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.1 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.2 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.3 Despite the abundance of genes involved in HSP, many of the genes impact on the same molecular pathways. These include axonal transport (KIF5A, KIF14), endoplasmic reticulum formation (ATL1, SPAST, REEP1), endosomal and lysosomal trafficking (SPG11, AP4B1), and mitochondrial function (SPG7, mATP6, HSPD1).2

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.4 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.5 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/tru-sight_exome_product_files.html.

DNA samples were diluted to 5 ng/µl and were then target-fragmented according to the Trusight Enrichment DNA Sample Preparation Guide. Libraries were sequenced on 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, BSC1, HSPDI, GJC, KIAA0196, KIF5A, NIPA1, PLP, PNPLA6, REEP1, RTN2, SPG11, SPG7, CYP7B1, FAZH. DNA was diluted to 250 ng and procedures followed the TruSeq Custom Amplicon Library Preparation Guide. Libraries were sequenced on 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.

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 Sequence 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 Variant Server and an internal database of over 600 exomes. All novel variants 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 Sequence 3 software (Applied Biosystems).

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Novel variants identified

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. 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. 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 pedigrees. 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 onset | Urinary symptoms | LL sensory disturbance | Neuropathy | Cognitive impairment | TCC | WML | Cerebellar signs | Additional features |
|--------|-------------|------------|-----|-----------|--------------|------------------|------------------------|------------|---------------------|-----|-----|------------------|---------------------|
| 1      | X-linked    | HSP1       | M   | Pure      | 27           | +                | −                     | −          | −                   | −   | −   | −                | −                   |
| 2      |             | HSP8       | F   | Pure      | NA           | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 3      |             | HSP9       | M   | Pure      | 40           | +                | −                     | −          | −                   | −   | −   | −                | −                   |
| 4      | Sporadic    | HSP11      | M   | Pure      | 60           | +                | +                     | −          | −                   | −   | −   | −                | −                   |
| 5      |             | HSP12      | M   | Pure      | 35           | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 6      |             | HSP13      | M   | Complicated | 60           | −                | +                     | +          | −                   | −   | −   | +                | −                   |
| 7      |             | HSP14      | M   | Pure      | 14           | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 8      | Unknown     | HSP16      | M   | Complicated | 40           | +                | +                     | +          | −                   | −   | −   | −                | −                   |
| 9      |             | HSP17      | M   | Complicated | 40           | −                | −                     | +          | −                   | −   | −   | −                | −                   |
| 10     | Sporadic    | HSP19      | M   | Pure      | 9 m          | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 11     | Unknown     | HSP20      | F   | Pure      | 63           | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 12     | Sporadic    | HSP21      | M   | Complicated | 10           | −                | +                     | −          | −                   | −   | −   | −                | −                   |
| 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            | −                | +                     | −          | −                   | −   | −   | +                | −                   |
| 20     | AR          | HSP34      | M   | Complicated | 10           | +                | −                     | −          | −                   | −   | −   | −                | −                   |
| 21     | AD          | HSP35      | F   | Complicated | 13           | −                | +                     | +          | −                   | −   | −   | +                | −                   |
| 21     | AD          | HSP37      | F   | Pure      | NA           | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 22     | Sporadic    | HSP40      | M   | Complicated | 6            | −                | +                     | +          | −                   | −   | −   | +                | −                   |
| 22     | Sporadic    | HSP41      | F   | Pure      | 27           | −                | +                     | −          | −                   | −   | −   | +                | −                   |
| 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           | −                | −                     | +          | +                   | +   | +   | +                | + Dysesthesia        |
| 27     | AD          | HSP49      | M   | Pure      | 46           | −                | +                     | −          | −                   | −   | −   | +                | −                   |
| 27     | AD          | HSP50      | M   | Pure      | 22           | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 28     | AD          | HSP51      | M   | Pure      | 22           | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 29     | AD          | HSP52      | M   | Pure      | 25           | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 30     | AD          | HSP55      | M   | Pure      | 12           | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 31     | AD          | HSP62      | M   | Pure      | 8            | −                | +                     | −          | −                   | −   | −   | +                | −                   |
| 32     | Sporadic    | HSP64      | M   | Complicated | 12           | +                | +                     | +          | −                   | −   | −   | +                | −                   |
| 33     | AD          | HSP65      | M   | Pure      | 10           | −                | +                     | −          | −                   | −   | −   | −                | −                   |
| 34     | AD          | HSP66      | F   | Pure      | 18 m         | −                | −                     | +          | −                   | −   | −   | −                | −                   |
| 34     | AD          | HSP70      | M   | Complicated | 18 m        | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 35     | Sporadic    | HSP67      | M   | Complicated | 5            | −                | +                     | −          | −                   | −   | −   | +                | −                   |
| 35     | Sporadic    | HSP68      | F   | Pure      | 25           | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 36     | AD          | HSP69      | M   | Pure      | 49           | −                | −                     | −          | −                   | −   | −   | +                | −                   |
| 37     | AD          | HSP71      | F   | Pure      | 50           | −                | −                     | −          | −                   | −   | −   | −                | −                   |
| 38     | Sporadic    | HSP73      | F   | Complicated | 15           | −                | −                     | +          | +                   | +   | −   | +                | −                   |
| 39     | Sporadic    | HSP74      | M   | Pure      | NA           | −                | −                     | −          | −                   | −   | −   | −                | −                   |

