

# The phenotypic manifestations of chromosome 17p11.2 duplication

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## Summary

Clinical and electrophysiological investigations and nerve biopsies were carried out on 61 patients shown to have a chromosome 17p11.2 duplication (hereditary motor and sensory neuropathy—HMSN Ia). Of these, 50 showed a Charcot–Marie–Tooth (CMT) phenotype and eight could be classified as having the Roussy–Lévy syndrome. Of the patients with a CMT phenotype, three had associated pyramidal signs and of these one had ‘complicated’ HMSN and also signs of cerebellar and bulbar involvement. Diaphragmatic weakness was present in three severely affected cases, one of whom also had denervation of the anal sphincter associated with faecal incontinence. One unusual case presented in middle life with incapacitating muscle cramps associated with calf hypertrophy and only mild clinical signs of neuropathy. Prominent distal sensory loss was a consistent feature in one family, resulting in

acrodystrophic changes in several members. Concurrent focal peripheral nerve lesions were seen with both the CMT and Roussy–Lévy phenotypes, in seven patients. Upper limb motor nerve conduction velocity was  $19.9 \text{ m/s} \pm 1.3$  (SEM), range 5–34 m/s. This corresponds to values previously obtained for autosomal dominant HMSN I. This series consisted mainly of older patients with more advanced disease. In contrast to the findings in younger patients, in their nerve biopsies, myelin thickness tended to be relatively reduced for axon size, indicating remyelination and/or hypomyelination; there was also regression of the onion bulbs. It is concluded that the possession of two copies of the peripheral myelin protein 22 gene within the duplicated region on chromosome 17p gives rise to a range of phenotypes and not solely to a CMT syndrome, and that the pattern of histological change in the peripheral nerves alters with advance of the disease.

**Keywords:** Charcot–Marie–Tooth disease; hereditary motor and sensory neuropathy; hypertrophic neuropathy; peripheral myelin protein 22; Roussy–Lévy syndrome

**Abbreviations:** CIDP = chronic inflammatory demyelinating polyneuropathy; CMT = Charcot–Marie–Tooth disease; HMSN = hereditary motor and sensory neuropathy; HNPP = hereditary neuropathy with liability to pressure palsies; MNCV = motor nerve conduction velocity; PMP22 = peripheral myelin protein 22; SAP = sensory nerve action potential

## Introduction

The commonest form of inherited demyelinating neuropathy has been given the name of type I hereditary motor and sensory neuropathy (HMSN I) (Thomas *et al.*, 1974; Dyck, 1975) or type I Charcot–Marie–Tooth disease (CMT 1) in the gene mapping literature. Inheritance is usually autosomal dominant (Dyck and Lambert, 1968a; Harding and Thomas,

1980a). One gene for families with this phenotype was initially mapped to chromosome 1 (Bird *et al.*, 1982; Guiloff *et al.*, 1982; Lebo *et al.*, 1991), the disorder being referred to as HMSN Ib (or CMT 1B). It was subsequently found that linkage to chromosome 17p11.2 was substantially more frequent (Vance *et al.*, 1989; Raeymaekers *et al.*, 1989;

Middleton-Price *et al.*, 1990; Hallam *et al.*, 1992). This form was termed HMSN Ia (or CMT 1A). Other unidentified loci also exist (Chance *et al.*, 1990).

In 1991, two independent reports appeared describing a large segmental duplication within band 17p11.2, involving ~1.5 Mb of DNA (Lupski *et al.*, 1991; Raeymaekers *et al.*, 1991). The duplication is known to include the gene for peripheral myelin protein 22 (PMP22) (Matsunami *et al.*, 1992; Patel *et al.*, 1992; Timmerman *et al.*, 1992; Valentijn *et al.*, 1992a).

The clinical features of HMSN I were analysed by Harding and Thomas (1980b) and compared with those of HMSN II in which the underlying pathology is an axonopathy (Dyck and Lambert, 1968b; Behse and Buchthal, 1977; Berciano *et al.*, 1986). That investigation would predominantly have included examples of HMSN Ia rather than Ib as it is likely that the majority of cases with autosomal dominant HMSN I carry the chromosome 17p11.2 duplication (Harding, 1995). Their distinction was not possible at that time. The present study has examined the clinical and electrophysiological features and nerve biopsy findings in a series of cases with the duplication to assess the range of phenotypic manifestations that may be encountered. A preliminary report of the findings has appeared in abstract form (Marques *et al.*, 1996).

## Patients and methods

### Patient ascertainment

A total of 66 blood samples referred either to the Institute of Child Health or Institute of Neurology, London since the introduction of testing showed a chromosome 17p11.2 duplication. In five, adequate clinical information was not available. The 61 patients analysed were derived from the National Hospital for Neurology and Neurosurgery, London (47 cases), the Royal Free Hospital, London (11 cases) and Ipswich Hospital (three cases). In each, a possible diagnosis of HMSN I had been made on clinical and electrophysiological grounds. Most were examined by two of the authors (either P.K.T. or A.E.H.); otherwise the clinical details were obtained from the case notes.

### DNA analysis

DNA was extracted from blood samples by standard methods. At the Institute of Child Health initially DNA from all individuals was tested for a 17p duplication by digestion with *MspI* and probing with D17S122 (VAW409R3). In cases which were heterozygous for the 2.7 and 2.8 kb alleles, a dosage difference was detectable by visual inspection (Hallam *et al.*, 1992). Samples which were uninformative for the RFLP (restriction fragment length polymorphism) were tested by dosage analysis. Later, duplications of the loci D17S122 and D17S125 were detected by dosage analysis of Southern blots using methods described previously (Hoogendijk *et al.*, 1992; Hensels *et al.*, 1993). Briefly, DNA was digested

with *EcoRI* and hybridized with probes VAW409R3a and VAW412R3a (supplied by C. van Broeckhoven) and a reference probe E3.9 which maps to chromosome 22 (supplied by P. Bolhuis) (Hoogendijk *et al.*, 1992; Hensels *et al.*, 1993). These probes hybridized to DNA fragments of 2.0, 4.5 and 3.9 kb. Filters were exposed to a Phosphor Imager (Molecular Dynamics) storage screen at room temperature for 1–4 days, and signals were then measured in the Phosphor Imager. VAW409R3a/E3.9 and VAW412R3a/E3.9 signal ratios were calculated for each sample. At least four normal control samples were analysed simultaneously on each filter, and the mean ratios for these were calculated. Patient sample ratios were expressed as percentages of the control mean value for each filter so that samples from different experiments could be compared. A total of 76 control experiments, using samples from 52 normal subjects, was used to define the normal ranges of these ratios. The distributions of control ratios for VAW409R3a/E3.9 and VAW412R3a/E3.9 were normally distributed, with respective means  $\pm$  standard deviations (SDs) and ranges of  $1.0 \pm 0.09$  and  $0.78\text{--}1.20$ , and  $1.01 \pm 0.08$  and  $0.79\text{--}1.20$ . The ranges correspond closely to 99% confidence limits of the means ( $\pm 2.58 \times \text{SD}$ ), which were  $0.77\text{--}1.24$  and  $0.79\text{--}1.22$ , respectively. The presence of a duplication of this region of 17p11.2 was defined by both ratios falling above the 99% confidence limits of the normal range. More recently, duplications were detected using a probe hybridizing to the repeat that flanks the duplication/deletion of 17p11.2 in HMSN Ia/HNPP (hereditary neuropathy with liability to pressure palsies; Chance *et al.*, 1994), respectively. Subclone pNEA102 (provided by Dr J. Lupski), which contains a 1.8-kb *EcoRI* fragment, was used to probe *EcoRI* digests. This normally detects a 7.8-kb fragment from the proximal duplication monomer and a 6.0-kb fragment from the distal repeat. The signal intensity of these bands was determined using a Phosphor Imager and the 6.0 kb : 7.8 kb ratio calculated. The patient sample ratios were normalized to the mean ratio value of at least three normal samples run on each gel. The HMSN Ia duplication normally includes the 6.0-kb fragment, leading to an increased 6.0 kb : 7.8 kb ratio. The mean 6.0 kb : 7.8 kb ratio in 85 healthy control subjects was 1.02; range, 0.85–1.19; and 99% confidence limits, 0.79–1.24. Again, the presence of a duplication was defined by a ratio above the 99% confidence limits to the normal range.

