

Autosomal dominant juvenile amyotrophic lateral sclerosis

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Summary

Juvenile amyotrophic lateral sclerosis (ALS) is a form of chronic motor neuron disease characterized by combined upper and lower motor neuron symptoms and signs with onset prior to age 25 years. We report the clinical and electrodiagnostic findings in 49 affected family members and neuropathological findings from two autopsies of a Maryland kindred with autosomal dominant juvenile ALS linked to the chromosome 9q34 region (ALS4). Patients ranged in age from 12 to 85 years (mean 45 years) and the mean age of onset was 17 years. Distal weakness and atrophy was associated with pyramidal signs (43/49) and normal sensation (44/49). Motor conduction studies ($n = 8$) showed reduced evoked amplitudes and normal conduction parameters. Sensory conduction studies ($n = 8$), quantitative sensory testing ($n = 4$) and intracutaneous sensory fibres in skin biopsies ($n = 6$) were normal in all

patients tested. Electromyography showed distal more than proximal chronic partial denervation and reinnervation ($n = 8$). Post-mortem spinal cord tissue demonstrated atrophic spinal cords with marked loss of anterior horn cells and degeneration of corticospinal tracts, as well as loss of neurons in the dorsal root ganglia and degeneration of the posterior columns. Axonal spheroids were present in the grey matter of the spinal cord, the dorsal root entry zones and the peripheral nerves. Motor and sensory roots, as well as peripheral nerves, showed significant axonal loss. Swellings were prominent around motor neurons, probably representing changes in presynaptic terminals. These studies define autosomal dominant juvenile ALS linked to the chromosome 9q34 region (ALS4) and extend the clinical, pathological and genetic heterogeneity of familial ALS and juvenile ALS.

Keywords: amyotrophic lateral sclerosis; neuropathology; genetic disease

Abbreviations: ALS = amyotrophic lateral sclerosis; HSP = hereditary spastic paraplegia; MRC = Medical Research Council

Introduction

Motor neuron disease refers to a group of neurodegenerative disorders characterized by progressive weakness and atrophy of skeletal muscle due to the selective degeneration of motor neurons. Amyotrophic lateral sclerosis (ALS) is a form of motor neuron disease that is usually fatal, and it is characterized by clinical and pathological features of upper and lower motor neuron degeneration. Approximately 90% of ALS cases are sporadic and 10% are familial (Emery and Holloway, 1982). Up to 20% of families with familial ALS carry a mutation in the superoxide dismutase gene (*SOD1*) (Rosen *et al.*, 1993). For the majority of families with familial ALS, the gene defect and pathogenesis are unknown.

Progressive upper and lower motor neuron signs are also found in a group of juvenile-onset motor neuron disorders with different clinical and genetic patterns. In general, the term juvenile ALS has been used for patients with onset of disease prior to age 25 years and prolonged survival (Ben Hamida *et al.*, 1990). Most cases of juvenile ALS are autosomal recessive, although autosomal dominant inheritance patterns have been described (Ben Hamida *et al.*, 1990). Recessive forms of juvenile ALS have been mapped to chromosome regions 2q33 (ALS2) (Hentati *et al.*, 1994a; Siddique *et al.*, 1996) and 15q12–21 (Hentati *et al.*, 1994a, 1997).

Four years prior to the publication of Dyck and Lambert's

classification scheme for Charcot–Marie–Tooth disease (Dyck and Lambert, 1968*a, b*), Myriantopoulos and co-workers described three families with this disease (Myriantopoulos *et al.*, 1964). Family D was a large Maryland kindred with an autosomal disorder characterized by weakness, atrophy and pyramidal signs. Re-examination of two members of this family who had previous diagnoses of Charcot–Marie–Tooth disease showed distal weakness and amyotrophy, hyper-reflexia and normal sensation, findings not consistent with the current concept of this disease. In this report, we describe the findings of our re-examination of this large Maryland family, including the first two autopsies. Genetic studies have shown linkage to the 9q34 region (Chance *et al.*, 1998). As a result of this investigation, we reclassify this family as having juvenile ALS. These studies broaden the spectrum of disease classified as juvenile ALS to include a non-fatal form with autosomal dominant inheritance (termed ALS4) and further define the clinical and pathological heterogeneity seen in heritable forms of motor neuron disease.

Methods

Patients

This study was approved by the ethics committee of Johns Hopkins University School of Medicine and Johns Hopkins Hospital, and informed consent was obtained from all participating family members or their legal surrogates prior to inclusion in the study.

All patients were examined by at least two of the authors, either at Johns Hopkins or at a family reunion in June 1994. In total, 150 family members have been examined to date. Criteria for inclusion as affected were: (i) corticospinal tract signs; (ii) distally predominant weakness and amyotrophy; and (iii) absence of confounding neurological illness. Previous medical records were reviewed and used to aid classification of individual patients. Motor strength was graded by the Medical Research Council (MRC) scale (Medical Research Council, 1986). Tendon reflexes were graded as 0–4: 0 = absent; 1 = hypoactive; 2 = normal; 3 = brisk; 4 = pathological with clonus.

Electrodiagnostic studies

Electrodiagnostic studies were performed with standard techniques. Quantitative sensory testing was performed using the Case IV machine (WR Electronics, Stillwater, Minn., USA).

Skin biopsy

Skin biopsies were performed on six individuals for evaluation of cutaneous nerve fibres (McCarthy *et al.*, 1995), a technique particularly useful for visualizing the

small sensory nerve fibres within the epidermis (Holland *et al.*, 1998).

