

# **Hereditary spastic paraplegia with mental impairment and thin corpus callosum in Tunisia: SPG11, SPG15 and further genetic heterogeneity.**

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## ABSTRACT

**Background:** Complicated forms of hereditary spastic paraplegia (HSP) with autosomal recessive (AR) transmission frequently associate slowly progressive spastic paraparesis and mental deterioration with thin *corpus callosum* (TCC). This association has been found in families linked to six different HSP loci; *SPG7*, *SPG11*, *SPG15*, *SPG21*, *SPG32* and the locus of HSP with epilepsy. **Objective:** To perform a clinical and genetic study of Tunisian families with AR-HSP-TCC. **Methods:** We recruited and examined 73 subjects from 33 “apparently” unrelated Tunisian AR-HSP families. Families with AR-HSP-TCC were subsequently tested for linkage to the corresponding loci using microsatellite markers from the candidate intervals, followed by direct sequencing of the *KIAA1840* gene in families linked to *SPG11*. **Results:** We identified eight Tunisian families (8/33=24%), including 19 affected patients, fulfilling the clinical criteria for HSP-TCC. In seven families, linkage to either *SPG11* (62.5%) or *SPG15* (25%) was suggested by haplotype reconstruction and positive LOD score values for microsatellite markers. The identification of two recurrent mutations (R2034X and M245VfsX) in the *SPG11* gene in five families validated the linkage results. The neurological and radiological findings in *SPG11* and *SPG15* patients were relatively similar. The remaining family, characterized by an earlier age at onset and the presence of cataracts, was excluded for linkage to the six known loci, suggesting further genetic heterogeneity. **Conclusions:** AR-HSP-TCC is a frequent subtype of complicated HSP in Tunisia, and is clinically and genetically heterogeneous. *SPG11* and *SPG15* are the major loci for this entity, but at least another genetic form with unique clinical features exists.

**Keywords:** Hereditary spastic paraplegia, Autosomal recessive, Thin *corpus callosum*, Linkage, *SPG11*, *SPG15*.

## INTRODUCTION

Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by slowly progressive spasticity of the lower extremities. In addition to pure forms, complicated forms involving additional neurologic features such as mental retardation, ataxia, peripheral neuropathy, retinopathy, optic atrophy, deafness and ichthyosis have also been reported.<sup>1,2</sup> Pathologically, HSP is characterized by axonal degeneration in the long descending and ascending tracts of the spinal cord, especially in their terminal portions.<sup>3</sup> The corticospinal tracts are mainly affected, but the Goll and spinocerebellar tracts may also be involved.

Autosomal dominant (AD), autosomal recessive (AR) and X-linked forms of HSP have been identified.<sup>4</sup> To date, more than 33 HSP loci and 15 spastic paraplegia genes (SPG) have been identified.<sup>4-7</sup> The corresponding proteins are often involved in axonal trafficking or mitochondrial metabolism.<sup>8</sup>

Hereditary spastic paraplegia with mental impairment and thin *corpus callosum* (HSP-TCC) is a frequent subtype of complicated HSP, often inherited as an AR trait. A frequency of 35% among recessive forms has been reported in Brasil.<sup>9</sup> Mental deterioration usually starts during the first or second decade of life, associated with various signs of complicated HSP.<sup>10</sup>

The purpose of this study was to establish, through clinical and paraclinical investigations of a large series of AR-HSP patients, the relative frequency of complex forms associating TCC and mental impairment (HSP-TCC) and its molecular bases in Tunisia. We report eight Tunisian AR-HSP-TCC families and confirm the phenotypic and genetic heterogeneity of this particular form of HSP.

