Autosomal recessive spastic paraplegia (SPG30) with mild ataxia and sensory neuropathy maps to chromosome 2q37.3

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The hereditary spastic paraplegias (HSPs) are a clinically and genetically heterogeneous group of neurodegenerative diseases characterized by progressive spasticity in the lower limbs. Twenty-nine different loci (SPG) have been mapped so far, and 11 responsible genes have been identified. Clinically, one distinguishes between pure and complex HSP forms which are variably associated with numerous combinations of neurological and extra-neurological signs. Less is known about autosomal recessive forms (ARHSP) since the mapped loci have been identified often in single families and account for only a small percentage of patients. We report a new ARHSP locus (SPG30) on chromosome 2q37.3 in a consanguineous family with seven unaffected and four affected members of Algerian origin living in Eastern France with a significant multipoint lod score of 3.8. Ten other families from France (n = 4), Tunisia (n = 2), Algeria (n = 3) and the Czech Republic (n = 1) were not linked to the newly identified locus thus demonstrating further genetic heterogeneity. The phenotype of the linked family consists of spastic paraparesis and peripheral neuropathy associated with slight cerebellar signs confirmed by cerebellar atrophy on one CT scan.

Keywords: SPG30; chromosome 2q37.3; autosomal recessive spastic paraplegia; linkage

Abbreviations: HSP = hereditary spastic paraplegia


Introduction

The hereditary spastic paraplegias (HSPs) are a clinically and genetically heterogeneous group of neurodegenerative diseases characterized by progressive spasticity in the lower limbs. The mode of inheritance may be autosomal dominant, autosomal recessive (ARHSP) or X-linked. Twenty-nine different loci (SPG) have been mapped so far, and 11 responsible genes identified. The corresponding proteins are often involved in axonal trafficking or mitochondrial metabolism (Reid, 2003).

Clinically, one distinguishes between pure and complex forms of HSP (Durr and Brice, 2000; Tallaksen et al., 2001). In pure forms, clinical features consist of isolated pyramidal signs, such as brisk reflexes, Babinski sign, spasticity and motor deficit, which can be associated with sphincter disturbances and deep sensory loss. In the complex forms of HSP, the disease is variably associated with numerous combinations of neurological and extra-neurological signs such as cerebellar ataxia, dysarthria, mental retardation, peripheral neuropathy, optic atrophy, retinitis pigmentosa, hearing loss or thin corpus callosum.

Approximately 40% of dominant cases are explained by the known loci. Less is known about ARHSP since the mapped
loci have been identified in few, often single, families and account for only a small percentage of patients. ARHSP is usually associated with clinically complex phenotypes but SPG5, SPG24 and SPG28 are considered to be pure forms of the disease (Bouslam et al., 2005; Hodgkinson et al., 2002; Meijer et al., 2004; Wilkinson et al., 2003).

In this study we report a new ARHSP locus on chromosome 2q37.3 in a consanguineous family of Algerian origin.

**Patients and methods**

**Patients**

Eleven families with ARHSP were selected (eight with a pure form and three with a complex form of ARHSP). They included 64 individuals, 27 of whom were affected. After written consent, all individuals were examined by a neurologist. Standardized charts were used for the clinical evaluations of all participants. An Algerian family (FSP-546; Fig. 1), with seven unaffected and four affected members, was included in a genome-wide scan. The other ten families originated from France (n = 4), Tunisia (n = 2), Algeria (n = 3) and the Czech Republic (n = 1).

DNA was extracted from blood samples using a standard protocol. Mutations in the SPG7 gene (paraplegin) (Casari et al., 1998) were excluded in the index patients of all families by dHPLC screening (Elleuch et al., 2006).

**Genotyping**

Microsatellite markers were amplified with fluorescent primers and the fragments resolved on an ABI-3730 sequencer (Applied Biosystems, Foster City, CA, USA). Genotypes were determined with the GeneMapper 3.5 software (Applied Biosystems).

The genome-wide scan in family FSP-546 was performed using 400 microsatellites spaced ~10 cM (centimorgans) apart on all chromosomes. Pairwise and multipoint linkage analyses were performed using Fastlink 3.0 (Cottingham et al., 1993) and Allegro 1.2c software (Gudbjartsson et al., 2000). The disease was considered to be a fully penetrant autosomal recessive trait, with a disease allele frequency of 0.00005 and equal recombination fractions for males and females. Genetic distances were those of the Marshfield Centre for Medical Genetics and map positions were verified on the human genome sequence draft (NCBI and Ensembl centres).

**Candidate gene analysis**

Direct sequencing of all coding exons, their flanking splice sites and at least 50 bp of intronic sequence on each side of the STK25 gene was done using the BigDye terminator chemistry on an ABI-3730 sequencer (Applied Biosystems). The chromatogram profiles were analysed using Seqscape 2.5 software (Applied Biosystems). PCR primers and annealing conditions are available upon request.

