

Phenotype of autosomal dominant spastic paraplegia linked to chromosome 2

A. Dürr,^{1,2} C.-S. Davoine,³ C. Paternotte,⁵ J. von Fellenberg,⁶ S. Cogilnicean,^{1,2} P. Coutinho,⁷ C. Lamy,⁴ S. Bourgeois,³ J.-F. Prud'homme,⁵ C. Penet,¹ J.-L. Mas,⁴ J.-M. Burgunder,⁶ J. Hazan,⁵ J. Weissenbach,⁵ A. Brice^{1,2} and B. Fontaine^{2,3}

¹INSERM U289, the ²Fédération de Neurologie, the ³INSERM U134, Hôpital de la Salpêtrière, the ⁴Service de Neurologie, Hôpital Sainte Anne, Paris, the ⁵Généthon and CNRS URA 1922, Evry, France, the ⁶Neurologische Klinik, Inselspital, Bern, Switzerland and the ⁷Serviço de Neurologia, Hospital Geral de Santo António, Porto, Portugal

Correspondence to: Dr Alexandra Dürr, INSERM U289, Hôpital de la Salpêtrière, 47, Boulevard de l'Hôpital, 75651 Paris Cedex 13, France

Summary

We report the clinical features of 12 families with autosomal dominant spastic paraplegia (ADSP) linked to the SPG4 locus on chromosome 2p, the major locus for this disorder that accounts for ~40% of the families. Among 93 gene carriers, 32 (34%) were unaware of symptoms but were clinically affected. Haplotype reconstruction showed that 90% of the asymptomatic gene carriers presented increased reflexes and/or extensor plantar responses independent of

age at examination. The mean age at onset was 29 years, ranging from 1 to 63 years. Intra- as well as inter-familial variability of age at onset was important, but did not result from anticipation. Phenotype-genotype correlations and comparison with SPG3 and SPG5 families indicated that despite the variability of age at onset, SPG4 is a single genetic entity but no clinical features distinguish individual SPG4 patients from those with SPG3 or SPG5 mutations.

Keywords: spastic paraplegia; SPG4; linkage analysis; anticipation; genetic heterogeneity

Abbreviation: ADSP= autosomal dominant spastic paraplegia

Introduction

Spastic paraplegia, a hereditary disease first described by Strümpell (1880) in two brothers, is characterized pathologically by degeneration of the corticospinal tracts and thinning of the columns of Goll and spinocerebellar tracts. A 'pure' form of the disease has been distinguished, but its pattern of inheritance is variable. In patients with ADSP in addition to pyramidal signs in the lower limbs, sphincter disturbances and diminished vibration sense are frequently observed (Polo *et al.*, 1993; Dürr *et al.*, 1994). Even in the 'pure' form, paraclinical investigations revealed asymptomatic multisystem involvement of the nervous system (Tedeschi *et al.*, 1991; Dürr *et al.*, 1994). Age at onset is highly variable, and Harding (1981) used this parameter in a series of 61 patients to distinguish two types of ADSP. In type I onset ranges from 20 to 40 years, in type II from 40 to 65 years. Sensory loss and urinary symptoms were more frequent in the latter. Both

types may be observed in a single family (Polo *et al.*, 1993; Dürr *et al.*, 1994).

'Pure' ADSPs are genetically heterogeneous. There are at least three loci, designated SPG3–5. The first was assigned to chromosome 14q (SPG3) in a single French family (Hazan *et al.*, 1993). A German and a Tunisian family were found to be linked to the same locus (Hentati *et al.*, 1994; Gispert *et al.*, 1995). Genetic linkage to chromosome 2p (SPG4) was demonstrated in 12 families, seven French and one Dutch (Hazan *et al.*, 1994), three North American and one Tunisian (Hentati *et al.*, 1994). In a North American family with ADSP, the locus was mapped to chromosome 15q (SPG5) (Fink *et al.*, 1995b).

In this study, the clinical features of ADSP genetically linked to SPG4 were analysed in 12 families of different geographic origins to define the characteristics of this disorder, and their variability among a genetically homo-

geneous group of patients, compared with SPG3 and SPG5 kindreds.

Methods

Patients

One hundred and seventy-seven individuals from 12 families with spastic paraplegia were included in this study. The kindreds are shown in Fig. 1, except the already published families: 618, 624, 625 (Fontaine *et al.*, 1995) and family A (Hazan *et al.*, 1993). Two published families, 627 and 645 were re-examined: the status of individuals 627-III-1, 627-IV-1, 627-IV-3 and 645-IV-8 had changed, and a new patient 645-III-6 was added (Fig. 1). Seven families were French (618, 624, 625, 627, 645, 4014, A), three Swiss (2992, 5330, 5226), one Polish (1620) and one Portuguese (3266).

The clinical inclusion criteria for the index cases, prior to the linkage study, were the existence of progressive spastic paraplegia with pyramidal signs in the lower limbs and a pattern of segregation of the disease in the family, compatible with autosomal dominant inheritance. The patients were classified: definitely affected if they had spasticity, increased reflexes in the lower limbs and an extensor plantar response; probably affected if only increased reflexes in the lower limbs or extensor plantar response were present; possibly affected if they had brisker reflexes in the lower than in the upper limbs. Disability was assessed on a three-point scale: 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 chairbound. A severity score related to disease duration was obtained by dividing the disability score by disease duration in years and multiplying by 100. Age at onset calculations included eight deceased patients at the time of the study for whom clinical data were available.