Neuropathology

Portions of brain and spinal cord tissues from patients VII-10 and VIII-18 were collected, and each specimen was divided into three portions: one was fixed in 4% paraformaldehyde for paraffin sections, one was fixed in 5% glutaraldehyde and postfixed in osmium tetroxide for plastic embedding and one was frozen in dry ice–isobutane for cryostat sections. Sections stained with haematoxylin and eosin were prepared from each paraffin block of spinal cord and brain tissue. Selected sections were then processed with the following special stains: haematoxylin and eosin with Luxol fast blue counterstain; Hirano (modified Bielschowsky) silver stain; Masson trichrome stain; Congo red stain; modified Thioflavin S stain. Immunohistochemical stains were prepared using antibodies to identify the following antigens: glial fibrillary acidic protein (Dako, Carpinteria, Calif., USA), ubiquitin (Dako), and phosphorylated and non-phosphorylated epitopes of the 200 kDa neurofilament protein recognized by the antibodies SMI-31 and SMI-32, respectively (Sternberger Monoclonals, Inc., Baltimore, Md., USA). Plastic sections of spinal cord, ventral and dorsal roots, dorsal root ganglia and peripheral nerve were stained with toluidine blue. Thin sections were stained with lead citrate and uranyl acetate.

Results

Clinical findings

We examined 150 individuals from a large Maryland kindred. This family has traced its American roots back to the 1600s, when its ancestors arrived from England. Forty-nine patients had clinical signs (Table 1). There were 26 females and 23 males, and males and females both had a mean age of symptom onset of 17 years (Table 2). However, if clinically affected but asymptomatic patients are included in this analysis, the overall age of onset rises to 23 years (27 years for females and 15 years for males).

Distal amyotrophy was present in 90% (44/49) of the patients. Although most patients had upper (90%) and lower (92%) limb weakness on examination, lower limb weakness was usually more severe (Table 1). Pathological hyper-reflexia was present in 84% (41/49) and clonus was seen in 20% (10/49). Extensor plantar responses were documented in 18% of the patients (9/49) during their clinical course.

Six of our patients did not have upper motor neuron signs. Five of these patients (VII-10, VIII-8, VIII-18, VIII-20 and IX-43) had weakness and atrophy too severe at the time of initial examination for these signs to be elicited. The one remaining patient (IX-11) had a normal examination, but because his son (X-12) appeared affected

Table 1 Clinical features of autosomal dominant juvenile ALS (ALS4)

Patient	Age at exam (years)	Onset age (years)	Sex	Amyotrophy	Arm strength				Leg strength				Reflexes			Plantar response	Sensation		
					D	B	Ta	W	I	Q	H	T	G	B	T			P	A
VII-10	85	20	F	+	3	4	4	4	2	2	2	1	1	2	1	0	0	NR	nl
VII-19	73	6	F	+	5	5	5	5	4	5	5	4	4	3	3	3	0	FP	dec
VIII-8	66	28	M	+	3	3	0	0	1	3	0	0	0	0	0	0	0	NR	nl
VIII-12	62	35	F	+	4	4	4	5	3	4	4	3	4	3	3	3	1	FP	nl
VIII-15	62	40	F	+	5	5	5	5	4	5	5	5	4	3	3	3	3	FP	nl
VIII-17	51	18	M	+	5	5	5	4	4	4	4	4	4	3	3	3	3	FP	nl
VIII-18	71	10	M	+	2	1	2	2	0	0	0	0	0	2	2	0	0	NR	dec
VIII-20	71	24	F	+	4	4	4	2	2	3	3	0	0	1	0	0	0	FP	nl
VIII-21	71	AS	F	-	5	5	5	5	5	5	5	4	5	3	3	3	3	FP	nl
VIII-23	66	12	F	+	5	3	3	4	3	2	2	0	2	3	3	3	3	FP	nl
VIII-24	43	12	M	+	5	4	4	5	4	4	4	4	5	4	3	3	3	FP	nl
VIII-28	41	12	M	+	5	5	5	4	4	5	5	4	5	3	3	3	3	EP	nl
VIII-31	34	AS	F	+	5	5	5	5	4	5	5	4	5	3	3	3	3	FP	nl
VIII-39	63	63	F	+	5	5	5	5	5	5	5	5	4	3	3	3	4	FP	nl
VIII-36	67	20	M	+	1	2	3	0	0	1	1	0	0	1	0	2	0	EP	nl
IX-1	45	15	F	+	5	5	5	5	4	5	5	0	4	3	3	3	0	FP	nl
IX-3	44	13	F	+	5	5	5	5	4	5	5	0	4	3	3	3	2	FP	nl
IX-5	60	35	M	+	4	5	4	1	1	2	2	0	1	4	1	1	0	FP	nl
IX-10	64	AS	F	+	5	5	5	5	4	5	5	4	5	3	3	3	2	EP	nl
IX-11	62	AS	M	-	5	5	5	5	5	5	5	5	5	2	2	2	2	FP	nl
IX-13	58	12	F	+	5	5	5	5	4	5	5	5	5	2	2	3	3	FP	nl
IX-16	53	9	F	+	4	4	4	3	4	3	3	0	3	4	2	0	0	FP	nl
IX-18	60	AS	F	-	5	5	5	5	4	5	5	5	5	2	3	3	3	EP	nl
IX-20	41	31	F	+	4	4	4	5	4	4	4	4	5	2	2	2	3	FP	dec
IX-21	39	33	M	+	4	5	5	5	4	4	4	4	4	3	3	3	1	FP	nl
IX-23	34	10	M	+	5	4	4	4	3	2	3	0	0	3	3	3	2	EP	nl
IX-24	28	AS	F	+	4	5	5	5	4	5	5	4	4	4	3	3	3	EP	nl
IX-26	47	20	F	+	5	5	5	5	4	4	4	4	5	4	3	2	0	FP	nl
IX-29	44	10	M	+	4	4	4	2	2	2	2	0	1	3	3	3	0	FP	nl
IX-31	31	15	F	+	5	5	5	5	5	5	5	4	5	3	3	3	3	FP	nl
IX-36	15	14	M	+	5	5	5	5	4	5	5	4	5	3	3	3	4	FP	nl
IX-37	12	AS	M	+	5	5	5	5	4	5	5	4	5	3	3	3	3	FP	nl
IX-39	44	6	M	+	5	5	4	4	3	4	2	0	0	3	3	3	0	NR	dec
IX-41	35	AS	F	+	5	5	5	4	4	5	5	3	4	3	3	2	3	FP	nl
IX-43	65	9	F	+	3	4	4	4	1	4	4	0	0	2	2	0	0	FP	nl
X-1	24	14	M	+	5	5	5	5	4	5	4	3	5	3	3	2	3	FP	nl
X-5	35	AS	F	-	5	5	5	5	4	4	4	4	4	3	3	4	2	FP	nl
X-11	35	13	M	+	5	5	4	5	4	5	5	3	5	3	3	3	2	FP	nl
X-12	36	18	M	+	5	5	5	5	5	5	5	0	1	3	3	3	0	FP	dec
X-13	38	7	F	+	5	5	4	4	4	5	4	0	0	3	3	2	0	FP	nl
X-14	33	5	M	+	5	5	4	4	4	3	4	3	4	3	3	3	2	FP	nl
X-18	27	5	F	+	5	5	5	5	5	5	5	4	5	3	3	3	4	FP	nl
X-19	19	1	M	+	5	5	5	4	4	5	5	4	4	3	3	2	1	EP	nl
X-22	37	AS	F	-	5	5	5	5	5	5	5	5	5	3	3	3	3	FP	nl
X-23	32	15	F	+	5	5	5	5	4	4	4	4	5	4	3	3	3	EP	nl
X-24	41	14	M	+	4	4	4	3	2	4	3	0	0	3	3	1	0	NR	nl
XI-1	13	10	M	+	5	5	5	4	4	4	4	1	4	3	3	3	2	FP	nl
XI-4	15	AS	M	+	4	4	4	4	4	5	5	4	5	3	3	3	3	FP	nl
XI-5	14	12	M	+	5	5	5	5	4	5	5	4	5	3	3	3	3	FP	nl