**Results**

**Mapping of SPG30**

After exclusion of linkage to several known loci for ARHSP (SPG5, SPG11, SPG21, SPG24, SPG27, SPG28) and amyotrophic lateral sclerosis (ALS2 and ALS5) and in the
absence of mutations in the SPG7 gene, a genome-wide screen in family FSP-546 provided evidence of linkage at two consecutive markers on chromosome 2 with a multipoint lod score of 3.8. Six other possible locations with multipoint lod scores >1 were detected on chromosomes 2, 7, 10, 11, 12 and 20, but were excluded when 25 additional markers were used (data not shown).

Analysis of nine additional microsatellite markers on chromosome 2 generated significant pairwise lod scores >3 (Table 1) at markers D2S2285 ($z = 3.1$) and D2S125 ($z = 3.2$). A maximal and significant multipoint lod score of 3.8 was obtained in the D2S2338–D2S2585 interval (Fig. 2), in agreement with haplotype reconstruction showing that all markers in this 5.1 cM interval were homozygous in affected patients (Fig. 1). This new locus was named SPG30 according to the HUGO nomenclature. This interval spans a 4 Mb region and contains 62 genes, one of which, STK25, that encodes a protein kinase involved in the response to environmental stress and in protein transport, did not have mutations/polymorphisms in the coding exons, in patients.

The interval between markers D2S2338 and D2S2585 was excluded in the other 10 ARHSP families by haplotype reconstruction and/or linkage analysis with multipoint lod scores below the threshold of −2 (data not shown).

### Clinical features in family FSP-546

In the nuclear family FSP-546 (Fig. 1), there were nine siblings (six men, three women) born of parents who were first cousins. All 11 members were examined and sampled for DNA extraction. Four of the siblings (three men, one woman) were clinically affected and neurological examination was normal in the remaining children and both parents. The mean age at onset was $17.5 \pm 4$ years (12–21 years).

The overall picture was spastic gait with variable associated distal wasting, sensory neuropathy and cerebellar ataxia (Table 2).

The index patient (FSP-546-004) was a 35-year-old man who first noticed stiff legs at age 20. At age 25, he was unable to run and had difficulty going down the stairs. Examination showed increased reflexes in the lower limbs (LL) and flexor plantar responses. Reflexes were normal in the upper limbs. Spasticity was moderate on gait and at rest with moderate weakness in the proximal LL. Muscle wasting in

### Table 1 Pairwise lod scores calculated in family FSP-546 between the disease locus and 9 microsatellite markers on chromosome 2

<table>
<thead>
<tr>
<th>Marker</th>
<th>LOD score at $	heta$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>D2S338</td>
<td>$\infty$</td>
</tr>
<tr>
<td>D2S345</td>
<td>-2.35</td>
</tr>
<tr>
<td>D2S2338</td>
<td>-2.45</td>
</tr>
<tr>
<td>D2S2285</td>
<td>3.11</td>
</tr>
<tr>
<td>D2S125</td>
<td>3.23</td>
</tr>
<tr>
<td>D2S395</td>
<td>1.35</td>
</tr>
<tr>
<td>D2S1378</td>
<td>2.80</td>
</tr>
<tr>
<td>D2S140</td>
<td>1.42</td>
</tr>
<tr>
<td>D2S2585</td>
<td>$\infty$</td>
</tr>
</tbody>
</table>

LOD: logarithm of odds.

Fig. 2 Multipoint linkage analysis. Lod scores are plotted according to the genetic map of chromosome 2q. In bold are indicated the markers used for the genome scan. cM = centimorgan.
both legs was evident. Vibration sense and pinprick sensation were normal, and there were no sphincter disturbances or cerebellar signs.

His brother (FSP-546-009) complained at age 17 of a pricking sensation in the upper limbs. When he was examined at age 27 he could not stand with his feet in tandem position. There was moderate spasticity in his lower limbs at gait and rest. He had increased reflexes in his knees but not in his ankles and plantar reflexes were extensor. There was mild distal wasting in the lower limbs and some distal weakness. The finger–nose test showed cerebellar clumsiness. The patient also described mild sphincter disturbances. Distal touch/pinprick sensitivity was decreased in the legs, but vibration sense at the ankles was observed. Ocular gaze was normal except for saccadic pursuit. Decreased sensory but physiological motor amplitudes were recorded during nerve conduction studies (Table 3). Needle EMG showed a reduced recruitment pattern with some potentials firing at an increased rate, which is a sign of denervation. Cerebral CT scan performed at age 29 showed mild diffuse cerebellar atrophy.

