Mitochondrial cytopathy presenting as hereditary sensory neuropathy with progressive external ophthalmoplegia, ataxia and fatal myoclonic epileptic status

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Summary

We present six adult patients from three separate families, with a remarkably uniform heredo-ataxic syndrome, developing in three stages and ending in early death. The initial stage is determined by severe sensory neuropathy. The second stage is characterized further by progressive external ophthalmoplegia (PEO), probably caused by ocular myopathy, and progressive ataxia. During a short last stage there is epilepsia, and particularly myoclonic status epilepticus, of which four patients died unexpectedly. Sural nerve biopsies showed severe loss of myelinated fibres in a rather early stage of disease. Skeletal muscle biopsies (and a specimen of ocular muscle) revealed ragged-red fibres. Autopsy examination in two patients revealed multisystemic involvement of the nervous system, with, in particular, degeneration of spinal dorsal columns and spinocerebellar tracts. Pedigree data were compatible with an autosomal recessive disorder. Additional findings, particularly elevation of CSF lactate, suggested mitochondrial cytopathy as an essential feature of the multisystem degeneration in these patients.

Keywords: sensory neuropathy; progressive external ophthalmoplegia; ataxia; myoclonus epilepsia; mitochondrial encephalomyopathy

Abbreviations: COX = cytochrome *c* oxidase; CPEO-plus = chronic progressive external ophthalmoplegia with multisystemic involvement; KSS = Kearns–Sayre syndrome; MEM = mitochondrial encephalomyopathy; MERRF = myoclonus epilepsy with ragged-red fibres; mtDNA = mitochondrial DNA; PEO = progressive external ophthalmoplegia

Introduction

Without knowing the underlying molecular genetic mechanisms, the classification of hereditary multisystem disorders, presenting as heredo-ataxic syndromes, has traditionally been the subject of controversy. The establishment of a growing number of underlying inborn errors of metabolism, of which the primary mitochondrial dysfunction currently attracts much attention, resulted in the lumping together of disorders, that were previously distinguished on clinical grounds (Rosenberg and Grossman, 1989; Marsden *et al.*, 1990; Harding, 1993). On the other hand, genetically or biochemically defined mitochondrial diseases seem to have an expanding number of non-specific, clinical features, including ophthalmoplegia,

myopathy, fatigue, polyneuropathy, ataxia and seizures (Lombes *et al.*, 1989; DiMauro and Moraes, 1993). Progressive sensory axonal neuropathy is a common finding in heredo-ataxic disorders, particularly spinocerebellar degenerations (Dyck, 1993) and, to a lesser degree, has been reported in mitochondrial encephalomyopathic (MEM) syndromes (Yiannikas *et al.*, 1986; Mizusawa *et al.*, 1991).

We now present six adult patients from three families with a progressive neurological disorder, characterized by insidious onset at adolescence of severe sensory neuropathy. These patients subsequently developed PEO, gait ataxia, with slight cerebellar symptoms, and myoclonic status epilepticus in a

Table 1 *Clinical and biochemical characteristics*

	Patient no.						Normal values
	A.S3	A.S4	A.S6	B.S6	B.S9	C.Fl	
Age of onset (years)	20	<20	18	20	17	<18	
Age of death (years)		44	32	36		32	
Neuropathy	+++	+++	+++	+++	+++	+++	
CPEO	+++	++	++	+++	+++	++	
Dysarthria	++	++	+	+	++	+	
Epilepsy	–	Myocl status	Myocl status	Myocl status	Absences	Myocl status	
Blood lactate (μmol l^{-1})	2556	3130	1300*	?	2575	1340*	700–1800
Urine lactate (μmol mmol creatine^{-1})	31	1012	73	?	92	30	<60
CSF lactate (μmol l^{-1})	2982 <40>	2850 <25>	2500 <21>	?	2110 <45>	2131* <24>	140–1900
CSF protein (mg l^{-1})	361	650	685	633	420	550	180–580

Blood and CSF lactate values in resting condition. + = mild; + + = moderate; + + + = severe; ? = not available; < > = age of investigation. *The normal values for the laboratory conditions concerned were given as 600–1200 and 1200–1600 μmol l^{-1} in blood and CSF, respectively.

short last stage. This multisystem degeneration neither fits in any of the heredo-ataxic syndromes in the most recent classification (Harding, 1993), nor could it be identified as one of the previously established MEM syndromes. Additional findings suggest mitochondrial involvement as an essential feature.

Case summaries (Table 1)
Family A
Three patients from this family (Fig. 1) were affected. The parents of these patients exhibited no symptoms, neither did they suffer from any complaints. Patient A.S4 had complaints of progressive unsteady gait from the age of 20 years onward, but had clumsy performance before that. On clinical examination at the age of 31 years, there were severe sensory neuropathy (with loss of vibration and position sense but intact nociception), areflexia, ataxia with slight cerebellar signs (particularly dysarthria and slight dysmetria), and ophthalmoparesis (particularly upward gaze) with symmetric ptosis. At the age of 32 years she had two grand mal insults, followed by gradually progressive myoclonus, particularly of the right side of the body. At the age of 33 years there was repeated myoclonic epileptic status (see the EEG trace, Fig. 2A), of which she died at the age of 34 years; autopsy was performed.

Patient A.S6 also had sub-optimal motor performance at school age, and complained of muscle stiffness and pains in the legs, with fatigue and unsteady gait, from the age of 18 years onward. She was admitted at a psychiatric hospital because of anxiety and severe psycholability with 'delusions' for several years. At the age of 21 years there were ataxia, dysarthria, kyphoscoliosis, pes cavus and partial external ophthalmoplegia with asymmetric ptosis. Autonomic neuropathy was considered because of orthostatic hypotension, hyperhydrosis, keratitis sicca and a tendency to urine-retention, with recurrent cystitis. She was confined to a

wheelchair by the age of 29 years, with complete external ophthalmoplegia, visual acuteness of 0.5, areflexia and slight muscular weakness. At the age of 29 years she gradually developed myoclonic jerks in the legs, initially particularly at night. At the age of 31 years grand mal epilepsy with myoclonus of both hands developed, followed by repeated epileptic insults and fatal myoclonic epileptic status after 8 months. No autopsy was performed.

Patient A.S3 developed numbness in the legs, fatigue and chronic episodic diarrhoea, after delivery of a healthy boy when she was aged 25 years. Endoscopy and radiology of the gastrointestinal tract revealed no abnormalities. Bedside testing of autonomic functions, including orthostatic changes of blood pressure, ECG changes of heart rate at rising, Valsalva manoeuvre and hyperventilation (McLeod and Tuck, 1987) showed normal responses. From the age of 30 years onward there were severe sensory ataxia, PEO (without visual loss), dysarthria with slight discoordination, areflexia, mild muscular weakness, distal loss of sensibility for all qualities, and loss of position, vibration and discrimination sense from the trunk extending down the legs. The feet were high-arched and there was thoracolumbar scoliosis. At the age of 40 years she could not walk without aid, there was complete external ophthalmoplegia, but no clinical signs of epilepsia. The EEG showed recurrent paroxysmal bitemporal sharp waves, with a tendency to spread, yet without clinical signs of epilepsy.