Patient numbers correspond to those in the paper by Chance *et al.* (Chance *et al.* 1998). Onset age: AS = asymptomatic. Strength was measured using the MRC scale. Arm strength: D = deltoid; B = biceps; T = triceps; W = wrist extensors; I = interossei. Leg strength: Q = quadriceps; H = hamstring; Ta = tibialis anterior; G = gastrocnemius. Reflexes are graded on a scale of 0–4: 0 = areflexia; 1 = reduced reflex; 2 = normal; 3 = brisk; 4 = clonus. Deep tendon reflexes: B = biceps; T = triceps; P = patella; A = ankle. Plantar responses: FP = flexor plantar; EP = extensor plantar; NR = no response. Sensation: nl = normal; dec = decreased.

we have tentatively classified him as an obligate carrier who is so far unaffected.

Increased vibratory thresholds at the great toes were present in five (10%) of our patients (VII-19, VIII-18, IX-

20, IX-39 and X-12), although none had sensory symptoms. Only two patients (VIII-20 and X-19) had cranial nerve dysfunction; both of these patients had facial weakness. No patient had respiratory or bulbar weakness.

Table 2 Summary of clinical data from affected autosomal dominant juvenile ALS individuals

Characteristic	Female	Male	Total
Number of patients	26	23	49
Age at time of examination (mean, years)	51	38	
Age at symptom onset (mean, years)*	17	17	
Amyotrophy	22 (85%)	22 (96%)	44 (90%)
Upper limb weakness	21 (81%)	23 (91%)	44 (90%)
Lower limb weakness	23 (88%)	22 (96%)	45 (92%)
Pyramidal signs	23 (88%)	20 (87%)	43 (88%)
Sensory involvement	2 (8%)	3 (13%)	5 (10%)
Cranial nerve dysfunction	1 (4%)	1 (4%)	2 (4%)
Asymptomatic	8 (31%)	3 (13%)	11 (22%)

*Age at symptom onset includes only symptomatic patients (see text).

Interestingly, 22% (11/49) of the patients had unambiguous upper and lower motor neuron signs but were asymptomatic at the time of initial evaluation. The eight asymptomatic female patients (VIII-21, VIII-31, IX-10, IX-18, IX-24, IX-41, X-5 and X-22) ranged in age from 35 to 71 years. They account for 16% of all the patients and 31% of the female patients. Of the asymptomatic male patients, two are teenagers in whom it may be too early for them to notice symptoms, and the other is patient IX-11, mentioned above.

Electrodiagnostic studies

Nerve conduction studies were performed on eight patients (summarized in Table 3). All had normal sural and median sensory responses. Of 16 motor nerves tested, five had reduced amplitudes of evoked motor compound muscle action potential (four in the deep peroneal and one in the median nerve). Electromyography revealed chronic partial denervation–reinnervation in a graded pattern of distal muscles that were affected more severely than proximal muscles in the upper and lower limbs. Quantitative sensory testing, performed using the CASE IV machine on patients IX-23, IX-26, X-14 and X-19, was normal.

Skin biopsies

Six individuals, ranging in age from 19 to 60 years, underwent skin biopsies to examine intracutaneous nerve fibres. All had normal densities and morphology of the intra-epidermal nerve fibres.

Case reports

Patient IX-5

This patient was 60 years old when examined. His symptoms began in his early 30s with difficulty in walking. His disease progressed over the next 25 years to the point where he was unable to walk and had weakness in his hands. At the time of examination he denied dysarthria, dysphagia or dyspnoea. He had MRC grade 4 strength proximally in the arms and

MRC grade 1 strength in the intrinsic hand muscles. Proximal lower limb strength was MRC grade 2 with flaccid paralysis distally. Deep tendon reflexes were grade 4 in the upper limbs, grade 1 at the knees with crossed adductors and absent at the ankles. His toes were unresponsive to plantar stimulation and sensation was normal. He had four sisters, two unaffected and two mildly affected, and three brothers, all asymptomatic. However, one of his siblings (IX-11) with a normal neurological examination had an affected son (X-12).

Patient X-12

This patient was a nephew of patient IX-5. He was 36 years old when examined. With the exception of a club foot at birth, he had normal development. Symptoms began at age 18 years with weak ankles and development of a limp. His disease progressed slowly, and he eventually noticed weakness in his upper limbs. There was knee flexion weakness (MRC grade 4), severe weakness in the ankle plantar flexors (MRC grade 1) and paralysis of the ankle dorsiflexors. In the upper limbs, proximal strength was normal but intrinsic hand muscles showed mild weakness (MRC grade 4). Tendon reflexes were grade 3 in the arms, grade 3 at the knees with crossed adductor reflexes, and absent at the ankles. Plantar responses were flexor.

