

ARTICLE

# Hereditary spastic paraplegia in Greece: characterisation of a previously unexplored population using next-generation sequencing

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Hereditary Spastic Paraplegia (HSP) is a syndrome characterised by lower limb spasticity, occurring alone or in association with other neurological manifestations, such as cognitive impairment, seizures, ataxia or neuropathy. HSP occurs worldwide, with different populations having different frequencies of causative genes. The Greek population has not yet been characterised. The purpose of this study was to describe the clinical presentation and molecular epidemiology of the largest cohort of HSP in Greece, comprising 54 patients from 40 families. We used a targeted next-generation sequencing (NGS) approach to genetically assess a proband from each family. We made a genetic diagnosis in >50% of cases and identified 11 novel variants. Variants in *SPAST* and *KIF5A* were the most common causes of autosomal dominant HSP, whereas *SPG11* and *CYP7B1* were the most common cause of autosomal recessive HSP. We identified a novel variant in *SPG11*, which led to disease with later onset and may be unique to the Greek population and report the first nonsense mutation in *KIF5A*. Interestingly, the frequency of HSP mutations in the Greek population, which is relatively isolated, was very similar to other European populations. We confirm that NGS approaches are an efficient diagnostic tool and should be employed early in the assessment of HSP patients.

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## INTRODUCTION

Hereditary spastic Paraplegia (HSP) refers to a heterogeneous group of disorders characterised by slowly progressive limb spasticity and weakness.<sup>1</sup> Spasticity can occur in isolation (pure HSP), or can be complicated by additional features including learning disability, seizures, neuropathy or ataxia (complicated HSP). Approximately two-thirds of patients have a family history of the disorder, with the remainder occurring sporadically.<sup>2</sup>

All modes of inheritance are known to cause HSP and at least 45 genetic types are known, although a smaller number of genes account for the majority of cases.<sup>3</sup> Despite the abundance of genes involved in HSP, many of the genes impact on the same molecular pathways. These include axonal transport (*KIF5A*, *KIF1A*), endoplasmic reticulum formation (*ATL1*, *SPAST*, *REEPI*), endosomal and lysosomal trafficking (*SPG11*, *AP4B1*), and mitochondrial function (*SPG7*, *mATP6*, *HSPD1*).<sup>2</sup>

The clinical heterogeneity of HSP and the diversity of genetic causes can make it difficult to achieve a genetic diagnosis in many patients. In most clinical settings, a small number of genes can be sequenced by conventional Sanger methods owing to the cost and low frequency of mutations in many genes. Overall, >50% of autosomal dominant (AD) HSP cases and 70% of autosomal recessive (AR) HSP cases never receive a genetic diagnosis.<sup>4</sup> HSP occurs in all populations, and there are regional variations in the frequency of causative genes. However,

no study has evaluated the genetic spectrum of HSP in Greece. In this study, we present the largest Greek cohort of HSP patients, comprising 54 patients from 40 families.

The development of massively parallel sequencing has revolutionized genetics, making it possible to simultaneously sequence thousands of genes, faster and at lower cost than traditional Sanger sequencing.<sup>5</sup> There are a number of different NGS technologies, including whole-genome, exome, targeted exome and panel-based sequencing. Although sequencing a whole-genome provides unparalleled genetic information, the interpretation of these variants is difficult and time consuming. In addition, when only single probands are available for sequencing, genome or exome sequencing may reveal thousands of potentially pathogenic variants. Targeted exome sequencing and panel-based sequencing offer some advantages owing to cost savings and the ease and speed of data interpretation.

In this study, we used a combination of targeted exome sequencing and panel sequencing to characterise the mutational spectrum of HSP in probands from 40 Greek HSP families, providing insights into the genetics of HSP in this population for the first time.

## MATERIALS AND METHODS

### Patient recruitment

This study included consecutive patients referred with suspected HSP to the Neurogenetics Unit, 1st Department of Neurology, University of Athens

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Medical School, Eginition Hospital, over an 18-year period (1995–2013). The Athens Neurogenetics Unit is the only unit of its kind in Greece and has uniquely offered molecular diagnostic testing for adult patients with neurogenetic disorders within the framework of the Greek public health service. It receives referrals for clinical assessment and/or molecular genetic testing in patients with suspected neurogenetic disorders from all regions of the country. In the case of HSP, the Unit has not offered diagnostic molecular testing, but has assessed patients clinically and stored DNA for future analysis. Patients included in the present study originate from various regions of Greece, were in most cases referred by other neurologists in the Department and gave informed consent for molecular genetic testing.

### Targeted exome sequencing

Nine patients were sequenced on an Illumina Trusight Exome panel, which targets 2731 genes known to cause human disease. These include 34 genes associated with HSP (see Supplementary Table). The complete list of targeted genes is available online from [http://support.illumina.com/downloads/trusight\\_exome\\_product\\_files.html](http://support.illumina.com/downloads/trusight_exome_product_files.html).