Statistical significance was determined with the Yates corrected χ^2 test and with the Mann-Whitney *U* comparison. Parent-offspring and sibship resemblance was tested by intraclass and interclass analysis.

Genetic linkage analysis

Informed consent was obtained from each family member and each control individual. Blood samples from the family members and from unrelated control individual were drawn, and genomic DNA was prepared according to standard procedures (Gusella, 1986). Polymerase chain reaction amplification and genotyping of the dinucleotide repeats were performed as described in Hazan *et al.* (1992). The dinucleotide repeats used in this study were D2S352, D2S400 and D2S367 (Gyapay *et al.*, 1994). Two newly identified dinucleotide repeats D2S2347 and D2S2351 were also used (Dib *et al.*, 1996). Both loci map within the 3 cM interval flanked by D2S352 and D2S367 (Fig. 2).

Pairwise lod-scores were calculated with the M-LINK program of the computer package LINKAGE (version 5.1)

(Lathrop *et al.*, 1985). A frequency of 0.0001 was assumed for ADSP. To perform linkage analysis, liability classes were determined from the cumulative age at onset curve for the studied families: 0–19 years, 0.17; 20–24 years, 0.32; 25–30 years, 0.55; 31–51 years, 0.92; >51 years old, 0.99 (Fontaine *et al.*, 1995). Equal allele frequencies were used. Recombination fractions were assumed to be equal between men and women. For linkage analysis, definitely affected individuals were considered as affected, the status of probably and possibly affected individuals was considered to be undetermined. Genetic homogeneity was tested with the program HOMOG (Ott, 1991).

Results

Clinical examination revealed spastic paraparesis in 95 patients, including 83 with definite, 10 with probable and two with possible spastic paraplegia as defined above (Table 1).

Linkage analysis

Lod-scores for the tested markers were positive in the 12 families (Table 2). For D2S2351 and D2S2347, total peak-lod-scores were 19.16 and 21.30, respectively, for a recombination fraction of $\theta = 0.00$. HOMOG analysis confirmed the genetic linkage of the families to chromosome 2, with posterior probabilities of linked type ranging from 0.75 to 0.99 for D2S2351. Similar results were obtained for the other three markers (data not shown).

Genotypes of probable and possible cases

Phenotype-genotype correlations confirmed the clinical suspicion in nine out of 12 probable or possible cases (Table 3). Three probably affected patients did not carry the disease haplotype: a 54-year-old woman and a 51-year-old man, both with hyper-reflexia in the lower limbs and flexor plantar response, and a 6-year-old girl with clearly increased reflexes in the lower limbs but no extensor plantar response. No other neurological abnormalities were noticed in either patient. In another woman with a diagnosis of probable spastic paraplegia, leg spasticity began at age 26 years, but at the time of examination, at age 42 years, she had experienced relapsing episodes of motor weakness. Cerebrospinal fluid and MRI studies were suggestive of multiple sclerosis, but she, as well as the two possibly affected individuals, all carried the disease haplotype. Among the 82 at-risk individuals with normal neurological examination only one, aged 57 years, carried the disease haplotype.

Clinical analysis of 83 patients with definite spastic paraplegia

Mean age at examination was 45 ± 20 years, ranging from 3 to 85 years. Functional impairment was absent or slight in

Table 1 Twelve families with pure autosomal dominant spastic paraplegia linked to the SPG4 locus on chromosome 2p

Family	No. of patients with spastic paraplegia			Mean age (years)	
	Definite*	Probable	Possible	at examination (n = 95)	at onset (n = 61)
618	10 (3)	0	0	32±20	24±17 (17–51)
624	4 (1)	1	0	50±25	37±10 (25–45)
625	6 (3)	0	0	40±21	25
627	8 (4)	2	1	38±21	37±12 (25–53)
645	7 (3)	4	1	48±19	37±21 (5–63)
1620	8 (1)	0	0	47±17	22±22 (2–62)
4014	6 (3)	2	0	26±12	12±11 (1–22)
A	7 (0)	1	0	43±19	21±10 (4–30)
2992	6 (0)	0	0	52±16	31±7 (20–40)
3266	8 (4)	0	0	45±17	43±7 (35–50)
5330	9 (1)	0	0	49±15	30±14 (1–45)
5226	4 (0)	0	0	67±19	33±16 (10–45)
Total	83 (23)	10	2	43±20 (3–85)	29±15 (1–63)

*Unaware of symptoms.

27% ($n = 22$), moderate with inability to run in 41% ($n = 34$) and severe in 32% ($n = 27$), who walked with help or were wheelchair-bound. The overall clinical picture is presented in Table 4. There was no significant clinical difference between men ($n = 42$) and women ($n = 41$), except the frequency of sphincter disturbances, more often mentioned in women ($P < 0.05$).