At the Institute of Neurology, duplication analysis was performed using fluorescent quantitative PCR (polymerase chain reaction) of microsatellite markers from within the region of chromosome 17p11.2 known to be duplicated in HMSN Ia: D17S122, D17S839, D17S921, D17S955 and D17S1358. Analysis was carried out using an ABI373 automatic DNA sequencer and Genescan software. The presence of a duplication was determined either by the presence of three alleles at one or more marker loci (88% of cases) or by gene dosage (12%). The presence of a duplication by dosage was defined as an allelic peak height

ratio of  $> 60 : 40$  as compared with values between  $53 : 47$  and  $50 : 50$  observed in 47 controls.

### **Nerve biopsy**

Fascicular biopsy specimens were obtained under local anaesthesia from standard sites from the sural or radial nerves posterior to the lateral malleolus or just proximal to the styloid process of the radius, respectively. The specimens were fixed in 3% glutaraldehyde in PIPES (piperazine-*N-N'*-bis 2-ethane sulphonic acid) buffer, postosmicated and, after dehydration, embedded in Araldite or Durcupan. Semithin sections ( $0.5 \mu\text{m}$ ) were stained with thionin and acridine orange (Sievers, 1971). Ultrathin sections were contrasted with methanolic uranyl acetate and lead citrate and examined in a Zeiss EM902 electron microscope.

## **Results**

### **Patients**

Of the 61 patients, 32 were male and 29 female. Their mean ages at the time of DNA testing were 31.4 (range 3–74) and 41.6 (7–74) years, respectively, and 36.7 (3–74) years for the total sample. A positive family history consistent with autosomal dominant inheritance was present in 36. The remainder were either sporadic (eight patients) or of uncertain inheritance (17). In two patients without a family history, DNA testing was undertaken on both parents; in both instances, neither parent possessed a duplication.

### **Age at onset and presenting features**

Evidence of HMSN was initially detected within the first decade in 46 patients (75%) and in six (10%) in the second. Symptoms were initially noticed after the age of 20 years in only four patients (7%), two in the third and two in the fourth decade. In five the age of onset was uncertain.

Ten cases were initially brought to medical attention because of developmental motor delay manifested by a failure to begin walking independently until after the age of 18 months. In 21 instances the initial symptom was difficulty in walking and/or running, without earlier developmental delay. Another 20 presented because of foot deformity and one because of chest deformity (pectus excavatum). In seven cases there were nonspecific motor problems which led to the relatives being suspicious that they were affected because of experience with other affected family members. In single instances, presentation was with muscle cramps, with an acute polyneuropathy, and with leg weakness developing during a pregnancy.

### **Clinical features**

#### **Individual features**

The most consistent clinical abnormality was muscle wasting and weakness which uniformly had a distal emphasis. It

affected both the upper and lower limbs in 45 patients and the lower limbs alone in eight. The upper limbs were never involved in isolation or to a greater extent than the lower. In three cases the diaphragm was affected, giving rise to dyspnoea when lying flat and to nocturnal hypoventilation. Two cases had weakness of the bulbar musculature. In one it was manifested by dysphagia and dysarthria; diaphragmatic weakness was also present. In the other there was involvement of the laryngeal muscles with selective weakness of the vocal cord abductors and stridor; there was no associated dysphagia or dysarthria. In one case there was weakness of the pelvic floor muscles resulting in faecal incontinence; this patient also had diaphragmatic weakness. Two other single cases had weakness of the bulbar and pelvic floor muscles, respectively, both of whom also had diaphragmatic weakness, as did one other patient. In seven patients there was no detectable weakness on clinical examination.

In eight patients there was postural upper limb tremor. This was most evident on holding the arms outstretched and was also present during movement, for example in the finger–nose test, without terminal exaggeration. There was no accompanying head or lower limb tremor.

The tendon reflexes were absent both in the upper and lower limbs in 46 patients. In 13 some tendon reflexes were lost or depressed, others being preserved. In two cases they were normal, both in the lower and upper limbs. The plantar responses were flexor in 55 patients, definitely extensor in three and unobtainable in two.

Sensory loss on clinical assessment was evident in 43 patients. It affected the lower limbs only in 34 and both the upper and lower in nine. In 18 cases, no sensory loss was detectable. In one patient, and consistently in her relatives, sensory loss was particularly severe, leading to foot ulceration in several members of this kinship (*see* Appendix, Case 26).

Positive sensory symptoms were not a general feature of these cases. Neuropathic pain was not reported although pain of musculoskeletal origin was recorded in some instances. Patients with superimposed focal nerve lesions (carpal tunnel syndrome, ulnar neuropathy, meralgia paraesthetica) experienced localized paraesthesiae.

Foot deformity was present in 44 cases. This was usually pes cavus, often associated with an equinovarus deformity. Clawing of the fingers was evident in four patients and scoliosis in eight. Acetabular dysplasia was evident in four cases and pectus excavatum in two.

One patient, with the Roussy–Lévy syndrome, developed hypertrophic cardiomyopathy (Case 45; *see* Appendix).

### **Composite clinical picture**

The overall clinical features are summarized in Table 1. In 34 cases there was a classical CMT syndrome, namely with distally accentuated weakness in the limbs, most evident in the lower limbs, usually associated with loss of tendon reflexes and foot deformity with or without distally accentuated sensory impairment which, if present, was of

**Table 1** Summary of clinical features

Phenotype	No. of cases
Classical CMT syndrome	34
CMT syndrome with additional features	
CNS signs	3
Associated focal peripheral nerve lesions	5
Prominent muscle cramps	4
IgM paraproteinaemia	1
Diabetes mellitus	1
Parkinsonism/torticollis	2
Roussy-Lévy syndrome	
Alone	5
Associated focal peripheral nerve lesions	2
Hypertrophic cardiomyopathy	1
Neuropathy with prominent sensory loss	1
Neuropathy with cramps, calf hypertrophy and atypical neuromyotonia	1
Asymptomatic	1
Total	61

only mild or moderate severity. All sensory modalities could be affected. Autonomic features were not found. In 16 patients a CMT syndrome was accompanied by a variety of other neurological features. Recurrent muscle cramps were a prominent feature in four cases. There were associated peripheral nerve lesions in five patients (two carpal tunnel syndrome, one carpal tunnel syndrome and meralgia paraesthetica, two ulnar neuropathy). Single patients also had torticollis, dopa-responsive Parkinson's disease, diabetes mellitus and a monoclonal IgM kappa paraproteinaemia.