Patient IX-26

This patient was 45 years old when examined. Her symptoms began when she was a teenager, with decreasing ability to run or jump. Her weakness progressed to the point where she was unable to walk up stairs at the time of examination. She noticed weakness in her hands and had great difficulty performing tasks requiring fine motor control. She had distal amyotrophy, normal strength proximally in the upper limbs, MRC grade 4 strength in the intrinsic hand and proximal lower limb muscles, grade 4 ankle dorsiflexor strength and grade 4 ankle plantar flexor strength. Tendon reflexes were grade 4 at the biceps, grade 3 at the triceps, grade 2 at the

Table 3 Nerve conduction studies of autosomal dominant juvenile ALS patients

Patient	Nerve	Distal latency	Distal amplitude (µv)	Conduction velocity (m/s)	F-wave latency
VIII-40	Sural (s)		20	41	
	Deep peroneal (m)	5.8	2000	41	54.5
	Median (s)		35	59	
IX-26	Median (m)	3.4	9500	59	31.9
	Sural (s)		15	44	
	Deep peroneal (m)	4.0	2400	46	48.0
X-19	Median (s)		39		57
	Median (m)	2.9	5900	53	30.3
	Sural (s)		19		58
X-14	Deep peroneal (m)	4.4	700	35	NT
	Median (s)		34	67	
	Median (m)	3.6	6700	61	31.1
IX-23	Sural (s)		30	53	
	Deep peroneal (m)	4.3	990	46	NT
	Median (s)		41	61	
VIII-24	Median (m)	3.1	5200	54	NT
	Sural (s)		23	42	
	Deep peroneal (m)	5.6	130	42	NT
X-23	Median (s)		39	64	
	Median (m)	4.5	200	36	NT
	Sural (s)		11	44	
VIII-12	Deep peroneal (m)	3.3	1100	49	NT
	Ulnar (s)		13	56	
	Ulnar (m)	2.4	6000	58	31.3
X-23	Sural (s)		13	42	
	Deep peroneal (m)	5.1	2000	50	49.0
	Median (s)		61	55	
VIII-12	Median (m)	3.1	13 400	55	27.0
	Sural (s)		20	40	
	Deep peroneal (m)	5.1	2000	40	54.9
VIII-12	Median (s)		20	56	
	Median (m)	3.6	4700	56	30.8

s = sensory; m = motor; NT = not tested. Normal values: sensory amplitude >9 µV; sensory conduction velocity >39 m/s in legs and >49 m/s in arms; motor amplitude >2000 µV in the legs and >4000 µV in the arms; motor conduction velocity >39 m/s in the legs and >49 m/s in the arms; distal latency (motor), deep peroneal <5.6 ms, median <4.3 ms, ulnar <3.5 ms; F wave latency: deep peroneal <55 ms, median <32 ms, ulnar <33 ms.

knees with crossed adductor reflexes, and absent at the ankles with extensor plantar responses. Sensation was normal.

Patient X-19

This patient, who was the son of patient IX-26, had onset of symptoms early in life, with weakness first noticed by his mother at age 1 year. His clinical course since then has been one of slow but steady progression. He was 19 years old when examined. There was facial weakness, normal strength proximally and MRC grade 4 strength in the distal limbs. Deep tendon reflexes were grade 3 in the arms, grade 2 at the knees and grade 1 at the ankles. He had bilateral extensor plantar responses. Sensation was normal.

Neuropathology

Autopsies limited to the nervous system were performed on two patients. Patient VII-10 died of respiratory failure shortly after an ischaemic stroke in the left middle cerebral artery

territory at age 88 years. Patient VIII-18 died of pneumonia at age 75 years.

Patient VII-10

Brain. Brain weight prior to fixation was 1110 g. On gross examination, the most striking finding was a large area of acute ischaemic infarction involving the territories supplied by the left middle cerebral artery, particularly the left frontal and parietal lobes. However, ischaemic damage was also evident in the left cingulate gyrus, the caudate nucleus, the occipital pole and the CA1 region of the hippocampus. In the brainstem, the most striking pathological changes were in the dentate and nucleus gracilis, where ubiquitin-positive axonal swellings (spheroids) were numerous. Spheroids were also present in the intracranial parts of the third and fourth cranial nerves. The hypoglossal nucleus was normal.

Spinal cord. The corticospinal tracts showed only mild degeneration (Fig. 1A). The posterior columns were pale,

