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Hereditary demyelinating neuropathy of infancy
A genetically complex syndrome

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Summary
Nine cases are described of a demyelinating peripheral neuropathy that had an onset in infancy. The clinical features conformed to those of type III hereditary motor and sensory neuropathy or Dejerine–Sottas disease. All showed a severe neurological deficit and had profoundly reduced nerve conduction velocities. Amongst these cases we identified four novel point mutations in the peripheral myelin protein 22 (PMP22) gene. These were Ser72Trp, Ser76I1e and Leu80Pro. The Ser72Trp mutation was dominantly inherited by a mother and son, both severely affected. Two novel mutations in the gene for P_0 myelin protein were also detected. These were Ile134Thr in exon 3, and a complex rearrangement in exon 4. The remaining three patients had presumed autosomal recessive inheritance. In these, no abnormality for the PMP22 and P_0 genes was detected and a mutation at another locus or loci seems probable. On nerve biopsy the final two cases were shown to be examples of hereditary neuropathy with focally folded myelin sheaths. One showed both bulbar and diaphragmatic involvement. It is concluded that hereditary demyelinating neuropathy of infancy is genetically heterogeneous. Mutational screening for the PMP22 and P_0 genes and nerve biopsy are therefore merited in patients with a childhood demyelinating neuropathy that is more severe than usual and in whom a chromosome 17 duplication is not present.

Keywords: hereditary motor and sensory neuropathy; demyelination; peripheral myelin protein 22; myelin protein zero

Abbreviations: HNFFM = hereditary neuropathy with focally folded myelin sheaths; HNPP = hereditary neuropathy with liability to pressure palsies; HMSN = hereditary motor and sensory neuropathy; MNCV = motor nerve conduction velocity; PCR = polymerase chain reaction; PMP22 = peripheral myelin protein 22; P_0 = P zero myelin protein; SSCP = single stranded confirmation of polymorphism

Introduction
The current classification of the hereditary motor and sensory neuropathies largely had its origins in the study by Dyck and Lambert (1968*a, b*) in which the syndrome of peroneal muscular atrophy was subdivided, on the basis of combined clinical, genetic, electrophysiological and nerve biopsy findings into two broad categories. The first consisted of demyelinating neuropathies and was itself subdivided into hypertrophic neuropathy of the Charcot–Marie–Tooth type, usually with autosomal dominant inheritance, and a more severe type with presumed autosomal recessive inheritance referred to as hypertrophic neuropathy of infancy or Dejerine–Sottas disease. The second category comprised the neuronal type of peroneal muscular atrophy. The nonspecific term hereditary motor and sensory neuropathy (HMSN) was introduced by Thomas *et al*. (1974) to cover this group of conditions. It broadly, but not precisely, encompasses both Charcot–Marie–Tooth disease and Dejerine–Sottas disease as employed in the gene mapping literature. In the study by Dyck and Lambert (1968*a, b*) and that by Harding and Thomas (1980*a, b*), cases with X-linked inheritance were not identified but these were recognized later (*see* Hahn, 1993). The more benign form of demyelinating HMSN was

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categorized as HMSN I and both autosomal dominant and, less commonly, autosomal recessive, families were described (Harding and Thomas, 1980*b*, *c*). The severe childhood form was termed HMSN III by Dyck (1975), although it was later pointed out that there are difficulties in distinguishing between HMSN III and autosomal recessive HMSN I (Ouvrier *et al.,* 1987).

Molecular genetic studies have demonstrated that the majority of patients with autosomal dominant HMSN I have a large (1.5 Mb) duplication on the short arm of chromosome 17 (17p11.2) which results in affected individuals carrying three copies in this region instead of the normal two (Lupski *et al.,* 1991; Raeymakers *et al.,* 1991). Evidence that the disorder, referred to as HMSN Ia (Charcot–Marie–Tooth disease type 1A), results from a dosage effect, rather than a disruption of a gene during duplication, was obtained from the observation that a more severe phenotype was shown by a patient whose parents both carried the duplication and he himself carried four copies (Lupski *et al.,* 1991). Secondly, a small number of patients have been reported who carry a much larger, cytogenetically visible, duplication or translocation, with breakpoints well outside the critical region and who still show signs of HMSN Ia (Chance *et al.,* 1992; Lupski *et al.,* 1992; Upadhyaya *et al.,* 1993). The initial evidence that the gene for a myelin protein (PMP22) could be the responsible gene within the duplication came from observations on a mouse model for demyelinating neuropathy, the Trembler mouse. Point mutations within the PMP22 gene were found in two allelic Trembler mutants, Tr and Tr-J (Suter *et al.,* 1992*a, b*). Confirmation of the pathogenetic role of PMP22 came from a family in which affected individuals carried the same mutation as the Tr-J mouse (Valentijn *et al.,* 1992). However, the overexpression of mRNA for peripheral myelin protein 22 (PMP22) was found to be increased in only ~50% of nerve biopsies from patients with HMSN Ia (Hanemann *et al.,* 1994; Yoshikawa *et al.,* 1994).

HMSN Ia is a relatively benign condition most commonly with an onset in the first or second decades, and shows progression throughout adult life. Other families with the HMSN I phenotype have been shown to be due to mutations in the P_0 (P zero myelin protein) gene (Kulkens *et al.,* 1993; Su *et al.,* 1993) on chromosome 1q22–23 (Hayasaka *et al.,* 1993*a*). This disorder has been categorized as HMSN Ib (Charcot–Marie–Tooth disease type 1B). The severe HMSN III phenotype has recently been shown to consist of more than one entity. Some cases are probably nongenetic and examples of chronic inflammatory demyelinating polyneuropathy (Gabreëls-Festen and Gabreëls, 1993). Others have been shown to be due to *de novo* mutations of the PMP22 (Roa *et al.,* 1993*a*) or P_0 (Hayasaka *et al.,* 1993*b*) genes. They have been referred to as Dejerine–Sottas disease A and B, respectively, in the gene mapping literature. The present study documents a group of nine patients with the HMSN III phenotype in which the onset was in early childhood and in whom the disorder led to severe disability.

Case reports

Case 1 (9937)

Female aged 39 years. There was no family history of neurological disorder and her parents were not consanguinous. Her pregnancy and birth were normal but she was observed to be hypotonic at the age of 1 month. Her early motor milestones were delayed. She did not walk until aged 5 years. Slow deterioration of motor function in all four limbs subsequently occurred, but she is still able to walk for short distances with elbow crutches. Increasing weakness of her hands became apparent over the past 7–8 years, but manual function was improved by bilateral tendon transfer operations to improve thumb opposition. She has been aware of distal sensory loss in the upper and lower limbs and has to guide hand movement by vision. Hearing has been impaired for ~10 years.

Examination

The cranial nerves were normal apart from bilateral mild facial weakness and moderate sensorineural deafness. There was mild weakness of neck flexion, distal wasting in the upper limbs, with weakness of all muscle groups below the elbows and severe weakness of the small hand muscles. She showed a mild postural tremor and upper limb ataxia. In the lower limbs there was generalized wasting and weakness with total muscle paralysis below the knees. All tendon reflexes were absent and no plantar responses were obtainable. All sensory modalities were impaired both in the lower and upper limbs. Her peripheral nerves were not thickened.

Investigations

Haematological and biochemical screening were normal, including serum lipids, phytanic acid and white cell enzymes, as was the CSF. Ulnar motor nerve conduction velocity (MNCV) was 3 m/s (1963). Neuro-otological testing in 1971 revealed bilaterally absent caloric responses and normal audiometry. When repeated in 1985, bilateral high-tone deafness was present. Brainstem auditory evoked potentials were poorly reproducible and wave V was delayed bilaterally. Speech discrimination scores were severely reduced. Evoked otoacoustic responses were not obtained from either ear. Sural nerve biopsy was performed in 1963 (*see* Results).

Case 2 (12868)

Female aged 21 years. The pregnancy and birth were normal. She did not walk until the age of 2 years. She was always unsteady on her feet and was never able to run. Scoliosis developed at the age of 12 years and spinal fusion was performed at 14 years. By the age of 16 years she required support to walk and she had developed distal upper limb

weakness. When seen at age 21 years her condition had stabilized.

Examination

Apart from mild weakness of eye closure, the cranial nerves were normal. There was diffuse wasting and weakness in all four limbs. Her tendon reflexes were all absent and her plantar responses flexor. She showed impairment for all sensory modalities distally in the limbs. There was a prominent kyphoscoliosis and mild bilateral pes cavus. There was no definite nerve thickening.