Family B
Patient B.S9 had absence-like attacks at the time of menarche, for which she received anticonvulsants for some years. From the age of 17 years onward she complained of painful, hypersensitive and abnormal temperature sensations in both legs. Examination at the age of 20 years revealed pes cavus, sensory ataxia with normal muscle strength and nystagmoid ophthalmoplegia (with symmetric ptosis). There was loss of discrimination and vibration sense for the lower part of the
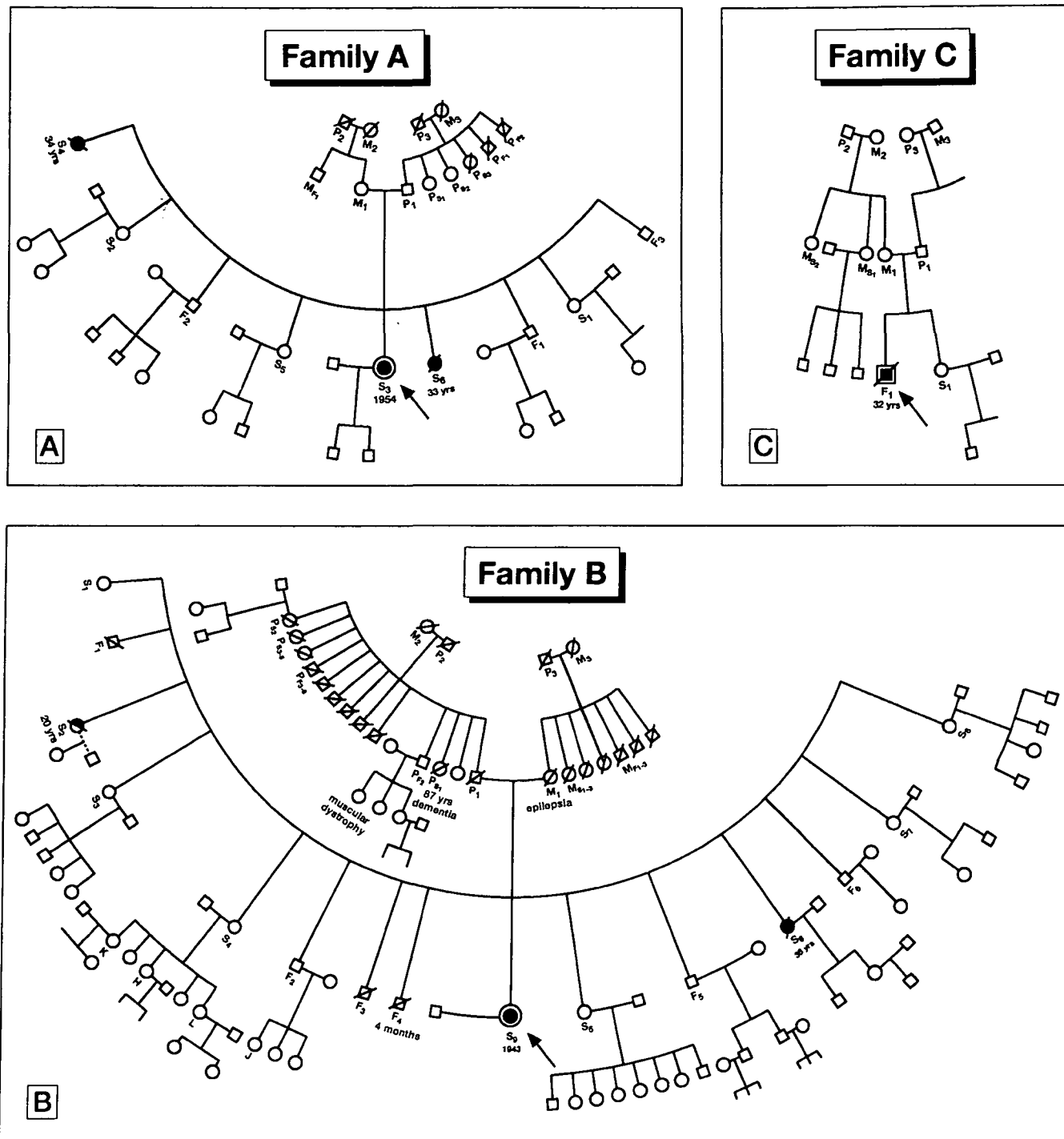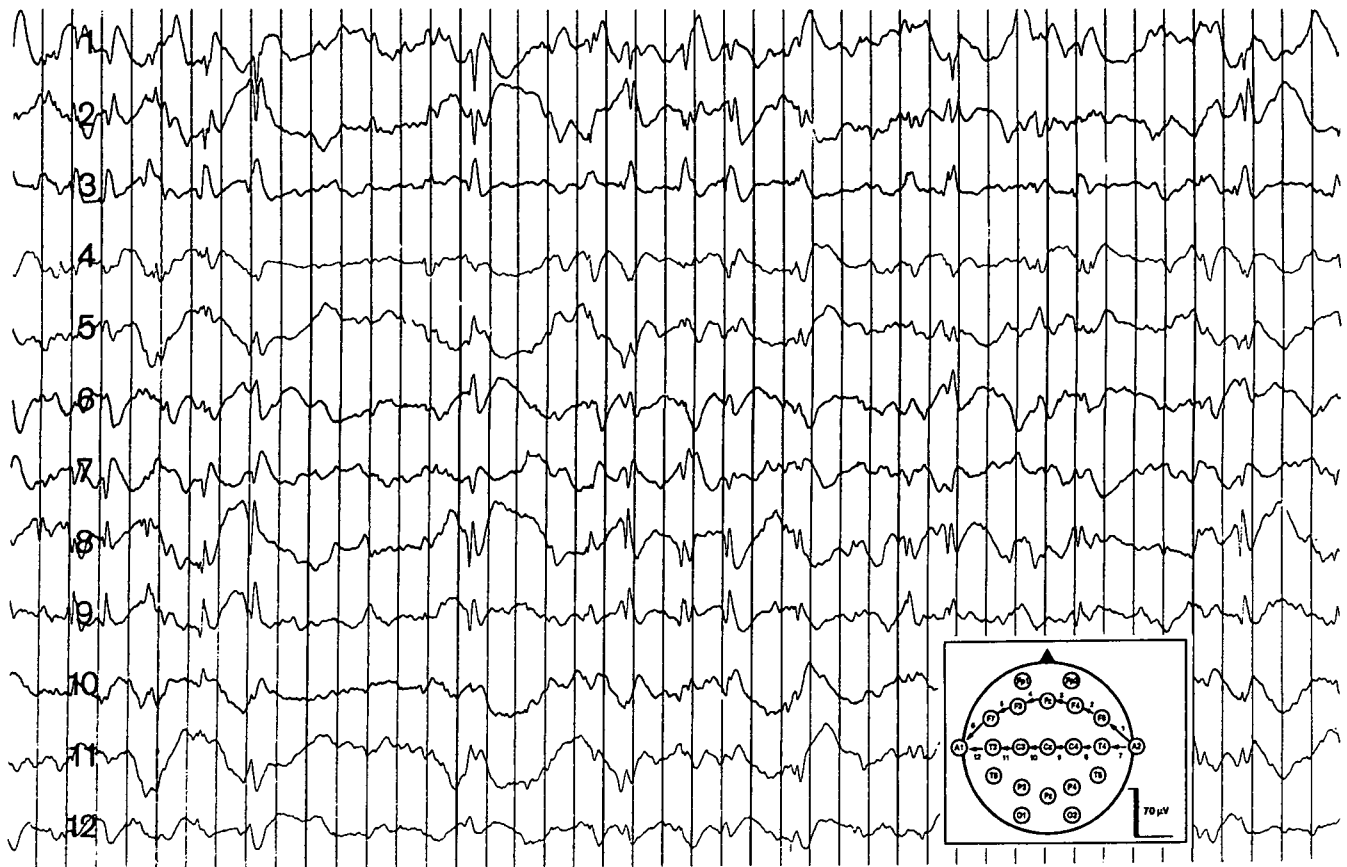
Fig. 1 Pedigrees of families A, B and C. Arrow = index patient.