## **METHODS**

### **Patients and families**

During the last 15 years, we assessed 73 affected patients from 33 “apparently” unrelated Tunisian families of Arab origin in the department of Neurology of the University Hospital of Sfax, the reference centre in Neurological disorders in South Tunisia. After obtaining informed consent, all available affected and apparently unaffected family members were assessed neurologically. Age at symptom onset was obtained from the parents or the available medical records. Disability was assessed on a 7-point scale in which 1 indicates minimal disability (slight stiffness of the legs); 2, mild disability (unable to run, but full autonomy); 3, moderate disability in walking (reduced perimeter, frequent falls); 4, severe disability (unilateral assistance required to walk); 5, bilateral assistance required to walk; 6, wheelchair bound; and 7, bedridden. Mental impairment was assessed by the mini mental state examination (MMSE) and was considered as mild (MMSE between 21 and 26), moderate (MMSE between 16 and 20) or severe (MMSE < 15). TCC was assessed by brain magnetic resonance imaging (MRI, 1.5 T) by experienced neurologists by comparison with normal MRI after a mean disease duration of  $17.8 \pm 9.1$  (range 3-39) years.

Only families meeting the following criteria were diagnosed as having AR-HSP-TCC: (1) inheritance consistent with an AR trait, (2) slowly progressive spastic paraparesis and mental impairment in at least one affected patient, (3) thinning of the *corpus callosum* as revealed by brain MRI in at least one member of the family, and (4) exclusion of other disorders by brain and spinal MRI and other laboratory tests.<sup>11</sup>

### **Genetic studies**

DNA was extracted from blood using a standard protocol. According to the clinical presentation and transmission mode, we tested the selected families for linkage to several loci previously

described for AR-HSP associated with mental impairment and TCC using appropriate microsatellite markers (list available upon request to the authors): *SPG7*, *SPG11*, *SPG15*, *SPG21/MAST*, *SPG32* and HSP-TCC-epilepsy.<sup>12-17</sup> Genotypes were determined using standard methods and linkage analysis was performed using Allegro (DECODE Genetics, Reykjavik, Iceland) assuming a fully penetrant recessive disease with similar male-female recombination frequencies and equal allele frequencies. Gene frequency values of 0.0005 or 0.002 were used and did not affect the LOD score results. Young unaffected individuals (below the mean age at onset of the affected patients in the family) were considered to have unknown status for LOD score calculations. Haplotypes segregating at each locus were reconstructed manually by minimizing the number of recombination events.

The index patient from each family with putative linkage to *SPG11* was screened for mutations in all coding exons and splice junctions of the *KIAA1840* gene, as described previously.<sup>6</sup>

## RESULTS

### Frequency and clinical features of AR-HSP-TCC in Southern Tunisia

Among the 33 families with AR-HSP that could be assessed, we identified eight that fulfilled the criteria for HSP-TCC (24%). Consanguinity was present in seven families. Two cases were apparently sporadic but both were consanguineous. Onset in 19 patients (10 men, 9 women) from the 8 AR-HSP-TCC families occurred at  $10.6 \pm 5.1$  years (range 2-16). In all patients, the presenting symptoms were gait abnormalities due to the insidious appearance of stiffness and weakness in the lower extremities (Table 1).

After a mean disease duration of  $18.9 \pm 7.8$  years (range 6-33), cognitive decline was present in all affected individuals except one who had only 7 years of evolution, in addition to lower limb (LL) hyperreflexia and bilateral Babinski signs. Others signs related to pyramidal tract dysfunction were found in several patients: pseudobulbar dysarthria, upper limb (UL) spasticity and bladder

dysfunction. Additional signs were occasionally observed such as cerebellar ataxia (7/19), LL distal amyotrophy (5/19) cataract (5/19) and decreased vibration sense (2/19).

MRI was performed at the mean age of  $26.5 \pm 5.4$  years (range 20-31) and after a mean disease duration of  $16.3 \pm 6.4$  years (range 6-26). Consistent with the clinical findings, all of the nine patients studied by brain MRI had TCC, most prominently in the rostrum, genu and body, associated with cerebral and cerebellar atrophy (Table 2). White matter changes were found frequently (6/9 patients, 66%). In three individuals, hyperintense T2-weighted lesions were restricted to occipital and frontal regions, but in the other cases there were diffuse abnormalities involving periventricular regions (Figure 1). Cortical cerebral atrophy with frontal predominance seemed to be a late feature, since it was absent in patient TUN17-168 who had the shortest disease duration when examined (6 years). Electroneuromyograms (ENMG) showed electrophysiological signs of predominantly axonal motor peripheral neuropathy in 4/8 patients tested (Table 2). Muscle and nerve biopsies of these 4 patients showed signs of axonal degeneration associated with chronic neurogenic changes without specific alterations.

