. Eight other members of 24 additional available members were found to be heterozygous for this mutation.

Patient *F1-A* age 35 years had learning disability during primary school and could not attend secondary school. He had normal motor milestones, but when he was 18 years old motor disability became apparent and he started to fall frequently. He became unable to walk without assistance at approximately 28 years of age and is now severely dysarthric, mentally impaired and can walk barely with assistance. Neurological examination revealed spastic quadriparesias (more marked in the lower limbs), hand muscle atrophy, mild bilateral postural hand tremor, increased muscle tone, brisk tendon reflexes-non sustained patellar clonus, dysarthric speech, dysidiadochokinesia, reduced vibration sensation (more marked in his lower limbs), and flexor plantar reflexes. He had no urinary problems. His mini mental test score was 15/30 and sensory and motor nerve conduction studies were consistent with axonal sensory motor polyneuropathy. MRI showed thin corpus callosum with periventricular white matter changes and moderate diffuse cortical atrophy. His sister (*F1-B*) is now 28 years old and she is less severely affected, being able to walk without assistance. She also has a learning disability and could not attend secondary school but had less severe cognitive impairment than her brother. Her gait disturbance began when she was 20 years old and then progressed. Neurological examination revealed mild spastic paraparesis, ataxic gait, brisk tendon reflexes (non sustained patellar and ankle clonus), dysidiadochokinesia, flexor plantar reflexes, reduced vibration sensation (more marked in her lower limbs) and flexor plantar reflexes. She had no urinary problems. Her mini mental test score was 18/30. MRI showed thin corpus callosum, periventricular white matter changes and moderate cortical atrophy. The third patient, *F1-C*, presented with walking difficulties at age 17 years progressing slowly; now, at age twenty seven he cannot walk and is bed ridden. No significant cognitive deficiency was reported, however his speech is barely understandable and he exhibits spastic dysarthria. His understanding is mildly affected. He left school during the first year of high school. Urinary incontinence is present. MRI data is not available.

Family F2—A homozygous A insertion (c.1838insA) was detected in exon 9 of *KIAA1840* gene. This insertion leads to a frameshift and a premature stop codon at the 619 amino acid position (p.L613HfsX619). This mutation was found in the 2 HSP patients, and both parents, who were healthy first-degree cousins, were found to be heterozygous for this mutation. Patient *F2-A*, a 30 year old man, presented with motor disability, recognized by his basketball coach at age of 20. Initially he was falling frequently and had difficulty running. One of the major

complaints at first admission was leg cramps. These symptoms slowly worsened, and currently he can still walk with assistance. He has had urinary incontinence for the last one and half years. Neurological examination revealed spastic paraparesis (4/5), increased muscle tone, hyperreflexia of lower extremities, patellar clonus, extensor plantar responses and mild hand tremor. His mini mental test score was 24/30. Cerebellar tests and sensory examination findings were all normal. His 31 year old sister (*F2-B*) began to experience walking difficulties at the age of 17 years and was unable to walk without assistance at age of 27; she is now wheelchair bound. She had urinary incontinence for the last two and half years and she also developed progressive mental deterioration. Neurological examination showed spastic quadriparesia (more marked in the lower limbs), hand muscle amyotrophy, hand tremor, dysarthric speech, brisk tendon reflexes, and bilateral Babinski sign. Her mini mental test score was 20/30. Brain MRI from both patients showed thin corpus callosum.

Family F3—This family, living in UK, is of Indian ancestry. Four affected family members were found to carry a homozygous T deletion at 5769 nucleotide position in exon 30 (c.5769delT), which also creates a frameshift change and a premature codon stop at 1950 aa position (p.S1923RfsX1950). Three unaffected family members were found to carry this mutation in heterozygous status.

The family tree is complex with multiple consanguineous loops. All four family members presented with an onset in childhood or teenage years of a progressive complex recessive hereditary spastic paraplegia. The proband was a late developer in both walking and did not talk until 3 years old. She was always slow at school and poor at sports. In her late teens she developed problems with walking and stiffness. All aspects of her clinical presentation continue to deteriorate. On examination she has cognitive problems with anterior and subcortical dysfunction, on WAIS-R verbal IQ at the age of 23 years was 67 and performance IQ 78. She walks with a spastic scissoring gait and wears ankle foot orthosis. She has a right ptosis, jerky hypometric saccades, a skew deviation and poor up gaze. She has a slow tongue with a spastic dysarthria. There was marked increased in tone in all four limbs with brisk reflexes and extensor planters and normal sensory examination. MRI is similar in all four affected family members with mild global atrophy, patchy white matter changes and a thin corpus callosum. Nerve conduction studies and EEG were normal.