O = alive not affected
● = alive affected
Ø = dead, not affected
⬮ = dead affected
◉ = index patient
⌀ = probably affected

S = sister
F = brother
M = mother/grandmother
P = father/grandfather

(A)

(B)

body and loss of nociception of the feet. Furthermore, there were keratitis sicca, suboptimal vision (0.8/0.7), but no retinal degeneration, chronic recurrent cystitis and uncomprehended precordial sensations. At the age of 50 years she was wheelchair bound, and there were near-total external ophthalmoplegia, areflexia, slight dysarthria, some distal muscle waste and relatively good strength of proximal muscles. The EEG (Fig. 2B) demonstrated evidence of generalized paroxysmal 3 s^{-1} spike-and-wave discharges on hyperventilation, without clinical seizures ever since puberty. The patient was treated with coenzyme Q10 and experienced remarkable subjective improvement of well-being.

The case history of her sister, patient B.S6, has been reported before as an atypical case of chronic PEO with multisystemic involvement (CPEO-plus) with Friedreich's ataxia (Bastiaensen *et al.*, 1977). She suffered from increasing psycholability and pain in the legs, with ataxia and PEO, from the age of 25 years onward. These signs became evident after her first, and more markedly after the second pregnancy, at the age of 28 years. She gradually developed myoclonic epilepsy at the age of 36 years, and died in myoclonic epileptic status ~1 year later. Findings at post-mortem examination were similar to those reported in case A.S4. Her sister, Patient B.S2, died at the age of ~20 years, probably of myoclonic epileptic status, some time after delivery of a healthy child. Retrospectively she also had shown an abnormal gait and may have suffered from the same disorder. The mother (B.M1) suffered from benign idiopathic epilepsy, without signs of neuropathy or ophthalmoplegia at an old age; the father was in good health.

Family C

Patient C.F1 was seen at the age of 7 years by a neurologist, because of clumsy performance, but no abnormalities were shown. Sensory neuropathy, with slight cerebellar signs, particularly dysarthria, were reported from the age of 18 years onward. Later on he also complained of dysphagia, precordial sensations and episodic diarrhoea. Repeated cardiological examination revealed no signs of cardiomyopathy. He was referred to our department at the age of 24 years, because of PEO with symmetric ptosis, polyneuropathy with areflexia and some wasting of peroneal muscles and intrinsic muscles of hands and feet. Myoclonic epilepsy manifested unexpectedly at the age of 32 years, without previous signs of epilepsy. He was admitted to an intensive care unit, where he died after 1 month of treatment for myoclonic epileptic status. No permission for post-mortem

examination was given. His mother, sister and nephew were examined, but they did not show any related signs. His father was not available for examination, but was reported by his ex-wife to be in good health.

Results of additional investigations
Laboratory investigation (see also Table 1)
Metabolic, endocrinological, immunological or chronic infectious diseases were excluded by appropriate investigations. More particularly the serum creatine kinase, myoglobin, ammonia, phytanic acid, purines and pyrimidines, lysosomal enzymes, transferrine, copper, caeruloplasmin, vitamins B and E, paraprotein and immunoglobulin concentrations were normal. Tests for hypo-betalipoproteinaemia and disorders of fatty acid metabolism, including carnitine, revealed normal results. Serum organic acids and amino acids were normal, apart from the variably elevated lactate, and glycine or alanine in Patients A.S3, A.S6 and B.S9. Examination of CSF consistently showed increase of lactate, at an early age, in all patients, with normal cell counts, no elevation of immunoglobulins and mild elevation of protein in some cases. Lactate concentrations in blood and 24 h urine samples, obtained under normal conditions, were less consistently elevated.

Electrophysiological studies (Table 2)
For stimulation and registration of nerve conduction velocities, maximal compound motor axon potential and sensory nerve action potential, surface electrodes were used according to standard methods. Examination of the peripheral nerves showed marked reduction of sensory nerve action potentials (and to a lesser degree compound motor axon potentials), with relatively mild slowing of conduction velocities and distal latencies. Involvement of proximal nerves and nerve roots was suggested by slowing and amplitude reduction of Hoffmann reflexes. Concentric needle EMG of at least two muscles (proximal and distal) showed mild signs of denervation or neurogenic dysfunction in all cases, but no significant myopathic changes. The EMG of the ocular muscles in case B.S6, however, suggested ocular myopathy.

Sural nerve biopsies (Table 3; Figs 3 and 4)
A whole sural nerve biopsy was performed at the mid-calf level, in all patients except A.S3, and prepared for light and electron microscopic examination, including teased fibre

Fig. 2 EEG tracing (paper speed 30 mm s^{-1}, time constant 1.0 s, and Hf filter 70 Hz) of patients A.S4, taken at the time of progressive myoclonic epilepsy (A) and B.S9 (B), at the ages of 34 and 50 years, respectively. (A) Note the undifferentiated background activity with fast activity of variable frequencies superimposed. Repeated spike and spike-and-wave discharges with paroxysmal desynchronization. (The picture shown has been limited to just 12 channels for reasons of good reproducibility, as is shown by the insert.) (B) Note the slowing of background activity with paroxysmal generalized 3 s^{-1} spike-and-wave discharges. The patient, however, at that moment showed no clinical evidence of myoclonus or epilepsy.