### **Genetic findings**

We first evaluated linkage to known genes (*SPG7*, *SPG11* and *SPG21*) and subsequently looked for mutations in linked families. Further explorations in non-mutated and non-linked families included loci *SPG15*, *SPG32* and the locus for HSP-TCC-epilepsy.

In five families (62.5%), linkage to *SPG11* was suggested by identical homozygosity at several markers and positive multipoint LOD score values from 1.1 to 2.5 reaching the maximal expected values in the pedigrees (table 3). Patients in families TUN 3, TUN 4 and TUN 22 had partially similar haplotypes encompassing the *SPG11* gene, suggesting inheritance of a common mutated ancestral chromosome. Similarly, families TUN 9 and TUN 12 shared the same homozygous alleles for the whole *SPG11* interval. In accordance with these results, direct

sequencing of all exons and splice junctions of the *SPG11* gene in the five chromosome 15q-linked families revealed two recurrent mutations: c.733\_734delAT (exon 4) / p.M245VfsX in families TUN 3, TUN 4 and TUN 22 as well as c.6100C>T (exon 32) / p.R2034X in families TUN 9 and TUN12 (Figure 2). The mutations segregated with the disease in all families but were also present in the homozygous state in individual TUN 3-42 who was still unaffected at 11 years of age. This individual, only had brisk reflexes on examination and is still younger than the age at onset of his affected relatives. Because of his young age, no further investigations could be performed.

In all three non-SPG11 families, haplotype reconstructions excluded linkage to *SPG7*, *SPG21*, *SPG32* and the locus for HSP-TCC-epilepsy because there were no haplotypes identical by descent in the affected patients of each kindred and because the multipoint LOD scores for the candidate intervals were negative (Table 3).

The exclusion of other known HSP-TCC loci and the co-segregation with the disease of chromosome 14q-haplotypes between markers *DI4S1038* and *DI4S270* in two families (TUN 8 and TUN 17, 25%) suggested their linkage to *SPG15* (Figure 3A). Homozygosity was observed in the whole interval in family TUN 17 and between markers *DI4S981* and *DI4S1002* in family TUN 8. In addition, linkage to *SPG15* was supported by positive LOD scores of 2.0 and 1.8 in families TUN 8 and TUN 17, respectively. The haplotypes segregating with the disease were different in the two families, suggesting the possibility of different allelic mutations.

In the remaining family (TUN 35, Figure 3B), linkage to the six known loci of HSP-TCC was excluded (Table 3), suggesting the existence of another genetic locus for this condition.

### **Phenotype-genotype correlations**

The mean age at onset (12.4 *versus* 14.3 years) and the clinical profile of patients with SPG11 (n=11) and SPG15 (n=3) were similar, but there were a few subtle differences between them. SPG11 patients tended to have a more severe disease than SPG15 patients (mean severity score



5.18 *versus* 4.33), but this was correlated with the disease duration (mean disease duration 16.54 *versus* 13 years). Muscle atrophy or cerebellar signs were never observed in SPG15 patients. Indeed, electrophysiological analysis of the two SPG15 patients studied showed no evidence of peripheral neuropathy, and radiological analysis showed less cerebellar atrophy and slighter white matter lesions that were restricted to the frontal and occipital horns. Not surprisingly the phenotypes of SPG11 patients with the M245fsX or the R2034X mutations were undistinguishable.

The phenotype of family TUN 35 was clearly different. Although the age at onset was earlier (mean  $4.4 \pm 3.5$  years), the cognitive decline was mild, progression was less severe (mean severity score 3) and patients were still able to walk after a mean disease duration of 27.6 years. In addition, all patients had cataracts and cerebellar ataxia, and *pes cavus* was frequent (4/5). Patient TUN 35-27/07 had facial dyskinesia without other extrapyramidal signs, associated with mild hand tremor. ENMG was normal in a single patient. Cerebellar atrophy was more severe on brain MRI and white matter abnormalities were unremarkable.