Investigations

Haematological and biochemical screening, including serum lipids and phytanic acid, and white cell enzymes, was normal, as were cranial and spinal MRIs and an ECG. Electromyography demonstrated denervation. Motor nerves were all inexcitable, even at high stimulus strengths, and sensory nerve action potentials were unrecordable. Sural nerve biopsy was performed (*see* Results).

Family history

12868's parents, who were nonconsanguinous, were asymptomatic and normal on neurological examination. She had a twin brother and an older sister who were healthy and a son (Case 3) who is affected.

Case 3 (13533)

Male aged 3 years, son of Case 2. He did not sit unsupported until after age 1 year and was still not walking at age 3 years. Weakness, maximal distally was evident in all four limbs, and greater in the legs. He was areflexic and showed no skeletal deformity. The peripheral nerves were not thickened.

Case 4 (12687)

Female aged 28 years. The pregnancy and birth were normal. She was hypotonic at birth, sat unsupported at 18–20 months and did not walk until 3–4 years. Her gait was always unsteady and she was never able to run. Progressive deterioration occurred and she became unable to walk when aged 11 years. Weakness of her upper limbs had also been present throughout her life. Foot deformity and kyphoscoliosis developed during childhood. The patient was adopted and no family history was available.

Examination

There was mild weakness of eye closure but otherwise cranial nerve function was normal. She showed diffuse muscle wasting and weakness in the upper limbs which was severe distally and greater on the left where her fingers were clawed. Her cough was weak, as was her diaphragm, with paradoxical abdominal wall movement on breathing. There was diffuse wasting in the legs with no voluntary movement. She was areflexic; plantar responses were unobtainable. All sensory modalities were impaired distally in the limbs. There was severe kyphoscoliosis and bilateral equinovarus foot deformities. Her peripheral nerves were possibly thickened.

Investigations

Haematological and biochemical screening was negative. Her peripheral nerves could not be stimulated at high stimulus strengths. Her vital capacity fell from 2.5 l when sitting to 1.5 l on lying. A sleep study showed normal blood gas values. Spinal MRI demonstrated diffuse thickening of nerve roots, particularly in the cervical region.

Case 5 (12274)

Female aged 6 years. The pregnancy and birth were normal. She did not walk until aged 2 years. She currently has difficulty in walking for long distances and falls readily. She can run in an ungainly manner. Upper limb function has been satisfactory. The patient's parents were healthy and nonconsanguinous with no abnormality on neurological examination and normal nerve conduction. She had two healthy older siblings.

Examination

The cranial nerves were normal. Her limbs were hypotonic. Upper limb motor function was normal but there was diffuse lower limb weakness. The tendon reflexes were all absent and her plantar responses were flexor. There was no detectable sensory deficit. She showed an excessive lumbar lordosis but there was no other skeletal abnormality and her peripheral nerves were not enlarged.

Investigations

Ulnar MNCV was 8 m/s. No response was obtained from the peroneal nerve.

Case 6 (14257)

Female aged 25 years. Her developmental motor milestones were delayed. When first assessed neurologically at the age of 21 years, she had weakness in all four limbs, generalized tendon areflexia and flexor plantar responses. Corrective operations for foot drop were performed during childhood. Progressive deterioration subsequently occurred so that currently she is confined to a wheelchair and has considerable difficulty with her upper limbs. The patient's parents were healthy and nonconsanguinous.

Examination
The cranial nerves were normal. There was generalized wasting and weakness in the limbs, maximal distally and greater in the lower than in the upper limbs. Her tendon reflexes were absent and the plantar responses were flexor. There was sensory loss below both knees. She had no foot deformity.

Investigations
Electromyography showed severe denervation of distal upper and lower limb muscles. Ulnar MNCV was 6 m/s. The abductor pollicis brevis and extensor digitorum brevis muscles were inexcitable. The latency of the response in tibialis anterior on stimulation of the peroneal nerve at the neck of the fibula was 24 ms (normally <5 ms). No upper or lower limb sensory action potentials were detectable. Muscle biopsy (*see* Results) was performed.

Case 7 (12692)
Female aged 42 years, product of a consanguinous (father/daughter) relationship. Her birth and early development were normal. When aged 2.5 years her left foot became 'floppy' and inverted on walking. A corrective operation was performed without benefit. Progressive deterioration occurred with the development of distal weakness and wasting in all limbs associated with tendon areflexia. Callipers for walking were provided at the age of 12 years. Progressive deterioration continued with increasing weakness and distal sensory loss in the limbs. Scoliosis developed during early adolescence and mild bilateral deafness at the age of 35 years. She has been confined to a wheelchair for the past 12 years. Her parents were stated to have been normal as is a daughter of the patient. Her sister, by the same parentage, is mentally retarded but shows no evidence of neuropathy on examination and has normal motor and sensory nerve conduction.

Examination
On neurological examination, the cranial nerves were normal apart from bilateral sensorineural deafness. There was diffuse severe wasting and weakness in all four limbs, total tendon areflexia and unobtainable plantar responses. She showed a distally accentuated loss for all sensory modalities both in the arms and legs. Her peripheral nerves were not enlarged. Examination of other systems revealed bilateral xanthelasmata and a severe kyphoscoliosis.

Investigations
Haematological and biochemical screening was normal, including serum lipids, phytanic acid and vitamin E levels. White cell enzyme screening showed pseudodeficiency for aryl sulphatase A (homozygous for PD allele). Hexosaminidase and β-galactosidase activity was normal. Electromyography demonstrated widespread denervation in lower and upper limb muscles. Ulnar MNCV was 3 m/s; no values were obtained for the lower limbs. Upper and lower limb sensory action potentials were absent. An EEG, cranial CT scan and an ECG were normal.

Case 8 (10994)
Male aged 5 years. The pregnancy and birth were normal. He began to walk when aged 1 year, but unsteadily. He progressively developed a bilateral dropfoot gait. His hands had been noted to be clumsy and his fingers to be held in a flexed posture. His speech was slightly indistinct. The patient's parents were healthy and normal on neurological examination, but were consanguinous (first cousins). He had an older brother aged 10 years, who was normal, but had been diagnosed as having vitamin D deficiency in early childhood.

Examination
The cranial nerves were normal apart from a mild slurring dysarthria. There was bilateral wasting of the small hand muscles with clawing of the fourth and fifth digits, and distal upper limb weakness which was severe for the intrinsic hand muscles. In the lower limbs there was bilateral anterior tibial wasting, distal weakness and a dropfoot gait. His tendon reflexes were all absent and his plantar responses were obligatorily flexor. No sensory loss was detected but examination was difficult. There was no skeletal deformity and the peripheral nerves were not thickened.

Investigation
Haematological and biochemical screening was negative. His CSF was normal except for a protein concentration of 1.34 g/l. Median MNCV was 17 m/s with a distal motor latency of 5.8 ms and an F wave latency of 69 ms. Ulnar MNCV was 15 m/s with a distal motor latency of 4.2 ms. No response was obtained in extensor digitorum brevis on stimulating the common peroneal nerve, and no sural sensory action potentials were detected. Sural nerve biopsy was performed (*see* Results).

Case 9 (14324)
Male aged 16 years. He was born by a high forceps delivery following prolonged labour. His mother noticed that he had an abnormal cry as a baby. His early developmental milestones were not delayed. He sat unsupported at 6 months. He walked at 13 months, but it was noticed that if he fell he was 'reluctant' to get up. Problems with walking soon became evident and he was referred to the Hammersmith Hospital at the age of 3 years when his limbs were noted to be thin and hypotonic and no tendon reflexes were elicitable. Shortly

Fig. 1 Case 9 showing bilateral weakness of facial and jaw muscles.

after this bilateral vocal cord paralysis with stridor became evident. Limb weakness became progressively more evident so that he is now confined to a wheelchair and has considerable difficulty with the use of his upper limbs. His stridor improved but he continued with difficulty with phonation and articulation. He has problems with chewing but none with swallowing. When aged 16 he began to develop frequent chest infections and symptoms of nocturnal hypoventilation consisting of morning headaches and diurnal drowsiness. Overnight oximetry demonstrated significant oxygen desaturation, early morning capillary blood gases showing high bicarbonate and PCO_2 levels. He was started on a nighttime bipap facial mask ventilator with resolution of these symptoms.

The patient's parents are both normal on neurological examination but are first cousins once removed. He has a healthy younger brother. Nerve conduction studies were normal in all three individuals.

Examination

In the cranial nerves, there was bilateral facial and jaw weakness (Fig. 1) and wasting of the tongue. His voice was hypophonic and dysarthric. There was mild stridor. There was generalized wasting and weakness in the limbs with total paralysis below the knees. His tendon reflexes were all absent and no plantar responses were obtainable. Pain appreciation was impaired distally in all four limbs. Vibration sense was lost in his feet and joint position sense impaired in his toes. There were bilateral flexion contractures of his fingers, but no other skeletal deformity. His peripheral nerves were not thickened.