Age at onset and anticipation

The age at onset could be determined for 61 patients, 23 were unaware of symptoms but were definitely affected, and one, aged 77 years, could not remember when his symptoms first began. The mean age at onset was 29 ± 15 years, ranging from 1 to 63 years. In more than half of the patients, 37 out of 61, the disease started before the age of 35 years. Mean age at onset was not statistically different among families ($P = 0.15$), but decreased in successive generations (Table 5). Mean age at onset was significantly earlier in generation III than in generation I or II ($P < 0.001$), but the proportion of gene carriers, asymptomatic or unaware of their symptoms, significantly increased in successive generations ($P < 0.001$), reaching 100% in generation IV. In 33 parent-offspring pairs, age at onset was a mean 7 years earlier in offspring ($P < 0.005$); the mean age at onset in parents was 35 ± 13 years and in their children of 28 ± 15 years. The differences ranged from 58 years earlier to 32 years later than the age at onset of their parents. There was a positive correlation between age at onset of parents and anticipation ($r = 0.57$, $P < 0.001$). This suggested an observation bias, since the likelihood of observing an earlier age at onset in children is greater when the parent is older (Fig. 3); however, among the 33 parent-child pairs, 20 showed anticipation and 12 anti-anticipation and in one, the age at onset was the same in the parent and the child. This distribution was not significantly different from the null hypothesis that half of the offspring had an earlier and half a later onset than the parent ($P = 0.22$).

Clinical signs according to age at onset and disease duration

In accordance with Harding's classification (1981), we divided the patients in two groups, with onset before and after age 35 years. There was no significant difference in the frequency of the clinical signs associated with spastic paraplegia or in the severity of the disease. Disease duration, as expected, influenced the clinical picture. Spasticity and muscle weakness were more severe in advanced stages of the disease. The frequency of urinary urgency and decreased vibration sense increased with the duration of the disease. In contrast, hyper-reflexia in the upper limbs did not increase with evolution. To evaluate differences in the clinical course according to the age at onset, a severity index was calculated for each individual. The correlation between the age at onset and the severity index was significant ($r = 0.26$, $P = 0.035$). The older the onset the more severe the course of the disease, but this correlation is not individual (Fig. 4).

Phenotype-genotype correlations

Comparison between these 12 SPG4 families and 4 SPG4 kindreds reported in the literature (Hentati *et al.*, 1994) confirmed the wide variability of age at onset, ranging from 1 to 75 years, but no other clinical information was available for comparison (Table 6). Three SPG3 kindreds had an earlier mean age at onset but the range was still large (2–50 years) (Hazan *et al.*, 1993; Hentati *et al.*, 1994; Gispert *et al.*, 1995). The course of the disease was reported to be more benign in one family (Hazan *et al.*, 1993), but 40% of the patients needed help walking in the other (Gispert *et al.*, 1995). The single SPG5 family was unusual in that age at onset was less variable, ranging from 12 to 35 years, and all patients had pes cavus. As in SPG4 kindreds, vibration sense was often decreased (percentage was not indicated) (Fink *et al.*, 1995a).