In eight patients the clinical features could be categorized as the Roussy-Lévy syndrome in that they displayed a prominent upper limb postural tremor associated with tendon areflexia, pes cavus and variable distal weakness and sensory loss. Two patients had associated peripheral nerve lesions (one carpal tunnel syndrome, one neuropathic lesion of peroneal nerve). A description for one of the Roussy-Lévy cases is given in the Appendix.

Reference has already been made to the occurrence of a family with acrodystrophic neuropathy in which a CMT syndrome was associated with severe distal sensory loss in the lower limbs leading to a mutilating acropathy in several members. One patient (Case 44) had a complex neurological syndrome. A CMT syndrome with severe lower and upper limb and diaphragmatic weakness, and bilaterally extensor plantar responses were combined with bulbar weakness and evidence of cerebellar dysfunction (*see* Appendix).

One unusual patient (*see* Case 56; Appendix) presented in middle life with incapacitating muscle cramps which had begun in early adult life. He showed calf muscle hypertrophy and only trivial evidence of neuropathy on examination. Neurophysiological investigation demonstrated probable neuromyotonia.

Two patients reported troublesome lower limb paraesthesiae. In one, a female (Case 15) they were mainly nocturnal and sufficiently severe to interfere with sleep. This symptom

was partially relieved by nocturnal clonazepam but was considerably lessened by repeated courses of high dose intravenous human immunoglobulin. Because of this, a diagnosis of superimposed chronic inflammatory demyelinating polyneuropathy (CIDP) was questioned but nerve biopsy failed to demonstrate inflammatory infiltrates. The second patient, a male (Case 46) developed an acute paralytic illness at the age of 39 years, necessitating assisted ventilation. He made a good recovery over 6–7 months but remained with slightly impaired balance and difficulty in running. He was treated with high dose corticosteroids without benefit. A sural nerve biopsy at that stage showed a hypertrophic neuropathy but no inflammatory infiltrates. His condition was relatively static until the age of 55 years, when he began to deteriorate, developing bilateral footdrop and weakness of his hands. At the age of 59 years he underwent plasma exchange and is stated to have improved. At the age of 67 years his limb weakness again increased and he experienced burning paraesthesiae in his feet and hands. Plasma exchange was again undertaken following which the paraesthesiae improved. He has remained clinically stable since. A further patient had a history of an acute episode, diagnosed as 'infective peripheral neuritis', before the onset of symptoms of her HMSN (Case 44; Appendix).

### Clinical neurophysiology

Motor nerve conduction velocity (MNCV) was measured in the upper limbs, usually for the median nerve on recording from abductor pollicis brevis. The mean value was  $19.9 \text{ m/s} \pm 1.3$  (SEM) m/s with a range of 5–34 m/s. A value for the lower limbs was less frequently obtained because of complete denervation of the small foot muscles. It was either for the peroneal or tibial nerve on recording from extensor digitorum brevis or abductor hallucis, respectively. The mean value was  $17.0 \pm 1.2$  m/s (range 10–22 m/s). Sensory nerve action potentials were usually absent or of severely depressed amplitude. When sensory conduction velocity was measurable, the reduction was of the same order as for MNCV (mean  $22.9 \pm 1.6$  m/s).

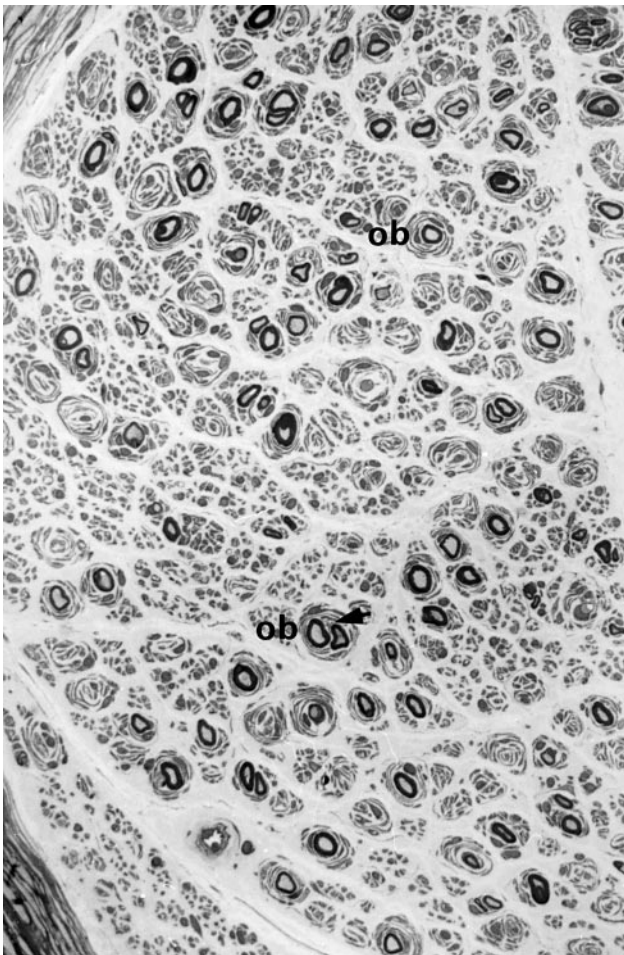
### Nerve biopsy

Sural or radial nerve biopsy was performed in 10 cases. All showed a depletion in the myelinated nerve fibre population, the magnitude of which correlated with the clinical assessment of disease severity (Table 2). The hypertrophic changes in less severe cases and the extensive endoneurial fibrosis in the more advanced examples will have contributed to the reduced fibre density. Hypertrophic changes of onion bulb type were most evident in the cases with a higher myelinated fibre density, diminishing progressively with reduction in density (Table 2; Figs 1 and 2). Well developed onion bulbs had the typical appearance of concentrically proliferated Schwann cells surrounding a central myelinated axon or a small cluster of regenerating axons (Fig. 3) or sometimes a