Their sister (FSP-546-010) a 26-year-old woman, first remarked unsteadiness and stiff legs at age 21. She suffered of a painful knee and spasticity was moderate in her lower limbs predominantly in her left leg, on gait and at rest. Clinical examination revealed increased reflexes in all limbs and a plantar extensor reflex on both sides. Finger–nose test was slightly cerebellar on the left side. Vibration sense was described as normal at the ankles. Pinprick sensation was decreased in both feet.

The youngest brother (FSP-546-11) fell accidentally at age 11, and thereafter unsteady gait and stiff legs symptoms developed. At age 19, he was moderately impaired but unable to run. Spasticity was severe at gait and muscle tonus increased at rest. He had increased reflexes in all limbs with ankles clonus and bilateral extensor plantar reflexes. There was mild wasting in the upper limbs. He reported having mild urinary urgency and painful legs. Ocular pursuit was saccadic.

**Discussion**

We mapped a novel ARHSP locus (SPG30) on chromosome 2q37.3 in a consanguineous Algerian family living in Eastern France. After exclusion of candidate SPG and ALS loci and of the other regions with a lod score $>1$ in the genome-wide scan, a single candidate region remained on chromosome 2. Fine mapping using nine additional markers and haplotype reconstruction narrowed the candidate region to a 4 Mb interval. Linkage to SPG30 was also excluded in 10 other ARHSP families who did not carry mutations in the SPG7 gene.

This is the first ARHSP locus found on chromosome 2 where two forms of ADHSP, SPG4—the most frequent—and SPG13, have also been located (Durr et al., 1996; Hansen et al., 2002). ALS2 (Alsin) (Yang et al., 2001) and a genetic form of a spastic cerebral palsy (McHale et al., 1999) also map to chromosome 2, although to different regions and differ clinically from SPG30 by the occurrence...
<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Chromosomal location</th>
<th>Gene product</th>
<th>Phenotype</th>
<th>Age at onset (years)</th>
<th>Cerebellar signs and/or atrophy</th>
<th>PNP</th>
<th>Additional signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPG5 (Hentati et al., 1994; Wilkinson et al., 2003)</td>
<td>8q–11q13</td>
<td>Pure</td>
<td>1–40</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>SPG7 (Casari et al., 1998; DeMichele et al., 1998; Wilkinson et al., 2004)</td>
<td>16q24.3</td>
<td>Paraplegin</td>
<td>Pure, complex</td>
<td>11–42</td>
<td>Yes</td>
<td>Yes</td>
<td>Pes cavus, optic atrophy</td>
</tr>
<tr>
<td>SPG11 (Martinez Murillo et al., 1999)</td>
<td>15q13-q15</td>
<td>–</td>
<td>Pure, complex</td>
<td>1–50</td>
<td>No</td>
<td>–</td>
<td>Mental retardation, pes cavus, thin CC</td>
</tr>
<tr>
<td>SPG14 (Vazza et al., 2000)</td>
<td>3q27-q28</td>
<td>–</td>
<td>Complex</td>
<td>~30</td>
<td>No</td>
<td>Yes</td>
<td>Pes cavus, mental retardation, visual agnosia, memory deficiency</td>
</tr>
<tr>
<td>SPG15 (Hughes et al., 2001)</td>
<td>14q22-q24</td>
<td>Complex</td>
<td>13-23</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
<td>Intellectual deterioration, pigmented macula, CC and brainstem atrophy</td>
</tr>
<tr>
<td>SPG20 (Patel et al., 2002)</td>
<td>13q12.3</td>
<td>Spartin</td>
<td>Complex</td>
<td>Early childhood</td>
<td>Yes</td>
<td>–</td>
<td>Mental retardation, shortness of stature</td>
</tr>
<tr>
<td>SPG21 (Simpson et al., 2003)</td>
<td>15q21-q22</td>
<td>Maspardin</td>
<td>Complex</td>
<td>20–40</td>
<td>Yes</td>
<td>Yes</td>
<td>Extrapyramidal syndrome, dementia, thin CC, periventricular white matter hyperintensities, cataract, dystonia, chorea, hand muscle atrophy, Abnormalities of skin and hair pigmentation, facial and skeletal dysmorphism, postural tremor, cognitive impairment</td>
</tr>
<tr>
<td>SPG23 (Blumen et al., 2003)</td>
<td>1q24–q32</td>
<td>–</td>
<td>Complex</td>
<td>Early childhood</td>
<td>No</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SPG24 (Hodgkinson et al., 2002)</td>
<td>13q14</td>
<td>–</td>
<td>Pure</td>
<td>1</td>
<td>No</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SPG25 (Zortea et al., 2002)</td>
<td>6q23-q24.1</td>
<td>–</td>
<td>Complex</td>
<td>30–46</td>
<td>No</td>
<td>Yes</td>
<td>Multiple disc herniation, bilateral cataract, congenital glaucoma</td>
</tr>
<tr>
<td>SPG26 (Wilkinson et al., 2005)</td>
<td>12p11.1–12q14</td>
<td>–</td>
<td>Complex</td>
<td>22–42</td>
<td>No</td>
<td>Yes</td>
<td>Emotional lability, tongue tremor, mild intellectual impairment</td>
</tr>
<tr>
<td>SPG27 (Meijer et al., 2004; Ribai et al., 2006)</td>
<td>10–q22.1–q24.1</td>
<td>–</td>
<td>Pure, complex</td>
<td>2–45</td>
<td>Yes</td>
<td>Yes</td>
<td>Mental retardation, microcephaly, facial dysmorphism, blepharophimosis, skeletal dysmorphism</td>
</tr>
<tr>
<td>SPG28 (Bouslam et al., 2005)</td>
<td>14q21.3–q22.3</td>
<td>–</td>
<td>Pure</td>
<td>6–15</td>
<td>No</td>
<td>–</td>
<td>Pes cavus and scoliosis in 1 patient</td>
</tr>
<tr>
<td>SPG30</td>
<td>2q37.3</td>
<td>–</td>
<td>Complex</td>
<td>12–21</td>
<td>Yes</td>
<td>Yes</td>
<td>–</td>
</tr>
<tr>
<td>Spastic cerebral palsy (McHale et al., 1999)</td>
<td>2p24–25</td>
<td>–</td>
<td>Complex</td>
<td>Early childhood</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