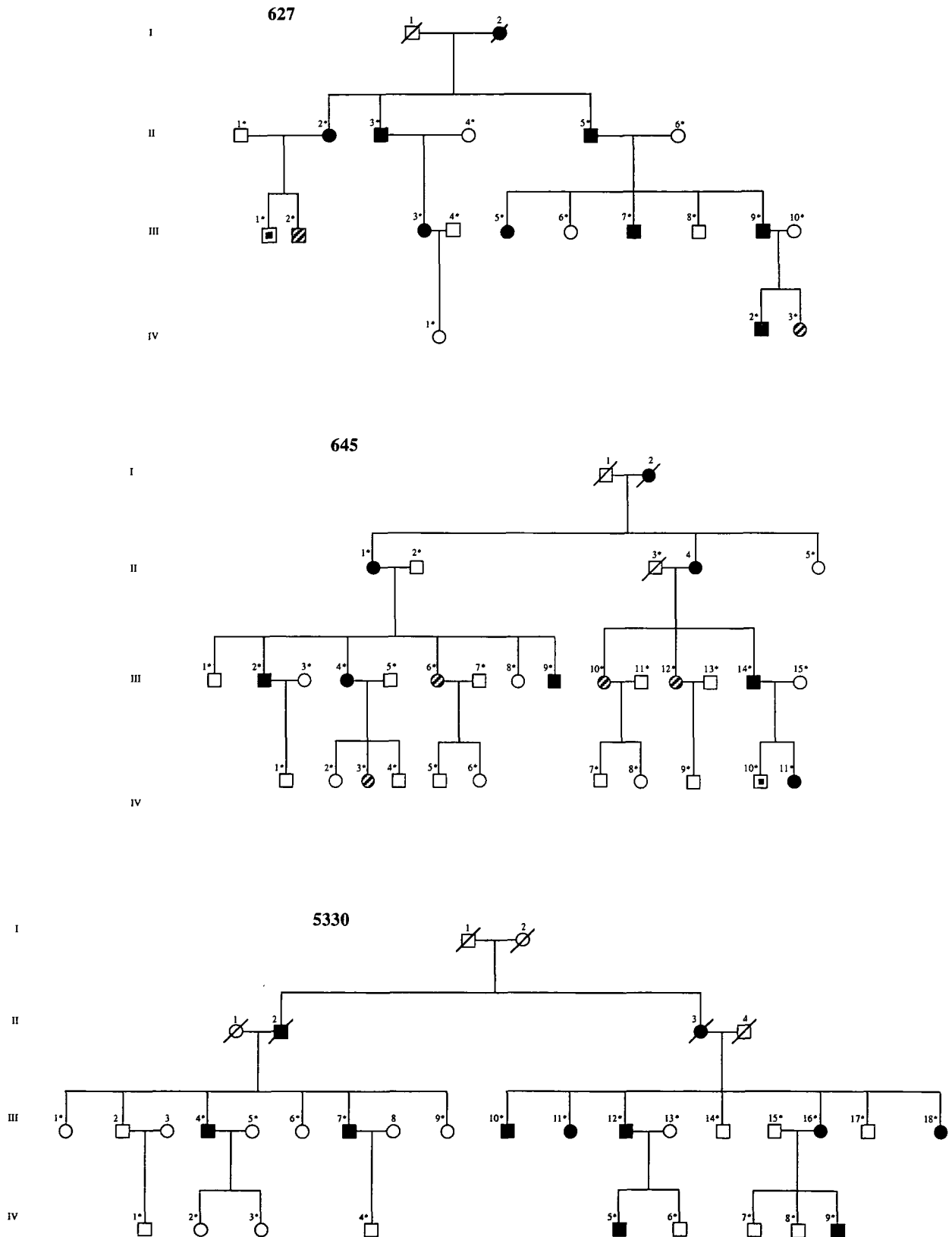
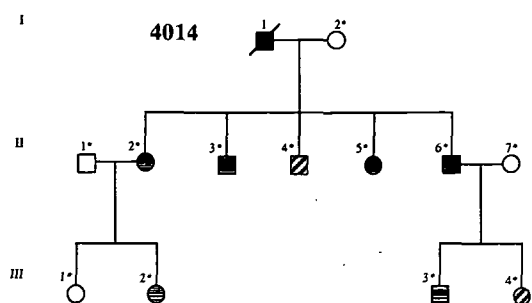
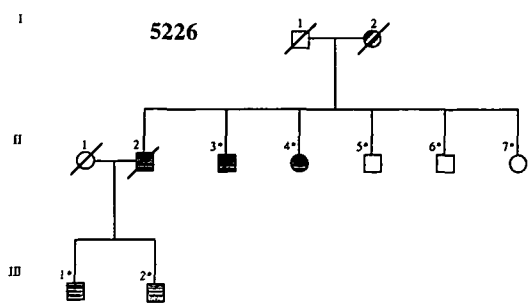
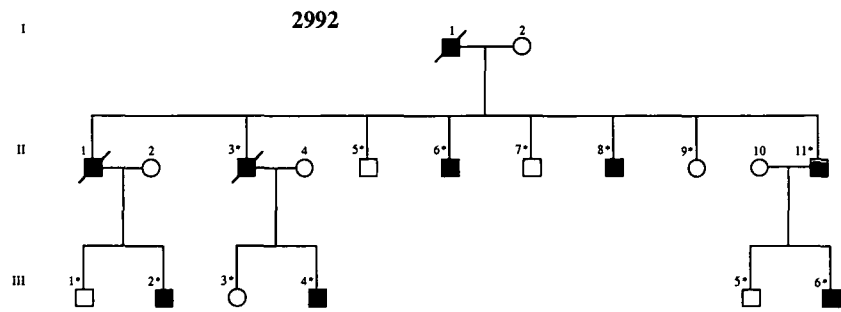
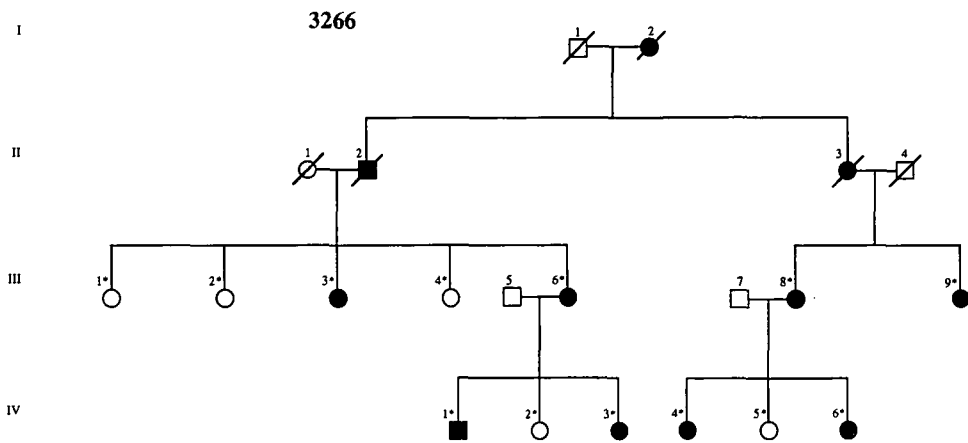
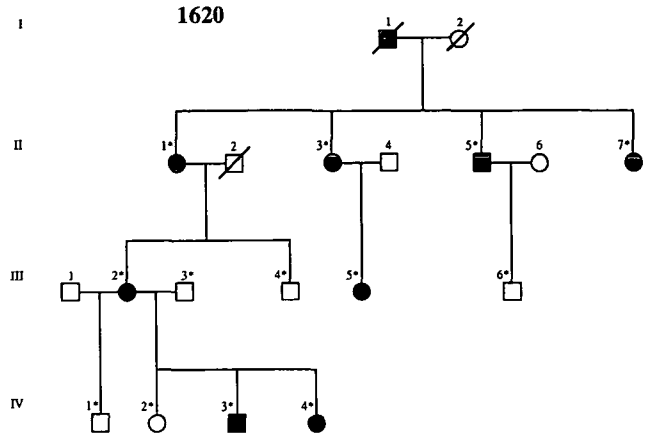


Fig. 1 Pedigrees of families with autosomal dominant spastic paraplegia linked to SPG4. Definitely affected individuals are represented by filled symbols, probably affected ones by hatched symbols and possibly affected ones by small black symbols. Asterisks indicate examined and sampled individuals.