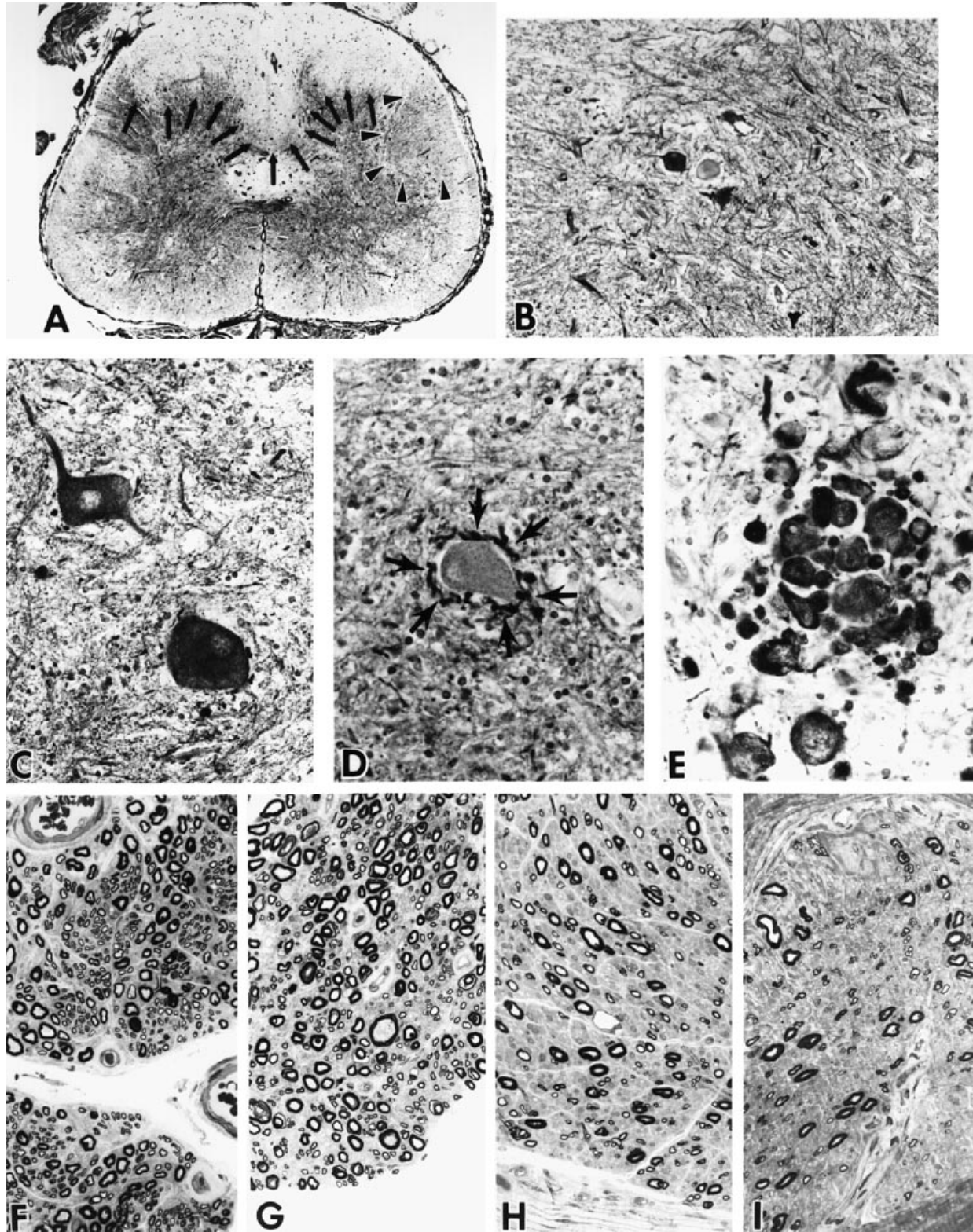


Fig. 1 Neuropathology of spinal cord and peripheral nerve from patient VII-10. (A) Spinal cord section at the lumbar level demonstrating myelin pallor in fasciculus gracilis of the dorsal columns (arrows) as well as corticospinal tracts (arrowheads) (magnification $\times 18$). Bielschowsky silver stain. (B) Section from the lumbar anterior horn demonstrating reduced numbers of motor neurons. Of the three in the centre of this section, one is pale and chromatolytic ($\times 140$). (C) Two large motor neurons in the anterior horn of the lumbar spinal cord stain intensely with the anti-phosphorylated neurofilament antibody SMI-31 ($\times 468$). (D) A large neuron in the lumbar anterior horn is surrounded by SMI-31-positive neurites ($\times 560$). (E) SMI-31-positive dystrophic neurites ($\times 504$). (F–I) Toluidine blue-stained plastic sections of thoracic ventral root (F), lumbar dorsal root (G), peroneal nerve (H) and sural nerve (I) ($\times 243$). Note the reduced large fibre densities in F and G and reduced densities of all myelinated fibres in H and I.

with marked loss of myelin staining (Fig. 1A). There were only a few anterior horn cells remaining in the thoracic and lumbar ventral horns (Fig. 1B and C). Some of these motor neurons showed chromatolysis, but no intracytoplasmic inclusions were found. Dystrophic neurites were present in the anterior horns and they often surrounded the remaining motor neurons (Fig. 1D and E). Ubiquitin-positive spheroids were readily identified at all levels of the cord but were more abundant in the lumbosacral roots than the thoracic roots and were particularly prominent in the dorsal horns and dorsal root entry zones. Markedly thickened blood vessel walls were evident in the spinal roots as well as in the spinal cord. Amyloid stains were negative.

Peripheral nerves. The ventral and dorsal roots, as well as the peroneal and sural nerves, showed loss of both large and small myelinated fibres but no ongoing Wallerian-like degeneration (Fig. 1F–I). Ultrastructural examination of the axonal swellings demonstrated numerous membrane-bound organelles within them (Fig. 2). There were no neuro-filamentous swellings.

Patient VIII-18

Brain. Brain weight prior to fixation was 1180 g. Coronal sections demonstrated mild ventricular enlargement. Patchy gliosis was seen in the grey and white matter. Mild to moderate neuron loss was present in the hypoglossal nucleus and dorsal motor nucleus of the vagus. Large cytoplasmic inclusions were present in some neurons of the hypoglossal nucleus. These inclusions stained with both the Hirano silver stain and the ubiquitin immunostain.

Spinal cord. The spinal cord was small in calibre, with particularly small ventral roots. Microscopic examination demonstrated marked loss of anterior horn cells, the few remaining neurons appearing shrunken (Fig. 3A–C). The severity of the cell loss varied from section to section, but was generally greater at the lumbosacral level (Fig. 2C). Axonal spheroids were present in the dorsal horns and dorsal root entry zones, as well as in the surrounding remaining motor neurons. There was almost complete loss of myelin staining in one of the corticospinal tracts, with significant pallor on the contralateral side (Fig. 3A and B). The dorsal columns were pale, with gliosis and rare axonal spheroids. Occasional spheroids were also present within the dorsal horns and around large neurons in the anterior horns. They were more numerous in the ventral and dorsal roots, where they stained with haematoxylin and eosin and silver and reacted with the antibodies directed against phosphorylated neurofilament (SMI-31) and ubiquitin (Fig. 3D and E). Blood vessels were thickened but Congo red stain for amyloid was negative. Marked fibre loss was seen in both ventral and dorsal roots. Ubiquitin-positive axonal swellings were more prominent in the dorsal roots than in the ventral roots.

