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Spastic Paraplegia, Optic Atrophy, and Neuropathy: New Observations, Locus Refinement, and Exclusion of Candidate Genes

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Summary

SPOAN is an autosomal recessive neurodegenerative disorder which was recently characterized by our group in a large inbred Brazilian family with 25 affected individuals. This condition is clinically defined by: 1. congenital optic atrophy; 2. progressive spastic paraplegia with onset in infancy; and 3. progressive motor and sensory axonal neuropathy. Overall, we are now aware of 68 SPOAN patients (45 females and 23 males, with age ranging from 5 to 72 years), 44 of which are presented here for the first time. They were all born in the same geographic micro region. Those 68 patients belong to 43 sibships, 40 of which exhibit parental consanguinity. Sixty-one patients were fully clinically evaluated and 64 were included in the genetic investigation. All molecularly studied patients are homozygotes for D11S1889 at 11q13. This enabled us to reduce the critical region for the SPOAN gene from 4.8 to 2.3 Mb, with a maximum two point *lod* score of 33.2 (with marker D11S987) and of 27.0 (with marker D11S1889). Three genes located in this newly defined critical region were sequenced, but no pathogenic mutation was detected. The gene responsible for SPOAN remains elusive.

Keywords: Spastic paraplegia, optic atrophy, neuropathy, SPOAN, linkage.

Introduction

Hereditary spastic paraplegias (HSP) are a heterogeneous group of genetic disorders clinically characterized by progressive spasticity and pyramidal weakness, predominantly in lower limbs. They can be inherited either as an X-linked or as an autosomal dominant or recessive trait. Two distinct phenotypes for HSP can be recognized: *pure*, with isolated spastic paraplegia of lower limbs, and *complicated*, associated with other neurological and extraneurological signs or symptoms such as skin abnormalities, dementia, optic atrophy, ataxia, epilepsy, neuropathy, retinopathy, and mental retardation (McDermott et al., 2000; Fink, 2008; Stevanin et al., 2008). In 2005, our group (Macedo-Souza et al., 2005) reported an autosomal recessive complicated form of spastic paraplegia with a previously undescribed phenotype characterized by: 1. non-progressive congenital optic atrophy; 2. spastic paraplegia with onset in infancy; and 3. juvenile progressive motor and sensory axonal neuropathy. Additional common findings were dysarthria, spine deformity, and joint retractions, as well as startle response following unexpected noises. This condition was named SPOAN (an acronym for spastic paraplegia, optic atrophy, and neuropathy). Linkage studies performed with 25 affected individuals resulted in a two point *lod* score of +14.43 at D11S1883.

Herein, we expanded the clinical and genetic investigation of SPOAN, including the additional study of 44 patients.

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Methods

Clinical Study and Diagnostic Tests

Individuals in which the diagnosis of SPOAN was suspected were initially evaluated by a medical geneticist, who was able to

confirm the diagnosis in 68 individuals and collect complete family histories. Complete neurological and ophthalmological examinations were performed in 61 patients. For rating disease severity, we used the Spastic Paraplegia Rating Scale, SPRS (Schüle et al., 2006), which has a maximum score of 52. In a small number of individuals, brain magnetic resonance image (MRI), nerve conduction velocity, electroretinogram, nerve biopsy, cerebrospinal fluid (CSF) analysis, and urine organic acid determination were also performed. This study was approved by Institutional Ethics Committee and patients or their caretakers consented to participate in this research.

Mapping and Linkage Refining

Genomic DNA from 64 patients was obtained following standard techniques. A search was performed using six informative microsatellites (D11S4191, D11S4076, D11S1883, D11S1889, D11S987, and D11S1314), located at 11q12–13, inside and flanking the 4.8 Mb critical region for SPOAN. These markers were amplified by polymerase chain reaction (PCR) using standard protocols. The PCR products were subjected to electrophoresis in the MegaBace 1000 DNA Sequencer (Amersham Biosciences, Little Chalfont, UK) and subsequently analyzed with Genetic Profiler software (Amersham Biosciences, Little Chalfont, UK). Fifty single nucleotide polymorphisms (SNPs), located in the critical region, some of them in candidate genes, were also investigated (list available on request).

Parametric two-point linkage analysis for each marker was performed using the MLINK software from the FASTLINK (version 5.1) software package (Cottingham et al., 1993). The disease was analyzed as an autosomal recessive trait with a disease allele frequency of 0.0001. Marker allele frequencies were assumed to be 1/N, N being the number of different alleles observed on the pedigree. Although assuming allele frequencies equal to 1/N could lead to an overestimation of the lod score values, we believed it was the best approach since there were not enough genotypic data from founder individuals or individuals from unaffected branches of the inbred SPOAN family.

