Mapping of a complicated familial spastic paraplegia to locus SPG4 on chromosome 2p

Olivier Heinzlef, Caroline Paternotte, Florence Mahieux, Jean-François Prud’homme, Joëlle Dien, Michel Madigand, Jean Pouget, Jean Weissenbach, Etienne Rouillet, Jamile Hazan

Abstract
Autosomal dominant familial spastic paraplegia (AD-FSP) is a degenerative disorder of the central motor system characterised by progressive spasticity of the lower limbs. AD-FSP has been divided into pure and complicated forms. Pure AD-FSP is genetically heterogeneous; three loci have been mapped to chromosomes 14q (SPG3), 2p (SPG4), and 15q (SPG6), whereas no loci responsible for complicated forms have been identified to date. Here we report linkage to the SPG4 locus in a three generation family with AD-FSP complicated by dementia and epilepsy. Assuming that both forms of AD-FSP are caused by mutations involving the same FSP gene, analysis of recombination events in this family positions the SPG4 gene within a 0 cM interval flanked by loci D2S2255 and D2S2347.

Keywords: familial spastic paraplegia (FSP); dementia; epilepsy; SPG4 locus

Familial spastic paraplegia (FSP) is a clinically and genetically heterogeneous group of neurodegenerative disorders mainly characterised by progressive spasticity of the lower limbs (spastic gait, hyperreflexia, clonus, and Babinski sign). The basic pathological features of FSP are degeneration of the crossed pyramidal tracts and thinning of the dorsal columns. FSP is commonly divided into two forms, depending on whether spasticity occurs in isolation (“pure” FSP, MIM 182600) or associated with a wide range of additional symptoms (“complicated” FSP), such as mental retardation (MIM 270850, 270950, 182690), ichthyosis, optic atrophy (MIM 182830), dementia (MIM 182830), epilepsy, ataxia, deafness (MIM 312910, 182690), and peripheral neuropathies (MIM 182800). FSP can be inherited in an autosomal dominant (AD-FSP), autosomal recessive (AR-FSP), or X linked (X-FSP) manner. Within each inheritance pattern, FSP exhibits both clinical variability and genetic heterogeneity. Genetic heterogeneity was first described for two different forms of X linked FSP; in a family manifesting X-FSP associated with mental retardation and optic atrophy, the disease gene was mapped to Xq28 markers (locus SPG1), while linkage to the Xq21-22 region (locus SPG2) was reported in a kindred with pure X-FSP. Pure AD-FSP and AR-FSP were also shown to be genetically heterogeneous. A locus for AR-FSP has been mapped to chromosome 8 (SPG5) and three loci responsible for pure AD-FSP have been localised on chromosomes 14q (SPG3), 2p (SPG4), and 15q (SPG6).

Compld forms of FSP are rare and are usually transmitted in an autosomal recessive pattern although some cases showing autosomal dominant or X linked inheritance have also been described. Here we report a three generation family with AD-FSP complicated by dementia and epilepsy, in which we found significant linkage of the disease gene to the SPG4 locus on chromosome 2p. Assuming that both forms of FSP result from mutations in the same gene, recombinant analysis in this complicated AD-FSP kindred allowed us to refine the SPG4 region and to place the gene within a 0 centimorgan (cM) interval flanked by loci D2S2255 and D2S2347.

Subjects and methods
PATIENTS
Fourteen members of a three generation French family (family CM, fig 1) were examined by the authors (OH, MM, JD, and JP) and seven met the disease criteria defined as follows: (1) subjects with progressive spastic paraplegia showing pyramidal signs in the lower limbs (spasticity, increased reflexes, and Babinski sign) and an asymptomatic patient with bilateral extensor plantar reflexes were considered to be affected; (2) the asymptomatic family members with normal neurological examination were considered unaffected. Disability was assessed on a three point scale as previously described: 1=normal gait or very slight stiffness in the legs; 2=unable to run, but able to walk without help; 3=unable to walk without help or wheelchair bound. Living affected members with memory impairment underwent the following neuropsychological tests: Mini-mental state (MMS) to
evaluate global cognitive performance, Rey-Osterrieth complex figure exploring constructional abilities, Wechsler memory scale, Raven’s progressive matrices which provide an evaluation of IQ based on non-verbal reasoning; and WAIS-R which consists of several subtests performed to determine verbal and non-verbal IQs. Informed consent was obtained from each family member before blood samples were drawn.