Table 2 *Electrophysiological findings*

	Patient no.					Normal values
	A.S3	A.S4	A.S6	B.S6	C.F1	
Age (years)	40	25	30	50	25	
N. suralis						
SNAP (μV)	0.0	0.0	0.0	0.0	0.0	
N. peroneus						
CV (m s^{-1})	39	41	37	30	–	>52
CMAP (mV)	2.4	NA	NA	0.2	0.0	>2.0
N. medianus						
CV* (m s^{-1})	56	49	45	55	51	>53
DL (ms)	2.6	2.7	2.9	3.5	3.7	<2.6
SNAP (μV)	0.0	2.0	2.0	0.0	0.0	>10
CMAP (mV)	13	NA	20	14.8	5.6	>3.0
H-reflex m. soleus						
H CMAP (mV)	6.5	15	29	6.0	0.3	
H–M interval (ms)	38.2	31.4	NA	32.6	32.3	<27

Values of left and right side were comparable. SNAP = sensory nerve action potential; CV = conduction velocity; CMAP = compound motor axon potential; NA = not available from reports or no response; DL = distal latency. *If no SNAP arousable, then CV calculated from motor response.

Table 3 *Morphological findings in sural nerve biopsies*

	Patient no.					Normal values
	A.S	A.S6	B.S6	B.S	C.F1	
Age (years)	25	34	37	36	24	(21–40)
MF density (1 mm^{-2})	1540	<500	<500	2005	1200	10 210±2200 (n = 9)
MF size histogram						
% MF <4 μm	71	100	100	85	97	40%±11% (n = 9)
% MF >6.5 μm	1	0	0	1	0	33%±4% (n = 9)
TTFA (mm^2)	0.36	NA	NA	0.28	0.19	0.85± 0.31 (n = 4)

MF = myelinated fibre; NA = not available; TTFA = total transverse fascicular area.
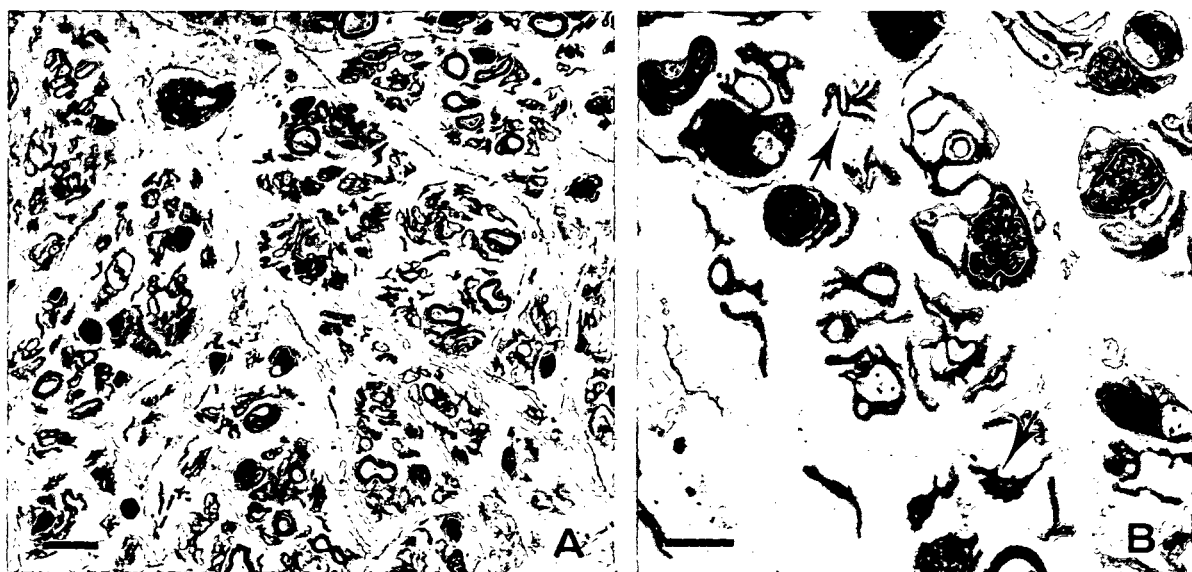

Fig. 3 Sural nerve biopsy specimen. (A) Low power electron micrograph of sural nerve of patient A.S4 showing severe loss of myelinated fibres of which only some small fibres are left, and massive increase of endoneural collagen. Bar = 10 μm. (B) Higher magnification showing many denervated stacks of Schwann cells, mostly of the unmyelinated type (arrows). Bar = 2 μm.
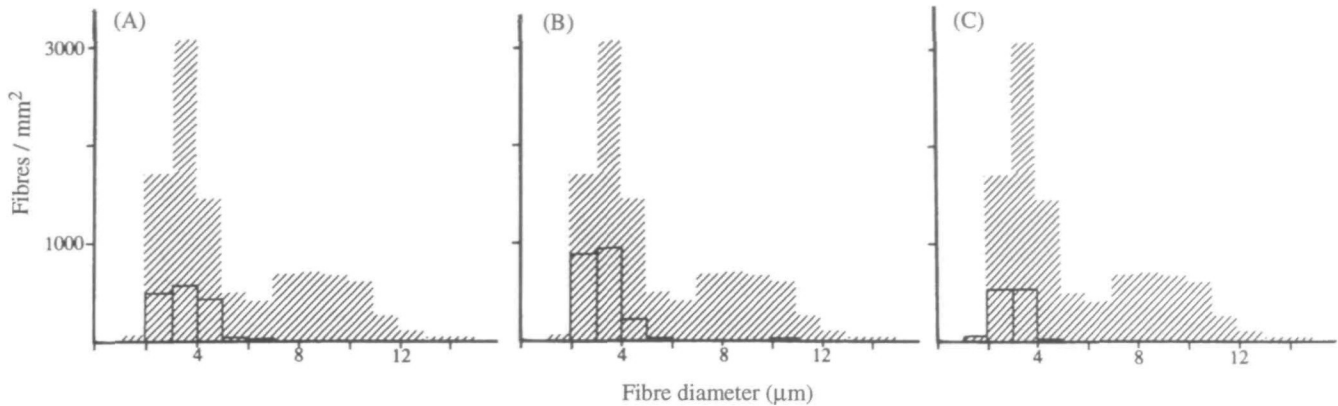
Fig. 4 Diameter histograms of myelinated fibres (outlined areas) of patient A.S4 (**A**), patient B.S9 (**B**) and patient C.F1 (**C**), in comparison with age-matched control (hatched areas).