Investigations

Haematological and biochemical screening was negative, including serum lipids, phytanic acid and vitamin E concentrations. Ulnar MNCV, when aged 5 years was 16 m/s. In a recent study, the small hand and foot muscles were found to be totally denervated. No evoked response was obtained from flexor carpi ulnaris even at very high stimulus strengths despite preservation of some voluntary motor unit activity. Upper and lower limb sensory action potentials were absent. Sural nerve biopsy was performed (*see* Results).

Methods

Molecular genetic studies

Testing for the 1.5 Mb duplication on chromosome 17p11.2 was performed as described previously (Hallam *et al.,* 1992; Hensels *et al.,* 1993; Chance *et al.,* 1994).

Sequencing and single stranded confirmation of polymorphism (SSCP)

PMP22 gene sequences were amplified from genomic DNA using the following primers. Exon 1: forward 5′AGAAACTCCGCTGAGCAGAA3′ (from the 5′ untranslated sequence), reverse 5′GGAACCCAGATGGGGAAG3′ (from intron 1). Exon 2: forward TTTCCTTCACTCCTCCCTCC3′ reverse 5′GTCTGAGGACAAGCTCACGG3′. Exon 3: forward 5′TGGCCAGCTCTCCTAAC3′, reverse 5′CACCCCGCTTCCACATG3′. Exon 4: forward 5′GCCATGGACTCTCCGTC3′, reverse (3′ nontranslated) 5′CCTATGTACGCTCAGAG3′. Each of the forward primers was biotinylated for sequencing. A nested sequencing primer 5′ATGCTCCTCCTGTTGCTGAGTATC3′ was used to sequence the 3′ end of the exon 1 product. The size of the polymerase chain reaction (PCR) products was 166 bp (base pairs) (exon 1), 194 bp (exon 2), 220 bp (exon 3) and 250 bp (exon 4).

P_0 primer sequences and nested sequencing primers were as described in Nelis *et al*. (1994*a*).

Two methods of SSCP analysis were used. Radioactive products were analysed on 6% polyacrylamide gels containing 5% glycerol and run both at room temperature and at 4°C. Nonradioactive products were analysed on 1×Hydrolink MDE gels (J. T. Baker) at 4°C with and without 8% glycerol. The

gels were stained with 0.012 M silver nitrate for 20 min after initial fixing and oxidizing steps with 10% ethanol for 5 min and 1% nitric acid for 3 min. After developing the bands to optimum intensity with 0.28 M N_2CO_3 in 0.019% formaldehyde, the reaction was stopped with 10% acetic acid for 2 min. After a final rinse step with distilled water, the gel was reduced in size with 50% ethanol and dried.

Biotinylated PCR products were purified using Dynabeads (Dynal, UK) and single stranded products sequenced using the USB Sequenase version 2.0 kit. PCR amplified fragments from exon 4 of the P_0 gene in Case 6 were cloned using an Invitrogen TA cloning kit. Fifteen individual clones were sequenced.

Nerve biopsy

Sural nerve fascicular biopsies were obtained from a standard retromalleolar site. They were processed by routine techniques using aldehyde fixation, postosmication and dehydration through an ascending alcohol series, followed by resin embedding. Semithin sections were stained with thionin and acridine orange (Sievers, 1971) or with methylene blue, azure II and basic fuchsin (Humphrey and Pittman, 1974) for examination by light microscopy. Ultrathin sections for electron microscopy were contrasted with uranyl acetate and lead citrate.

Results

Molecular genetics

All nine patients formed part of a study to establish the clinical phenotype associated with the duplication of chromosome 17p11.2 found in patients with HMSN Ia (Marquez *et al.,* 1996). They were selected for sequencing of the PMP22 and P_0 genes after it was shown that they did not possess a duplication or deletion.

PMP22 mutations

Sequence analysis of all four exons and splice site sequences showed changes resulting in amino acid missense mutations in four patients (Cases 1–4). Cases 2 and 3 are mother and son (Fig. 2). The sequence changes were Ser76Ile, Ser72Trp and Leu80Pro, all in exon 3 (*see* Table 1). The changes were confirmed by restriction enzyme digestion, as Taq I (Ser72Trp) and Bsm I (Ser76Ile) sites are removed. All three changes were clearly demonstrable using SSCP and no such SSCP change was found in 52 other individuals, indicating that they are not polymorphisms.

P_0 mutations

A substitution of isoleucine by threonine at position 135, as a result of a T→C change, was observed in Case 5 (Fig. 2D). As a more complex change involving >1 bp was observed in Case 6, the PCR products were cloned and several individual clones sequenced. A G→C change at position 1068 within codon 167 followed by a deletion of a C position at position 1071 were found in one strand (Fig. 3). This would result in a frame shift and a highly altered amino acid sequence, but as no new termination codons would be produced, an extended protein (full length plus two amino acids) would be found (Fig. 4). Each sequence change was confirmed by restriction-enzyme digestion of a PCR product amplified from genomic DNA. The deletion of C (1071) destroyed a *BSa*J1 site (CCTCGG to TCTCGG) (Fig. 3B) and the G→C change creates an *Msp*1 site (Fig. 3C).

Cases 7–9

In these patients the coding sequence for the PMP22 gene was normal and no SSCP or sequence change was found in any of the six exons of P_0.

Nerve biopsies

Case 1

Sural nerve biopsy was performed at the age of 8 years. Only paraffin-embedded material was available. There was a loss of myelinated fibres, surviving fibres possessing uniformly thin myelin sheaths, and excess endoneurial collagenization. No onion bulbs were seen.

Case 2

Sural nerve biopsy was performed at the age of 15 years. The specimen consisted of two small fascicles with an area of only 0.22 mm^2 (age-matched controls 0.5–0.6 mm^2 (Jacobs and Love, 1985). The nerve was largely occupied by onion bulb formations, some having demyelinated or thinly myelinated axons at their centres (Fig. 5). There was increased endoneurial collagen and some oedema. Electron microscopy showed concentrically arranged Schwann cell processes occasionally enclosing an axon (Fig. 6). There was often loss of Schwann cell cytoplasm from the innermost processes leaving paired strands of basement membrane (Figs 5 and 6).

Case 6

A vastus medialis muscle biopsy was performed at the age of 3 years. This showed little abnormality apart from mild type II fibre atrophy. Intramuscular nerve bundles were severely abnormal, containing both large and small axons without detectable myelin sheaths. Minor hypertrophic (onion-bulb) change was evident.

Case 7

Sural nerve biopsies were performed at the age of 22 and 37 years and a radial nerve biopsy at the age of 39 years. All three showed a reduced myelinated nerve fibre density with