## COMMENT

We report the clinical and genetic study of eight Tunisian families with HSP-TCC including 19 patients, the largest group of North African patients of Arab origin investigated so far. HSP-TCC with mental impairment was common in our AR-HSP families (8/33 or 24% of all families) but less than in a Brazilian cohort (35%).<sup>9</sup>

AR-HSP-TCC is a subtype of complicated HSP that is clinically and genetically heterogeneous. This condition was originally described in Japanese patients and has been found in many countries.<sup>6,9,13,18,19,20</sup> AR-HSP-TCC is characterized clinically by slowly progressive spastic paraparesis and mental deterioration that begins mainly in the second decade of life.<sup>21</sup> Cerebellar ataxia and impaired vibration sense are also observed in some patients. Additional manifestations include spasticity and hyperreflexia in the UL, distal amyotrophy, *pes cavus*, urinary disturbance,

dysarthria, nystagmus, congenital cataracts, seizures and extrapyramidal signs.<sup>10,11,21</sup> On brain MRI, progressive thinning of the *corpus callosum* is the neuroradiologically distinctive feature of this syndrome.<sup>9</sup> Periventricular white matter changes and late cortical atrophy are additional and frequent features. Cerebral and cerebellar atrophy are slowly progressive and abnormalities of cerebral white matter with frontal predominance have been observed in long-standing cases.<sup>22</sup>

It is clear from molecular genetic analyses that there are several underlying causes of this syndrome,<sup>23</sup> with five genetic loci identified (*SPG11*, *SPG15*, *SPG21*, *SPG32* and *HSP-TCC-epilepsy*). *SPG7* has also been associated with a similar phenotype. The major locus is, however, *SPG11* (62% in Tunisia), a frequency similar to Japanese (77%, 11/13), Mediterranean (60%, 6/10) and Italian (41%, 5/12) families.<sup>11,13,24,25</sup> Results of linkage analysis were confirmed by the subsequent identification of two recurrent mutations: R2034X and M245VfsX, in two and three Tunisian families, respectively, suggesting founder effects.<sup>6</sup> R2034X-segregating haplotypes were similar in a previously published Moroccan kindred with the same mutation, suggesting inheritance of a common ancestral mutation.<sup>6</sup> On the contrary, the segregating haplotypes in French and Italian kindreds carrying the M245VfsX mutation were different indicating independent ancestral events or a very ancient mutation.<sup>6,26</sup> *SPG15*, responsible for 25% of AR-HSP-TCC in our series, was the second most important locus, in agreement with a frequency of 15% reported in another study of families from the Mediterranean basin.<sup>27</sup> The similarity between the neurological and radiological findings in *SPG11* and *SPG15* patients suggests that the responsible genes may be functionally related. Although a few subtle differences were observed, such as the absence of LL/UL amyotrophy, peripheral neuropathy, or cerebellar signs in some *SPG15* patients, who also had severe lesions of the cerebellum, these features may be present in other *SPG15* patients,<sup>27</sup> and cannot be used to distinguish *SPG11* from *SPG15*-linked families on a clinical basis.

One family in our series was not linked to any of the loci tested, suggesting that an as yet unmapped locus is responsible for their disease. Interestingly, this family, which represents a new

genetic entity, also had a unique phenotype, that differed from that of SPG11 and SPG15 by an earlier onset and a slower progression of spastic gait, as well as the presence of cataracts and cerebellar ataxia.

In conclusion, we describe a group of Arab families from North Africa with AR-HSP-TCC. We demonstrate that this clinical-radiological syndrome is a frequent form of HSP and that *SPG11* and *SPG15* are the major loci for this clinical entity. In addition, we show the existence of a novel genetic form of AR-HSP-TCC with unique and different clinical features.