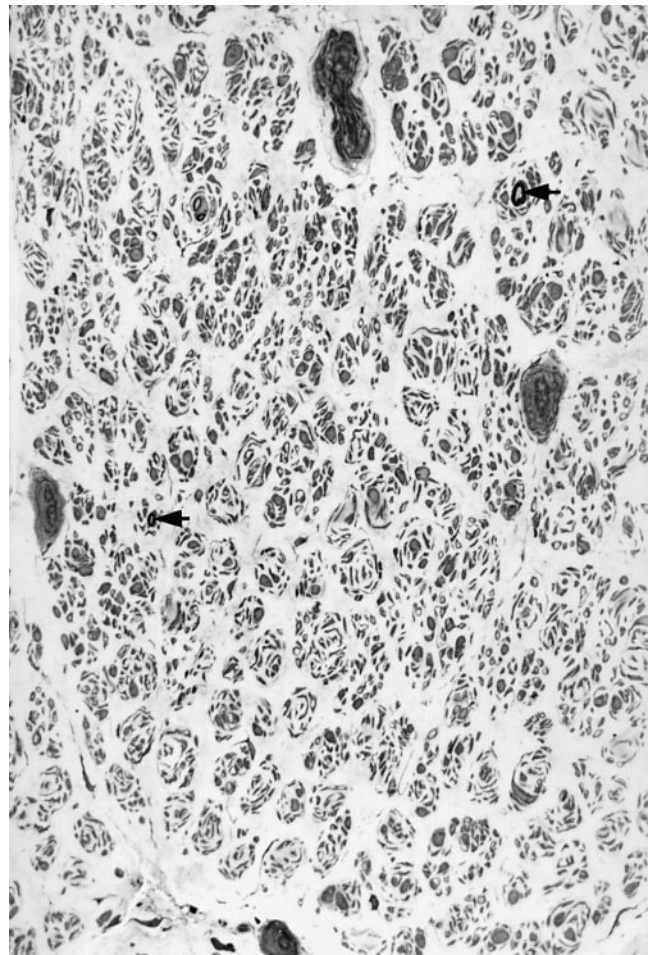
**Table 2** Nerve biopsy findings

Case	Age	Myelinated fibre density per mm <sup>2</sup>	g ratio			Clinical severity*	Frequency of onion bulbs <sup>†</sup>
			<0.4 (%)	>0.7 (%)	Mean		
15	25	781	0	25	0.62	++	++
26	53	457	0	46	0.70	++	+++
30	47	26	0	54	0.70	+++	+
32	48	715	0	56	0.70	++	++
35	42	377	0	46	0.68	+++	+
42	36	877	1.3	24	0.63	+	+++
44	39	967	3.4	28	0.63	++	+++
45	52	844	1.2	43	0.68	++	+++
56	55	1089	3.5	14	0.60	+	+++
57	13	1273	2.1	32	0.64	+	+
Controls <sup>‡</sup>		9716 ± 655	11.0 ± 1.4	7.3 ± 0.53	0.576 ± 0.005		

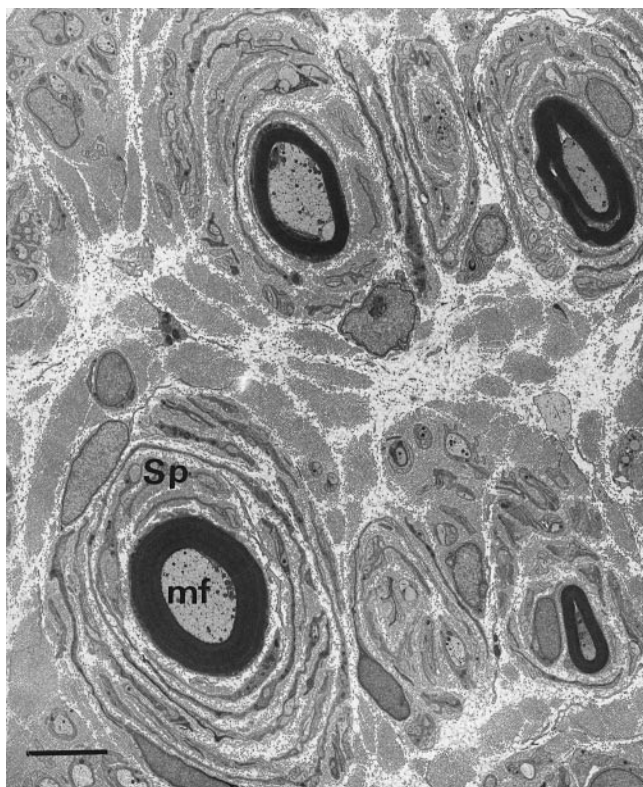
\*Severity: + = fully ambulant; ++ = severe weakness but ambulant; +++ = wheelchair bound. <sup>†</sup>The relative lack of onion bulbs in case 57 is probably attributable to his young age. <sup>‡</sup>Mean values ± SEM, *n* = 5.



**Fig. 1** Case 56. Transverse section through sural nerve biopsy specimen showing depleted myelinated fibre population and multiple onion bulbs (ob), some of which contain several regenerating myelinated axon sprouts (arrow). Thionin and acridine orange. Magnification ×380



**Fig. 2** Case 30. Transverse section through sural nerve biopsy specimen showing only two myelinated fibres (arrows). Multiple groups of Schwann cells are present, some of which show a residual indication of a concentric arrangement. Thionin and acridine orange. Magnification ×380

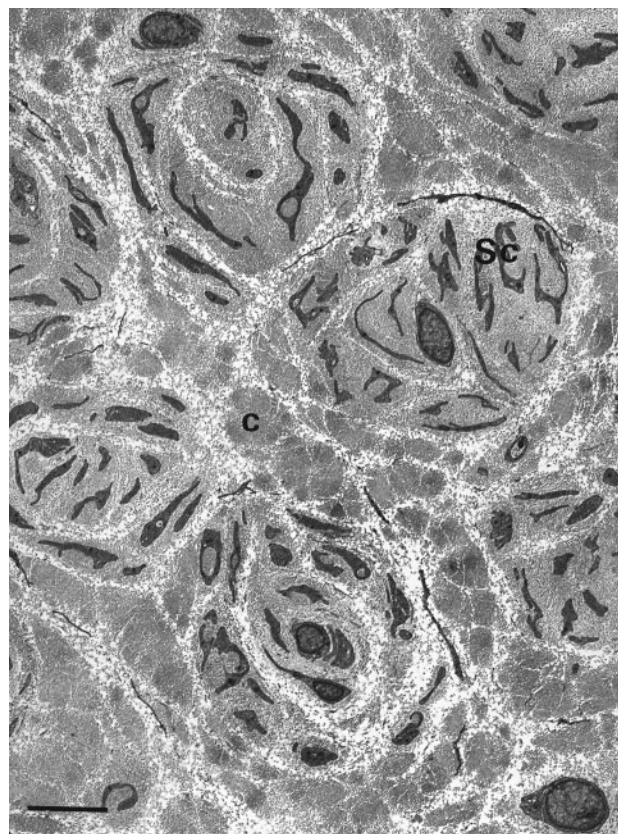


**Fig. 3** Case 46. Electron micrograph of transverse section through sural nerve biopsy specimen showing myelinated nerve fibres (mf) surrounded by concentric arrays of Schwann cell processes (Sp). Bar represents 5  $\mu$ m.

group of Schwann cell processes associated with one or more unmyelinated axons. The Schwann cell lamellae of the onion bulbs were regularly associated with small nonmyelinated axons, but not with myelinated axons. In the cases with a severe loss of myelinated axons, a few onion bulbs remained with small central myelinated or unmyelinated axons, but most of the intrafascicular compartment of the nerve was occupied by collections of Schwann cells embedded in dense endoneurial collagen deposits. These collections of Schwann cells sometimes showed a minor degree of concentric orientation to indicate their derivation from former onion bulbs (Fig. 4), but frequently this was not evident. These Schwann cells were not consistently associated with axons. Evidence of active demyelination was only seen in a single fibre from one patient (Case 44).