Mutational Analysis

We sequenced exonic regions of three genes which are expressed in the brain in the newly defined candidate region for SPOAN: *LRFN4* [leucine-rich repeat and fibronectin type III domain-containing 4 (Morimura et al., 2006)], which is a membrane spanning glycoprotein which plays a role in the development of the central nervous system; *KLC2* [kinesin light chain 2 (Rahman et al., 1998)], which is an ATP-dependent molecular motor involved in vesicle and organelle traffic along microtubules; and *CCS* [copper chaperone for SOD1 (Culotta et al., 1997)], whose product is responsible for intracellular copper loading of superoxide dismutase 1 (SOD1).

Results

Clinical Study and Diagnostic Tests

Clinical information regarding 22 patients have been already published (Macedo-Souza et al., 2005), but one of them, a 63-year-old woman assigned previously as affected, was reevaluated and excluded because she had no optic atrophy or neurophysiologic evidence of peripheral neuropathy. Clinical data regarding 21 previously reported SPOAN patients and 40 new observations are summarized in Table 1. Severity of spastic paraplegia, as measured by SPRS is presented in Figure 1. Complete family pedigree, with 12 different family branches, is depicted in Supplementary Figures S1 to S7. The number of evaluated affected individuals in each family varied from 1 (26 families) to 4 (1 family). No significant intrafamilial clinical variability was detected in all adult patients, and all three main features of SPOAN were always present after adolescence. All patients are from the same geographic area and probably share a common ancestor.

Brain MRI was performed in five patients, with normal results. Four-limb nerve conduction velocity was performed in five individuals by the same examiner at 5, 14, 25, 26, and 59 years of age. The youngest patient was the only one with normal results; all others presented a severe motor and sensory neuropathy with axonal pattern. Electroretinogram was performed in two patients, CSF analysis in four, and urine organic acid determination in one individual, all with normal results. Most patients did not perform diagnostic tests because they live in a region with very scanty health resources.

Mapping and Linkage Refining

The identification of additional cases of SPOAN and the exclusion of a patient previously accounted as affected allowed us to recalculate the *lod* score and to restrict the candidate region. We analyzed six microsatellites located in 11q12–13 from 64 patients and 85 unaffected relatives, and obtained with marker D11S987, previously excluded at $\theta = 0.0$, the highest two-point *lod* score, with a Zmax of 33.2 at $\theta = 0.0$, followed by marker D11S1889 with a Zmax of 27.00 at $\theta = 0.0$ (Table 2). Markers D11S987 and D11S1889 are only 580 Kb apart, and D11S987 is more polymorphic than D11S1889, which explains the higher *lod* score yielded by the former, even though all patients are homozygous only at D11S1889. We further analyzed 50 single nucleotide polymorphisms (SNPs) and selected a single informative one, allowing us to reduce the candidate region from approximately 4.8 to 2.3 Mb, now flanked by SNP rs1939212 and by microsatellite D11S987, at 11q13. Informative affected individuals which allowed us to define the candidate region for SPOAN are depicted in Figure 2.

Characteristic	Macedo-Souza, 2005 n = 21	Present study n = 40	Total (%)
Sex			
Male	7	12	19 (31.2)
Female	14	28	42 (68.8)
Age at ascertainment (y)			
<10	1	1	2 (3.3)
10 to <20	2	4	6 (9.8)
20 to <30	4	11	15 (24.6)
30 to <40	7	11	18 (29.5)
40 to< 50	5	6	11(18)
>50	2	7	9 (14.8)
Age of onset of motor symptoms (y)			
<1	17	5	22 (36.1)
1<2	1	14	15 (24.6)
2<6	0	11	11 (18)
6<8	0	6	6 (9.8)
Not known	3	4	7 (11.5)
Optic Atrophy			
Present	21	37	58 (95.1)
Evaluation impossible (cataracts)	0	3	3 (4.9)
Fixation nistagmus			
Absent	4	11	15 (24.5)
Spine deformity			
Scoliosis and/or kyphosis	14	23	37 (60.6)
Absent	7	17	24 (39.4)
Joint retractions			
Absent	4	1	5 (6.7)
Present	17	39	56 (93.3)
Distal wasting			
Absent (all < 20 years)	3	3	6 (9.8)
Present	18	37	55 (90.2)
Tactile, vibratory and arthrestesic sense reduction			
Lower limbs only	6	1	7 (11.5)
Upper & lower limbs	14	32	46 (75.4)
Not known	1	7	8 (13.1)
Dysarthria			
Absent	7	14	21 (34.4)
Present	14	26	40 (65.6)
Extrapyramidal signs			
Absent	16	37	53 (86.9)
Present	5	3	8 (13.1)
Acoustic startle			
Absent	0	12	12(19.7)
Present	21	28	49 (80.3)

Table 1 Clinical Data Summary and Inventory of Complicating Signs and Symptoms in 61 patients with SPOAN

Mutational Analysis

No pathogenic mutation was found in the coding region or intron-exon boundaries of genes *KLC2, CCS* and *LRFN4*.