Peripheral nerves. The most prominent features seen in the brachial plexus were axonal swellings and occasional degenerating fibres. The peroneal, phrenic and sural nerves showed marked loss of both large and small myelinated fibres accompanied by prominent axonal swellings (Fig. 3F–H). While there was some evidence of demyelination and remyelination, there was no evidence of active degeneration. Occasional regenerating clusters were present.

Discussion

The Maryland kindred described in this report has an autosomal dominant, early-onset, neurodegenerative disease characterized by progressive amyotrophy, weakness, pathological hyper-reflexia with abnormal spread of reflexes, and extensor plantar responses. Sensation was normal in 90% of the subjects, the rest having minimal, exclusively distal sensory signs. Anterior horn cell dysfunction was evident in patients who underwent electrodiagnostic testing but there was no evidence of peripheral neuropathy from electrophysiological studies, quantitative sensory testing or skin biopsy. Thus, affected individuals have typical clinical features of ALS, viz. progressive, unambiguous upper and lower motor neuron dysfunction in the absence of significant sensory abnormalities or ataxia. Given the age of onset of 17 years and the chronicity of the disease, this disorder should now be classified as juvenile ALS.

Analysis of two autopsy specimens demonstrated marked loss of spinal motor neurons in the anterior horns and less pronounced degeneration of the corticospinal tracts. Surprisingly, we also found myelin pallor in the posterior columns, marked axonal degeneration in peripheral nerve (sural, peroneal and phrenic nerves), dorsal and ventral roots and dorsal root ganglia. Together, these findings extend the clinical and pathological boundaries of juvenile ALS.

The patients described in this report, as well as the ALS2 patients described by Ben Hamida and co-workers with autosomal recessive juvenile ALS from Tunisia (Hentati *et al.*, 1994a) and those previously reported by Refsum (Refsum and Skillicorn, 1954), all have early-onset diseases that are characterized by amyotrophy, weakness and pyramidal dysfunction but appear to be clinically and genetically distinct. Many ALS2 patients develop bulbar and respiratory weakness (Hentati *et al.*, 1994b), whereas none of the ALS4 patients examined so far has significant respiratory or bulbar signs or symptoms. The relative lack of bulbar and respiratory muscle weakness in most of our patients also distinguishes them from sporadic ALS (Charcot and Joffroy, 1869) and familial ALS (Kurland and Mulder, 1955; Figlewicz and Rouleau, 1994). Interestingly, we and others have not yet been able to demonstrate accumulation of ubiquitinated inclusions in the cytoplasm of neurons of juvenile ALS patients (Matsumoto *et al.*, 1993). However, ubiquitin-positive material is present in axonal spheroids (see Results).