**GENOTYPING**

DNA was extracted from whole blood using standard procedures. Polymerase chain reactions (PCR) were carried out as previously described. Four amplification products, generated with separate primer sets on identical DNA samples, were co-precipitated and co-migrated in a single lane of 6% polyacrylamide denaturing gel. Separated products were then transferred to Pall membranes and hybridised successively with no-radio labelled (ECL, Amersham) PCR primers as previously reported.

**LINKAGE ANALYSIS**

Two point lod scores between FSP and SPG4 markers were calculated by MLINK of the LINKAGE package (version 5.1) under the assumption of an autosomal dominant FSP gene with a frequency of 10\(^{-5}\) and equal female and male recombination rates. Based on the clinical evaluation, we chose to estimate the pairwise lod scores using penetrance values of 100% and liability classes. Five liability classes were obtained from the cumulative age of onset previously designed for subjects from 0 to 19 years old, 0.32 for 20-24 years, 0.55 for 25-30 years, 0.92 for 31-51 years, and 0.9 for subjects over 51 years. Marker allele frequencies were assumed to be equal. Simulation analyses with extensive alterations of the allele frequencies did not modify the conclusions of the linkage analyses.

**Results**

**CASE REPORTS**

The clinical characteristics of the affected family members are shown in table 1. In the kindred presented here, seven subjects are affected with FSP; four of these have cognitive impairment or dementia and three have epilepsy. It should be noted that none of the unaffected subjects complained of memory loss, had abnormal Mini-mental state (MMS), or had epilepsy.

**Patient II.6 (proband)**

This 69 year old woman had been unsteady since she was 52 and was first referred when she was 62. At that time she could not walk for more than 10 minutes. She was treated for two years for urinary urgency. She had slight memory impairment. Examination showed severe spasticity of the legs with brisk reflexes in the lower (+++) and upper limbs (+), and clonus of the ankles. There was no motor or sensory deficit. She had bilateral Babinski

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**Table 1** Summary of clinical features in affected members

<table>
<thead>
<tr>
<th>Age at onset</th>
<th>Age at first examination</th>
<th>Severity of SP</th>
<th>Cognitive impairment</th>
<th>Epilepsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>II.6 (proband)</td>
<td>52</td>
<td>65</td>
<td>3</td>
<td>Dementia</td>
</tr>
<tr>
<td>II.3</td>
<td>56</td>
<td>67</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>II.2</td>
<td>45</td>
<td>60</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>III.6</td>
<td>30</td>
<td>36</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>III.11</td>
<td>Unknown*</td>
<td>34</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>II.2</td>
<td>32-35</td>
<td>32</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>III.5</td>
<td>20</td>
<td>30</td>
<td>2</td>
<td>+</td>
</tr>
</tbody>
</table>