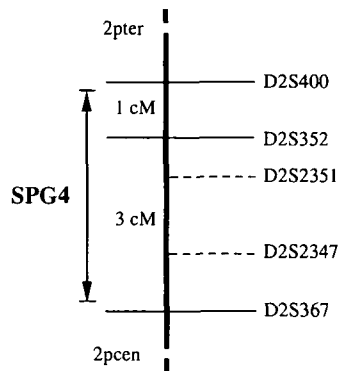


Fig. 2 Abridged map of the chromosome 2p interval containing the SPG4 locus.

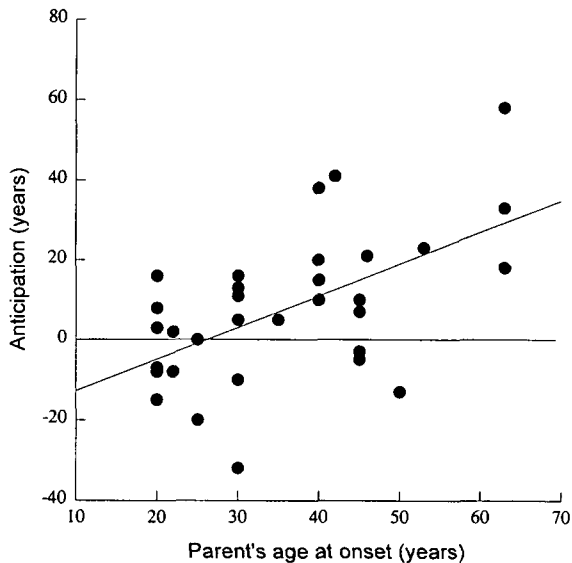


Fig. 3 Correlation between anticipation and the parent's age at onset. The positive correlation suggests an observation bias since the likelihood of observing an earlier age at onset in children is greater the later the parent's age at onset.

Discussion

Families with ADSP were screened for genetic linkage to chromosome 2p (SPG4). Approximately 40% of the tested families showed linkage to SPG4, establishing SPG4 as a major locus for ADSP. We report 12 ADSP families linked to chromosome 2p (SPG4) with 83 definitely affected individuals. This represents the largest series of genetically homogeneous ADSP which enabled us to analyse, in detail, the clinical characteristics of the disorder. Haplotype reconstruction confirmed that the SPG4 gene was located in the interval flanked by D2S400 and D2S367 (Hazan *et al.*, 1994). Haplotype reconstruction also confirmed the genetic status of the 12 probably or possibly affected individuals, nine of whom carried the haplotype segregating with the disease. This indicates that the disease may be suspected in at-risk individuals on clinical grounds, before they become aware of symptoms. For example, only increased and spread reflexes were present in a 10-year-old girl and a 54-year-old woman, both gene carriers. However, three other individuals

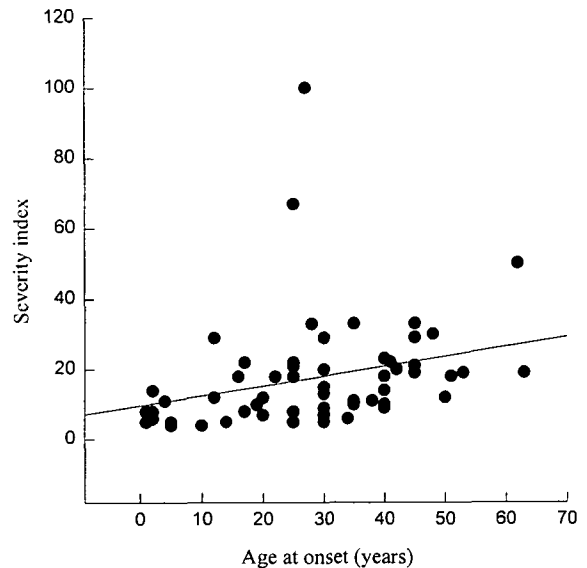


Fig. 4 Correlation between the severity index, defined as the disability score divided by disease duration in years and multiplied by 100, and the age at onset in ADSP patients.

with increased reflexes were not gene carriers. Conversely, a 57-year-old man with a normal neurological examination turned out to be a gene carrier. Despite some exceptions, this study demonstrates the presence and the high specificity of neurological abnormalities in asymptomatic gene carriers, that should be taken into account in genetics studies or counselling.

Adult onset was observed in most patients but intrafamilial variability, and particularly its range (1–63 years), was considerable, as already reported in 'pure' ADSP (Harding, 1981; Polo *et al.*, 1993; Dürr *et al.*, 1994). These figures do not reflect the whole population of patients, however, since onset could only be assessed in 61 out of 93 gene carriers (66%). The others, with age at examination ranging from 3 to 74 years, were either unaware of their symptoms or completely asymptomatic. Over one-third of gene carriers are therefore unaware of their status.

Is the variability in age at onset due to anticipation? Although the differences in age at onset between generation I or II and III, and in parent–offspring pairs, suggest anticipation, it is only apparent, and results from an observation bias. There are several reasons for this: (i) no age at onset can be attributed to one-third of the gene carriers, especially in the youngest generations, who may become symptomatic later than their parent; (ii) awareness of the disease in families can result in diagnosis at an earlier age; (iii) anticipation is strongly correlated with the age at examination of the transmitting parent, with a tendency for regression to the mean; (iv) the distribution of parent–child pairs in which onset in the child is earlier or later than the parent is not significantly different from the null hypothesis which postulates absence of anticipation.