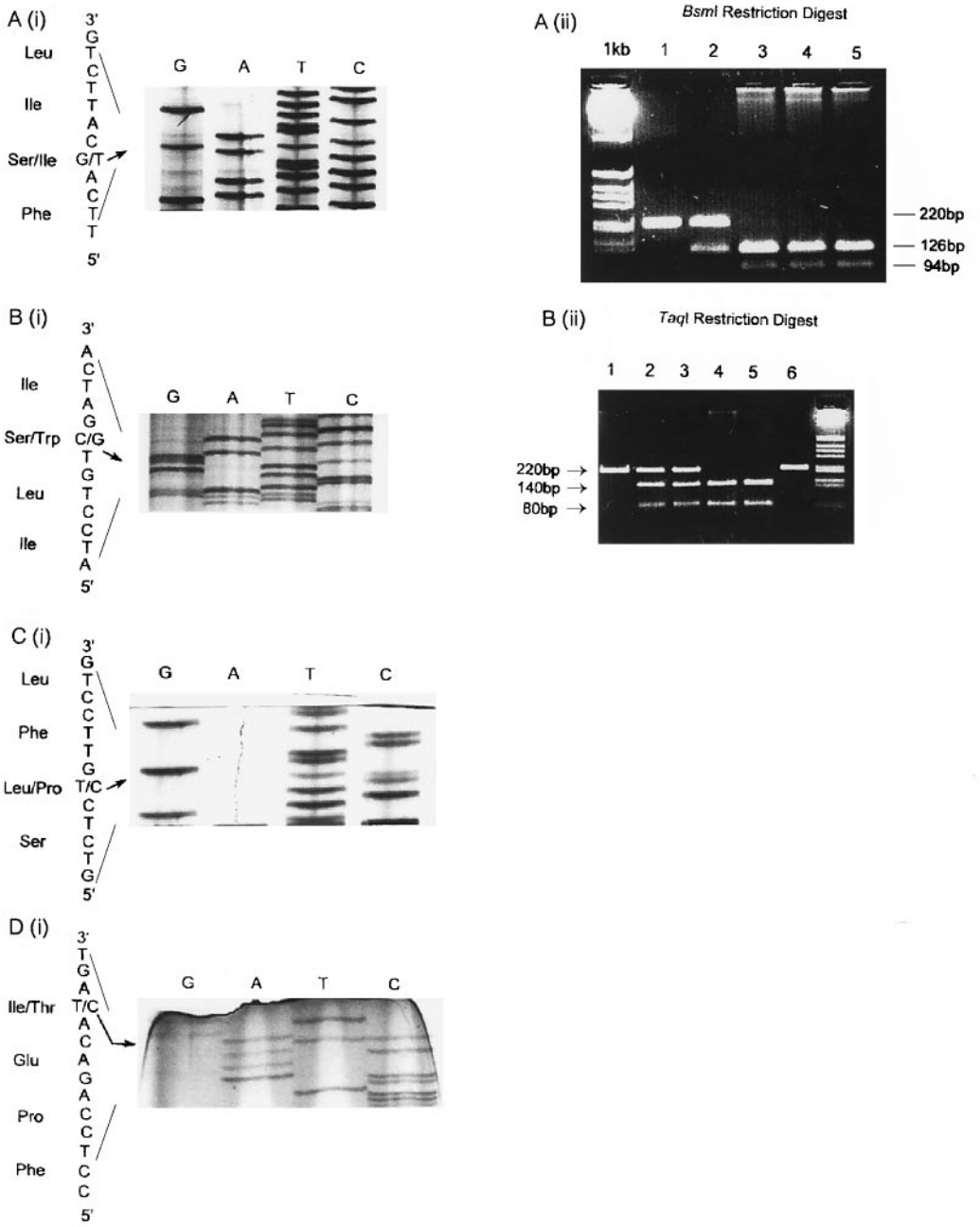

Fig. 2 Sequence changes in exon 3 of the PMP22 gene (**A**–**C**) and in exon 3 of P_0 (**D**). (**A**) Case 1: Ser76Ile mutation (**Ai**) and loss of a *Bsm*I restriction site (**Aii**). (**B**) Case 2: Ser72Trp mutation in 12868. (**Bi**) Removal of *Taq*I restriction enzyme site. (**Bii**) Undigested DNA lanes 1 and 6, *Taq*I digested DNA from 12868 (lane 2), 13533 (lane 3), normal controls (lanes 4 and 5), Molecular weight marker (end lane). (**C**) Case 4: Leu80Pro mutation. No restriction enzyme site change. (**D**) Case 5: Mutation in exon 3 of P_0 Ile135Thr (**D**i). No restriction enzyme site change.

Table 1 *Novel mutations in the PMP22 gene*

Case	Amino acid	Mutation	Restriction site
1	76	Ser (A**G**C)→Ile (A**T**C)	*Bsm* I
2/3	72	Ser (TG**C**)→Trp (TG**G**)	*Taq* I
4	80	Leu (C**T**G)→Pro (C**C**G)	None

uniformly thin myelin sheaths around surviving axons (Fig. 7). In transverse sections viewed by light microscopy, myelinated and demyelinated fibres were surrounded by a largely acellular zone of dense collagen, delimited by fibroblasts with encircling processes (Fig. 7). These changes became more evident in the successive biopsies. Staining for metachromatic material was consistently negative. Electron microscopy confirmed these appearances and demonstrated the presence of onion bulbs

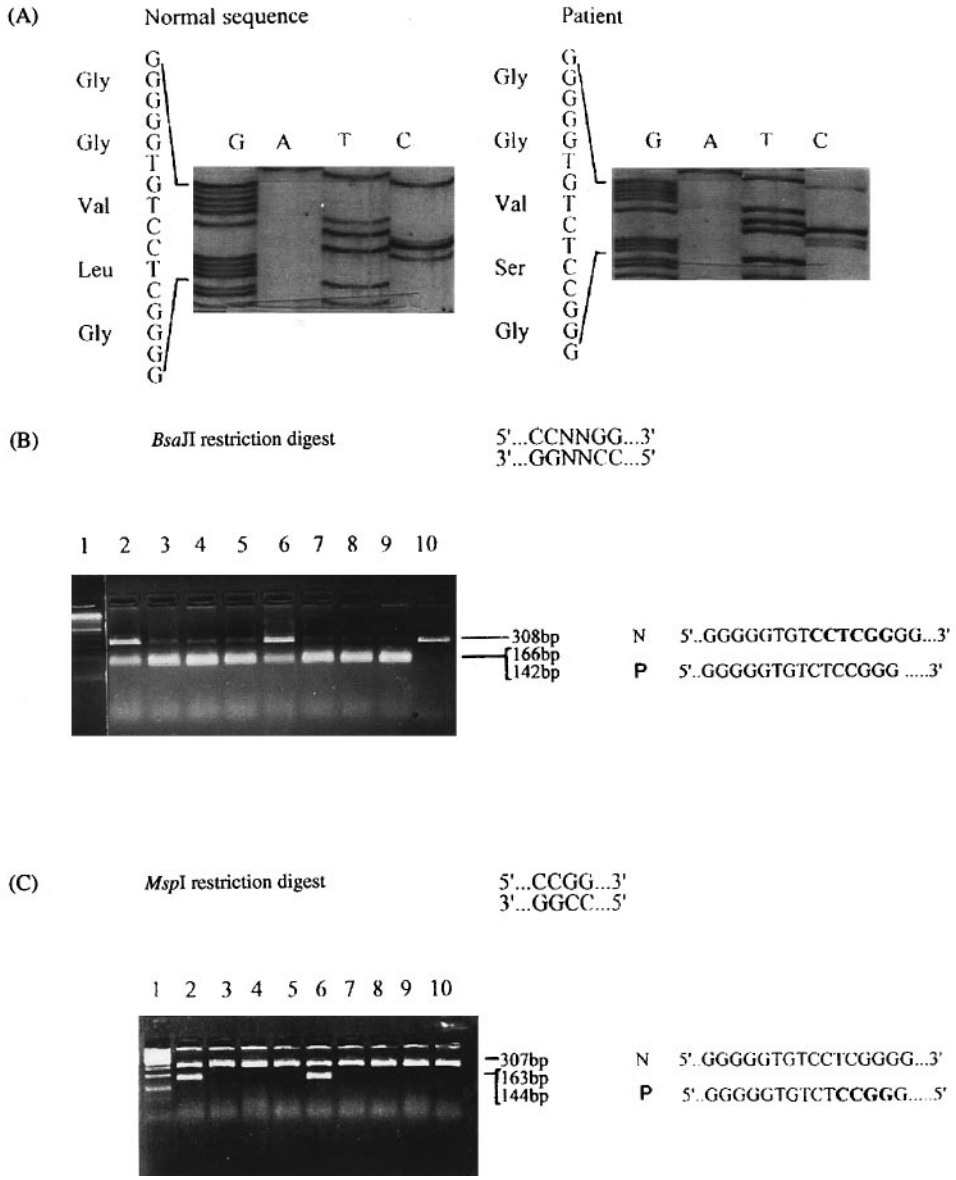

Fig. 3 DNA sequence changes in individual clones (**A**) and restriction site alterations in PCR products from genomic DNA (**B** and **C**) in 14257. (**B**) *Bsa*JI digestion of PCR amplified exon 4. Track 1 is a 100 bp ladder, tracks 2 and 6 are from Case 6, tracks 3–5 and 7–9 from normal controls, and track 10 is undigested exon 4 product. The 308 bp product is cut into 166 and 142 bp fragments. The *Bsa*JI site is shown in bold in the normal sequence. (**C**) *Msp*I digest of PCR amplified exon 4. Track 1 is a 100 bp ladder; tracks 2 and 6 are from Case 6, and tracks 3–5 and 7–9 from normal controls. Track 10 shows undigested exon 4 products. The creation of an *Msp*I site is shown in bold in the sequence for Case 6. P = patient; N = normal.

with sparse Schwann cells (Fig. 8A) and, more frequently, basal laminal onion bulbs (Fig. 8B and C). For many fibres there was an inner zone of persisting or disintegrating basal laminae intermingled with fine collagen fibrils, surrounded by a dense zone of larger, longitudinally oriented collagen fibrils. Some Schwann cells contained accumulations of nonspecific dense bodies (Fig. 8D).