DNA samples were diluted to 5 ng/μl and were then tagged and fragmented according to the Trusight Enrichment DNA Sample Preparation Guide. Libraries were sequenced with an Illumina HiSeq 2500. Sequences were aligned using BWA and variants called with GATK. Mean target coverage was 42, and 87% of bases were read 10 times. There was an average of 7065 variants called per sample.

### Truseq custom amplicon panel sequencing

Thirty-one patients were sequenced using a custom HSP amplicon panel, which was designed to target the coding regions of the following genes implicated in HSP: *ATL1*, *SPAST*, *BSCL2*, *HSPD1*, *GJC*, *KIAA0196*, *KIF5A*, *NIPA1*, *PLP*, *PNPLA6*, *REEP1*, *RTN2*, *SPG11*, *SPG7*, *CYP7B1*, *FA2H*. DNA was diluted to 250 ng and procedures followed the Truseq Custom Amplicon Library Preparation Guide. Libraries were sequenced on an Illumina Miseq and variants were called on the Illumina BaseSpace platform. Mean coverage of the Custom Panel was 592 reads per targeted base. A graphical representation of coverage per patient and coverage per gene may be found in Supplementary Figure 1.

### Sanger sequencing

All potentially pathogenic variants identified by next-generation sequencing (NGS) were confirmed by conventional Sanger sequencing. The exon carrying the variant was PCR amplified using flanking intronic primers (primer sequences available on request). The PCR product was purified and then sequenced in both directions using Big Dye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequencing products were purified and read on an ABI 3730 DNA Analyzer (Applied Biosystems). Sequences were analysed using Seqscape 3 software (Applied Biosystems).

Variants are described with reference to the following transcripts: *SPAST*: NM\_014946; NG\_008730.1, *KIF5A*: NM\_004984; NG\_008155.1, *REEP1*: NM\_001164732.1; NG\_013037.1, *ATL1*: NM\_015915; NG\_009028.1, *CYP7B1*: NM\_004820.3; NG\_008338.1, *SPG11*: NM\_025137; NG\_008885.1, *SPG7*: NM\_003119; NG\_008082.1, *GJC2*: NM\_020435; NG\_011838.1, *ZFYVE26*: NM\_015346; NG\_011836.1. All variants detected have been deposited in the Leiden Open Variation Database found at <http://databases.lovd.nl/shared/genes> (Patient IDs 39349–39359 and 46990–47000).

## RESULTS

### Clinical characterisation

We identified 54 patients from 40 families affected by HSP. The clinical descriptions of the patients and families can be found in Table 1. Thirty-six patients (67%) presented with pure HSP and 18 (33%) with complicated HSP.

Pure forms: the majority of pure HSP occurred in families with an AD mode of inheritance (55%), followed by sporadic inheritance (31%) and AR inheritance (7%). X-linked dominant inheritance occurred in one family (3%). Just over two-thirds of the patients

with pure HSP were men. The mean age of onset was 32 years. The most frequent symptoms described in addition to spastic paraplegia were lower limb sensory disturbance (28%) and urinary urgency/frequency (19%). Cerebellar signs and cognitive impairment occurred rarely. White matter lesions on MRI were described in three patients.

Complicated forms: the most frequent mode of inheritance in families with a complicated HSP phenotype was AD (38%), sporadic (30%) and AR (23%). The mode of inheritance was unknown in 15%. There was a significant excess of men with a complicated phenotype (77%) compared with women (23%). The mean age at onset was 23 years. The most frequently reported additional symptoms were lower limb sensory disturbance (61%), cognitive impairment (44%), cerebellar signs (28%) and neuropathy (28%). Urinary symptoms occurred in 17%. Three patients had thinning of the corpus callosum on MRI and four patients had white matter lesions.

### Molecular characterisation

We performed genetic analysis on one proband from each HSP family (40 total), using the Truseq custom HSP panel (targeting 16 of the most frequently mutated HSP genes) or Trusight Exome. MLPA analysis was not performed. This may underestimate the frequency of some genes owing to genomic deletions.

A genetic diagnosis was made in 6/9 patients using the Trusight Exome and 15/31 patients using the Truseq custom HSP panel.

A genetic diagnosis was achieved in 13 of the 30 patients with pure HSP (43%) and in 8 of the 10 patients with complicated HSP (80%). Where a clear AR pattern of inheritance was given, a genetic diagnosis could be made in all cases. Single heterozygous variants found in known AR HSP genes are given in Supplementary Table 1.