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## FIGURES LEGENDS

**Figure 1:** Brain MRI (1.5 T) in three patients with AR-HSP-TCC. A) Sagittal T1-weighted, axial and coronal T2-weighted brain MRI in patient TUN 9-97 (*SPG11*), performed at the age of 36 years and after a disease duration of 20 years, showing TCC, diffuse white matter abnormalities involving occipital, frontal and periventricular regions, cortical cerebral atrophy with frontal predominance and mild cerebellar atrophy. B) Sagittal and axial T1-weighted and axial T2-weighted brain MRI in patient TUN 8-91 (*SPG15*), performed at the age of 29 years and after a disease duration of 13 years, showing TCC with mild cortical and cerebellar atrophy and slight white matter abnormalities restricted to the frontal and occipital horns. C) Sagittal, axial and coronal T2-weighted and axial T1-weighted brain MRI in patient TUN 35-27/07), performed at the age of 30 years and after a disease duration of 28 years, showing TCC, moderate cortical cerebral atrophy with frontal predominance and cerebellar atrophy.

**Figure 2:** Pedigrees and segregation of the mutations detected in *KIAA1840* in five *SPG11* Tunisian families with AR-HSP-TCC. Haplotype reconstructions for flanking microsatellite markers are shown. Square symbols are men, and circles are women. The filled symbols are affected individuals. The numbers are an internal reference for each sampled individual. Asterisks indicate sampled subjects. Inferred haplotypes are bracketed. m = mutation; + = wild type. The correspondence between the numbering of alleles and their size in bp is indicated.

**Figure 3:** Pedigrees of non-*SPG11* Tunisian families with AR-HSP-TCC. A) Haploidentity in affected individuals from two families with suggestive linkage to the *SPG15* locus. The region of homozygosity is highlighted by black bars in patients. B) Pedigree of family TUN 35. The code numbers of all sampled (\*) individuals are given below the symbols. Closed circles

(women) and squares (men) indicate affected members. Arrowheads indicate the positions of recombination events.



Locus/gene (Mutation)	SPG11 (M245VfsX)						SPG11 (R2034X)						SPG15			Unknown				
	TUN 3		TUN 4	TUN 22			TUN 9		TUN 12			TUN 8		TUN 17	TUN 35					
Consanguinity (degree)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	Yes (3)	Yes (1)	No	No	Yes (2)	Yes (2)	Yes (2)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	
Individual N° (sex)	38 (M)	40 (M)	48 (F)	201 (M)	206 (F)	212 (M)	97 (M)	98 (M)	125 (M)	129 (F)	130 (F)	91 (M)	92 (M)	168 (F)	27/07 (M)	26/07 (F)	32/07 (F)	33 (F)	36/07 (F)	
Age at examination, years	25	21	24	36	21	37	36	34	31	34	21	29	33	20	30	32	35	31	32	
Age at onset, years	15	14	8	13	4	10	16	15	15	14	13	16	13	14	2	2	2	6	10	
Disease duration, years	10	7	16	23	16	27	20	19	16	20	8	13	20	6	28	30	33	25	22	
Disability scale	4/7	3/7	4/7	6/7	6/7	5/7	6/7	5/7	6/7	6/7	6/7	4/7	6/7	3/7	2/7	3/7	4/7	3/7	3/7	
LL spasticity	S	S	S	S	S	M	S	S	S	S	S	Mo	S	Mo	S	Mo	S	S	M	
LL reflexes	+++	+++	+++	+	+	+	+++	+++	+++	+++	+++	+++	+	+	+++	+++	+++	+++	+++	
LL weakness	Mo	Mo	-	Mo	Mo	M	S	S	S	S	S	Mo	S	Mo	Mo	Mo	Mo	M	Mo	
LL distal amyotrophy	-	-	M	M	Mo	-	-	-	Mo	M	-	-	-	-	-	-	-	-	-	
Extensor plantar reflexes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
UL spasticity	-	-	M	-	-	-	Mo	M	-	-	M	-	-	M	M	M	M	Mo	M	
UL reflexes	N	N	+	N	N	N	+	+	+	+	+	+	N	N	+	+++	+	+	+	
UL weakness	-	-	-	-	-	-	M	-	-	M	-	-	-	-	M	-	-	-	-	
UL distal amyotrophy	-	-	-	M	-	-	-	-	M	M	-	-	-	-	-	-	-	-	-	
Cognitive decline	Mo	-	Mo	Mo	Mo	Mo	Mo	Mo	Mo	S	Mo	Mo	Mo	M	M	M	M	M	M	
Cerebellar signs	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+	+	