CC = corpus callosum; PNP = peripheral polyneuropathy.
of bulbar and pseudobulbar signs (ALS2), mental retardation, epilepsy and quadriplegia (spastic cerebral palsy). The refined region contains 62 genes, several of which encode proteins potentially involved in HSP according to their physiological function (proteins involved in the molecular trafficking and mitochondrial metabolism). Point mutations in the STK25 gene, which encodes serine/threonine kinase 25, were excluded by direct sequencing. The screening of other candidate genes is under way.

In contrast to autosomal dominant HSP where two genes, SPG3 encoding atlastin-1 and SPG4 encoding spastin, account for a significant proportion of patients, ARHSP seems to be caused by a multiplicity of genes, as suggested by a number of new ARHSP loci published recently (Hodgkinson et al., 2002; Meijer et al., 2004; Bouslam et al., 2005; Wilkinson et al., 2005). SPG7, the most frequent form of ARHSP reported so far, which encodes paraplegin, explains only part of the ARHSP cases (Elleuch et al., 2006).

The phenotype of the SPG30 family consists of early onset and slowly progressive spastic paraparesis associated with slight cerebellar signs, such as saccadic ocular pursuit, finger–nose clumsiness, difficulty with tandem standing and cerebellar atrophy on CT scan, when performed. In addition, electrophysiological examination in one patient showed the presence of a peripheral polyneuropathy which was found clinically in another patient. Finally, disease progression seemed slow as all patients were able to walk after disease durations up to 15 years.

Phenotypical variability is observed in several ARHSP and the phenotype of SPG30 might be larger than observed in this Algerian family. Most of the families tested in the present study showed a pure ARHSP phenotype, whereas most of the known ARHSP forms, including SPG30, are described as complicated with additional neurological or other clinical features (Table 4). This may explain why we did not find other families linked to the SPG30 locus. Both, pure and complicated forms may, however, be linked to the same locus, as families linked to the SPG30 locus. Both, pure and complicated forms (Table 4). This may explain why we did not find other families linked to the SPG30 locus. Both, pure and complicated forms may, however, be linked to the same locus.

Although spastic paraparesis is clearly the major sign in SPG30, superficial examination could miss the associated neurological signs (e.g. cerebellar signs) leading to the initial diagnosis of pure and not complicated HSP. This finding is important in clinical practice since many forms of ARHSP present with additional cerebellar signs (6/14 ARHSP loci, Table 4) which may be overlooked when examining patients with prominent spasticity. The mild cerebellar involvement which was found in this new form of ARHSP is similar to that observed in patients with SPG7 (paraplegin) mutations, which was also initially thought to be a pure ARHSP (De Michele et al., 1998). Recent studies have shown, however, that mild cerebellar signs and/or cerebellar atrophy on brain imaging are almost constant in SPG7 (Elleuch et al., 2006). Several other forms of ARHSP are also associated with neuropathy (Table 4) and its association with cerebellar ataxia is not specific to SPG30 but can also be found in SPG7, SPG21 and SPG27.

In conclusion we have mapped a novel locus (SPG30) to chromosome 2q37.3 that is responsible for a new autosomal recessive form of complicated HSP. This is the first step towards the identification of a new gene crucial for understanding the underlying pathophysiology of HSP.

**Electronic sources**

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