Discussion

Our results confirm our previous report regarding the SPOAN clinical phenotype, but the onset of motor symptoms might be more delayed than appreciated in our first

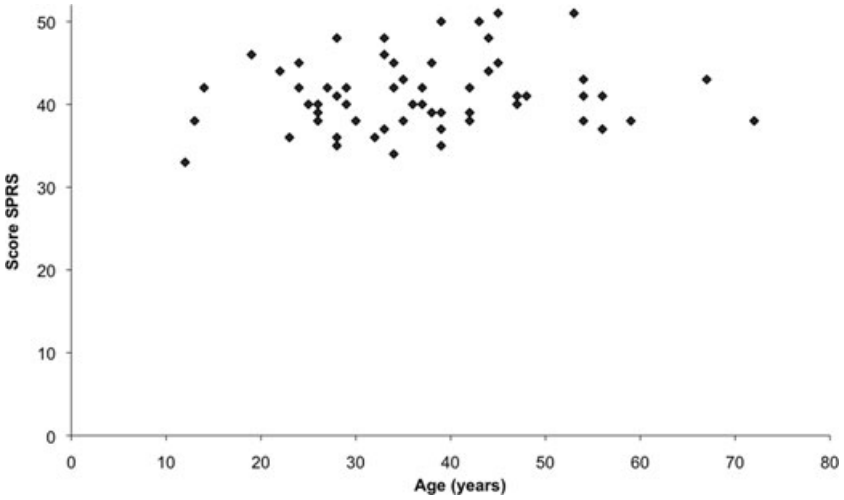

Figure 1 Age and severity score of spastic paraplegia (Schüle et al., 2006 in 59 patients with SPOAN.

Table 2 Two-point lod score for linkage of microsatellite markers of chromosome 11

	Lod Score at θ						
Markers	0.00	0.01	0.05	0.01	0.20	0.30	0.40
D11S4191	$-\infty$	8.91	13.80	13.69	10.23	5.93	2.23
D11S4076	$-\infty$	20.43	21.29	18.64	12.28	6.93	2.54
D11S1883	$-\infty$	24.33	23.50	20.60	13.92	7.63	2.78
D11S1889	26.99	26.29	23.55	20.11	13.35	7.34	2.75
D11S987	33.18	32.15	29.14	25.07	16.95	9.15	3.69
D11S1314	$-\infty$	9.86	14.91	14.74	10.91	6.22	2.25

publication (Macedo-Souza et al., 2005): for six patients (10%), it ranged from 6 to 8 years of age. The severity of spastic paraplegia, evaluated with the SPRS in 59 patients with more than 10 years of age, was high: an average of 39 points were obtained with patients from 10 to <20 years of age; of 41 points from 20 − <30 years; of 40.7 with 30 − <40 years; of 43.5 points from with 40 − <50; and of 41.1 points for individuals over 50 years. The minimum achieved score was 33 and the maximum was 51. Motor deficiency was always more severe in lower than in upper limbs, and spasticity became less severe with neuropathy progression. An apparently non-progressive optic atrophy was usually suspected at infancy, and was confirmed in all individuals, except in three older patients with cataracts, but with previous history of poor vision. Fixation nystagmus, commonly seen in congenital visual deficiency, was present in 46 patients and absent in 15. Startle to sudden noise was reported in 49 subjects. Distal amyotrophy, detected in 90.2% of patients, spine deformities such as kyphosis and scoliosis, seen in 60.6%, and joint retractions, present in 93.3%, were very common manifestations, probably secondary to motor and sensory imbalance. Progressive dysarthria was commonly seen after 20 years of age, impairing communication ability, especially after the fifth decade of life. Extrapyramidal signs such as tremor, parkinsonism and dystonia were infrequent, occurring in 13.1% of individuals with SPOAN.

The refined candidate region for SPOAN at 11q12 spans the region from markers rs1939212 to D11S987 and its size has now been narrowed down from 4.8 to 2.3 Mb. Silver syndrome, or SPG17, an autosomal dominant condition characterized by lower limb spasticity associated with hand and feet weakness and amyotrophy is now outside the candidate region. Overall, 72 genes and *bona fide* transcripts have been assigned to the 2.3 Mb current critical region, three of which, *LRFN4*, *KLC2*, and *CCS,* have been sequenced; however, no pathogenic mutation was detected. Therefore, the gene responsible for SPOAN remains elusive.