The biopsy in Case 32 (who had an associated IgM paraproteinaemic neuropathy) differed in that widely-spaced myelin was present and IgM deposition was demonstrated immunocytochemically on surviving myelin sheaths. This patient has previously been reported independently (Gregory *et al.*, 1993), as has Case 35 (Thomas *et al.*, 1996), who had concurrent diabetes mellitus. The latter patient showed the most severe fibre loss, with a myelinated fibre density of only 26/mm<sup>2</sup>.

The values for the measurement of the *g* ratio (axon diameter:total fibre diameter) are provided in Table 2. A



**Fig. 4** Case 30. Electron micrograph of transverse section through sural nerve biopsy specimen. No myelinated nerve fibres are present in this field. Previous onion bulbs are represented by groups of Schwann cells (Sc) with a residual partial concentric arrangement of their processes. There is extensive deposition of endoneurial collagen fibrils (c). Bar represents 5  $\mu$ m.

mean *g* ratio is given, together with the proportion of fibres having a ratio  $>0.7$ , indicating hypomyelination and with a ratio of  $<0.4$ , indicating hypermyelination. In comparison with five organ-donor control cases, the values for the patients indicated that the fibres were thinly myelinated with no excess of thickly myelinated fibres. In this analysis, Case 32 with an accompanying IgM paraproteinaemia and Case 35 with diabetes mellitus have been excluded. The mean proportion of fibres with a *g* ratio  $>0.7$  in the remaining eight cases was  $33.3 \pm 1.5$  (SEM)%; for those with a ratio  $<0.4$  it was  $1.5 \pm 0.2\%$ . The corresponding values for control cases were  $11.0 \pm 1.4\%$  and  $7.3 \pm 0.53\%$ , respectively. Both of these differences are statistically significant ( $P = 0.0013$  and  $0.0014$ ; Welch's modification of *t* test).

Very occasional examples of abnormal folding of the myelin sheath of the type seen in tomacula were observed on electron microscopy. Teased-fibre studies were only feasible in Case 44. No tomacula were seen.

## Discussion

It is now evident that ~90% of families with autosomal dominant HMSN I have a chromosome 17 duplication (Brice

*et al.*, 1992; Wise *et al.*, 1993; Patel and Lupski 1994; Harding, 1995). This probably applies to all ethnic groups. The duplication has been demonstrated in several patients lacking affected relatives and who had genetically normal parents (Hoogendijk *et al.*, 1992; Wise *et al.*, 1993), suggesting *de novo* mutation. This was true for two patients in the present series. The duplication is extensive, involving ~1.5 Mb of DNA. It is flanked by repeated sequences 17–29 kb in length termed CMT 1A-REP. A *de novo* mutation is likely to arise from misalignment of the distal repeat during meiosis (Pentao *et al.*, 1992; Chance *et al.*, 1994). The duplications are sometimes of maternal origin (Mancardi *et al.*, 1994; Blair *et al.*, 1996) but mainly of paternal origin (Palau *et al.*, 1993) and are of constant length in the majority of patients (Wise *et al.*, 1993). A smaller duplication ~460 kb in length has been described in one family (Valentijn *et al.*, 1993; Patel and Lupski, 1994).

The 17p11.2 band on chromosome 17 is known to contain the gene for PMP22 (Matsunami *et al.*, 1992; Patel *et al.*, 1992; Timmerman *et al.*, 1992; Valentijn *et al.*, 1992a) and two copies are present on a duplicated chromosome. The pathogenetic role of PMP22 mutations was established by the occurrence of a demyelinating neuropathy related to point mutations in this gene (Valentijn *et al.*, 1992b; Roa *et al.*, 1993; Nelis *et al.*, 1994; and others). Patients with point mutations usually display a more severe phenotype than those with a duplication (Tyson *et al.*, 1997).

The present study has analysed the range of phenotypic manifestations in a series of patients with a chromosome 17p11.2 duplication. This proved to be wide, ranging from being severely affected at the ages of 34 and 40 years, as in Cases 30 and 44, described in the Appendix, to being asymptomatic at the age of 33 years, as in the brother of Case 44. Most patients (69%) had a CMT phenotype, sometimes with additional features which may or may not have been related. The onset of symptoms was most frequently within the first decade (75%), considerably less frequently during the second (10%), and rarely after then. This conforms with the findings of Harding and Thomas (1980b).

The clinical picture was of a distal length-related neuropathy affecting the lower limbs to a greater extent than the upper limbs, and motor function to a greater extent than sensory function. As noted previously for HMSN I (Harding and Thomas, 1980b), the anterolateral lower limb muscles were affected to a greater extent than the calf muscles, in contrast to HMSN II in which the calf musculature is often affected to an approximately equal extent. All sensory modalities could be affected but autonomic function, apart from pupillary abnormalities in some patients, was preserved.

A high proportion of patients had foot deformity, usually pes cavus and often an equinovarus deformity with shortening of the calf muscles. Scoliosis was present in 13% of our patients. This is consistent with the frequent onset of disability in the first decade, before the cessation of skeletal growth. Acetabular dysplasia was present in four cases and this is

therefore probably a significant association, as was pectus excavatum which was seen in two cases.

With advance of the disease, more proximal muscles became weak, but neither greater proximal than distal nor greater upper than lower limb involvement was encountered. In severe cases, the diaphragm was affected.; this was observed in three patients. Diaphragmatic weakness has previously been described, both in HMSN I and HMSN II (Hardie *et al.*, 1990). Involvement of the diaphragm is not unexpected in a distally accentuated neuropathy in view of the length of the phrenic nerve, neither is weakness of the laryngeal muscles, as occurred in Case 61, considering the length of the recurrent laryngeal nerve. What is somewhat surprising is involvement of the levator palati and the pharyngeal muscles at an early stage in the evolution of the disorder in Case 44 and of the innervation of levator ani in Case 30. These muscles are supplied by relatively short nerves.

An upper limb postural tremor characterized the cases with a Roussy–Lévy phenotype. The features of the tremor resemble those of familial essential tremor (Dyck, 1975) and also that seen in association with a demyelinating neuropathy in patients with an IgM paraproteinaemia (Smith *et al.*, 1983; Yeung *et al.*, 1991). A recent electrophysiological analysis of the tremor of IgM paraproteinaemic neuropathy (Bain *et al.*, 1996) suggested that the tremor is the result of a distorted and mistimed peripheral input reaching a central processor, probably the cerebellum which, although intact, is misled into producing tremor in certain parts of the body. In a study using PET (Brooks *et al.*, 1992), it was found that, in patients with either essential tremor or neuropathic tremor related to IgM paraproteinaemia, there was evidence of increased activity of the cerebellar connections. It would be of interest to know whether similar changes are detectable in patients with the Roussy–Lévy syndrome.

The present study has established that the Roussy–Lévy syndrome can be a manifestation of HMSN Ia and that it may be shown by one member of a family and not another; e.g. it was shown by Case 45 but not by his father (*see* Appendix). It is of interest that tremor and ataxia dominated the early clinical picture in Case 45, so much so that he was diagnosed as having Friedreich's ataxia; however, these features gradually disappeared during adult life.

One patient with the Roussy–Lévy syndrome (Case 45; *see* Appendix) also had a hypertrophic cardiomyopathy. This may well be a chance association, but we have previously encountered another Roussy–Lévy case with cardiomyopathy (Lascelles *et al.*, 1970). Cardiac involvement, if it occurs, must be a rare feature of HMSN Ia.