Genetic mapping studies have confirmed the heterogeneity

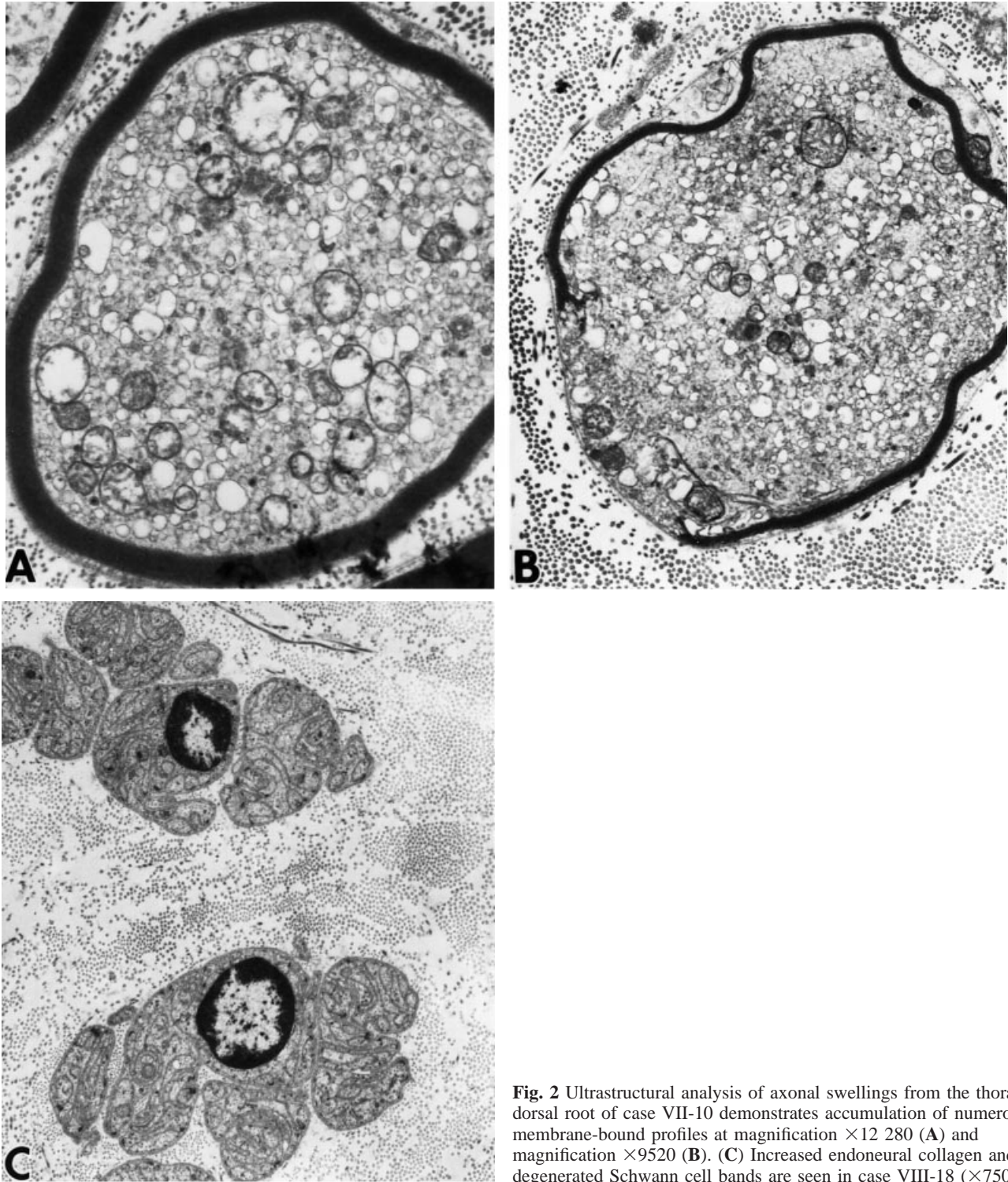


Fig. 2 Ultrastructural analysis of axonal swellings from the thoracic dorsal root of case VII-10 demonstrates accumulation of numerous membrane-bound profiles at magnification $\times 12\,280$ (A) and magnification $\times 9\,520$ (B). (C) Increased endoneurial collagen and degenerated Schwann cell bands are seen in case VIII-18 ($\times 7\,500$).

of the juvenile-onset diseases. We have previously reported linkage of autosomal dominant juvenile ALS (*ALS4*) to markers on chromosome 9q34 (Chance *et al.*, 1998), while the autosomal recessive forms are linked to markers on chromosomes 2q33–35 (Hentati *et al.*, 1994b; Siddique *et al.*, 1996) and 15q12–21 (Hentati *et al.*, 1997).

Although the clinical spectrum of familial ALS is much broader than that of sporadic ALS, we considered whether

or not this family represented a form of neurodegenerative disease other than juvenile ALS. Selective motor system disease involving pyramidal tract dysfunction in the absence of bulbar weakness is also seen in hereditary spastic paraplegia (HSP) (Harding, 1981). The pure form of HSP is genetically heterogeneous with early onset, autosomal dominant HSP families showing linkage to chromosomes 14q (Hazan *et al.*, 1993), 2p (Hazan *et al.*, 1994; Hentati

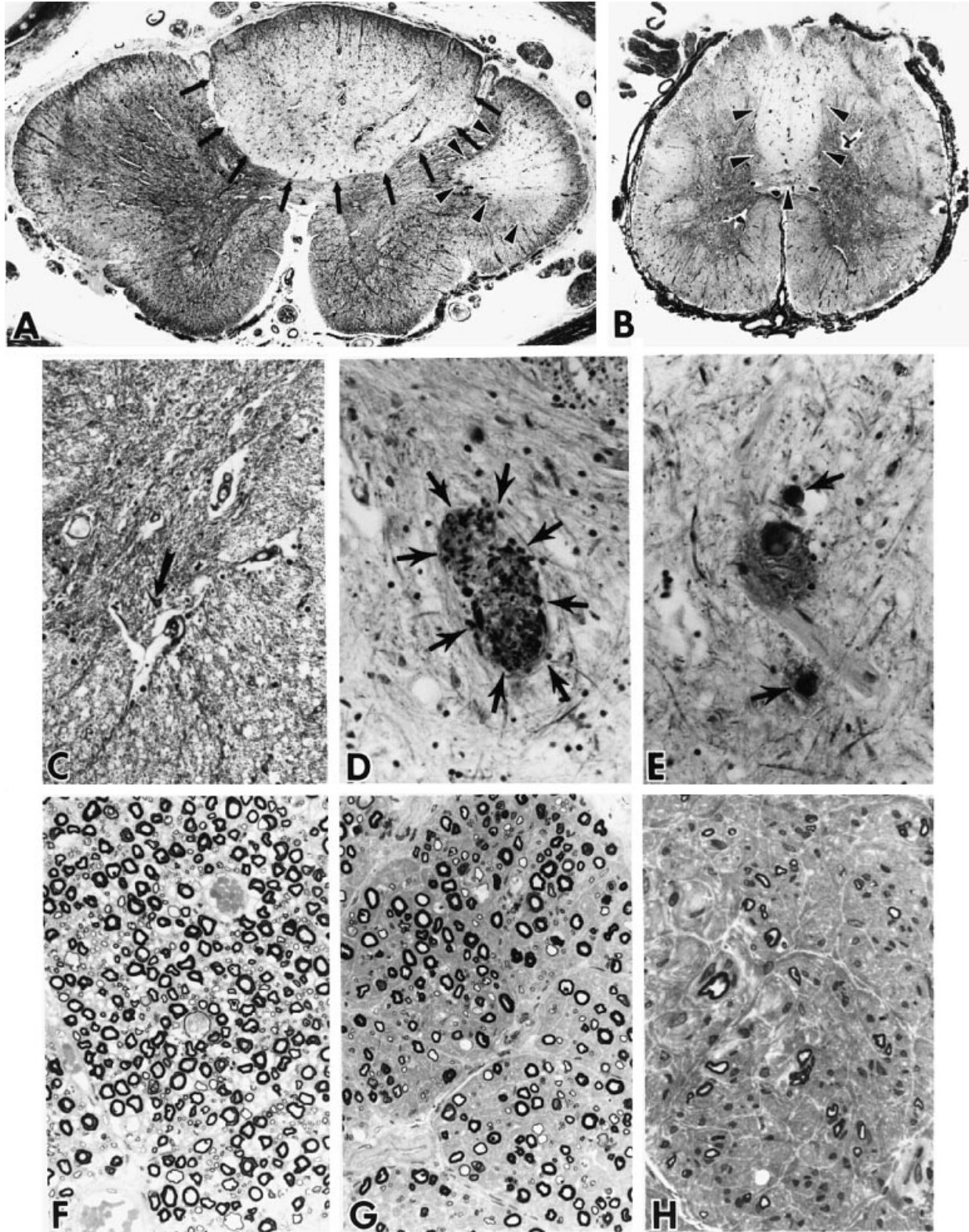


Fig. 3 Neuropathology of spinal cord and peripheral nerve from patient VIII-18. (A) Spinal cord section at the thoracic level demonstrating myelin pallor in the corticospinal tracts (arrowheads) and dorsal columns (arrows). Few anterior horn cells are present in the ventral horns. Bielschowsky silver stain (magnification $\times 27$). (B) Spinal cord section at the lumbar level demonstrating myelin pallor in fasciculus gracilis of the dorsal columns (arrowheads) as well as corticospinal tracts. Bielschowsky silver stain ($\times 18$). (C) Few anterior horn cells (arrow) are present in the ventral horn of this section of the lumbar spinal cord. Bielschowsky silver stain ($\times 126$). (D and E) Phosphorylated neurofilaments (visualized with SMI-31 antibody) accumulate in dystrophic neurites (arrows) within the ventral horn. Some of these neurites are probably surrounding degenerated motor neurons ($\times 504$). (F–H) Loss of myelinated fibres in peroneal nerve ($\times 243$) (F), phrenic nerve ($\times 243$) (G) and sural nerve ($\times 225$) (H). (F–H) Plastic sections stained with toluidine blue.