Table 4 *Morphological and biochemical findings in muscle biopsies related to mitochondrial involvement*

	Patient no.				
	A.S4	A.S6	B.S6	B.S9	C.F1
Age (years)	25	29	36	36	25
Muscle	Soleus	Quadriceps	Ocular	Soleus	Quadriceps
RRF (LM)	++	++	+++	0*	++
Fibre type grouping	++	0		++	+
Abnormal ultrastructure of mitochondria[†]	++	+	++	++	++
COX (nmol min⁻¹ mg protein⁻¹)[‡]	145	210[§]		103	307

0 = not present; + = present; ++ = markedly present; RRF = ragged red fibres; LM = light microscopic investigation. *Significantly increased number of subsarcolemmal mitochondria; [†]morphological changes and paracrystalline inclusions (Fig. 5); [‡]normal values: 73–284 nmol min⁻¹ mg protein⁻¹ in supernatant; [§]deficient COX activity in single muscle fibres (Fig. 5E and F).

studies and morphometry, using standard techniques (Gabreëls-Festen *et al.*, 1991). Sural nerves of all examined patients showed severe reduction of myelinated fibre density (Fig. 3A). Clusters of regenerated fibres were hardly present; active axonal degeneration was not observed. Teased fibre studies showed clustering of paranodal and segmental demyelination and remyelination along some fibres, suggestive of neuronal or axonal atrophy. Fibre diameter distribution was unimodal with a peak at 2–4 μm diameter and near total loss of large diameter fibres (Fig. 4). Electron microscopic studies revealed severe loss of myelinated and, apparently, also unmyelinated axons, leaving behind denervated, thin Schwann cell bands, among areas of collagen fibres (Fig. 3B). In case A.S4 sural nerve biopsy at the age of 26 years was compared with sural nerve obtained at post-mortem examination, at the age of 34 years. The process of axonal degeneration, involving myelinated and unmyelinated axons, had advanced to a stage of near total loss of all myelinated fibres. Teased fibre studies of the femoral nerve, examined post-mortem, showed clustering of (secondary) segmental de- and remyelination.

Skeletal muscle biopsies
Morphological changes (Table 4 and Fig. 5)
Muscle biopsy specimens of soleus and quadriceps, and in patient B.S6 left ocular superior rectus, muscles were stained according to standard methods (Dubowitz and Brooke, 1973). In cases A.S4, A.S6, B.S6 and C.F1, at ages between 25 and 36 years, 0.5–2% of the fibres were ragged-red fibres. In case B.S9 (the patient who was the least severely affected clinically) local elevation of succinate dehydrogenase activity suggested subsarcolemmal accumulation of mitochondria. In the light microscopic preparation of case A.S6, most of the ragged-red fibres were type I fibres, with absent cytochrome oxidase (COX) staining. About 15% of the normal looking type I fibres also showed decreased or absent COX activity (Fig. 5F). Electron microscopic examination additionally established abnormal mitochondria, with increased size, and disarrangement of cristae, and typical paracrystalline inclusions, particularly in the area of accumulated mitochondria, in Patients A.S4, A.S6, B.S6 and C.F1.

Biochemical findings (Table 4)
For biochemical examination, part of the fresh samples of skeletal muscle was prepared according to methods described previously (Fischer *et al.*, 1986; Trijbels *et al.*, 1988). Oxidation rates of various substrates were measured radiochemically in fresh muscle supernatant. Adenosine triphosphate and creatine phosphate production rates and oxidation rates of [1-¹⁴C]pyruvate and [U-¹⁴C]malate were determined in parallel incubations, according to proce-
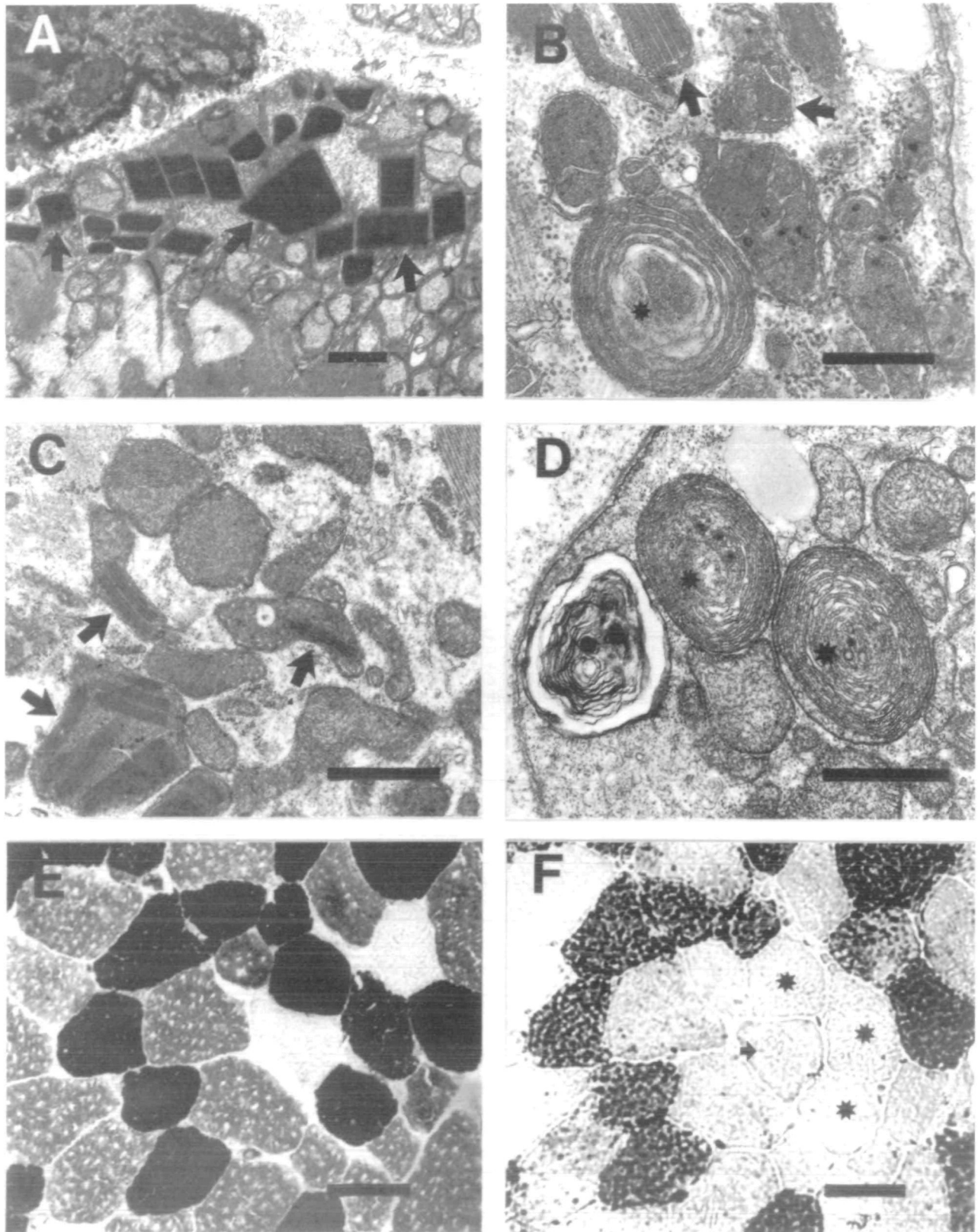
Fig. 5 Muscle biopsy specimens. Electronmicroscopical abnormalities of mitochondria (A–D) and deficient COX-activity in light microscopy (E and F). Paracrystalline inclusions in A, B and C (arrows); absence of cristae in some or many mitochondria in A, C and D; mitochondria with concentric cristae (star-symbol) in B and D; a degenerating mitochondrion in D (left). E and F represent consecutive sections stained for myofibrillar ATPase (preincubation at pH 4.6) and COX, respectively. F shows one type-IIA fibre (arrow) and three type-I fibres (star symbols) that are COX-deficient. Bars = 1 µm in A, C and D, 0.5 µm in B and 50 µm in E and F.