Decreased vibration sense	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Pseudo bulbar dysarthria	+	-	+	+	+	-	+	+	+	+	-	-	+	+	+	+	-	+	-
Pes cavus	-	-	+	-	-	-	-	-	+	+	-	-	-	+	+	+	+	+	-
Scoliosis	-	-	-	+	+	+	+	-	+	-	-	-	-	-	+	-	-	+	-
Urinary symptoms	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Others signs	None	None	Hand tremor	None	None	Hand tremor	Deafness and strabismus	Strabismus	Hand tremor	Pes equin	None	None	None	None	Facial dyskinesia, hand tremor and cataract	Cataract	Cataract and depression	Cataract and strabismus	Cataract

**Table 1:** Clinical characteristics of families with thin *corpus callosum*. M, male; F, female; +, present; -, absent; M, mild; Mo, moderate; S, severe; Reflexes, N, normal, +, brisk, +++, very brisk; UL, upper limbs; LL, lower limbs; PNP, peripheral polyneuropathy; ND, not done.

Locus/gene (Mutation)	Patients	Brain MRI			ENMG
		TCC	WMA	Atrophy	
<b>SPG11</b> (M245VfsX)	TUN 3-38	R, G, B, S	F, O, Pv	C, Cb	Normal
	TUN 4-48	R, G, B, S	F, O	C, Cb	Axonal motor PNP
	TUN 22-206	R, G, B, S	F, O, Pv	C, Cb	Axonal motor PNP
<b>SPG11</b> (R2034X)	TUN 9-97	R, G, B, S	F, O, Pv	C, Cb	Axonal motor PNP
	TUN 12-125	R, G, B, S	F, O	C, Cb	Axonal motor PNP
<b>SPG15</b>	TUN 8-91	R, G, B, S	F, O	C, Cb	Normal
	TUN 17-168	R, G, B	-	Cb	Normal
<b>Unknown</b>	TUN 35-27/07	R, G, B, S	-	C, Cb	Normal
	TUN 35-32/07	R, G, B, S	-	C, Cb	ND

**Table 2:** MRI and ENMG findings in index patients of eight families with HSP and thin *corpus callosum*. B, body; C, cerebral; Cb, cerebellar; F, Frontal; G, genu; ND, not done; O, occipital; PNP, peripheral neuropathy; Pv, periventricular; R, rostrum; S, splenium; TCC, thin *corpus callosum*; WMA, white matter abnormalities.