et al., 1994c) and 15q (Fink *et al.*, 1995), while some families with autosomal recessive HSP have shown linkage to markers on chromosome 8q12–13 (Hentati *et al.*, 1994b). Linkage to markers on Xq22 (PLP gene) and Xq28 (L1CAM gene) has also been shown (Keppen *et al.*, 1987). However, since pure HSP patients do not have amyotrophy or other signs of lower motor neuron dysfunction, our family does not have pure HSP. In addition, our previous genetic studies have excluded the known HSP loci (Chance *et al.*, 1998). Families with ‘complicated’ HSP have been reported with upper and lower motor neuron dysfunction (Garland and Astley, 1950; Cross and McKusick, 1967; Harding and Thomas, 1984), although many of these families have additional findings, such as optic atrophy, mental retardation and/or sensory signs (Webb *et al.*, 1998). ‘HSP with amyotrophy’ patients appear to differ from our patients by the presence of marked spasticity, particularly in the lower limbs, and amyotrophy found predominantly in the upper limbs. The patients we have described have little or no spasticity and marked amyotrophy is present in both upper and lower limbs. In addition, the neuropathologies of these diseases differ significantly. HSP is characterized pathologically by axonal degeneration of the distal portions of the longest axons in the CNS: corticospinal tracts to the legs, fasciculus gracilis and spinocerebellar fibres. Diffuse axonal swellings are not a prominent feature.

Silver described two families with early-onset HSP characterized by amyotrophy and weakness of the hands and relatively mild pyramidal disease in the legs (Silver, 1966). A similar disorder was reported by van Gent and co-workers (van Gent *et al.*, 1985). These patients had an early-onset (second decade) autosomal dominant disease characterized by pyramidal tract dysfunction associated with amyotrophy. Three of the 18 affected patients developed sensory symptoms late in their course and were thought to have either HSP with amyotrophy or hereditary motor and sensory neuropathy type V. Patients with the latter disease also have some features in common with ALS4 patients, including upper and lower motor neuron dysfunction (Harding, 1981, 1984). However, they have clear sensory signs and electrophysiological evidence of neuropathy rather than neuronopathy (Dyck, 1975). Another series of patients with an autosomal dominant, juvenile-onset disease characterized by upper and lower motor neuron dysfunction was reported in 1904 (Ormerod, 1904). The possibility that these patients, as well as those described by Silver and by van Gent, had a form of juvenile ALS similar to our family cannot be excluded by the information provided in either report, and was also considered by the authors (van Gent *et al.*, 1985; Silver, 1966). It is possible that genetic studies in these families would confirm the heterogeneity.

Although the finding of posterior column pathology was surprising given the lack of clinical signs or symptoms, the prominent involvement of non-motor systems, particularly the posterior columns, has been well described in familial ALS (Engel *et al.*, 1959; Hirano *et al.*, 1967; Metcalf and Hirano 1971; Tanaka *et al.*, 1984; Murayama *et al.*, 1989;

Takahashi *et al.*, 1994; Shibata *et al.*, 1996; Chudkowicz *et al.*, 1998). Patients with this form of familial ALS typically present with progressive muscular atrophy and weakness associated with pyramidal dysfunction that may have a very slow clinical course. Many of these patients have objective sensory findings. Some families with ‘familial ALS with posterior column involvement’ have been shown to carry the A4V (Shibata *et al.*, 1996; Chudkowicz *et al.*, 1998) and A4T mutations (Takahashi *et al.*, 1994) in *SOD1*. These patients may have associated clinical features of dysaesthesia, hypaesthesia and hypalgesia. Autopsy of a patient with the A4T mutation showed marked atrophy of the anterior horns, mild degeneration of the corticospinal tracts and prominent degeneration of the posterior columns and spinocerebellar tracts (Takahashi *et al.*, 1994). It is not known why the posterior columns are involved in some but not all *SOD1*-linked familial ALS individuals. Similarly, expression of the G37R, G93A and A4V superoxide dismutase 1 mutations in transgenic mice results in mice with similar motor neuron disease phenotypes but significantly different pathological findings (Dal Canto and Gurney, 1995; Wong *et al.*, 1995). These results highlight the fact that clinical expression of a specific, genetically defined disease is variable and depends on multiple genetic and environmental factors.

Why the pathological findings involving sensory pathways in our juvenile ALS patients are not associated with sensory symptoms or abnormalities on nerve conduction testing, quantitative sensory testing or skin biopsy remains unexplained. Only five patients had sensory signs, and these were solely of distal vibratory sensation loss. One possibility is that the pathological changes seen in sensory systems represent preclinical changes. Another possibility is that these changes are seen only late in the disease process or in end-stage disease. However, we have examined patients with severe disease and those late in life, and they have little or no sensory dysfunction.

In summary, we have described a large juvenile ALS family whose pathological findings include anterior horn cell and corticospinal tract degeneration, diffuse axonal swellings, degeneration in nerve roots and peripheral nerves, and prominent posterior column involvement. These findings extend the spectrum of familial ALS and juvenile ALS to include a slowly progressive, autosomal dominant, non-fatal but debilitating disease. Whether this family represents an intermediate form of motor neuron disease or a unique disease resulting from a primary abnormality of the axon must await further elucidation of the underlying defect in the gene responsible on chromosome 9q34.

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