Cases 8 and 9

The biopsy appearances in these two cases were closely similar and they will therefore be described together. The biopsy from Case 8 was obtained at the age of 5 years and that from Case 9 at the age of 16 years. Both specimens showed a severe depletion of myelinated nerve fibres. Fibre density was 3096/mm^2 in Case 8 and 910/mm^2 in Case 9 (normal range for these ages 8000–11 000/mm^2; Jacobs and Love, 1985). In transverse sections viewed by light microscopy, the myelin was frequently thrown into highly irregular conformations (Fig. 9A) and measurements of the g ratio (axon diameter/total fibre diameter) showed abnormally high values, indicating hypomyelination. Electron microscopy demonstrated that the myelin irregularity was related to abnormal outpouching of myelin (Fig. 10A and B). In teased fibre preparations and in

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Wild Type MAPGAPSSSPSPILAVLLFSSLVLSPAQAIVVYTDREVHGAVGSRVTLHCSFWSSE
   Mutant ..................................................................

          WVSDDISFTWRYQPEGGRDAISIFHYAKGQPYIDEVGTFKERIQWVGDPRWKDGSI
          ..................................................................

          VIHNLDYSDNGTFTCDVKNPPDIVGKTSQVTLYVFEKVPTRYGVVLGAVIGGVLGVV
          ..................................................... VIGGVSGWC

          LLLLLLFYVVRYCWLRRQAALQRRLSAMEKGKLHKPGKDASKRGRQTPVLYAMLD
          CCCCCFSTWFGTAGYAGRRPCRGGSVLWRRGNCTSQERTRRSAGGRRQCCMQC

          HSRSTKAVSEKKAKGLGESRKDKK
          WTTAEAPKLSVRRRPRGWGSLARIRNSG*
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Fig. 4 The effect on the amino acid sequence of the complex mutation of P_0 in patient 14257. The top line shows the normal sequence. Below is the predicted mutated sequence, starting at amino acid 167 and terminating four amino acids beyond the normal termination codon.

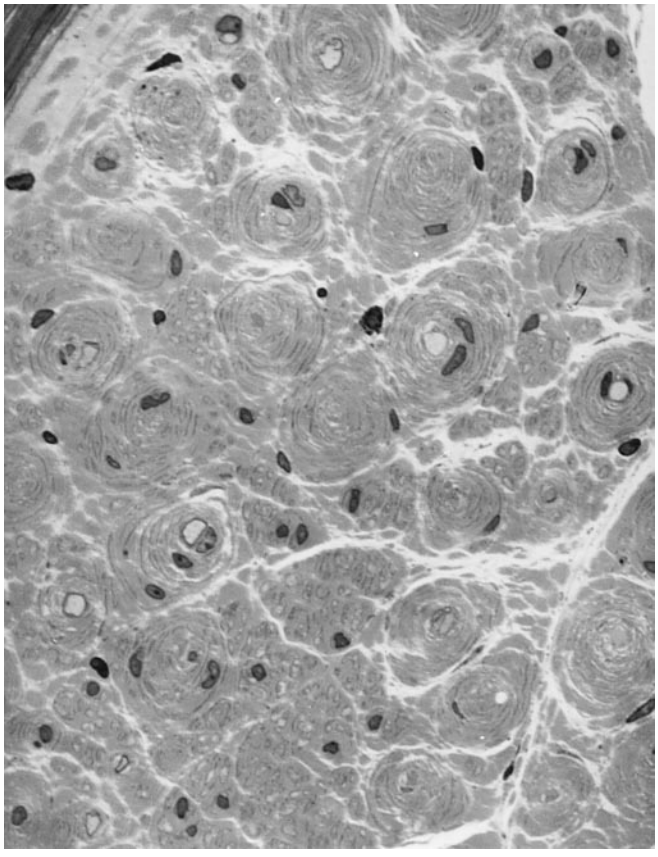

Fig. 5 Case 2. A semi-thin resin section of sural nerve showing onion bulb formations, some having demyelinated or thinly myelinated axons at their centre. Methylene blue, azure II, basic fuchsin (×570).

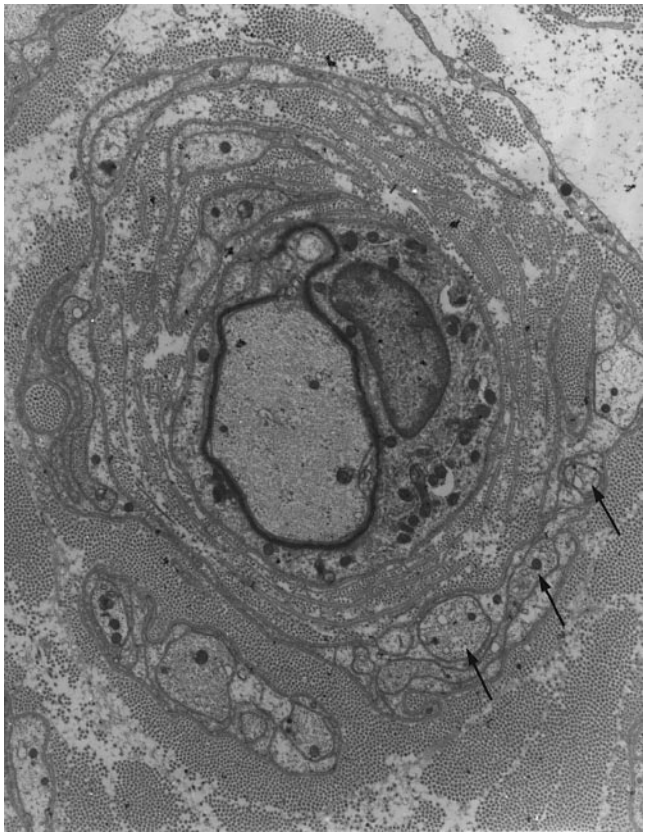

Fig. 6 Case 2. An electron micrograph of a transverse section of sural nerve showing a thinly myelinated axon surrounded by Schwann-cell processes enclosing an occasional axon (arrows). The innermost processes consist only of paired basement membranes (×300).

longitudinal semithin sections examined by light microscopy (Fig. 9B) the outpouchings were seen to occur in multiple restricted areas which were both paranodal and internodal.

Discussion

Dyck (1975) and Dyck *et al*. (1993) separated hypertrophic neuropathy of infancy as a distinct condition, designated as type III HMSN or the Dejerine–Sottas syndrome, probably inherited as an autosomal recessive trait. It was characterized by relatively stereotyped clinical, electrophysiological and neuropathological findings and a generally uniform clinical course. Typically, developmental motor milestones were delayed, nerve conduction velocity was severely reduced and the peripheral nerves were enlarged. Pathologically, there was a

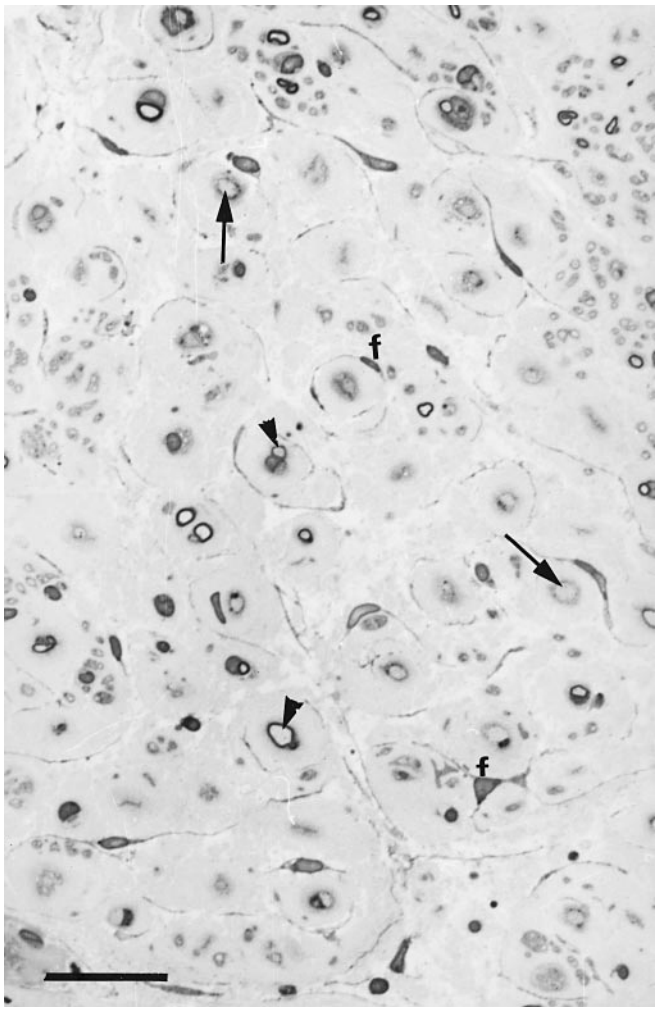

Fig. 7 Case 7. A transverse semithin section of sural nerve showing reduced myelinated fibre density. Surviving axons (arrow heads) either have thin myelin sheaths or are demyelinated (arrows). The fibres are surrounded by an amorphous collagenous zone delimited peripherally by circumferentially oriented fibroblasts (f). Thionin and acridine orange. Bar = 50 μm.

demyelinating/hypomyelinating neuropathy with hypertrophic 'onion-bulb' change. The disorder usually led to severe disability. There were early suggestions (Harding and Thomas, 1980*c*) that HMSN III was genetically heterogeneous. These suggestions have been vindicated recently by the demonstration that this syndrome can result from *de novo* mutations of the PMP22 (Roa *et al.,* 1993*a*) and P_0 (Hayasaka *et al.,* 1993*b*) genes. Such cases have been categorized in the gene mapping literature as Dejerine–Sottas disease A and B, respectively. The eponym is not entirely appropriate as in the original two cases reported by Dejerine and Sottas (1893), although onset of the disorder was in infancy in the son of unaffected parents, it was delayed until adolescence in his sister.