### Novel variants

Of the 21 potentially pathogenic variants we identified, 11 were novel. These are listed in Table 2. These include two nonsense mutations in *SPAST*, c.430C>T and c.575T>A, and one in *KIF5A* c.2590C>T. We also identified a novel frameshift mutation in *SPAST*, c.1591dupC. Of the remaining seven novel missense variants, all but one are absent in available databases, including the ExAC browser,<sup>6</sup> the Exome Variant Server and an internal database of over 600 exomes. All novel variants are predicted to be pathogenic by at least two *in silico* tools and all occur at conserved residues.

Only the *SPG11* c.5381T>C variant has a recorded frequency on control databases, with a reported frequency of five in 120 880 alleles (0.0004136) on the ExAC browser.<sup>6</sup> No homozygotes have been reported. This variant has maximally pathogenic scores on all three *in silico* tools and occurs at a highly conserved residue. It occurs in combination with another deleterious *SPG11* variant in three probands. In all cases the variant occurs with a frameshift or nonsense mutation and in one family, the same compound heterozygote mutations, c.[5470C>T]; [5381T>C] occur in two affected brothers. An unaffected brother carried only the c. 5381T>C variant. For these reasons, we believe that the c. 5381T>C variant in *SPG11* may be pathogenic when it occurs *in trans* with another pathogenic *SPG11* variant or perhaps in the homozygous state, and it is possible that this is a unique finding in the Greek population.

### Autosomal dominant mutations

*SPAST/SPG4*. Variants in *SPAST* were responsible for the majority of AD HSP in our cohort (see Table 3, Figure 1). We identified seven patients with potentially pathogenic variants in *SPAST*, of which four were novel and three previously described as pathogenic. The age of onset of HSP in patients with *SPAST* variants was highly variable

**Table 1 Clinical description of families and patients**

Family	Inheritance	Individual	Sex	Phenotype	Age at	Urinary	LL sensory	Cognitive		Cerebellar		Additional features
					onset	symptoms	disturbance	Neuropathy	impairment	TCC	WML	
1	X-linked	HSP1	M	Pure	27	+	-	-	-	-	-	
1	X-linked	HSP3	M	Pure	28	+	+	-	-	-	-	
1	X-linked	HSP4	M	Pure	20	-	+	-	-	-	-	
2	AD	HSP8	F	Pure	NA	-	-	-	-	-	-	
3	AD	HSP9	M	Pure	40	+	-	-	-	-	-	
4	Sporadic	HSP11	M	Pure	60	+	+	-	-	-	-	
5	AD	HSP12	M	Pure	35	-	-	-	-	-	-	
6	AR	HSP13	M	Complicated	60	-	+	+	-	-	+	
7	AD	HSP14	M	Pure	14	-	-	-	-	-	-	
8	Unknown	HSP16	M	Complicated	40	+	+	-	+	-	-	
9	AD	HSP17	M	Complicated	40	-	-	-	+	-	-	
10	Sporadic	HSP19	M	Pure	9 m	-	-	-	-	-	-	
11	Unknown	HSP20	F	Pure	63	-	-	-	-	-	-	
12	Sporadic	HSP21	M	Complicated	10	-	-	+	-	-	-	Sensorimotor axonal neuropathy
13	Sporadic	HSP22	M	Pure	24	-	-	-	-	-	-	
14	AD	HSP27	M	Pure	44	+	-	-	-	-	-	
15	Sporadic	HSP28	F	Pure	37	+	-	-	-	-	-	
16	Unknown	HSP29	M	Complicated	39	+	+	+	+	-	+	
17	Sporadic	HSP30	M	Pure	24	+	+	-	-	-	-	
18	Sporadic	HSP31	M	Pure	34	-	-	-	-	-	-	
19	AD	HSP32	F	Pure	52	-	-	-	-	-	-	
20	AR	HSP33	M	Complicated	8	-	+	-	-	-	-	Sensorineural hearing loss
20	AR	HSP34	M	Complicated	10	-	+	-	-	-	+	Sensorineural hearing loss
20	AR	HSP35	F	Complicated	13	-	+	-	-	-	+	Sensorineural hearing loss
21	AD	HSP37	F	Pure	NA	-	-	-	-	-	-	
21	AD	HSP40	M	Complicated	6	-	+	+	-	-	-	Sensory axonal polyneuropathy
22	Sporadic	HSP41	F	Pure	27	-	+	-	-	-	-	Parents 3rd cousins
23	AR	HSP45	M	Pure	19	-	-	-	-	-	-	
24	AD	HSP46	M	Pure	45	-	-	-	-	-	+	
25	AR	HSP47	F	Pure	41	-	-	-	-	-	+	
25	AR	HSP59	M	Pure	NA	-	-	-	-	-	-	
26	AR	HSP48	F	Complicated	15	-	-	-	+	+	+	Dysarthria
27	AD	HSP49	M	Pure	46	-	-	-	+	-	-	+
27	AD	HSP83	F	Pure	NA	-	-	-	-	-	-	
27	AD	HSP86	F	Pure	40	-	+	-	-	-	-	
27	AD	HSP87	M	Complicated	73	-	+	-	-	-	-	Chorea
27	AD	HSP88	M	Complicated	59	-	-	-	-	-	-	Chorea
27	AD	HSP89	M	Complicated	4	-	-	-	-	-	-	Epilepsy
27	AD	HSP90	M	Pure	16	-	-	-	-	-	-	
27	AD	HSP91	M	Pure	18	-	-	-	-	-	-	
28	AD	HSP50	M	Pure	22	-	-	-	-	-	-	
29	AD	HSP54	F	Pure	45	-	-	-	-	-	-	
30	AD	HSP55	M	Pure	NA	-	-	-	-	-	-	
31	AD	HSP62	M	Pure	8	-	+	-	-	-	-	
32	Sporadic	HSP64	M	Complicated	12	+	-	-	+	+	+	
33	AD	HSP65	M	Pure	10	-	+	-	-	-	-	
34	AD	HSP66	F	Complicated	18 m	-	+	-	+	-	-	
34	AD	HSP70	M	Complicated	18 m	-	+	-	+	-	-	
35	Sporadic	HSP67	M	Complicated	5	-	+	+	-	-	-	Sensorimotor axonal neuropathy
36	AD	HSP68	F	Pure	25	-	+	-	-	-	-	
37	Sporadic	HSP69	M	Pure	49	-	+	-	-	-	+	
38	AD	HSP71	F	Pure	50	-	-	-	-	-	-	
39	Sporadic	HSP73	F	Complicated	15	-	-	-	+	+	-	Ichthyosis
40	Sporadic	HSP60	M	Pure	NA	-	-	-	-	-	-	