		<b>Family</b>								
	<b>Relative map location (cM)</b>	<b>Marker</b>	<b>TUN 3</b>	<b>TUN 4</b>	<b>TUN 8</b>	<b>TUN 9</b>	<b>TUN 12</b>	<b>TUN 17</b>	<b>TUN 22</b>	<b>TUN 35</b>
<b>SPG7</b>	0	<i>D16S3023</i>	-infinity	<b>1.15</b>	-3.73	<b>1.09</b>	-3.17	-3.85	-3.56	-infinity
	0.75		-2.35	<b>1.14</b>	-4.01	<b>1.09</b>	-1.20	-2.58	-0.62	-1.76
	1.5		-1.76	<b>1.13</b>	-4.55	<b>1.09</b>	-2.01	-2.58	-0.33	-1.17
	2.25	<i>D16S303</i>	-1.41	<b>1.13</b>	-infinity	<b>1.10</b>	-3.18	-3.82	-0.16	-0.83
<b>SPG11</b>	0	<i>D15S781</i>	<b>1.93</b>	<b>1.75</b>	-infinity	<b>1.10</b>	<b>2.29</b>	-7.27	<b>2.48</b>	-11.12
	0.003	-	<b>1.93</b>	<b>1.75</b>	-10.69	<b>1.10</b>	<b>2.29</b>	-7.44	<b>2.48</b>	-11.47
	0.007	-	<b>1.93</b>	<b>1.75</b>	-10.68	<b>1.10</b>	<b>2.29</b>	-7.75	<b>2.48</b>	-12.07
	0.01	<i>D15S537</i>	<b>1.93</b>	<b>1.75</b>	-infinity	<b>1.10</b>	<b>2.29</b>	-infinity	<b>2.48</b>	-infinity
	0.55	-	<b>1.93</b>	<b>1.75</b>	-3.11	<b>1.10</b>	<b>2.29</b>	-5.46	<b>2.48</b>	-5.35
	1.08	-	<b>1.93</b>	<b>1.77</b>	-2.58	<b>1.10</b>	<b>2.29</b>	-5.18	<b>2.48</b>	-4.63
	1.62	<i>D15S516</i>	<b>1.93</b>	<b>1.77</b>	-2.26	<b>1.10</b>	<b>2.29</b>	-6.05	<b>2.48</b>	-4.32
	2.16	-	<b>1.93</b>	<b>1.79</b>	-2.29	<b>1.09</b>	<b>2.29</b>	-4.44	<b>2.47</b>	-4.21
	2.70	-	<b>1.93</b>	<b>1.80</b>	-2.44	<b>1.09</b>	<b>2.29</b>	-4.35	<b>2.46</b>	-4.28
	3.23	<i>D15S659</i>	<b>1.93</b>	<b>1.82</b>	-3.06	<b>1.08</b>	<b>2.29</b>	-5.84	<b>2.45</b>	-infinity
<b>SPG21</b>	18.81	<i>D15S1018</i>	-5.63	-3.28	-infinity	-1.35	-2.77	-5.21	-4.78	-infinity
	18.89	-	-5.64	-3.12	-8.76	-1.52	-2.93	-5.30	-4.53	-10.07
	18.96	-	-5.94	-3.12	-9.34	-1.80	-3.19	-5.41	-4.83	-10.56
	19.04	<i>D15S108</i>	-infinity	-3.27	-12.36	-2.90	-4.00	-5.71	-14.83	-infinity
	19.04	-	-11.82	-3.27	-12.87	-3.07	-4.00	-5.70	-8.74	-11.55
	19.05	-	-11.82	-3.27	-13.72	-3.37	-4.01	-5.68	-8.44	-11.25
	19.05	<i>D15S1507</i>	-infinity	-3.27	-infinity	-infinity	-4.01	-5.67	-8.27	-13.89
	19.42	-	-7.99	-2.95	-8.56	-3.87	-2.22	-5.23	-6.93	-5.77
	19.79	-	-7.99	-2.95	-7.97	-3.58	-1.93	-5.11	-6.94	-4.61
	20.16	<i>D15S1020</i>	-infinity	-3.29	-infinity	-3.42	-1.76	-5.30	-13.79	-3.93
<b>HSP-EP</b>	0	<i>D8S1820</i>			-infinity			-5.88		-infinity
	1.73	-			-3.75			-3.86		-6.77
	3.45	-			-3.52			-4.09		-6.65
	5.18	<i>D8S1769</i>			-infinity			-7.05		-infinity
	5.72	-			-1.37			-6.03		-7.30
	6.25	-			-1.68			-6.32		-6.78
	6.79	<i>D8S505</i>			-4.92			-infinity		-8.89
	8.47	-			-1.58			-1.23		-6.32
	10.14	-			-1.11			-0.49		-6.36
	11.82	<i>D8S535</i>			-0.84			-0.03		-infinity