Gabreëls-Festen *et al.* (1994) laid down criteria for the diagnosis of HMSN III which comprised a chronic sensorimotor neuropathy without CNS involvement, and with a congenital or infantile onset, a MNCV of $<$ 6 m/s and normal parents. The clinical features in the present series are summarized in Table 2. Cases 1–7 conformed to the HMSN III phenotype, apart from a mother and son both being affected (Cases 3 and 4). Onset was in infancy, nerve conduction velocity was markedly reduced or the peripheral nerves were totally inexcitable, and progression to severe disability occurred. The pattern of neurological involvement was similar in all cases, although severity varied. It was particularly severe in Case 9 who displayed bulbar involvement. A feature that differed from the description given by Dyck *et al*. (1993) was the lack of nerve thickening, although enlarged spinal roots were observed on myelography in Case 4. From their clinical features it would not have been possible to have predicted the underlying genetic defect. Gabreëls-Festen and Gabreëls (1993) emphasized that childhood chronic inflammatory demyelinating polyneuropathy can mimic HMSN III very closely. In the present series, Case 8 was originally referred to us as a possible instance of chronic inflammatory demyelinating polyneuropathy because of the electrophysiological finding of conduction block which was not confirmed in our own studies.

Cases 1, 4 and 9 displayed diaphragmatic weakness. This is known to occur in HMSN, both in types I and II (Hardie *et al.,* 1990) and phrenic nerve involvement has been described in Dejerine–Sottas disease (Felice *et al*., 1994). Case 9 also showed involvement of the laryngeal muscles. This is not surprising for severely affected individuals with a length-related neuropathy, in view of the length of the phrenic and recurrent laryngeal nerves. Two patients developed deafness (Cases 1 and 7), although whether this was part of the HMSN is uncertain. Deafness may occur in HMSN, either on its own (Satya-Murti *et al.,* 1979) or in conjunction with optic atrophy (Rosenberg and Chutorian, 1967). None of our cases showed visual loss.

Cases 1–4 in the present series were found to have missense mutations in the PMP22 gene. In all, these were novel mutations. There have now been a number of reports of mutations in the PMP22 gene in individuals without a chromosome 17p11.2 duplication. Nicholson *et al.* (1994) reported an individual with hereditary neuropathy with liability to pressure palsies (HNPP) who showed a 2 bp deletion, occurring at amino acid 7, resulting in a frame shift and a premature termination. This presumably acted as a null mutation and therefore had the same effect as a segmental deletion of chromosome 17p11.2 duplication which is known to result in HNPP (Chance *et al.,* 1993). Nelis *et al*. (1994*b*) reported a splice mutation comprising a G→A change at the first nucleotide of intron 3. It was not possible to carry out mRNA studies and it is therefore uncertain whether a mutant protein was produced, possibly with a skipped exon, or whether a reduced amount of normal protein was produced. The patient was diagnosed as having HMSN I and had an MNCV of 18 m/s. In a Dutch kindred in which the symptoms were severe (Hoogendijk *et al*., 1993) and in which most of the affected individuals would have been given a diagnosis of HMSN III, there was a leucine→proline mutation in amino acid 16, predicted to lie in the first transmembrane domain. Of

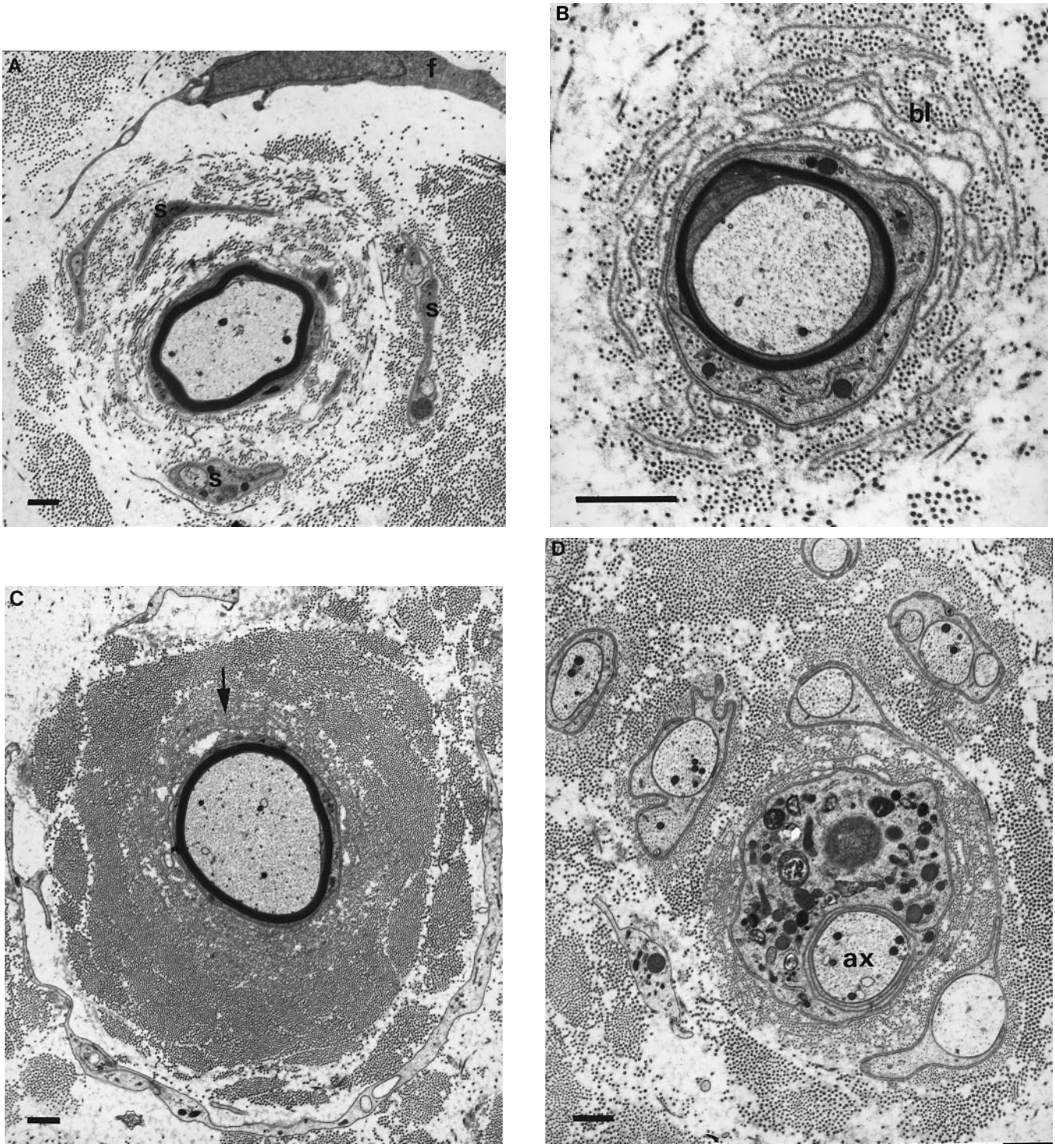

Fig. 8 Case 7. Transverse electron micrographs of sural nerve. (**A**) A myelinated axon surrounded by a zone of collagen fibrils, within which there are a few concentrically arranged Schwann cell processes (s) and fibroblasts (f). (**B**) A myelinated axon surrounded by multiple layers of fragmented basal lamina (bl). (**C**) A myelinated axon surrounded by a dense collagenous zone the inner part of which contains multiple layers of partially fragmented basal lamina (arrow). (**D**) An axon (ax) surrounded by a noncompacted spiral mesaxon. The associated Schwann cell contains multiple dense bodies. Bars = 1μm.

the six other missense mutations reported to date (Roa *et al.,* 1993*a–c*; Ionasescu *et al.,* 1995; Valentijn *et al.,* 1995), Thr118Met was found in a unique family in which recessive inheritance was uncovered by cosegregation with a 1.5 Mb deletion (Roa *et al.,* 1993*b*) and the remainder were described either as phenotypically type I (Ser79Cys; Roa *et al.,* 1993*c*) or type III (His12Glu, Met69Lys, Ser72Leu; Roa *et al.,* 1993*a*; Ionasescu *et al.,* 1995; Valentijn *et al.,* 1995).