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; Complicated, HSP with additional neurological features such as ataxia, neuropathy, cognitive impairment; M, male; F, female; NA, information not available; Pure, lower/upper limb slowly progressive spasticity ± back pain; +, present; -, absent; TCC, thinning of the corpus callosum; WML, white matter lesions.

**Table 2** Novel mutations detected with frequency data and *in silico* prediction score

Gene	Nucleotide change	Predicted AA change	Mutation type	Freq. (EXAC)	Freq. (EVS)	Freq. (internal db)	SIFT	Polyphen	Mutation taster	GERP++	Family segregation
SPAST	c.430C>T	p.(Q144*)	Nonsense	0	0	0	1	NA	1	4.6	Yes
SPAST	c.575T>A	p.(L192*)	Nonsense	0	0	0	1	NA	1	5.77	NA
SPAST	c.1508G>C	p.(R503P)	Missense	0	0	0	1	1	1	5.13	NA
SPAST	c.1591dupC	p.(Q531Pfs*9)	Frameshift	0	0	0	SIFTindel: Damaging		5.74	n/a	
KIF5A	c.745C>G	p.(L249V)	Missense	0	0	0	1	0.631	0.999	1.15	NA
KIF5A	c.2590C>T	p.(R864*)	Nonsense	0	0	0	1	NA	1	4.9	NA
REEP1	c.166G>A	p.(D56N)	Missense	0	0	0	1	0.98	0.63	5.07	Yes
SPG11	c.2278T>C	p.(C760R)	Missense	0	0	0	0.99	0.998	1	3.82	NA
SPG11	c.5381T>C	p.(L1794P)	Missense	0.00004	0.00015	0	0.99	1	1	5.8	NA
CYP7B1	c.1304T>C	p.(L435P)	Missense	0	0	0	1	1	1	5.55	NA
CYP7B1	c.1322C>T	p.(P441L)	Missense	0	0	0	1	0.99	0.48	5.09	NA

**Table 3** Autosomal dominant HSP genes

Individual	Inheritance	Gene	Nucleotide change	Predicted AA change	Phenotype	AAO	Additional features
HSP27	AD	SPAST	c.1591dupC	p.(Q531Pfs*9)	Pure HSP	44	Urinary urgency
HSP31	Sporadic	SPAST	c.1508G>C	p.(R503P)	Pure HSP	34	
HSP46	AD	SPAST	c.1536G>C	p.(E512D)	Pure HSP	45	Periventricular and subcortical white matter lesions
HSP62	AD	SPAST	c.575T>A	p.(L192*)	Pure HSP	8	Sensory disturbance (UL/LL)
HSP65	AD	SPAST	c.1322A>T	p.(D441V)	Pure HSP	10	Sensory disturbance (LL)
HSP66	AD	SPAST	c.430C>T	p.(Q144*)	Complicated HSP	18 m	MCI, sensory disturbance, pes cavus
HSP71	AD	SPAST	c.1417C>T	p.(Q473*)	Pure HSP	50	
HSP21	Sporadic	KIF5A	c.745C>G	p.(L249V)	Complicated HSP	10	Sensorimotor axonal neuropathy
HSP67	Sporadic	KIF5A	c.2590C>T	p.(R864*)	Complicated HSP	5	Sensorimotor axonal neuropathy, LL hemiatrophy, pes cavus
HSP50	AD	REEP1	c.166G>A	p.(D56N)	Pure HSP	22	Pes cavus
HSP55	AD	ATL1	c.1483C>T	p.(R495W)	Pure HSP	N/A	