Family F4—This family from the UK consists of two affected siblings. The parents were unrelated. Both affected were found to carry two heterozygous mutations, a heterozygous A insertion located in the exon 6 and a heterozygous AG deletion in exon 30. Both mutations create a frameshift change and a premature codon stop at 475 amino acid and 1828 amino acid position (p.I450NfsX475, p.E1819AfsX1828). The father was found to carry only the heterozygous AG deletion from exon 30, suggesting that the two mutations are located in *trans*.

Family F5—This family with a Pakistan origin consists of three affected siblings, whose parents were first cousins, however only one family member was available for study. The proband was found to carry a homozygous 4 base pair (bp) deletion and a homozygous 8 bp insertion at the same position (c.5399-407delAGATinsTGGAGGAG). Both changes lead to a frameshift that causes a stop codon at 1830 amino acid position (p.Q1800LfsX1830).

Sporadic Cases—Only one of 25 sporadic Spastic Paraplegia cases was found to carry mutations on *KIAA1840*. Patient 26011 carried a homozygous nonsense mutation (p.W89X) located at the beginning of the exon 2. This patient's family was from Pakistan and the parents were first cousins. No other affected members were identified in this family.

During the course of sequencing *KIAA1840* we also identified fourteen additional variants (table E-2), of which three are already described as polymorphisms (rs3759871, rs3759873, rs3759874), 2 were found to occur in patients who already carried *SPG11* mutations (G6R and V793M in 13317 and 26011 patients), 6 are silent (L636L, L1140L, T1526T, G2110G, Y2341Y and K2399K), 2 cause an amino acid change (Y886H, K2419R) and the last one leads to a leucine insertion at 2312 amino acid position (2312insL).

DISCUSSION

Recently it has been reported that mutations in *SPG11* are a major cause of familial SPTCC⁵. We have screened a large number of sporadic and familial complex HSP cases and provide data that further confirms *SPG11* as the most frequent mutated gene for SPTCC. We have identified six *SPG11* mutations segregating with disease in five different families and an additional *SPG11* variant in a sporadic case.

Out of the 20 complex ARHSP families we analyzed five carried *KIAA1840* mutations. A total of seven families had thinning of the corpus callosum although imaging in five families was limited to a CT scan (table 1). All *SPG11* families had a typical early onset presentation. Clinical features were typically walking difficulties due to spastic paraplegia, cognitive decline with IQ levels between 65-75, axonal peripheral neuropathy and ataxia. Dysarthria and eye signs were also common features.

Here we report the first analysis of *SPG11* in sporadic spastic paraplegia cases and have identified one case bearing an *SPG11* mutation, suggesting that mutations on *KIAA1840* may be also involved in apparently sporadic spastic paraplegia cases although they seem to be rarely present in sporadic SPG patients. This sporadic case had typical complex HSP without a neuropathy and with no family history although parental consanguinity suggested the role of recessive mutation in disease in this case. The MRI of the head and spine were normal as were nerve conduction studies. This case suggests that there is clinical heterogeneity in cases with *SPG11* mutations and that imaging of the brain and corpus callosum can be normal.

The mutations described here bring the total number of *KIAA1840* disease causing mutations described thus far to 17. These mutations are located across the *KIAA1840* gene (figure E-2) and have been described in patients from Asia and Europe. *KIAA1840* contains 40 exons and the reported mutations are located in several exons of the gene (exons: 1, 2, 3, 4, 6, 9, 16, 30, 31, 32, 34, and 39), suggesting that complete screening of *SPG11* is necessary rather than targeted resequencing of specific exons.

From the mutations identified so far it is difficult to draw any genotype/phenotype correlations. Although the cases with mutations usually had a full house of neurological features with SPG, ataxia, axonal neuropathy, cognitive decline and cranial nerve signs there were clear clinical differences between and within families (Table 1). The radiological sign of TCC and white matter abnormalities is an important clinical sign of HSP-TCC but from the screening of complex HSP cases at least two sporadic and four familial cases had this sign and were *KIAA1840* negative.

In addition to the 7 *SPG11* mutations identified here, we also describe 11 additional variants, of which 3 are known as common variants (rs3759874, rs3759873 and rs3759871). We have considered the remaining 8 as benign variants because either they do not cause an amino acid change or were found along with homozygous *KIAA1840* stop mutations.