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; Complicated, HSP with additional neurological features such as ataxia, neuropat hy, 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.
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 another deleterious variant had a significant trend for an earlier age of onset in truncating mutations at the 5' end of the gene.

### 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 | 1 | 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*) | Missense | 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 | 5.32 | NA |
| SPG11 | c.5381T>C | p.(L1794P) | Missense | 0.00004 | 0.00015 | 0 | 0.99 | 1 | 1 | 5.8 | NA |
| KIF5A | c.1304T>C | p.(L435P) | Missense | 0 | 0 | 0 | 1 | 1 | 1 | 5.56 | NA |
| KIF5A | c.1322C>T | p.(P441L) | Missense | 0 | 0 | 0 | 1 | 1 | 1 | 5.56 | 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 | Perventricular 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 | 18m | 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 | AT1L | c.1483C>T | p.(R495W) | Pure HSP | N/A | |

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

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.

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 that mutations in CYP7B1 may be associated with sensorineural deafness.

One 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 40s, 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. 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.

Patient HSP47 was found to have a homozygous novel mutation in CYP7B1 (c.5470C>T;[c.5381T>C]). This patient had 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          | CYP7B1| c.250delC         | p.(L84F*7)          | Complicated HSP       | 8   | Hearing loss, sensory disturbance (LL), symmetric frontoparietal WML | Same mutation found in two affected siblings |
| HSP45      | AR          | CYP7B1| c.1322C>T         | p.(P441L)           | Pure HSP              | 19  | –                                             | NA                |
| HSP41      | Sporadic    | CYP7B1| c.(1304T>C(;)1460dupT) | p.(L435P) p. (L487F*11) | Pure HSP              | 27  | Sensory disturbance (LL)                       | NA                |
| HSP73      | Sporadic    | SPG11 | c.2278T>C         | p.(C760R)           | Complicated HSP       | 15  | MCI, ichthyosis, TCC, WML                      | NA                |
| HSP29      | Unclear     | SPG11 | 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 UL hypereflexia, LL hyporeflexia, dysdiadochokinesis | N/A               |
| HSP13      | AR          | SPG11 | c.(4307,4308del();5381T>C) | p.(Q1436Fs*7); p. (L1794P) | Complicated HSP       | 60  | –                                             | N/A               |
| HSP47      | AR          | SPG11 | c.5470C>T         | 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          | SPG11 | c.6856C>T         | p.(R2286*); p. (V677G*11) | Complicated HSP       | 15  | Cognitive impairment, hearing loss, dysarthria, TCC, periventricular WML | NA                |
| HSP16      | Unclear     | SPG7  | c.1369C>T         | p.(R457*)           | Complicated HSP       | 40  | Urinary urgency, distal UL weakness, rest tremor, MCI, sensory disturbance (LL), UL hypereflexia | NA                |

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

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

**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%.11 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.12 However, two KIF5A variants outside the motor domain have been found,13,14 and the mechanism with which these cause disease has not been identified.15 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.16,17 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 ABDC1 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.18 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.19

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 naïve 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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