**Family**

	Relative map location (cM)	Marker	Family								
			TUN 3	TUN 4	TUN 8	TUN 9	TUN 12	TUN 17	TUN 22	TUN 35	
<b>SPG15</b>	0.00	<i>D14S1038</i>			-infinity				<b>-5.06</b>		-infinity
	0.93	-			-0.62				<b>1.29</b>		-9.57
	1.87	-			-0.60				<b>1.61</b>		-9.84
	2.80	<i>D14S1012</i>			-infinity				<b>1.81</b>		-infinity
	3.38	-			<b>1.38</b>				<b>1.81</b>		-14.99
	3.95	-			<b>1.72</b>				<b>1.82</b>		-14.92
	4.53	<i>D14S981</i>			<b>1.94</b>				<b>1.83</b>		-infinity
	4.74	-			<b>1.96</b>				<b>1.83</b>		-16.88
	5.51	-			<b>2.03</b>				<b>1.83</b>		-16.81
	5.84	<i>D14S1069</i>			<b>2.06</b>				<b>1.83</b>		-infinity
	5.90	-			<b>2.06</b>				<b>1.83</b>		-21.39
	5.96	-			<b>2.06</b>				<b>1.83</b>		-21.97
	6.02	<i>D14S1065</i>			<b>2.06</b>				<b>1.83</b>		-infinity
	6.51	-			<b>2.04</b>				<b>1.83</b>		-15.87
	7.01	-			<b>2.03</b>				<b>1.83</b>		-15.59
	7.50	<i>D14S588</i>			<b>2.02</b>				<b>1.83</b>		-infinity
	7.69	-			<b>2.01</b>				<b>1.83</b>		-12.77
	7.89	-			<b>2.00</b>				<b>1.83</b>		-11.99
	8.08	<i>D14S1029</i>			<b>1.99</b>				<b>1.83</b>		-16.05
	8.55	-			<b>1.81</b>				<b>1.83</b>		-12.59
	9.03	-			<b>1.50</b>				<b>1.83</b>		-12.79
	9.50	<i>D14S1002</i>			-4.48				<b>1.83</b>		-infinity
	10.62	-			0.22				<b>1.82</b>		-7.63
	11.73	-			0.32				<b>1.82</b>		-6.61
	12.85	<i>D14s1266</i>			0.25				<b>1.82</b>		-6.19
	14.71	-			-0.76				<b>1.82</b>		-6.32
	15.64	-			-8.65				<b>1.82</b>		-6.91
	15.64	<i>D14S270</i>			-8.18				<b>1.82</b>		-infinity
<b>SPG32</b>	0.00	<i>D14S264</i>			-4.52				-7.81		-infinity
	1.53	-			-3.28				-1.51		-5.14
	3.05	-			-3.59				-1.63		-5.82
	4.58	<i>D14S80</i>			-infinity				-8.68		-infinity
	5.60	-			-4.56				-1.78		-10.77
	6.62	-			-4.33				-1.84		-10.76
	7.64	<i>D14S1021</i>			-5.12				-6.30		-infinity
	11.60	-			-2.65				-3.15		-5.59
	15.57	-			-2.63				-3.53		-5.56
	19.53	<i>D14S70</i>			-infinity				-infinity		-infinity
	19.99	-			-4.89				-5.31		-7.94
	20.46	-			-4.68				-4.76		-8.25
	20.93	<i>D14S1017</i>			-5.21				-4.46		-infinity
	23.17	-			-2.10				-4.14		-3.22
	25.41	-			-1.92				-4.49		-1.81
	27.65	<i>D14S1009</i>			-4.31				-infinity		-1.02

**Table 3:** Results of multipoint linkage analysis with markers covering the *SPG7*, *SPG11*, *SPG15*, *SPG21*, *SPG32* and HSP-TCC-Epilepsy (HSP-EP) candidate intervals. The multipoint LOD scores calculated with ALLEGRO are indicated for each family relative to the position of the genetic markers. Positive LOD score values are indicated in bold.

*Nota Bene:* Families TUN 3, TUN 4, TUN 9, TUN 12 and TUN 22, in which causative mutations in *SPG11* segregated with the disease, were not tested for linkage to *SPG15*, *SPG32* and the locus for HSP-TCC-epilepsy.