The presence of true anticipation would facilitate identification of the SPG4 gene, by analogy with the five

Table 2 Pairwise lod-scores with four chromosome 2p markers in 12 families with autosomal dominant spastic paraplegia

Family	Recombination fraction							θ_{\max}	z_{\max}
	0.00	0.01	0.05	0.10	0.20	0.30	0.40		
Locus D2S352									
618	2.42	2.38	2.20	1.96	1.45	0.88	0.30	0.00	2.42
624	0.74	0.72	0.64	0.54	0.34	0.17	0.05	0.00	0.74
625	2.38	2.33	2.15	1.92	1.41	0.87	0.32	0.00	2.38
627	1.35	1.36	1.36	1.28	1.01	0.64	0.24	0.02	1.37
645	No information								
1620	1.21	1.18	1.06	0.91	0.61	0.32	0.09	0.00	1.21
2992	1.99	1.96	1.82	1.63	1.21	0.73	0.23	0.00	1.99
3266	2.73	2.68	2.47	2.19	1.61	0.97	0.34	0.00	2.73
5226	0.64	0.61	0.52	0.43	0.28	0.15	0.05	0.00	0.64
5330	No information								
4014	1.02	1.00	0.92	0.81	0.60	0.39	0.19	0.00	1.02
A	0.19	0.19	0.17	0.15	0.11	0.06	0.02	0.00	0.19
Total	14.67	14.41	13.31	11.82	8.63	5.18	1.83	0.00	14.67
Locus D2S2351									
618	2.31	2.27	2.09	1.85	1.34	0.79	0.27	0.00	2.31
624	No information								
625	1.13	1.10	1.00	0.86	0.58	0.29	0.07	0.00	1.13
627	1.93	1.89	1.74	1.55	1.15	0.72	0.28	0.00	1.93
645	2.13	2.09	1.93	1.73	1.28	0.78	0.28	0.00	2.13
1620	1.55	1.52	1.38	1.21	0.85	0.51	0.21	0.00	1.55
2992	3.44	3.38	3.13	2.81	2.11	1.33	0.47	0.00	3.44
3266	2.01	1.96	1.78	1.55	1.08	0.62	0.23	0.00	2.01
5226	1.63	1.60	1.46	1.28	0.90	0.50	0.14	0.00	1.63
5330	1.72	1.69	1.57	1.43	1.09	0.66	0.23	0.00	1.72
4014	0.66	0.65	0.59	0.51	0.36	0.22	0.10	0.00	0.66
A	0.65	0.64	0.58	0.51	0.39	0.28	0.15	0.00	0.65
Total	19.16	18.79	17.25	15.29	11.13	6.70	2.43	0.00	19.16
Locus D2S2347									
618	1.18	1.16	1.06	0.93	0.65	0.35	0.10	0.00	1.18
624	0.27	0.26	0.23	0.19	0.12	0.06	0.01	0.00	0.27
625	2.38	2.33	2.15	1.92	1.41	0.87	0.32	0.00	2.38
627	1.14	1.12	1.03	0.92	0.67	0.40	0.13	0.00	1.14
645	0.45	0.44	0.39	0.34	0.22	0.12	0.05	0.00	0.45
1620	2.17	2.13	1.95	1.73	1.25	0.75	0.30	0.00	2.17
2992	3.19	3.13	2.90	2.60	1.96	1.23	0.44	0.00	3.19
3266	2.39	2.35	2.15	1.90	1.36	0.78	0.25	0.00	2.39
5226	1.78	1.75	1.60	1.42	1.02	0.58	0.17	0.00	1.78
5330	3.69	3.62	3.37	3.03	2.31	1.51	0.64	0.00	3.69
4014	0.89	0.87	0.80	0.71	0.53	0.35	0.18	0.00	0.89
A	2.22	2.17	1.98	1.73	1.19	0.63	0.13	0.00	2.22
Total	21.75	21.33	19.61	17.42	12.69	7.63	2.72	0.00	21.75
Locus D2S367									
618	3.56	3.50	3.25	2.92	2.21	1.42	0.59	0.00	3.56
624	1.33	1.30	1.19	1.04	0.74	0.45	0.20	0.00	1.33
625	-3.89	-0.22	0.36	0.49	0.45	0.27	0.08	0.13	0.50
627	1.37	1.39	1.38	1.30	1.02	0.65	0.25	0.02	1.39
645	2.03	1.99	1.86	1.68	1.26	0.78	0.28	0.00	2.03
1620	2.18	2.14	1.96	1.73	1.26	0.77	0.32	0.00	2.18
2992	2.14	2.10	1.94	1.72	1.25	0.74	0.23	0.00	2.14
3266	2.21	2.16	1.96	1.71	1.18	0.65	0.21	0.00	2.21
5226	-3.89	-0.24	0.36	0.52	0.51	0.35	0.13	0.14	0.55
5330	0.85	0.84	0.78	0.71	0.53	0.34	0.15	0.00	0.85
4014	0.53	0.52	0.48	0.43	0.32	0.21	0.10	0.00	0.53
A	2.63	2.58	2.41	2.18	1.67	1.10	0.49	0.00	2.63
Total	11.05	18.06	17.93	16.43	12.40	7.73	3.03	0.02	18.27

Table 3 Phenotype-genotype correlations in probably and possibly affected individuals with spastic paraplegia