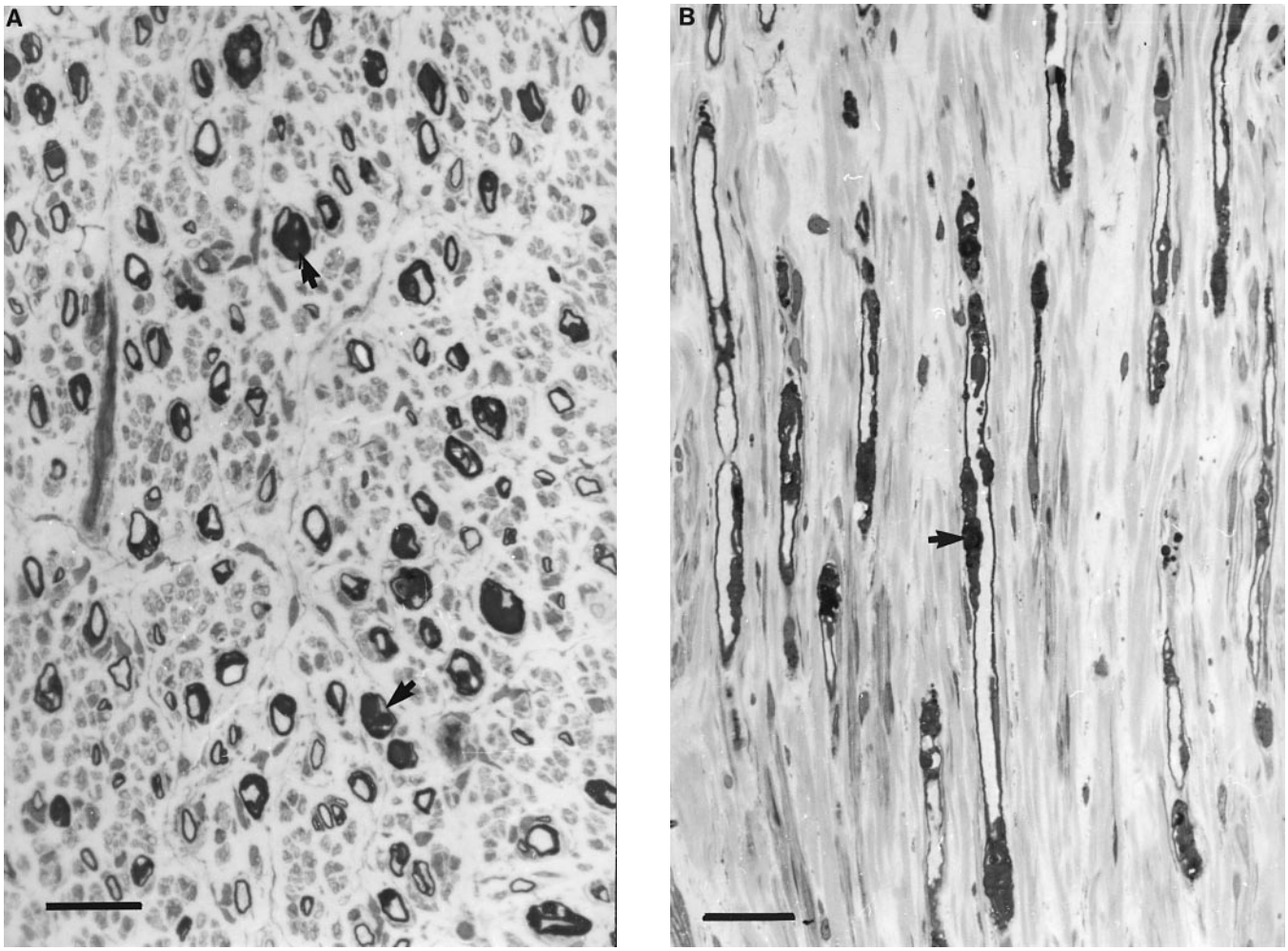

Fig. 9 Case 8. (**A**) A transverse semi-thin section showing reduced myelinated fibre density. Some fibres possess highly contorted myelin sheaths (arrows). (**B**) A longitudinal semi-thin section. The myelinated fibres possess thin myelin sheaths which are frequently expanded into focal irregular zones (arrowed). Bars = 20 μm.

At least 17 point mutations have been described for the P_0 gene. These have largely been missense mutations within exons 2 and 3 (Hayasaka *et al.,* 1993*a–c*; Kulkens *et al.,* 1993; Mitsui *et al.,* 1994; Ohnishi *et al.,* 1994; Rautenstrauss *et al.,* 1994; Latour *et al.,* 1995) but include two non-sense mutations in exon 6 (Hoogendijk *et al.,* 1993). It is not clear how the complex mutation found in our Case 6, involving both a G→C change and a deletion within 4 bp, could have arisen. A frame shift occurs but, surprisingly, no termination codon is created until two amino acids beyond the normal termination codon. In its effect on protein structure, this is similar to a read through mutation previously described (Rautenstrauss *et al.,* 1994) which resulted from a 2 bp insertion at amino acid 222 in exon 6. This case was also defined clinically as HMSN III.

How mutations affecting the PMP22 and P_0 genes result in a demyelinating neuropathy is not yet understood. The putative functions of these two myelin proteins have been reviewed by Snipes and Suter (1995) and Thomas *et al*. (1996). PMP22 is present in compact myelin (Snipes and Suter, 1995), but at a relatively low concentration. This suggests it does not play a structural role. It could be involved in cell cycle regulation (Suter *et al.,* 1993) or in adhesion between axons and Schwann cells (Snipes *et al.,* 1993). P_0 is an abundant myelin protein that is probably involved in homophilic linking between adjacent myelin lamellae (Snipes *et al.,* 1992). Observations in patients with differing genetic defects, combined with those in the Trembler mouse and in transgenic models are likely to contribute to the elucidation of the function of these two myelin proteins. Gabreëls-Festen *et al*. (1995) compared the morphological features seen in nerve biopsies from patients with autosomal dominant HMSN Ia with chromosome 17p11.2 duplications with those in patients with point mutations of the gene. In patients with the duplication, the mean g ratio (axon diameter:total fibre diameter) was significantly lower than normal despite the presence of remyelinating fibres, indicating increased myelin thickness relative to axon diameter. This may well be a gene dosage effect (*see* Introduction). Patients with point mutations in the PMP22 gene had extremely high g ratios, indicating hypomyelination. Hypertrophic onion-bulb change was profuse from an early age in such cases whereas this developed more gradually in the duplication cases. Nerve biopsies from our Cases 1 and 2, both with PMP22 point