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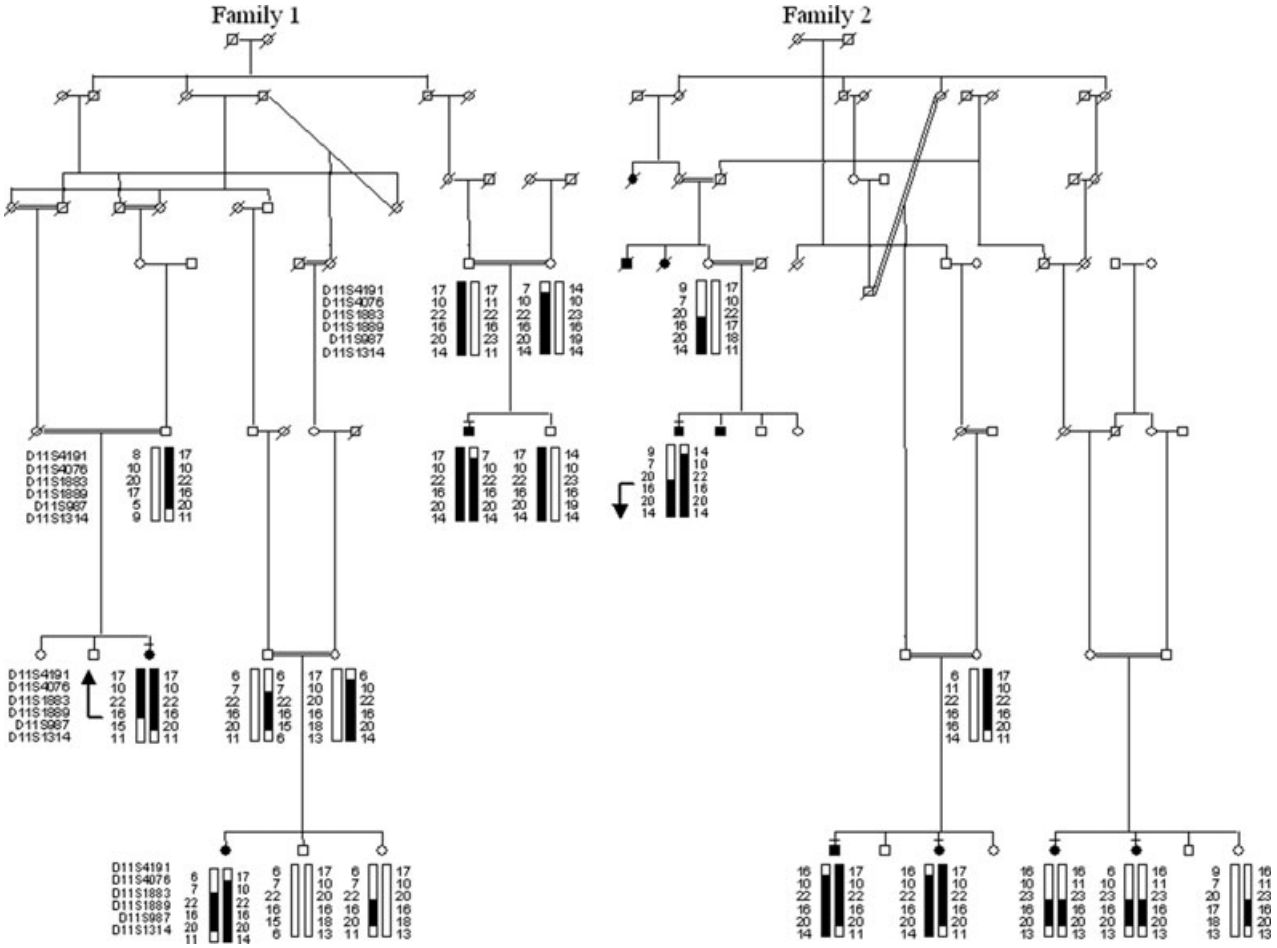

Figure 2 Informative haplotype which defines the limits of the candidate region for SPOAN. Family 1 (previously reported heredogram Macedo-Souza et al., 2005) and family 2 represents the set of new patients added to the original family tree. The individuals with a little *bar* over their symbols were clinically evaluated. *Arrows* demark the region where recombination occurred.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 to S7 – Complete families pedigree. In total, 12 branches with 43 families were investigated, all from the same geographic area.

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