dures described previously (Fischer *et al.*, 1986). In cases A.S4, A.S6, B.S9 and C.F1 COX and citrate synthase activities were determined to document respiratory chain activity. Fatty acid oxidation rates were based on the $[1-{}^{14}C]/[U-{}^{14}C]$palmitate ratio and found to be normal. Both oxydative phosphorylation and respiratory chain activity appeared normal.

Post-mortem neuropathological examination (Fig. 6)

The brains weighed 1050 and 1235 g (normal 1200–1400 g) in cases A.S4 and B.S6, respectively. In both cases the spinal cord showed degeneration, with marked myelin pallor of the posterior columns, particularly the fasciculi graciles, and, to a lesser extent, of spinocerebellar tracts and dorsal roots (Fig. 6A). Only minor other pathological changes were found in case B.S6, who has been extensively described previously (Bastiaensen *et al.*, 1977). Gross examination of case A.S4 revealed a normal pattern of cerebral gyri, and a relatively small cerebellum and brainstem. At microscopic examination, local vacuolar change of the neuropil, accompanied by astrocytosis and loss of neurons was seen in the occipital cortex (Fig. 6B). Occasional blood vessels in the lentiform nucleus showed mineralization of the vessel wall. In the cerebellum, marked loss of neurons was noted in the dentate nucleus (Fig. 6C), whereas the Purkinje cell layer showed mild loss of cells. There were no pathological changes of nuclei pontes, pyramidal tracts and optical systems. The dorsolateral part of the substantia nigra and the inferior olivary nuclei showed moderate neuronal loss. Involvement of inferior olivary nuclei was confirmed by central chromatolysis and vacuolation of the cytoplasm of some remaining neurons, astrocytosis and loss of myelin in surrounding white matter (Fig. 6D).

Genetic aspects

For mitochondrial DNA (mtDNA) analysis blood samples were obtained from the index patients from all families. Screening for mtDNA gross rearrangements, depletion and the point mutations 3460 (Leber), 4160, 11.778, 14.484, 15.257, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) 3243, 3271, 3252, Leigh/neuropathy, ataxia and retinitis pigmentosa (NARP) 8993, and myoclonus epilepsy with ragged-red fibres (MERRF) 8344, was negative in each family. No linkage with Friedreich's ataxia marker FD1 and D9S15 (Duclos *et al.*, 1993) was detected.

None of the parents were consanguinous. The families had not been related for several generations and lived far apart. Autosomal recessive inheritance is the most likely mode of inheritance in our patients (Fig. 1): the disease was restricted to patients from one generation, there was no evidence of maternal inheritance and the segregation ratio was close to

0.25, with an early age of onset and consistent pattern of clinical signs.

Discussion

Clinicopathological differentiation

We present six patients from three unrelated families, with hereditary multisystem degeneration of the nervous system, with insidious onset at adolescence and suspected autosomal recessive inheritance. Symptoms and signs of the initial stage appeared to be the consequence of severe progressive sensory neuropathy, leading to a near total loss of myelinated fibres. Stage 2 is characterized by PEO, caused by ocular myopathy. Spinocerebellar degeneration might have contributed to the progressive ataxia at this stage, resulting in wheelchair dependency after 10–20 years. Motor nerve involvement and myopathy remained sub-clinical. Stage 3 is characterized by the gradual evolvement of myoclonic jerks and the sudden onset of grand mal epilepsy, resulting in myoclonic epileptic status, which was the cause of premature death in four patients in the course of 1–3 years. Two patients (A.S3 and B.S9) apparently had a milder course of disease, with later onset (at about the age of 20) years and no seizures at the age of 40 and 51 years, respectively. Their EEG tracings, however, showed mild slowing of background with intermittent paroxysmal bitemporal sharp waves (Patient A.S3) or generalized slow-wave and spike-and-wave (3–4 s^{-1}) discharges (Patient B.S9), similar to the initial recordings in Patients A.S4 and B.S6, before clinical signs of epilepsy were noted. In none of the patients was there evidence of optic atrophy, short stature or facial dysmorphias, cataract, nystagmus, abnormal breathing, stroke-like episodes, cardiomyopathy or cardiac conduction block, involvement of pyramidal or extrapyramidal systems and classical retinitis pigmentosa with bone spicule formation (Mullie *et al.*, 1985). There were no significant atrophic changes of infratentorial structures, calcifications or leucodystrophy on CT or MRI of the head.