Abbreviations: AAO, age at onset; MCI, mild cognitive impairment; LL, lower limb; UL, upper limb.

ranging from early childhood to 50 years. We observed a trend for an earlier age of onset in truncating mutations at the 5' end of the gene (HSP62 and HSP66). All but one *SPAST* patient had a pure HSP phenotype, although sensory disturbance was common. One patient with early childhood onset had mild cognitive impairment in addition to spastic paraplegia (HSP66–c.430C>T variant) and another was found to have white matter lesions on MRI (HSP46–c.1536G>C variant).

**KIF5A.** *KIF5A* was the second most common AD HSP gene in our cohort. We identified two patients with *KIF5A* variants, both of which were novel. The c.2590C>T mutation is the first nonsense mutation identified in *KIF5A* to date. Both patients presented in early childhood with HSP complicated by a sensorimotor axonal neuropathy, the typical phenotype of SPG10.

A single patient with a novel *REEP1* variant and a single patient with a previously described pathogenic *ATL1* variant were also identified.

#### Autosomal recessive mutations

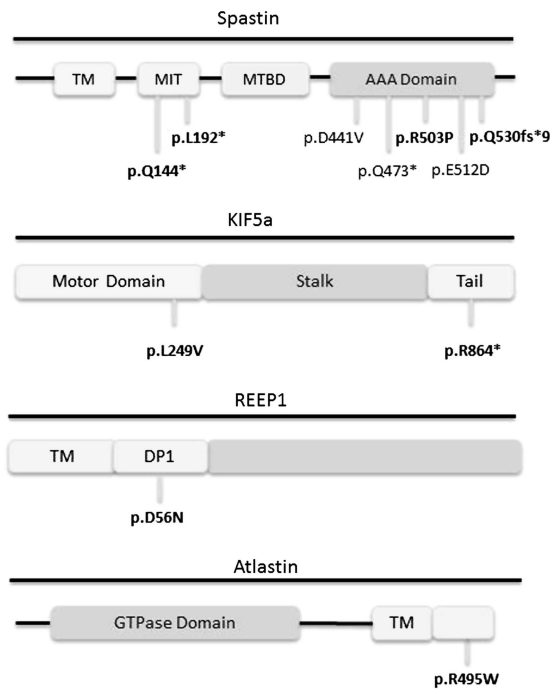
**SPG11.** We identified five probands with variants in *SPG11*. Two patients (HSP73 and HSP48) had a typical presentation of *SPG11* associated HSP with a complicated phenotype, thinning of the corpus callosum and onset below age 20 (see Table 4, Figure 2). These patients had a novel homozygous variant, c.2278T>C, and two known pathogenic variants c.[6856C>T (g) 2030\_2034del], respectively.

Interestingly, the three patients who had the c. 5381T>C variant in *SPG11* in addition to another deleterious variant had a significantly older age at onset. HSP29 developed symptoms at age 39. He had a complicated phenotype comprising spastic paraplegia, bilateral sensorineural deafness, diminished vibration sensation in the upper and lower limbs and mild sensory axonal neuropathy on Nerve Conduction Studies. MRI revealed mild ventricular and cortical sulci enlargement with diffuse periventricular T2 hyperintensity and a typical 'ears of the lynx' appearance at the frontal horn of the right lateral ventricle (see Figure 3). We identified a novel nonsense mutation c.255G>A in exon 1 of *SPG11* in addition to the c. 5381T>C variant, which we believe may be pathogenic. DNA was not available from other family members to assess if these variants occurred in *cis* or in *trans*.

HSP13 developed symptoms at age 60. He had two affected siblings. His parents were unaffected to our knowledge, implying an AR mode of inheritance. He presented with progressive spastic paraplegia, hyperreflexia in the upper limbs with hyporeflexia in the lower limbs. There was dysdiadochokinesis. We identified two variants in *SPG11*, a frameshift mutation in exon 25 (c.4307\_4308del) and the c. 5381T>C variant in exon 30.