Individual	Clinical classification	Sex	Age at examination	Lower limbs reflexes	Plantar reflex response	Haplotype
624-IV-3	Probable	F	21	+	Extensor	+
627-III-1	Possible	M	30	+	Indifferent	+
627-III-2	Probable	M	32	++	Flexor	+
627-IV-3	Probable	F	6	++	Flexor	-
645-III-6	Probable	F	51	++	Indifferent	-
645-III-10	Probable	F	54	++	Flexor	-
645-III-12	Probable	F	49	++	Flexor	+
645-IV-3	Probable	F	22	++	Indifferent	+
645-IV-10	Possible	M	24	+	Indifferent	+
4014-II-4	Probable	M	35	+++*	Unilateral	+
4014-III-4	Probable	F	10	++	Flexor	+
A-III-16†	Probable	F	42	++	Extensor	+

+ = normal, ++ = increased reflexes. *Unilateral hyper-reflexia; †spastic paraplegia at the age of 26 years followed at 42 years by relapsing episodes of motor weakness, then sphincter disturbances; oligoclonal gammaglobulin bands in CSF and MRI lesions supporting the diagnosis of multiple sclerosis were seen.

Table 4 Clinical picture in 83 patients with definite spastic paraplegia linked to SPG4 on chromosome 2p

Number of families	12
Number of patients	83
Mean age at examination (years)	45±20 (3-85)
Mean age at onset (years)	29±15 (1-63)
Number of affected individuals unaware of symptoms	23
Functional handicap	
Normal or slight	27%
Cannot run	41%
Walk with help or wheelchairbound	32%
Spasticity in the lower limbs	
None	23%
Mild	21%
Moderate	31%
Severe	25%
Hyper-reflexia in the upper limbs	22%
Extensor plantar reflexes	86%
Proximal or generalized weakness in the lower limbs	53%
Impaired vibration sense	56%
Urinary urgency or incontinence	34%
Pes cavus	12%
Dysarthria	4%
Supranuclear ophthalmoplegia	2%
Mental retardation	2%

other neurodegenerative disorders with anticipation in which the expansion of a translated CAG repeat has been demonstrated (Sutherland and Richards, 1995). Even in these disorders, however, anticipation calculated among generations or in parent-offspring pairs is overestimated due to an observation bias (Dürr *et al.*, 1996). Therefore, although the hypothesis of a CAG repeat expansion as the molecular basis of SPG4 is not supported by our data, it cannot be ruled out. Allelic heterogeneity at the SPG4 locus can also be excluded as the source of variability in age at onset, since variance within and among families is similar.

The SPG4 kindreds studied here do not conform to the classification of ADSP into two types according to age at onset, as proposed by Harding (1981). First, both type I and II patients were found in most SPG4 families making their classification impossible. Secondly, the frequency of associated signs, such as decreased vibration sense or urinary symptoms, did not vary as a function of age at onset. Thirdly, disease progression, variable and slightly more severe in late onset patients cannot be used to predict the progression of the disease.

The comparison of the phenotypes of SPG4 patients with published SPG3 and SPG5 patients (Hazan *et al.*, 1993; Hentati *et al.*, 1994; Gispert *et al.*, 1995; Fink *et al.*, 1995a) has brought certain differences to light. Although the number of SPG3 and SPG5 patients is too small for reliable genotype-

Table 5 Difference between age at examination and age at onset in different generations of gene carriers

Generations	Mean age at examination* (years)	Mean age at onset** (range) (years)	% unaware of their status**
I	72±11 (n = 10)	33±21 (10-63) n = 8	10
II	56±8 (n = 36)	35±13 (2-53) n = 31	19
III	31±6 (n = 38)	19±11 (1-35) n = 22	42
IV	10±5 (n = 8)		100

*P < 0.001 for generation II and I compared with generation III; **P < 0.001 between generations.

Table 6 Phenotype-genotype correlations in ADSP families

	Locus SPG3 (14q)			SPG5 (15q)	SPG4 (2p)	
	Hazan <i>et al.</i> , 1993	Hentati <i>et al.</i> , 1994	Gispert <i>et al.</i> , 1995	Fink <i>et al.</i> , 1995	Hentati <i>et al.</i> , 1994	Present study
Number of families	1	1	1	1	4	12
Number of patients	18	10	15	31	35	83
Mean age at onset (years)	6 (2-50)	<5 (5-14)	<13	22±5 (12-35)	>35 (2-75)	29±15 (2-63)
Patients with severe functional handicap	6%	ND	40%	29% (9/31)	ND	11% (9/83)
Decreased vibration sense	0%	ND	ND	Often mildly	ND	56%
Urinary symptoms	ND	ND	ND	10%	ND	34%
Pes cavus	ND	ND	ND	Always present	ND	12%

phenotype correlations, the mean age at onset was clearly earlier in the SPG3 than in the SPG4 or SPG5 groups, adult onset was still found in one out of the 18 SPG3 patients (Hazan *et al.*, 1993). True anticipation was not apparent in SPG4 and SPG5 families. However, in one SPG3 kindred the severity increased from one generation to the next (Hazan *et al.*, 1993). Decreased vibration sense and urinary symptoms were common in SPG4 and SPG5, but absent in SPG3 patients. On the contrary, pes cavus found in all SPG5 patients is rarely observed in those with SPG4. In spite of these differences, the variability in age at onset and clinical presentation of SPG4 patients prevent the application of phenotype-genotype correlation to single affected individuals.