An acrodystrophic neuropathy with foot ulceration was encountered in two cases. One patient had associated diabetes mellitus which may well have been responsible. The other was a member of the family of Case 26 in which prominent sensory loss in association with distal motor involvement was a regular feature and had been consistent over six



generations. In earlier generations, a diagnosis of lumbosacral syringomyelia had been made.

Case 56 was unusual in that presentation was with muscle cramps and painful 'muscle spasms'. Electrophysiological studies suggested atypical neuromyotonia, although this was not demonstrable on clinical examination. Neuromyotonia has previously been reported in HMSN II (Lance *et al.*, 1979) but not in HMSN I. The calf hypertrophy in Case 56 was presumably secondary to persistent fasciculation and recurrent muscle cramps. Calf hypertrophy was described as a consistent feature in affected members of a family with HMSN Ia related to a chromosome 17p11.2 duplication by Uncini *et al.* (1994).

It is of interest that two cases had a history of an acute paralytic illness diagnosed as an acute inflammatory neuropathy that preceded the onset of the symptoms of their HMSN. Whether these episodes are relevant is uncertain. In one of these patients (Case 46) and also another (Case 15), a diagnosis of superimposed CIDP was questioned because of the occurrence of positive sensory symptoms and an apparent response to plasma exchange in the first and to intravenous human immunoglobulin in the second. In neither was the diagnosis confirmed by nerve biopsy. Attention to an association of CIDP with HMSN was first drawn by Dyck *et al.* (1982). The occurrence of an accompanying benign monoclonal IgM kappa paraproteinaemia in Case 32, previously reported by Gregory *et al.* (1993), is probably not coincidental as two other unpublished cases of HMSN I with an IgM paraproteinaemia are known to the writers. The possible reasons for the association were discussed by Gregory *et al.* (1993).

Auditory involvement has been described as a feature of some examples of HMSN in the past (Satya-Murti *et al.*, 1979; Raglan *et al.*, 1987) but it was only present in one patient in the present series and was of only modest severity.

Associated focal peripheral nerve lesions were present in seven patients, one of whom had both the carpal tunnel syndrome and meralgia paraesthetica. Reciprocal segmental deletion at chromosome 17p11.2 results in the HNPP syndrome (Chance *et al.*, 1993) in which the characteristic histological abnormality is the presence of focal regions of myelin thickening termed tomacula (Behse *et al.*, 1972; Madrid and Bradley, 1975), although this change is not specific to HNPP. Tomacula have been described in HMSN Ib (Thomas *et al.*, 1994), this disorder being related to mutations in the gene for P<sub>0</sub> myelin protein (Hayasaka *et al.*, 1993; Kulkens *et al.*, 1993; Su *et al.*, 1993). Appearances in transverse section suggestive of tomacula were seen very occasionally on electron microscopy in the present series but they were clearly not a prominent feature. One contributory possibility for the occurrence of focal peripheral nerve lesions in HMSN Ia would be nerve enlargement, resulting in a greater liability to nerve entrapment.

Evidence for dysfunction of the corticospinal pathways was present in three patients. In one of these (Case 44), who had 'complicated' HMSN, there also was evidence of

cerebellar dysfunction with upper and lower limb ataxia in the face of normal joint position sense, interposed square wave jerks during voluntary pursuit eye movements and lack of suppression of caloric induced nystagmus. In none of the cases with pyramidal signs was there any indication of cord compression by enlarged spinal roots as may occur in some cases of hypertrophic neuropathy (Symonds and Blackwood, 1962). Many of such reported cases may well have had CIDP in which hypertrophic changes are more prominent in the spinal roots (*see* Ginsberg *et al.*, 1995). In a previous study of patients with HMSN and accompanying pyramidal signs, we found that in HMSN I but not in HMSN II central motor conduction time, assessed using magnetic stimulation of the motor cortex, was considerably prolonged, which suggested demyelination in the corticospinal pathways (Claus *et al.*, 1990). It is not known whether these patients had HMSN Ia. It has been shown that in the rat and mouse, *PMP22* mRNA expression in the CNS is restricted to spinal cord and brainstem motor neurons (Parmantier *et al.*, 1995). The nature of the changes in the CNS is therefore uncertain. The possibility has to be considered as to whether these patients could have developed an associated CIDP with multifocal CNS demyelination (Mendell *et al.*, 1987; Rubin *et al.*, 1987; Thomas *et al.*, 1987). In Case 44 there was no indication of this on MRI, and nerve biopsy in this patient showed no inflammatory infiltrates. In the other two cases with pyramidal signs MRI was not performed nor was nerve biopsy undertaken.

The severely reduced conduction velocity in the peripheral nerves in HMSN Ia is explicable in terms of the diffuse demyelinating pathology that is present. In the study by Harding and Thomas (1980*b*) a value of 38 m/s for upper limb MNCV was taken for separating types I and II HMSN. The mean value of 19.9 m/s with an upper limit of the range at 34 m/s in the present cases accords with this previous finding. It is likely that patients with intermediate values for MNCV to which attention was drawn by Davis *et al.* (1978) were examples of X-linked HMSN (Nicholson and Nash, 1993).

The reason for the occurrence of a demyelinating neuropathy in individuals who possess three copies of the *PMP22* gene is unknown. A more severe phenotype occurs if four copies are present, as in the patient reported by Lupski *et al.* (1992). Both parents were heterozygous and affected. This suggests a gene-dosage effect. Studies on *PMP22* mRNA expression in peripheral nerve biopsies are difficult to assess. Although expression has been found to be elevated (Yoshikawa *et al.*, 1994) it may vary with disease duration (Hanemann *et al.*, 1994). Nukada *et al.* (1983) found that in patients with HMSN I, myelin spiral length in the peripheral nerves was increased relative to axon size. It was concluded that this represented axonal atrophy. As *PMP22* is a myelin protein, it is more likely that the change is in myelin. Gabreëls-Festen *et al.* (1995) found that the mean *g* ratio (axon diameter/total diameter) was reduced in patients with HMSN Ia with a duplication in comparison with controls,



consistent with hypermyelination, whereas in cases with a point mutation in the *PMP22* gene, the *g* ratio was markedly elevated on almost all fibres, indicating hypomyelination.

The evolution of the hypertrophic changes in the peripheral nerves was analysed by Gabreëls-Festen *et al.* (1992). They showed that in young individuals with HMSN I, most of whom probably had HMSN Ia, active demyelination was evident and hypertrophic changes were not prominent. At later stages, active demyelination was no longer observed and hypertrophic changes with large onion bulbs had developed.

The nerve biopsy finding in the present series of cases showed a spectrum of changes ranging from the presence of exuberant onion bulbs in patients with a less severe reduction in myelinated fibre density to few onion bulbs in those with a low fibre density. The mean *g* ratio in the present series, excluding Cases 32 and 35 with an IgM paraproteinaemia and diabetes, respectively, was  $0.65 \pm 0.21$  (SEM). This finding diverges from that of Gabreëls-Festen *et al.* (1995) who obtained a mean value of  $0.56 \pm 0.04$  for their duplicated cases. This divergence is probably because of age differences. The patients examined by Gabreëls-Festen *et al.* (1995) ranged from 3.5 to 26 years whereas in the present series the span was from 13 to 53 years with a mean of 41 years. It therefore appears that in the later stages of HMSN Ia related to a chromosome 17p11.2 duplication the fibres become hypomyelinated. In view of the lack of evidence of continuing demyelination, this is probably a persistent condition and it could well represent arrested remyelination.