HSP47 had a pure HSP phenotype, and developed symptoms at age 41. MRI revealed periventricular and centrum semiovale white matter lesions. The same c.[5470C>T]; [5381T>C] variants were found in the proband and her affected brother. An unaffected brother carried only the c. 5381T>C variant, proving that the mutations occur *in trans* in these patients.

*CYP7B1*. We identified three probands with variants in *CYP7B1*/SPG5a. One patient with a homozygous frameshift variant (c.250delC), had onset of spastic paraplegia at age 8 with hearing loss and sensory disturbance in the lower limbs. Two siblings, who were similarly affected, were found to carry the same homozygous variant.



**Figure 1** Schematic representations of the spastin, KIF5a, REEP1 and atlastin proteins. Novel mutations are shown in bold. TM: transmembrane, MIT: microtubule interacting domain, MTBD: microtubule-binding domain, AAA: ATPase-associated with diverse cellular activity.

An affected sister, with onset of symptoms age 13 years, was found to have symmetric frontoparietal white matter lesions and gaze-evoked nystagmus. All three siblings had prominent impairment of vibration sensation and an affected brother also had ataxia. This finding confirms previous reports<sup>7</sup> that mutations in *CYP7B1* may be associated with sensorineural deafness.

Patient HSP45 was found to have a homozygous novel mutation in *CYP7B1* (c.1322C>T). This patient with pure HSP had onset of symptoms at age 19. Two siblings had later age of onset in the 30 and 40 s, although DNA was not available for testing.

Patient HSP41 was found to have the c.[1304T>C (;) 1460dupT] variants in *CYP7B1*. Although the c.1304T>C variant is novel, the c.1460dupT variant is known to cause HSP.<sup>8</sup> This patient, with a pure HSP phenotype, had onset of symptoms at age 27. Vibration and proprioception in the lower limbs was impaired, whereas imaging of the neuroaxis was normal.

*SPG7*. One patient (HSP16) was found to have a homozygous c.1369C>T variant in *SPG7*. This variant has previously been reported to cause HSP in compound heterozygous state.<sup>9</sup> This patient, with onset of symptoms at age 40, was found to have mild distal upper limb weakness, rest tremor, lower limb sensory symptoms and mild cognitive impairment. Although hand tremor has been reported in up to 10% of patients with *SPG4*, this is the first report of rest tremor occurring in a case of *SPG7*.<sup>10</sup>

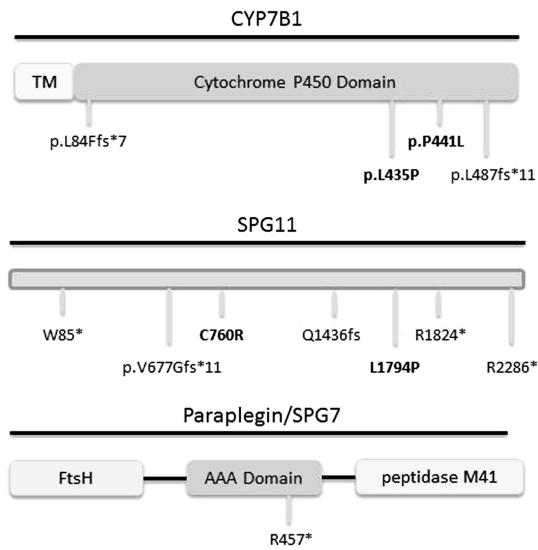
#### X-linked mutations

One patient was found to carry a small deletion in *ABCD1* (c.1174\_1178del: p.(Leu392SfsTer7), HSP4). This patient had been reported to have a pure HSP phenotype with possibly dominant inheritance. When the family history was reviewed, it was clear that males were affected severely, but females only mildly, and there was no male-to-male transmission of the disease, suggesting X-linked inheritance. The proband had onset of symptoms at age 20 with progressive