*Unaware of diagnosis. †Memory impairment but died before neuropsychological tests. SP=spastic paraplegia. 1=normal gait; 2=unable to run, able to walk without help; 3=unable to walk without help or wheelchair bound.
signs. Cerebral and medullary magnetic resonance imaging (MRI), visual and auditory evoked potentials, electromyography, and cerebrospinal fluid (CSF) analysis were normal. Sensory evoked potentials (SEPs) by stimulation of the nerve trunks of the lower limbs were consistent with spinal cord involvement but cervical SEPs were normal. Serum vitamin B12 and folates were normal; TPHA, VDRL, and HTLV-1 antibody tests were negative. Plasma long chain fatty acid analysis was negative. Muscle biopsy was normal. Neuropsychological examination disclosed a slight decrease in verbal ability (WAIS verbal IQ of 102 compared with an IQ of 124 on Raven’s progressive matrices) and poor verbal fluency. Her performance on the verbal memory scale was normal (memory quotient (MQ) of 124). During the next three years she experienced a gradual deterioration in memory and ability to walk. A new neuropsychological examination performed three years later was unchanged, except for a deterioration of memory abilities (MQ of 112). In April 1995 she had a generalised seizure and antiepileptic treatment was started. She walked with one stick and began to have spatial disorientation. Neuropsychological examination showed a reduction in conceptual and constructional abilities, while memory performance remained stable. Between April 1995 and June 1996 she had a marked decline in motor and cognitive abilities. Walking became impossible. She did not recognise her children. Examination showed bilateral grasping. Neuropsychological evaluation showed massive aphasia and visual agnosia. Memory impairment involved both short term and long term memory. There was marked temporospatial disorientation. The MMS was 9/30. She eventually met all DSM-III-R criteria for dementia. Repeat cerebral MRI showed widening of the cerebral sulci with dilatation of the ventricular structure.

Patient I.2
This woman died at the age of 80. She had progressive gait and walking difficulties in her 60s but was never examined by a neurologist.

Patient II.3
This 67 year old man had had walking difficulties since he was 56. He could not walk more than 5 metres, had urinary urgency, and complained of memory loss. On examination he had spasticity in the legs with pyramidal reflexes in all four limbs and impairment of short term and long term memory. He also had pes cavus and ichthyosis. The MMS was 29/30 but he refused extensive neuropsychological examination. He died suddenly two years later.

Patient II.2
This 63 year old woman had had walking difficulties since she was 45. When she was first examined in January 1994, she could walk 2 km with a stick. She did not complain of memory loss or urinary problems. Examination showed pes cavus, a proximal motor deficit of thigh flexion (4/5 on the MRC scale), spasticity, and brisk reflexes with spreading in the lower limbs and bilateral Babinski signs. There was no sensory deficit and the MMS was 30/30. On neuropsychological examination no abnormalities were found in verbal or constructional abilities. Her attention was slightly impaired, especially in the spatial component. There was an impairment of visual memory which was limited to delayed recall. In July 1994 she began to complain of urinary urgency with loss of control. At the same time, she developed dysarthria and language difficulties. A cerebral infarct was suspected but investigations were negative. CT scan of the head showed atrophy and slight leukoaraisis. There were generalised theta delta waves on the electroencephalogram. In August 1994 she was treated for partial motor epilepsy and in October 1994 developed a status epilepticus. MRI of the brain disclosed widening of the sulci with dilatation of the ventricular structures. The CSF was normal.

Patient III.2
This 35 year old woman was first seen in January 1994. She did not complain of any symptoms and her examination was normal. Although she again denied any symptoms, re-examination in January 1997 disclosed bilateral Babinski signs.

Patient III.5
This 30 year old man had a generalised seizure at 17 and began to have difficulties in walking at 20. He had urinary urgency and complained of memory loss. Examination showed slight spasticity in the lower limbs with bilateral Babinski signs. The MMS was normal. On neuropsychological examination he had impairment in episodic and semantic memory (the IQ was 105 on Raven’s progressive matrices) and his performance on the WAIS vocabulary subtest was impaired (age scaled score=5). On the Wechsler memory scale (revised), his index score was 86 for verbal memory, 126 for visual memory, 94 for attention, but only 80 for delayed recall. He was at the 90th centile for the copy test of the Rey figure and the 20th centile for recall.

Patient III.6
This 39 year old man began to complain of walking difficulties at the age of 30. Neuropsychological examination showed spasticity of the lower limbs without weakness, but with bilateral Babinski signs and brisk reflexes in the lower limbs.