At first, it was considered that the cardinal clinical features and post-mortem findings might largely apply to the heterogeneous spectrum of spinocerebellar degenerations (Rosenberg and Grossman, 1989; Subramony and Currier, 1991; Harding, 1993). However, none of the recently classified heredo-ataxic syndromes shows an identical progression and constellation of signs and cerebellar signs were only of minor importance in our cases. The loss of large myelinated nerve fibres is also a common feature in spinocerebellar ataxias (McLeod and Evans, 1981; Dyck, 1993), but ophthalmoparesis is only rarely seen (Werdelin, 1986; Harding, 1993). Reviewing the extensive literature on CPEO-plus, some incidental cases associated with ataxia and polyneuropathy were found, and these might have been clinically similar to our cases presented here (Stephens *et al.*, 1958; Jampel *et al.*, 1961; Drachman, 1968; Rosenberg *et al.*, 1968; Croft *et al.*, 1977; Bastiaensen, 1978; Ciucci *et al.*, 1988). However,
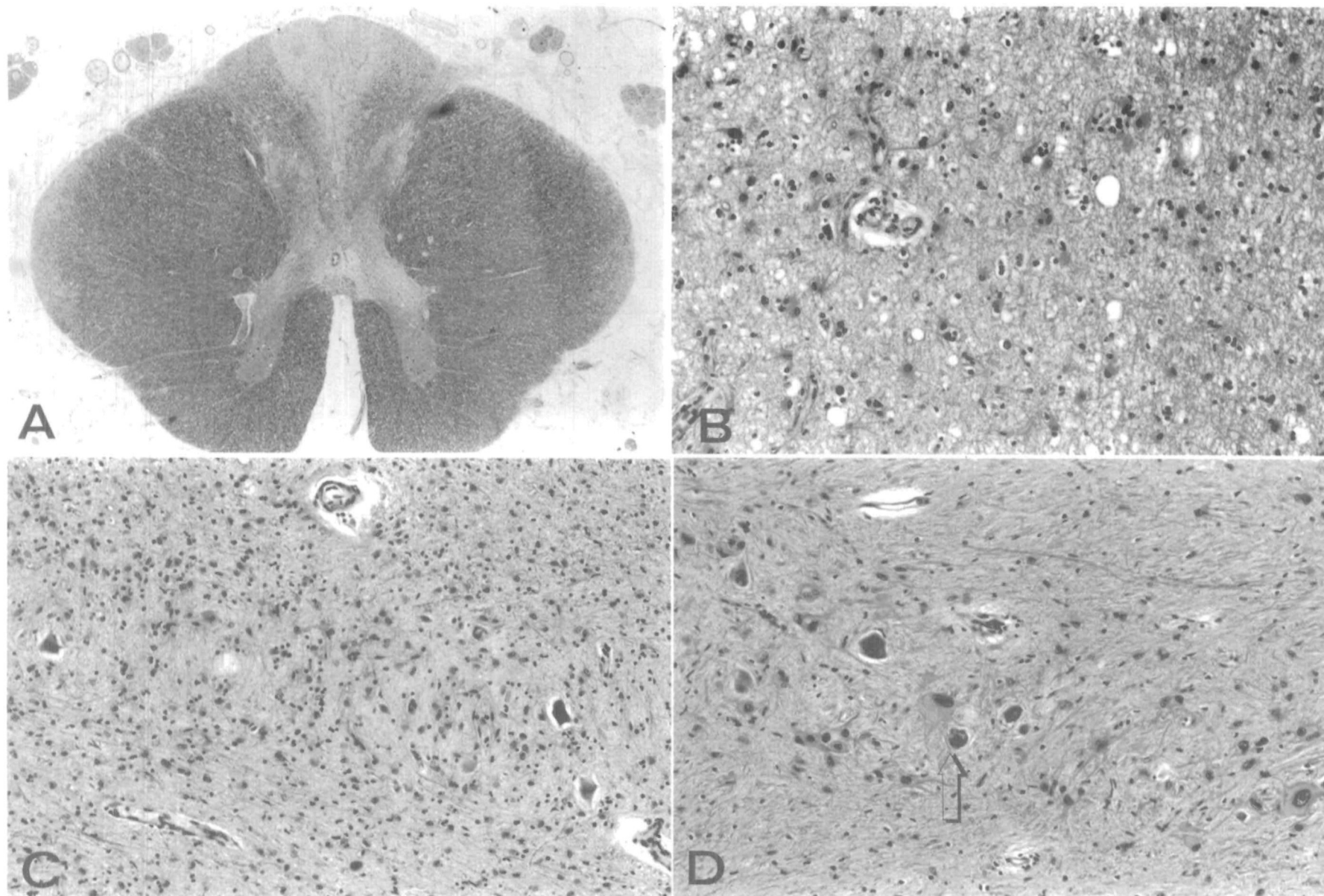
Fig. 6 Post-mortem findings in case A.S4. (**A**) Cervical spinal cord, with degeneration of fasciculus gracilis and, to a lesser extent, of fasciculus cuneatus and spinocerebellar tracts. Original magnification ×8, Klüver–Barrera staining. (**B**) Occipital cerebral cortex and white matter, showing astrocytosis, vacuolar change, neuronal loss in cortex (left side of picture) and loss of myelin in subcortical white matter (right side of picture). Original magnification ×80, Luxol fast blue/haematoxylin and eosin (LFB–HE) staining. (**C**) Dentate nucleus, with severe neuronal loss, accompanied by gliosis and loss of myelin in surrounding white matter. Original magnification ×80, LFB–HE. (**D**) Inferior olivary nucleus, showing neuronal loss (arrow: degenerated neuron showing microvacuolar change of cytoplasm, accompanied by hypertrophic reactive astrocyte), astrocytosis, loss of myelin in surrounding white matter. Original magnification ×80, LFB–HE.

they have been less completely specified, morphologically, biochemically or genetically, and did not result in myoclonic status epilepticus. Generally, CPEO-plus is associated nowadays most particularly with mitochondrial myopathy (Berenberg *et al.*, 1977; Mitsumoto *et al.*, 1983; Rowland, 1992).

The initial progressive ataxia, in our cases, seemed to be caused primarily by the progressive sensory neuropathy, resulting in an irregular broad-based gait. Although the morphological features of the sural nerve biopsies are qualitatively reminiscent of the neuronopathy in Friedreich's ataxia (Said *et al.*, 1986; Caruso *et al.*, 1987), clinical worsening in Friedreich's ataxia and the spinocerebellar ataxias is more specifically related to progressive involvement of spinal and cerebellar pathways, with relatively stable involvement of the peripheral myelinated fibres (Harding, 1981; Santaro *et al.*, 1990). Together with the ataxia, the painful dysaesthesias and the sensory impairment of the legs and lower trunk, the abnormalities of the sural nerves support pathogenetic similarity with the central-peripheral axonopathy, in which both centrally and peripherally directed axons of the primary sensory neurons degenerate, as a possible consequence of toxic/metabolic changes or defects in energy metabolism (Thomas, 1982; Cavanagh, 1984).

The peripheral neuropathy, reported so far in MEM syndromes, was not of an uniform nature. Sural nerve biopsies either suggested chronic axonal degeneration or showed primary demyelination (Yiannikas *et al.*, 1986; Mizusawa *et al.*, 1991). A demyelinating radiculopathy has been reported in some cases of Kearns–Sayre syndrome (KSS) (Groothuis *et al.*, 1980). Electrophysiologically the most common findings were a relatively mild slowing of conduction and decreased sensory nerve action potentials, particularly in the lower limbs (Peyronnard *et al.*, 1980; Eymard *et al.*, 1991). It may be difficult to derive from these data retrospectively whether the primary process leading to the neuropathy, in those patients, might have been caused directly by mitochondrial dysfunction, and whether the neuropathy is axonal in nature or primarily demyelinating. However, there is growing evidence of a more widespread incidence and range of polyneuropathy in MEM syndromes (Yiannikas *et al.*, 1986; Schröder and Sommer, 1991).