**Table 4** Autosomal recessive HSP genes

Individual	Inheritance	Gene	Nucleotide change	Predicted AA change	Phenotype	AAO	Additional features	Family segregation
HSP33	AR	<i>CYP7B1</i>	c.250delC	p.(L84Ffs*7)	Complicated HSP	8	Hearing loss, sensory disturbance (LL), symmetric frontoparietal WML	Same mutation found in two affected siblings
HSP45	AR	<i>CYP7B1</i>	c.1322C>T	p.(P441L)	Pure HSP	19	–	NA
HSP41	Sporadic	<i>CYP7B1</i>	c.[1304T>C (;) 1460dupT]	p.(L435P) (;) p.(L487Ffs*11)	Pure HSP	27	Sensory disturbance (LL)	
HSP73	Sporadic	<i>SPG11</i>	c.2278T>C	p.(C760R)	Complicated HSP	15	MCI, ichthyosis, TCC, WML	NA
HSP29	Unclear	<i>SPG11</i>	c.[255G>A (;) 5381T>C]	p.(W85*) (;) p.(L1794P)	Complicated HSP	39	Cognitive impairment, hearing loss, mild sensory axonal neuropathy, ventricular/cortical sulci enlargement, WML	N/A
HSP13	AR	<i>SPG11</i>	c.[4307_4308del (;) 5381T>C]	p.(Q1436Rfs*7) (;) p.(L1794P)	Complicated HSP	60	UL hyporeflexia, LL hyporeflexia, dysdiadochokinesis	N/A
HSP47	AR	<i>SPG11</i>	c.[5470C>T]; [5381T>C]	p.(R1824*); p.(L1794P)	Pure HSP	41	Periventricular, centrum semiovale WML	Same mutations found in affected brother, unaffected brother has only L1974P variant
HSP48	AR	<i>SPG11</i>	c.[6856C>T (;) 2030_2034del]	p.(R2286*) (;) p.(V677Gfs*11)	Complicated HSP	15	Cognitive impairment, hearing loss, dysarthria, TCC, periventricular WML	NA
HSP16	Unclear	<i>SPG7</i>	c.1369C>T	p.(R457*)	Complicated HSP	40	Urinary urgency, distal UL weakness, rest tremor, MCI, sensory disturbance (LL), UL hyporeflexia	NA

Abbreviations: AAO, age at onset; MCI, mild cognitive impairment; LL, lower limb; TCC, thinning of the corpus callosum; UL, upper limb; WML, white matter lesions.



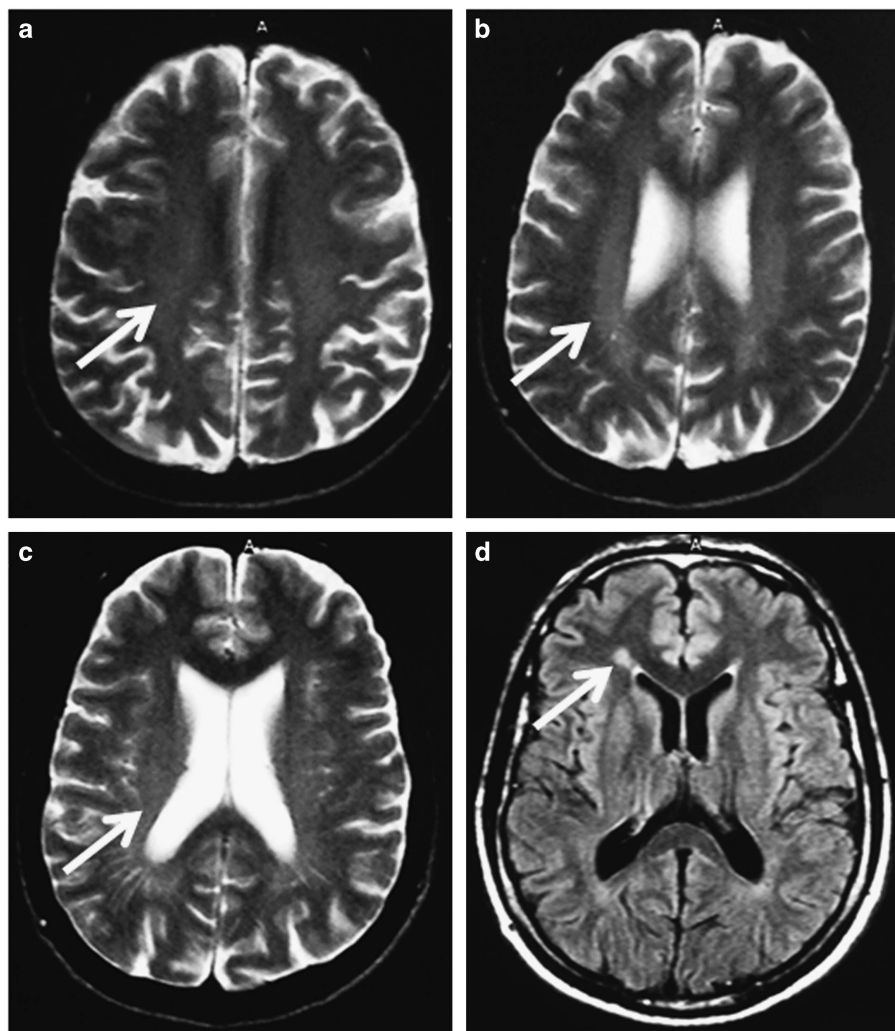
**Figure 2** Schematic representations of the SPG11, CYP7B1 and paraplegin proteins. Novel mutations are shown in bold. TM: transmembrane domain, FtsH: filamentous temperature sensitive H.

spastic paraplegia, urinary symptoms and sensory abnormalities in the lower limbs. Brain MRI was normal and thoracic spine MRI at age 55 revealed thinning of the thoracic spinal. Two cousins were similarly affected, and were also found to carry the same mutation. After the mutation was identified, very long chain fatty acids were tested in the proband and found to be within the range for adrenoleukodystrophy.