Patient III.11
This 34 year old man did not complain of walking problems but recognised that he had memory impairment. Examination showed bilateral Babinski signs, brisk reflexes in all four limbs, bilateral hypoaucousis, and pes cavus. The MMS was 27/30 and neuropsychological examination showed impairment in episodic and semantic memory while attention was preserved. His IQ was 110 on Raven’s progressive matrices, but his performance on the WAIS vocabulary subtest was poor (age scaled score=7). On the Wechsler memory scale
visual memory, AD-FSP loci different 50th centile in the Lod described in the Methods. the 15th centile Four liability in family recall, and delayed microsatellite markers some 14q entirely microsatellite markers (Zcalculated at different recombination fractions, assuming complete penetrance or using the liability classes (LC) described in the Methods.

**Table 2** Pairwise lod scores between SPG4 markers and FSP in family CM

<table>
<thead>
<tr>
<th>Locus</th>
<th>Penetrance</th>
<th>Recombination fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>D2S2283</td>
<td>100%</td>
<td>2.25</td>
</tr>
<tr>
<td>D2S352</td>
<td>100%</td>
<td>2.15</td>
</tr>
<tr>
<td>D2S2203</td>
<td>100%</td>
<td>3.47</td>
</tr>
<tr>
<td>D2S2351</td>
<td>100%</td>
<td>3.88</td>
</tr>
<tr>
<td>D2S2203</td>
<td>100%</td>
<td>3.13</td>
</tr>
<tr>
<td>D2S2351</td>
<td>100%</td>
<td>2.89</td>
</tr>
<tr>
<td>D2S325</td>
<td>100%</td>
<td>1.89</td>
</tr>
<tr>
<td>LC</td>
<td>100%</td>
<td>2.03</td>
</tr>
<tr>
<td>D2S2283</td>
<td>100%</td>
<td>3.49</td>
</tr>
<tr>
<td>D2S2347</td>
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<td>3.26</td>
</tr>
<tr>
<td>LC</td>
<td>100%</td>
<td>-4.73</td>
</tr>
<tr>
<td>D2S2351</td>
<td>100%</td>
<td>-4.06</td>
</tr>
</tbody>
</table>

Lod scores were calculated at different recombination fractions, assuming complete penetrance or using the liability classes (LC).

(revised), he obtained an index score of 84 for verbal memory, 87 for visual memory, 93 for delayed recall, and 101 for attention. He was at the 50th centile in the copy subtest and only the 15th centile in the recall subtest of the Rey figure.

**Haplotype and Linkage Analysis**

Four microsatellite markers from the three different AD-FSP loci were initially chosen from a published set21 and tested for linkage to FSP in family CM: D14S1055, D14S255, D14S269, and D14S1018 for locus SPG3; D2S252, D2S367, D2S2347, and D2S2283 for locus SPG4; and D15S1035, D15S128, D15S156, and D15S122 for locus SPG6. The chromosome 14q and 15q candidate regions were entirely excluded (Z < 2) by two point analyses in family CM, whereas maximum lod scores of 3.49 and 3.26 at θ = 0 were obtained with locus D2S2325, assuming complete penetrance and using liability classes, respectively. A total of 10 microsatellite markers spanning the SPG4 candidate interval were then analysed to confirm the linkage to this region. The pairwise data between AD-FSP and six informative chromosome 2p loci are presented in table 2.

The haplotype was constructed with all 10 loci and is shown in fig 1. The order of the markers presented in the haplotype is consistent not only with the physical map of the region (J Hazan, unpublished data) but also with the analysis of recombination events in 13 families displaying AD-FSP linked to the SPG4 locus.22 The analysis of recombination events within the haplotype positions the FSP causing gene in family CM within a 0 cM interval between loci D2S2255 and D2S2347.21 The distal border defined by the crossover at D2S2255 in unaffected subject III.3 is consistent with recombination events in affected members from the other SPG4 linked families (C Paternotte, unpublished data). The recombinant III.5 would narrow the critical interval on the proximal side at locus D2S2347 and thus would enable the refinement of the SPG4 candidate region.