Mitochondrial involvement

The multisystem disorder in our patients most particularly stands out from other heredo-ataxic syndromes for the consistent elevation of CSF lactate (with variable elevation of lactate in blood and urine), in an early stage. Exercise was not tolerated well, and all patients complained of fatigue and psychic lability. All but one patient showed ragged-red fibres, and morphological changes of muscle mitochondria. In the remaining patient (B.S9) the number of subsarcolemmal mitochondria was increased and paracrystalline inclusions were observed. Light microscopic examination of the muscle biopsy specimen in patient A.S6 revealed focal COX defi-

ciency (Fig. 5E and F). Assessment of oxidative phosphorylation and adenosine triphosphate production rate, revealed no further relevant biochemical changes in muscle specimens. A lack of correlation between the measurable metabolic defects and the clinical features in MEM syndromes is not uncommon (Lombes *et al.*, 1989; DiMauro and De Vivo, 1994). A characteristic clinical spectrum of mitochondriopathies does not exist, and increasing evidence suggests that nervous system involvement may overshadow the myopathy in MEM syndromes. Moreover, some uncomprehended heredo-ataxic syndromes are considered candidates for mitochondrial disease in the current literature (Blass *et al.*, 1988; Harding, 1991; Sorbi and Blass, 1992). Early degeneration of spinocerebellar tracts and Clarke's nucleus has been shown to be caused by experimental COX deficiency (Wong-Riley, 1989). Sensory axonal neuropathy has been reported previously in association with partial COX deficiency (Pezeshkpour *et al.* 1987). But further studies are required to substantiate a possible causal connection of mitochondrial disease with certain heredo-ataxic syndromes.

Both KSS and MERRF have some characteristic features in common with the syndrome in our patients. Progressive external ophthalmoplegia and myoclonus epilepsy are nonspecific clinical signs, occurring in a variety of disease states. The absence of retinitis pigmentosa, heart block, significant increase of CSF protein and the severe polyneuropathy with autosomal recessive inheritance, are evidence against the diagnosis of KSS (Berenberg *et al.*, 1977; Rowland, 1992). Progressive myoclonus in our patients was not a prominent sign, but preceded the myoclonic epileptic status for some time in three patients (A.S4, A.S6 and B.S6). Previous reports on heredo-ataxic syndromes with peripheral neuropathy and progressive myoclonus epilepsy mostly fell within the spectrum of MERRF 8344 (and partially overlapping neuropathy, ataxia and retinitis pigmentosa/Leigh 8993) mutant mtDNA (Berkovic *et al.*, 1989; Marsden *et al.*, 1990; Silvestri *et al.*, 1993; Sweeney *et al.*, 1994). From a morphological point of view, degeneration of brainstem nuclei (particularly inferior olivary nucleus), cerebellum (particularly dentate nucleus), spinal posterior columns and spinocerebellar tracts have been reported as additional neuropathological findings in both KSS and MERRF (Sparaco *et al.*, 1993). The vacuolation in cortical cells and brainstem neurons, with spongiform changes of the occipital cortex and siderocalcific deposits in basal ganglia, that were shown in our case A.S4, are similar findings to those in cases of KSS and other MEM syndromes (Berenberg *et al.*, 1977; Groothuis *et al.*, 1980; McKelvie *et al.* 1991; Sparaco *et al.*, 1993). But neither the mtDNA deletions, established in various tissues of cases with KSS (Moraes *et al.*, 1989; Degoul *et al.*, 1991), nor the mtDNA mutations of MERRF and neuropathy, ataxia and retinitis pigmentosa (Holt *et al.*, 1990) could be demonstrated in our cases.

Although the classification of MEM syndromes, based on clinical signs, is threatened by nosological chaos, clinical practice is rather insistent on symptoms that might corroborate

an association with mitochondrial disorder (DiMauro and Moraes, 1993). Apart from the mild visual impairment in Patients A.S4 and B.S6, sensorineural hearing loss in Patients B.S9 and C.F1, and atypical pigmentary changes of the retina in Patients B.S6 and B.S9, the severe episodic diarrhoea in Patients A.S3 and C.F1 was remarkable. Gastrointestinal signs (particularly pseudo-obstruction syndrome) in combination with ophthalmoplegia, axonal neuropathy, and potential CNS involvement is a well-defined autosomal recessive hereditary condition (Steiner *et al.*, 1987; Simon *et al.*, 1990). Although the pathogenesis might be obscure in these cases, mitochondrial involvement was evident in closely related syndromes, like the autosomal recessive myo-neuro-gastro-intestinal encephalopathy syndrome (Bardosi *et al.*, 1987; Hirano *et al.*, 1994), and ophthalmoplegia, sensorimotor peripheral neuropathy, intestinal pseudo-obstruction and MEM syndrome (Rowland, 1992; Uncini *et al.*, 1994). Generally speaking, episodic diarrhoea in mitochondrial disease, may be due to either smooth muscle involvement or autonomic nerve dysfunction (Simon *et al.*, 1990; Hirano *et al.*, 1994). Although not demonstrable at clinical examination (Patient A.S3), the hyperhidrosis, orthostatic hypotension (Patient A.S6), a tendency to urine retention with recurrent cystitis (A.S3, A.S4, A.S6, C.S9), and keratitis sicca (A.S6 and C.S9) might suggest that, in our patients, the autonomic nervous system was also involved. Furthermore, the aggravation after pregnancy (Patients A.S4, A.S3 and B.S6) and the ultimate culmination in untreatable myoclonic status epilepticus might be considered a consequence of further derangement at times of increased metabolic demand.

Further genetic considerations

The main aim of this study was to draw attention to this particular uniform clinical syndrome. Future studies might settle the possible pathogenetic background. It might be argued that mitochondrial function, or mtDNA, is non-specifically affected, similar to the suggested (age-related) involvement in neurodegenerative diseases (Beal *et al.*, 1993; Tritschler and Medori, 1993). But it is unlikely that the successive consistently developing peripheral neuropathy, PEO and progressive myoclonus epilepsy, all known within the spectrum of mitochondrial encephalomyopathies, together with the mitochondrial involvement at a young age, are merely secondary to a degenerative process alone. Therefore, autosomal recessive heredity and the confinement of (clinical) manifestations to a few tissues, support the existence of a primary nuclear DNA defect, in some way affecting oxidative phosphorylation at selective parts of the nervous system. The progress in understanding of mitochondrial disorders due to nuclear DNA lesions is slow (Shanske, 1992; Tritschler and Medori, 1993). Nucleus-driven multiple (heteroplasmic) deletions of mtDNA have been reported in autosomal dominant CPEO-plus syndromes, in which the initial percentage of abnormal mtDNA might be too small to be detected (Zeviani *et al.*, 1990; Cormier *et al.*, 1991; Servidei

et al., 1991; Ross *et al.*, 1994). Peripheral neuropathy was not an uncommon finding in such cases, the symptoms progressed with the increasing age and many patients died prematurely in their 40s or 50s, presumably due to a metabolic crisis (Haltia *et al.*, 1992).

In conclusion, we propose a nuclear DNA defect in some way affecting mitochondrial function, as the most likely molecular genetic background, in this disorder which cannot be categorized in current classifications.

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