In conclusion, the major locus for ADSP, SPG4 on chromosome 2, is characterized by a marked intrafamilial variability of age at onset and severity. This variability does not result from anticipation or allelic heterogeneity but could be due to other genetic or environmental factors. Careful clinical evaluation of at risk family members can identify most presymptomatic carriers, but accurate diagnosis can only be obtained by haplotype reconstruction. The high frequency of gene carriers unaware of their status is particularly important for genetic counselling.

Acknowledgements

We wish to thank the families for participating in this study. We also wish to thank Professors Olivier Lyon-Caen and Pascal Brunet, Drs Michel Serdaru and Catherine Allaire and the Association Strümpell-Lorrain for referring index cases, Christiane Penet, Yolaine Pothin, Claire Ponthieux and Jacqueline Bou for technical assistance and Dr Merle Ruberg and Professor Yves Agid for critical reading of the manuscript. The study was supported by the Association Française contre les Myopathies, the Groupement de Recherches et d'Études sur les Génomes and the Assistance Publique-Hôpitaux de Paris.

References

Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, et al. A comprehensive genetic map of the human genome based on 5264 microsatellites. *Nature* 1996; 380: 152-4.

Dürr A, Brice A, Serdaru M, Rancurel G, Derouesné C, Lyon-Caen O, et al. The phenotype of 'pure' autosomal dominant spastic paraplegia. *Neurology* 1994; 44: 1274-7.

Dürr A, Stevanin G, Cancel G, Duyckaerts C, Abbas N, Didierjean O, et al. Spinocerebellar ataxia 3 and Machado-Joseph disease: clinical, molecular and neuropathological features. *Ann Neurol* 1996; 39: 490-9.

Fink JK, Sharp GB, Lange BM, Wu C-B, Haley T, Otterud B, et al. Autosomal dominant, familial spastic paraplegia, type I: clinical and genetic analysis of a large North American family. *Neurology* 1995a; 45: 325-31.

Fink JK, Wu C-B, Jones SM, Sharp GB, Lange BM, Lesicki A, et al. Autosomal dominant familial spastic paraplegia: tight linkage to chromosome 15q. *Am J Hum Genet* 1995b; 56: 188-92.

Fontaine B, Rime C-S, Hazan J, Dürr A, Stevanin G, Penet C, et al. Exclusion of the candidate locus FSP1 in six families with late-onset autosomal dominant spastic paraplegia. *Neuromuscul Disord* 1995; 5: 11-17.

Gispert S, Santos N, Damen R, Voit T, Schulz J, Klockgether T, et al. Autosomal dominant familial spastic paraplegia: reduction of the FSP1 candidate region on chromosome 14q to 7cM and locus heterogeneity. *Am J Hum Genet* 1995; 56: 183-7.

Gusella JF. DNA polymorphism and human disease. [Review]. *Annu Rev Biochem* 1986; 55: 831-54.

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, et al. The 1993-94 Génethon human genetic linkage map [see comments]. *Nat Genet* 1994; 7 (2 Spec No): 246-339. Comment in: *Nat Genet* 1994; 7 (2 Spec No): 219.

Harding AE. Hereditary 'pure' spastic paraplegia: a clinical and genetic study of 22 families. *J Neurol Neurosurg Psychiatry* 1981; 44: 871-83.

Hazan J, Dubay C, Pankowiak M-P, Becuwe N, Weissenbach J. A genetic linkage map of the human chromosome 20 composed entirely of microsatellite markers. *Genomics* 1992; 12: 183-9.

Hazan J, Lamy C, Melki J, Munnich A, de Recondo J, Weissenbach J. Autosomal dominant familial spastic paraplegia is genetically heterogeneous and one locus maps to chromosome 14q. *Nat Genet* 1993; 5: 163-7.

Hazan J, Fontaine B, Bruyn RP, Lamy C, van Deutekom JC, Rime C-S, et al. Linkage of a new locus for autosomal dominant familial

spastic paraplegia to chromosome 2p. *Hum Mol Genet* 1994; 3: 1569–73.

Hentati A, Pericak-Vance MA, Lennon F, Wasserman B, Hentati F, Juneja T, et al. Linkage of a locus for autosomal dominant familial spastic paraplegia to chromosome 2p markers. *Hum Mol Genet* 1994; 3: 1867–71.

Lathrop GM, Lalouel JM, Julier C, Ott J. Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. *Am J Hum Genet* 1985; 37: 482–98.

Ott J. *Analysis of human genetic linkage*. Baltimore: Johns Hopkins University Press, 1991.

Polo JM, Calleja J, Combarros O, Berciano J. Hereditary 'pure'

spastic paraplegia: a study of nine families. *J Neurol Neurosurg Psychiatry* 1993; 56: 175–81.

Sutherland GR, Richards RI. Simple tandem DNA repeats and human genetic disease. [Review]. *Proc Natl Acad Sci USA* 1995; 92: 3636–41.

Strümpell A. Beiträge zur Pathologie des Rückenmarks. *Arch Psychiat Nervenkrankh* 1880; 10: 676–717.

Tedeschi G, Allocca S, Di Costanzo A, Carlomagno S, Merla F, Petretta V, et al. Multisystem involvement of the central nervous system in Strümpell's disease. *J Neurol Sci* 1991; 103: 55–60.

Received March 5, 1996. Accepted April 26, 1996