**Discussion**

We investigated AD-FSP complicated by cognitive impairment and epilepsy. Of the seven patients with FSP, four had cognitive impairment or dementia and three had epilepsy. The clinical pattern is consistent with the intrafamilial heterogeneity previously described in complicated FSP families.22 25 Few families with AD-FSP associated with epilepsy or an abnormal EEG have been reported to date.26-28

In a previous study of subclinical cognitive impairment in FSP patients, all the people affected with complicated AD-FSP had cognitive deficits on neuropsychological tests,29 such as lack of concentration, slowness to respond to the examiner, poor capacity for retaining new information, and temporospatial disorientation. On the other hand, pedigrees displaying AD- or AR-FSP complicated by dementia or clinically evident cognitive impairment are rare.22 27 28-30 In an Irish kindred reported previously,22 one affected member died of dementia and six had cognitive deficits with predominant visuospatial impairment in neuropsychological studies. Similarly, in family CM, one patient had severe dementia while three had cognitive impairment, but the neuropsychological deficit seems rather different from that described in the Irish kindred. Furthermore, cognitive impairment cosegregates with epilepsy in family CM. To our knowledge, the association of AD-FSP, dementia, and epilepsy has not been described previously.

In the present study, we report the first localisation of a gene responsible for a form of AD-FSP complicated by epilepsy and cognitive impairment. Our data clearly establish significant linkage between the disease causing gene in family CM and the SPG4 locus known to be associated with pure AD-FSP.9 28 At this stage of the study, three hypotheses might account for this result: (1) allelic heterogeneity, (2) locus heterogeneity involving distinct genes responsible for pure and complicated AD-FSP and located within the SPG4 region, and (3) a contiguous gene syndrome. (1) The two different forms of FSP may be the result of allelic heterogeneity at a single locus, as reported for X-FSP at the SPG2 locus.31 Similarly, in AD-FSP, allelic heterogeneity could explain the mapping of the gene responsible for this complicated form of FSP to the SPG4 locus.

(2) Within the SPG4 interval, there could be one gene responsible for pure AD-FSP and another one involved in the complicated form described here. (3) The coexistence of dementia and epilepsy with FSP in family CM may
also suggest a contiguous gene syndrome. A deletion spanning the AD-FSPG gene (SPG4) and one or several gene(s) involved in dementia and epilepsy could also account for the observed phenotype. However, no molecular microdeletion within the SPG4 region has been detected so far in affected members of this kindred (J Hazan, unpublished data).

Although none of these hypotheses has been confirmed or ruled out so far, allelic heterogeneity already shown for X-FSP at the SPG2 locus11 seems the most probable. Assuming this hypothesis, the analysis of recombination events in this pedigree places the SPG4 gene within a 0.0 cm interval flanked by loci D2S2255 and D2S2347. The reduction of the SPG4 interval from 4 cm to 0 cm is the result of a cluster of non-recombining markers in the last version of the published genetic map.12 However, the flanking markers of the cluster, D2S2255 and D2S2347, are separated by a physical distance estimated to be 2.5 Mb (J Hazan, unpublished data), while the physical distance spanning the former SPG4 interval flanked by loci D2S400 and D2S367 was estimated as 5 Mb.13

Inter- and intrafamilial variations in the age of onset have been observed in several families with AD-FSPG.14,15 It should be noted that the age of onset in family CM was earlier in generation III than in generation II. Anticipation of age at onset has been previously suggested,16,17 but may be explained by an observation bias.18 However, cognitive impairment seems to occur earlier in the third generation of the reported pedigree. Only the isolation of the SPG4 gene and the identification of the mutation(s) in different families will provide further insight into the questions of anticipation and allelic heterogeneity.

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