Although the Spatacsin function is unknown, the presence of a Leucine zipper motif and a Myb domain suggest this protein may be involved in the regulation of gene expression. In addition spatacsin may be act as a receptor or a transporter both because of the presence of

transmembrane domains and because mutations that cause defective trafficking have been described in other forms of HSP (SPG3A (ATL1), SPG10 (KIF5A), SPG7 (PGN), SPG4 (Spastin), SPG20 (Spartin))⁷.

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Table 1

Clinical features of all SPG cases with mutations on the KIAA1840 gene. The clinical features from 2 affected members from family 4, whose DNA were not available, are also shown

Origin	ID	Mutation	Age at onset (symptoms)	Age at exam	Severity	Muscle wasting	Ataxia	Reflexes	Planters	Neuropathy	Cognitive problems	Sphincter problems	MRI	EMG/NCS	Other
Turkey	F1-A (2023-08)	p.L1997MfsX2056	18 (walking)	35	moderate	moderate	yes	+++	Ext/Ext	yes	moderate	no	TCC, cortical atrophy and WM abnormalities	mild axonal PNP	spastic dysarthria
	F1-B (2023-09)	p.L1997MfsX2056	20 (walking)	28	mild-moderate	mild	yes	+++	Flex/Flex	yes	moderate	no	TCC, cortical atrophy and WM abnormalities	mild axonal PNP	
	F1-C (2023-12)	p.L1997MfsX2056	17 (walking)	44	severe	severe	yes	+++	Ext/Ext	yes	moderate	yes	NA	NA	severe spastic dysarthria, bed ridden
Turkey	F2-A (2024-03)	p.L613HfsX619	20 (walking)	30	moderate	mild	yes	+++	Ext/Ext	yes	mild	yes	TCC	mild axonal PNP	
	F2-B (2024-04)	p.L613HfsX619	17 (walking)	31	severe	moderate	yes	+++	Ext/Ext	yes	moderate	yes	TCC	mild axonal PNP	
India	F3-A (39691)	p.S1923RfsX1950	3 (walking and speech)	23	Moderate	Mild	yes	+++	Ext/Ext	No	Moderate MR	Yes	TCC, atrophy & WM abnormalities	Normal	Spastic dysarthria, gaze palsy, broken pursuit
	F3-B (39689)	p.S1923RfsX1950	3 (walking and speech)	18	Moderate	Mild	yes	+++	Ext/Ext	No	Moderate MR	Yes	TCC, atrophy & WM abnormalities	Normal	Spastic dysarthria, gaze palsy, broken pursuit
	F3-C (30949)	p.S1923RfsX1950	15 (walking)	23	Severe	Severe	yes	++	Ext/Ext	No	Moderate MR	Yes	TCC, atrophy & WM abnormalities	Normal	Spastic dysarthria
	F3-D (41536)	p.S1923RfsX1950	15 (tremor)	18	Mild	Mild	no	+	Ext/Flex	No	Moderate MR	Yes	TCC, atrophy & WM abnormalities	Normal	Spastic dysarthria
UK	F4-A (13316)	p.I450NfsX475 p.E1819AfsX1828	15 (dragging right foot)	34	Severe WB	Severe	no	++, + knee and absent ankle	Flex/Flex	mild	Moderate MR	yes	TCC, atrophy & WM abnormalities	PNP	Spastic dysarthria
	F4-B (13317)	p.I450NfsX475 p.E1819AfsX1828	1.8 (walking)	36	Severe WB	Severe	no	++, + knee and absent ankle	Flex/Flex	No	Mild MR	no	TCC, atrophy & WM abnormalities	Normal	Spastic dysarthria
Pakistan Parents first cousins	F5-A (30198)	p.Q1800LfsX1830	23 (walking)	30	moderate Severe	moderate	Yes	++, absent ankles	Ext/Ext	Yes	Moderate MR	no	TCC, atrophy & WM abnormalities	Mild Axonal neuropathy	Reduced visual acuity, broken

Origin	ID	Mutation	Age at onset (symptoms)	Age at exam	Severity	Muscle wasting	Ataxia	Reflexes	Platers	Neuropathy	Cognitive problems	Sphincter problems	MRI	EMG/NCS	Other
Pakistan Parents second cousins	Sp-1 (26011)	p.W89X	4 (sphincter problems)	28	Severe WB	Severe	Yes	+, absent ankle	Flex/Flex	No	Moderate MR	yes	Normal	Normal	frontal release signs, reduced visual acuity, slow tongue