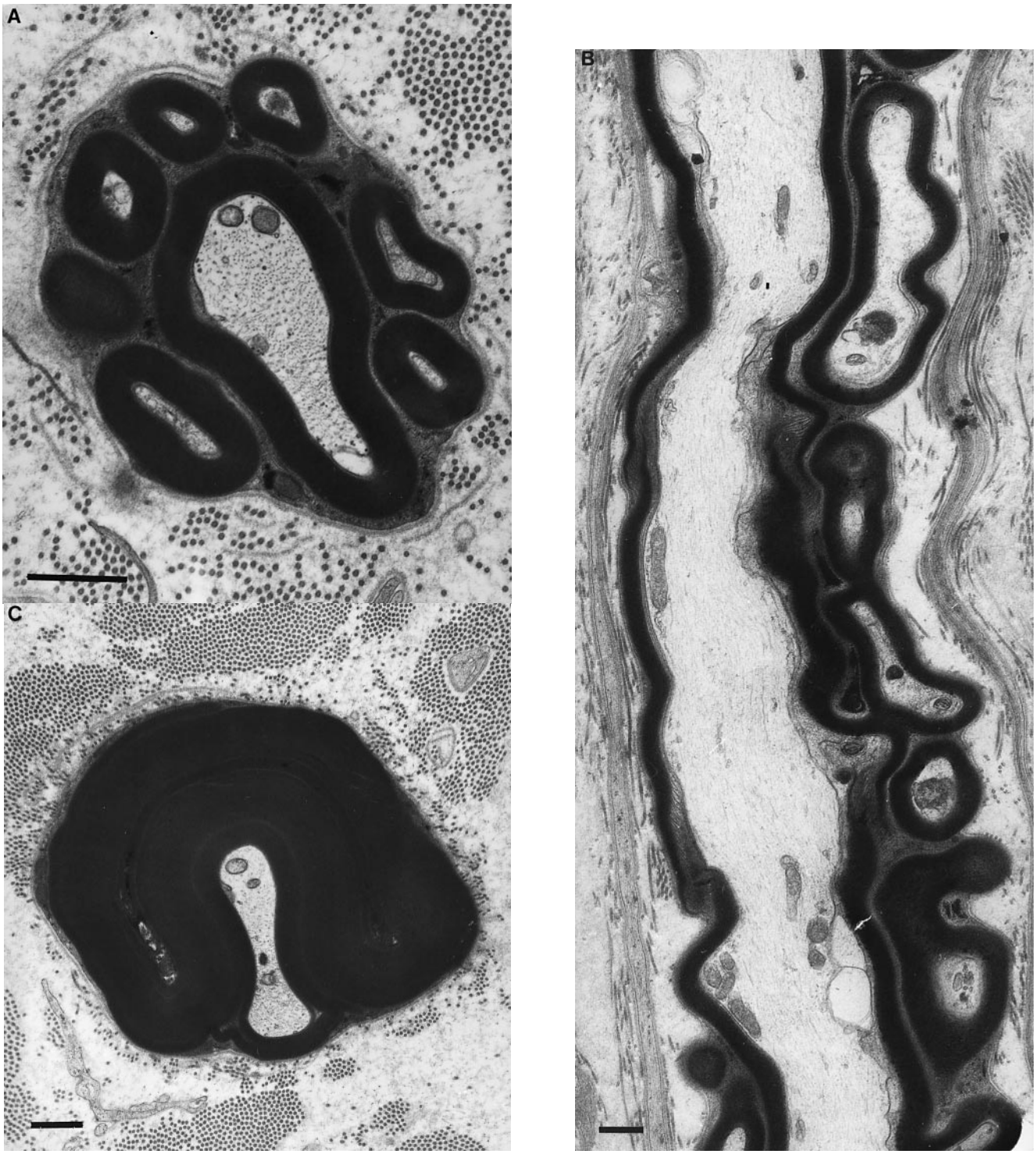

Fig. 10 Case 8. (**A**) An electron micrograph of transverse section through sural nerve showing multiple outpouchings of myelin, some of which contain axonal protrusions. (**B**) A longitudinal section showing multiple myelin outpouchings. (**C**) An electron micrograph of a tomaculum showing excess myelin lamellae. Bars = 1 μm.

mutations, were hypomyelinated. Case 2 showed florid hypertrophic changes whereas they were not seen in Case 1 (although this biopsy specimen was not processed by contemporary morphological techniques). Nevertheless, this indicates that a uniform type of pathology may not be expected in patients with PMP22 point mutations. In our Case 6 with a P_0 point mutation, intramuscular nerve fibres lacked detectable myelin sheaths, and only minor hypertrophic changes were evident.

Cases 7–9 are likely to be of autosomal recessive inheritance. In Case 7 there was close (father/daughter) parental consanguinity. In Cases 8 and 9 the parents were cousins. No

Table 2 *Summary of cases presented*

Case	Age (years)/ sex	Inheritance	Genetic defect	Clinical features		MNCV (m/s)	Pathology
				Onset	Current status		
1	39/F	AD*	PMP22 mutation	Neonatal hypotonia	Severe sensorimotor neuropathy; deafness	3	Hypomyelination; hypertrophic changes not detected
2	21/F	AD	PMP22 mutation	Delayed motor development	Severe sensorimotor neuropathy	Nerves inexcitable	Hypomyelination; 'classical' onion bulbs
3	2/M	AD	PMP22 mutation	Delayed motor development	Distal neuropathy	ND	ND
4	28/F	AD*	PMP22 mutation	Neonatal hypotonia	Severe sensorimotor neuropathy; kyphoscoliosis; pes equinovarus	Nerves inexcitable	ND
5	6/F	AD*	P_0 mutation	Delayed motor development	Motor neuropathy	8	ND
6	25/F	AD*	P_0 mutation	Delayed motor development	Severe sensorimotor neuropathy	6	Hypomyelination; minor hypertrophic changes
7	42/F	AR	Not known	Leg weakness from age 3 years	Severe sensorimotor neuropathy; deafness; kyphoscoliosis	3	Hypomyelination; basal laminal onion bulbs
8	5/M	AR	Not known	Abnormal gait from age 1 year	Severe sensorimotor neuropathy	15	Focally folded myelin sheaths
9	16/M	AR	Not known	Abnormal cry from birth; leg weakness from 13 months	Severe sensorimotor neuropathy; facial, bulbar and diaphragmatic weakness	16	Focally folded myelin sheaths

MNCV = motor nerve conduction velocity; AD = autosomal dominant; AR = autosomal recessive (presumed); ND = not done. *Presumed *de novo* mutation.

abnormality was detected on sequencing the PMP22 and P_0 genes in Case 7 and the disorder is thus likely to be related to a mutation at another unidentified locus. In recessively inherited Tunisian kindreds with a demyelinating neuropathy, the disorder was localized by homozygosity mapping to chromosome 8q23 (Ben Othmane *et al.,* 1993). Both in those cases and in our Case 7, basal laminal onion bulbs were observed. Such onion bulbs, originally described by Lyon (1969), consist of double layers of basal laminae, arranged concentrically around a central axon, from which the Schwann cell processes have presumably disappeared. Our Case 7 differs materially from those of Ben Othmane *et al*. (1993) in that MNCV was severely reduced from an early age, whereas in the Tunisian patients mean MNCV was substantially faster at 29 m/s. They are thus likely to represent different conditions.

In three patients with congenital hypomyelination neuropathy with basal laminal onion bulbs, Sawaishi *et al*. (1995) undertook immunohistochemical studies on peripheral nerve for myelin basic protein and P_0 and P_2 myelin proteins. They found no differences from controls in myelin basic protein and P_0, but P_2 staining was severely reduced. The P_2 protein gene was sequenced but found to be normal. It was therefore considered that the reduced P_2 expression could be a secondary phenomenon. It was not stated whether mutation of the PMP22 or P_0 genes had been excluded.

Patients diagnosed as having HMSN I with autosomal recessive inheritance are more severely affected than those with autosomal dominant HMSN I (Harding and Thomas, 1980*c*). Such cases may display basal laminal onion bulbs (Gabreëls-Festen *et al.,* 1992). Others may show classical onion bulbs with concentrically proliferated Schwann cells in association with hypomyelination, as in the two siblings reported by Harding and Thomas (1980*c*) and Thomas *et al*. (1996).

The histology in our Cases 8 and 9 was characteristic of hereditary neuropathy with focally folded myelin sheaths (HNFFM) (Ohnishi *et al.,* 1989; Gabreëls-Festen *et al.,* 1990). Reported cases so far, like ours, have been of presumed autosomal recessive inheritance, although there is one report of this histological pattern in a dominantly inherited kinship (Umehara *et al.,* 1993).

The classification of the hereditary motor and sensory neuropathies will need revision. As stated in the Introduction, the designation HMSN was introduced as a temporary expediency until such time as specific genetic defects could be identified. This is progressively taking place. The distinction between autosomal recessive HMSN I and HMSN III (or Dejerine–Sottas disease) is artificial, and mutational screening for PMP22 and P_0 genes is advisable in any case with demyelinating neuropathy without a chromosome 17p11.2 duplication, which is more severe than usual and in which a genetic basis seems probable. For clinical purposes, such cases

perhaps should now simply be referred to as hereditary demyelinating neuropathies, specified by the nature of the genetic defect or chromosomal linkage if established. Infantile hereditary demyelinating neuropathy is seen to be genetically complex. It can result from mutations of the PMP22 or P_0 genes or of genes at other unidentified loci. The histological features of HNFFM are characteristic, but the gene or genes responsible for this phenotype have not yet been located. A demyelinating neuropathy with an onset in infancy can also occur in metachromatic leukodystrophy (De Silva and Pearce, 1973) and Niemann–Pick disease (Landrieu and Said, 1984). The onset of classical Refsum's disease in infancy has not been documented and infantile Refsum's disease does not give rise to a demyelinating neuropathy.

The nature of examples of congenital neuropathy with amyelination (Karch and Urich, 1975; Kennedy *et al.,* 1977) is still uncertain. These present with severe hypotonia at birth or arthrogryposis (Charnas *et al.,* 1988) and survival is limited. It will be of interest to examine the PMP22 and P_0 genes in such cases.

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