The progressive loss of myelinated axons in our material correlated with increasing neurological disability and the regression of the onion bulbs. As suggested elsewhere (Thomas *et al.*, 1996), it is likely that the presence of unmyelinated axons in the Schwann cells of the onion bulbs is necessary for their persistence. There is evidence that Schwann cells atrophy and ultimately disappear if they are deprived of axonal contact (Weinberg and Spencer, 1978). The origin of the nonmyelinated axons in the Schwann cell lamellae of the onion bulbs is not established but there is some evidence that they may result from collateral sprouting from the central axons (Thomas and Lascelles, 1967). They would thus disappear with loss of the central axons in the onion bulbs.

The terminology used for the hereditary neuropathies is at present highly confused. The designation HMSN was introduced (Thomas *et al.*, 1974) as a nonspecific description of these disorders with the expectation that, as their molecular basis was identified, they would be labelled in terms of the genetic defect and be given an appropriate designation. Alongside the use of the HMSN label, the gene mapping literature has employed a classification system using the abbreviation CMT. This is perhaps less appropriate for clinical usage as it is clear from the present study that a significant proportion of patients with a chromosome 17p11.2 duplication do not have the CMT phenotype. Why there should be this interesting variety of phenotypes for a uniform genetic defect is obscure. Some phenotypes may result from interacting

environmental factors and others from differences in the unaffected *PMP22* allele or in genes at other loci. Neither of these explanations is likely to apply to the family of Case 26 in which a stable phenotype persisted over at least six generations. The solution will have to await elucidation of the precise mechanism of the demyelination for which observations on transgenic mice may be informative. It is possible that some of the phenotypic variation could be attributed to the effects of other genes within the duplicated region.

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## Appendix: selected case histories

### Advanced HMSN

#### Case 30

This 47-year-old woman had first developed an abnormal gait at the age of 11 years. This gradually increased in a somewhat stepwise manner. She had to use a wheelchair from the age of 34 years. Her hands had been weak from early life but this had become more severe from the age of 30 years. Her hearing became impaired during her fourth decade. At the age of 39 years she began to experience exertional dyspnoea and breathlessness on lying flat shortly afterwards. At about the same time she developed faecal incontinence without urinary disturbances. Her condition has continued to deteriorate so that mobility is now extremely limited and her hands have become useless.

In her family history, her father was similarly but less severely affected, as had been her paternal grandfather and two paternal aunts.

Examination showed normal cranial nerve function apart from moderate sensorineural deafness. There was generalized wasting and weakness in all four limbs which was virtually total distally. Neck flexion was weak. She showed paradoxical movement of the anterior abdominal wall on respiration. Her tendon reflexes were all absent and plantar responses were unobtainable. All sensory modalities were impaired in the legs and distally in the upper limbs. She had bilateral pes cavus and mild scoliosis. Her peripheral nerves were not thickened.

Routine haematological and biochemical tests were normal. MNCV in the ulnar nerve on recording from the flexor carpi ulnaris was 6 m/s. The intrinsic hand and foot muscles were all totally denervated and no diaphragmatic response was obtained on phrenic nerve stimulation. There was evidence of denervation in the anal sphincter (Dr C. J. Fowler). Visual (full field) cortical evoked potentials were delayed bilaterally at 121 and 122 ms. On brainstem auditory evoked potential recording, wave I was absent on both sides and later waves were difficult to discern. Upper and lower limb SAPs were absent as were somatosensory cortex evoked potentials following median nerve stimulation. Investigation of respiratory function indicated global weakness of the respiratory muscles with severe diaphragmatic involvement.

### Roussy-Lévy syndrome

#### Case 45

This 52-year-old man was born with bilateral talipes equinovarus. He began to walk at the age of 2 years but was unsteady. Neurological examination (P. H. Sandifer) at the age of 5 years showed no definite muscle wasting or weakness but there was ataxia of all four limbs and a wide-based unsteady gait. His tendon reflexes were all absent and his plantar responses equivocal. There was kyphoscoliosis, bilateral pes cavus and clawing of his toes. A diagnosis of atypical Friedreich's ataxia was made. Bilateral corrective surgery was performed on his feet. Subsequently progressive limb weakness developed. When reviewed at the age of 15 years he showed an upper limb postural tremor with distal weakness and wasting, generalized lower limb wasting and weakness, also maximal distally, tendon areflexia, absent plantar responses and impaired joint position sense in his toes. An electrocardiogram was stated to be compatible with Friedreich's disease.

On annual review of his neurological state since the age of 15 years, little symptomatic change was reported apart from gradual disappearance of the upper limb tremor. The most recent examination showed normal cranial nerve function. There was diffuse upper limb wasting with distal weakness but no tremor or ataxia. He also showed paradoxical movement of the anterior abdominal wall on respiration and diffuse lower limb muscle wasting and weakness which was virtually complete for the anterolateral lower limb group. He remained areflexic with absent plantar responses. All sensory modalities were impaired distally in the lower limbs but upper limb sensation was intact apart from reduced two-point discrimination on his fingers. Peripheral nerve thickening was evident.

At the age of 46 years the patient began to experience exertional dyspnoea and episodes of transient loss of consciousness. Cardiological investigation led to a diagnosis of hypertrophic cardiomyopathy with a probable paroxysmal dysrhythmia, although the latter was not confirmed by electrocardiographic monitoring. He has since been maintained on propranolol and Warfarin and has been free of attacks of unconsciousness.

In his family history, his father was affected with a typical CMT

syndrome and had kyphoscoliosis. A paternal aunt and her daughter were also affected.

MNCV in the median nerve at the age of 12 years was 23 m/s and 15 m/s in the peroneal nerve. SAPs were absent.

### **Acrodystrophic neuropathy**

#### *Case 26*

The patient, a girl aged 16 years, began to walk at the age of 18 months, but with an abnormal gait and with difficulty in running. Weakness in her legs developed during childhood and torticollis at the age of 14 years. Examination currently shows a torticollis to the left, mild wasting and weakness of her small hand muscles, more severe weakness of the anterolateral lower leg muscles, complete tendon areflexia and flexor plantar responses. Light touch sensation and joint position sense are preserved but pinprick and deep pain sensation is lost in her feet and vibration sense is impaired on her toes. There is bilateral pes cavus. Median MNCV is 12 m/s. MRI of the head and cervical spine is normal.