## DISCUSSION

In this paper we describe, for the first time, the clinical and genetic spectrum of HSP in Greece. We provide a clinical description of 54 cases from 40 families. Each of these families was genetically screened using a NGS approach. We made a genetic diagnosis in 21 families (52.5%). Our cohort consisted of a heterogeneous population with pure and complicated, sporadic and familial HSP. When variants in *SPAST* are excluded, our approach rendered a genetic diagnosis in 13 of 33 patients (39%), which is superior to the 25% diagnostic rate found by Kumar *et al.*,<sup>8</sup> who used a similar NGS panel to screen *SPAST*-negative patients in Australia.

Our study describes for the first time the mutational spectrum of HSP in Greece. The frequency of gene mutations in HSP varies considerably across populations, therefore a comprehensive



**Figure 3** (a–c) axial T2 images showing diffuse periventricular hyperintensity (arrows), along with ventricular and cortical sulci enlargement; (d) axial FLAIR image showing early 'flame-' or 'ears-of-the-lynx-' formation (arrow).

assessment of mutation frequency is essential to characterise the population and guide accurate genetic testing. We show that point mutations in *SPAST* are by far the most common cause of AD HSP in the Greek population, which is consistent with other studies. *KIF5A* variants were the second most common cause of AD HSP, and were the most frequent cause of complicated, early childhood onset HSP, consistent with previous reports estimating the frequency of *KIF5A* mutations in AD HSP at 10%.<sup>11</sup> In addition, we report the first nonsense mutation in *KIF5A* (c.2590C>T) in a patient with a typical SPG10 phenotype consisting of early-onset spastic paraplegia with sensorimotor axonal neuropathy. To date, most pathogenic *KIF5A* variants have been detected in the motor domain and are expected to impair protein function through a dominant-negative effect.<sup>12</sup> However, two *KIF5A* variants outside the motor domain have been found,<sup>13,14</sup> and the mechanism with which these cause disease has not been identified.<sup>15</sup> We therefore suggest that further functional work is required before *KIF5A* variants outside the motor domain can be excluded, particularly in patients with typical SPG10 or axonal CMT2 phenotypes.

*ATL1* and *REEP1* were relatively rare causes of AD HSP in our population, which is surprising in comparison with other Western European populations. No mutations in *NIPA1* were identified.

*SPG11* was the commonest cause of AR HSP, again in accordance with previous findings. Interestingly, we identified a novel c.5381T>C variant, which, when in combination with another deleterious *SPG11* variant, leads to a syndrome of complicated HSP with relatively late onset. This variant appears to be unique to the Greek cohort under study. The majority of *SPG11* variants identified to date lead to premature protein termination or splicing defects. However, at least two missense mutations have already been identified in families with a typical *SPG11* phenotype.<sup>16,17</sup> Clearly, further genetic and functional work will be required to determine whether and how missense mutations in *SPG11* lead to disease.

*CYP7B1* variants were the second most common cause of AR HSP, and we confirm that sensorineural deafness can occur as part of the spectrum of *CYP7B1*-associated HSP.

Surprisingly, we identified a family with a pathogenic deletion in *ABCD1* mimicking HSP and presenting with what was initially thought to be dominant inheritance. It has previously been shown that adrenoleukodystrophy can mimic HSP and even present with an apparently AR mode of inheritance.<sup>18</sup> It is essential to identify patients with adrenoleukodystrophy as they require careful monitoring of adrenal function and are potentially treatable with stem cell transplant should cerebral adrenoleukodystrophy develop.<sup>19</sup>

We did not detect potentially pathogenic variants in 19 probands. Sixteen of these were sequenced using the Truseq custom HSP panel and three were sequenced using the Trusight Exome. Of these 19, 9 had an AD family history of HSP, 8 had sporadic inheritance and the family history was unknown in 2. Seventeen had a pure HSP phenotype, with only two patients having a complicated phenotype. This raises interesting questions as to whether there are many more genes causing HSP that have not yet been identified. It will be necessary to perform whole-exome or whole-genome analysis on a large cohort of phenotypically similar pure HSP cases to address this.

This study shows that a NGS approach can be an efficient way to make a genetic diagnosis in a heterogeneous population naive to genetic testing. With one test, more than half of patients can be given a genetic diagnosis, allowing for resources to be concentrated on

investigating gene-negative patients and families. NGS approaches may be particularly relevant in resource poor settings, where biochemical and radiological testing may not be readily available or may be prohibitively expensive. This study confirms that NGS approaches are efficient and cost-effective and should be employed in the first line of investigation in HSP patients.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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