The patient's mother has a CMT phenotype with loss for all sensory modalities distally in the limbs, more marked in the feet. The patient's maternal grandmother, aged 53 years when examined (case IV.3, family 4 of Thomas *et al.*, 1974), had had difficulty with running and other athletic activities as a child. For the preceding 10 years she had noticed sensory loss in her feet and in her hands for 3 years. For 10 years she had had recurrent ulceration of the ball of her right foot. Examination revealed distal wasting and weakness in all four limbs, absent tendon reflexes, flexor plantar responses and loss of all sensory modalities distally in the lower limbs. Pinprick appreciation, joint position sense and two-point discrimination in her fingers were all impaired. She showed bilateral pes cavus and clawing of her toes and a chronic ulcer on the ball of her right foot. Her peripheral nerves were thickened. MNCV was 32 m/s in the median and 18 m/s in the peroneal nerve. SAPs were absent. This patient's mother and maternal grandfather and greatgrandfather had had ulcerated feet. A maternal uncle had been diagnosed as having lumbosacral syringomyelia and had had a right amputation below the knee for recurrent foot ulceration. His son had also had recurrent foot ulcers.

### **Muscle cramps and calf hypertrophy**

#### *Case 56*

This 55-year-old man had experienced leg cramps 'for as long as he could remember'. These affected his calves and the anterior tibial muscles. He had also been aware of twitching of these muscles throughout his life. In 1986 he developed an acute illness of uncertain nature while visiting Hong Kong during which he experienced severe stiffness and pain in his limb and trunk muscles. This subsequently recurred in attacks approximately thrice weekly lasting 2–3 h on each occasion. These tended to be provoked by exercise and emotional stress and were independent of the cramps. The attacks were relieved by lying down and by diazepam. His symptoms had become considerably worse in the preceding 3 years forcing him to give up his employment. He was initially investigated for an extrapyramidal disorder, but electrophysiological studies revealed severe slowing of motor and sensory conduction. His CSF protein content was slightly elevated at 0.7 g/l. Nerve and muscle biopsy findings were stated to have been normal. There was no family history of relevance.

When reinvestigated in 1991 his symptoms were as described

above. On examination his cranial nerves were normal apart from red/green colour blindness. There was no wasting or weakness of the limb or trunk musculature. His calf muscles were hypertrophied. There was widespread sparse fasciculation and prominent postcontraction fasciculation. Myotonia was not demonstrable but muscle cramps could be readily induced. There was no ataxia. Apart from sluggish biceps jerks, his tendon reflexes were absent. The plantar responses were flexor. Tactile sensation (cotton wool) was intact and there was no definite loss for pinprick. Joint position was normal but vibration sense was impaired in the fingers and below his knees, as was two-point discrimination in his fingers. His peripheral nerves were not thickened.

Routine haematological and biochemical investigations were normal. MNCV was 34, 27, 18 and 22 m/s in the median, ulnar, peroneal and tibial nerves respectively. Median and ulnar SAPs were absent. The radial and sural SAPs had amplitudes of 6 and 7  $\mu$ V and inflexion velocities of 24 and 25 m/s. Needle electrode sampling of upper and lower limb muscles showed multifocal spontaneous fasciculations occurring singly or in pairs. No fibrillation potentials or myokymic discharges were recorded. Motor unit recruitment patterns were slightly reduced, but motor unit potentials were of normal amplitude and morphology. Voluntary contraction and muscle contraction evoked by electrical nerve stimulation was followed by short bursts of motor unit activity associated with a slight delay in muscle relaxation. This was most evident in the small hand muscles.

The patient was treated with carbamazepine which abolished both his muscle cramps and episodes of pain and stiffness.

### **'Complicated' HMSN**

#### *Case 44*

This woman, now aged 51 years, had had normal development. At the age of 12 years she had awoken one day with general malaise, weakness of her legs and inability to walk. She was admitted to hospital and a diagnosis of 'infective peripheral neuritis' was made. No details are available. She recovered fully after 6 months. She then remained well until the age of 37 years, although she had always been poor at athletic activities. She then developed weakness in her legs on exertion, associated with muscle cramps. Two years later she found that her gait was becoming increasingly unsteady. She also developed weakness and clumsiness of her hands, a slurring dysarthria and dysphagia with nasal regurgitation of fluids.

On examination at the age of 40 years, her optic fundi and pupils were normal. Ocular pursuit movements were interrupted by square wave jerks. There was mild bilateral facial weakness, limited palatal and tongue movement and a nasal dysarthria. Brisk jaw and pout reflexes were elicited. There was distal wasting and weakness in all four limbs, mild postural tremor in the upper limbs, bilateral finger–nose and heel–shin ataxia and an ataxic gait. She had brisk biceps and brachioradialis tendon reflexes, depressed triceps jerks and absent lower limb tendon reflexes. Both plantar responses were extensor. The appreciation of light touch and pinprick was reduced distally in her feet and vibration sense was impaired on her toes. Joint position sense was intact. Her peripheral nerves were not enlarged and there was no skeletal deformity.

Extensive haematological and biochemical screening tests, including white cell enzyme studies, were negative. MNCV in the median, ulnar, peroneal and tibial nerves was 31, 23, 15 and 20 m/s respectively. SAPs were absent. The CSF protein concentration was 0.62 g/l but was otherwise normal, including IgG fractionation.

Electrocardiogram, EEG, VEPs, brainstem auditory evoked potentials and cranial MRI were normal. Upper limb stimulation revealed delayed somatosensory cortex responses but it was not possible to state whether central conduction was prolonged. On neurotological testing, electronystagmography confirmed the presence of square wave jerks during pursuit movements and failure of suppression of caloric-induced nystagmus was demonstrated, indicating a derangement of cerebellar control of visuovestibular interaction. Audiometry was normal. Denervation hypersensitivity of the pupils was shown by pilocarpine instillation. Quadriceps muscle biopsy revealed chronic partial denervation with reinnervation.

The patient has subsequently continued to deteriorate slowly with the development of increasing dysarthria and dysphagia, weakness of neck flexion and of the diaphragm and more severe limb weakness. The upper limb tendon reflexes have become absent. Tactile and pinprick sensory loss has also developed in the hands, but joint position sense remains normal in the fingers and toes.

In the family history, the patient has four brothers. All are asymptomatic. One aged 33 years when examined was normal apart from generally depressed tendon reflexes. MNCV was 24 and 29 m/s in the ulnar and peroneal nerves, respectively. Median and sural SAPs were absent and the ulnar SAP was 2  $\mu$ V in amplitude.

DNA testing confirmed a chromosome 17p11.2 duplication. The patient's mother, aged 60 years when examined, had had 'life long' weakness of her legs. She had never been able to run. Examination showed light-near dissociation of pupillary responses, jerky ocular pursuit movements and brisk jaw and pout reflexes. There was distal wasting and weakness in all four limbs, lower limb ataxia, generally absent tendon reflexes and flexor plantar responses. Light touch and pinprick sensation was reduced below midforearm and knee level. There was bilateral pes cavus. Her nerves were not enlarged. MNCV was 26 and 12 m/s in the ulnar and peroneal nerves, respectively, and SAPs were absent. The patient's father was normal on clinical and electrophysiological examination. A maternal aunt, aged 57 years when examined, had never been able to run but otherwise had had no neurological disability. Her pupils were slightly irregular with light-near dissociation and her ocular pursuit movements were jerky. She showed jaw and pout reflexes, mild weakness of the intrinsic hand muscles and of the anterolateral lower leg muscles, absent lower limb tendon reflexes and flexor plantar responses. Tandem walking was impaired. Pinprick sensation was lost to midforearm and knee level and vibration sensation was lost below the iliac crests. There was no skeletal deformity or nerve enlargement.