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Hereditary motor and sensory neuropathy—Lom, a novel demyelinating neuropathy associated with deafness in gypsies

Clinical, electrophysiological and nerve biopsy findings

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Summary

A previously unrecognized neuropathy was identified in Bulgarian gypsies, and was designated hereditary motor and sensory neuropathy-Lom (HMSNL) after the town where the initial cases were found. It was subsequently identified in other gypsy communities. The disorder, which is of autosomal recessive inheritance, was mapped to chromosome 8q24. It begins consistently in the first decade of life with gait disorder followed by upper limb weakness in the second decade and, in most subjects, by deafness which is most often first noticed in the third decade. Sensory loss affecting all modalities is present, both this and the motor involvement predominating distally in the limbs. Skeletal deformity, particularly foot deformity, is frequent. Severely reduced motor nerve conduction velocity indicates a demyelinating basis, which was confirmed by nerve biopsy. The three younger patients biopsied showed a hypertrophic 'onion bulb' neuropathy. The hypertrophic changes were not evident in the oldest individual biopsied and it is likely that they had regressed secondarily to axon loss. In the eight cases in which brainstem auditory evoked potentials could be recorded, the results suggested demyelination in the eighth cranial nerve and also abnormal conduction in the central auditory pathways in the brainstem. As no myelin genes are known to be located at chromosome 8q24, the disorder may involve a gene for a novel myelin protein or be due to an abnormality of axon–Schwann cell signalling.

Keywords: hereditary motor and sensory neuropathy—Lom; deafness; demyelinating neuropathy

Abbreviations: BAEP = brainstem auditory evoked potential; HMSN = hereditary motor and sensory neuropathy; HMSNL = hereditary motor and sensory neuropathy—Lom

Introduction

Hereditary motor and sensory neuropathy (HMSN) has increasingly been demonstrated to be genetically heterogeneous. Considerable advances in knowledge have been made in recent years as to the molecular genetics of autosomal dominant demyelinating HMSN (Harding, 1995). Examples with autosomal recessive inheritance (Harding and Thomas, 1980*a*) are less common but these are now beginning to be defined (Gabreëls-Festen *et al.*, 1992). One variety, identified in families in Tunisia (CMT 4A), has been mapped to chromosome 8q11–21.1 (Ben Othmane *et al.*, 1993, 1995). It is characterized by the presence of basal laminal onion bulbs on nerve biopsy. Another variety is recognized morphologically by the finding of focally folded myelin sheaths (Ohnishi *et al.*, 1989; Gabreëls-Festen *et al.*, 1990).

Linkage to chromosome 11q23 has been demonstrated in one family (Bolino *et al.*, 1996) but excluded in another. An example of a mutation in the gene for peripheral myelin protein 22 considered to show autosomal recessive inheritance is on record (Roa *et al.*, 1993), although this has subsequently been questioned (Nelis *et al.*, 1997). In two recently described families from Algeria with autosomal recessive demyelinating neuropathy (Kessali *et al.*, 1997) the disorder has been mapped to chromosome 5q23-q30 (LeGuern *et al.*, 1996). Patients from other kinships with presumed autosomal recessive inheritance who exhibit classic onion bulbs (Thomas *et al.*, 1996) still require genetic delineation.

We have recently reported a novel autosomal recessive demyelinating neuropathy associated with deafness in Bulgarian gypsies. As the first cases were identified in the town of Lom, the disorder was designated hereditary motor and sensory neuropathy—Lom (HMSNL). Using lod scores and linkage disequilibrium, the disease has been mapped to chromosome 8q24 (Kalaydjieva *et al.*, 1996). In the present communication, we document the clinical and electrophysiological features of the disorder and the findings on nerve biopsy.

Case ascertainment and investigation

The disorder was originally recognized in individuals living in the gypsy community of Lom in northwest Bulgaria by the late Dr Dimiter Abadjiev. In the present study, the cases were ascertained through hospital records and interviews with numerous members of the community, both from affected and unaffected families. A second cluster comprising three kindreds was then identified in an area 230 km east of Lom. During the period 1993–97 we identified affected gypsy families in several different European countries. This report is based on observations on 35 affected subjects and 10 carriers from these two areas in Bulgaria. A full neurological examination was performed in each individual. Observations are also included on a gypsy family from northern Italy with an identical syndrome affecting four members of a single sibship.

Electrophysiological investigations

Nerve conduction studies were undertaken in 23 affected individuals using standard techniques. Motor nerve conduction was examined in the ulnar, peroneal and tibial nerves in 20 patients and in the median nerve in seven. It was also examined in the facial nerve in 14 subjects and in the axillary and musculocutaneous nerves in 13. Sensory conduction was studied in 21 individuals. Nerve conduction studies were performed in 11 of the 12 carriers.

Auditory function tests and brainstem auditory evoked potential (BAEP) recordings were undertaken in 19 affected patients and nine carriers. The BAEPs were recorded using click stimuli at 80 dB hearing pressure level with the amplifier band frequency set at 200–2000 Hz.

Sural nerve biopsy

Sural nerve biopsy specimens were obtained with informed consent under local anaesthesia from a standard site posterior to the lateral malleolus. The specimens were fixed in 3% glutaraldehyde in PIPES [piperazine-*N*-*N*′-bis(2-ethane sulphonic acid)] buffer (Baur and Stacey, 1977) with 2% sucrose. After postosmication in 1% osmium tetroxide in PIPES containing 1.5% potassium ferricyanide, the specimens were dehydrated through increasing concentrations of ethanol and embedded in Durcupan via 1,2-epoxypropane. Semithin sections were stained with thionin and acridine orange (Sievers, 1971) for examination by light microscopy. Ultrathin sections were contrasted with lead citrate and uranyl acetate and examined in a Zeiss EM902C electron microscope (Zeiss, Oberkochen, Germany).

Morphometry

The myelinated nerve fibre size distribution was assessed on transverse sections stained with thionin and acridine orange using a Kontron IBAS AT image analysis system (Image Associates, Thame, UK). Measurements were made of axon diameter and total fibre diameter, i.e. to the external border of the myelin sheath. Unmyelinated axon density was assessed visually in montages of electron micrographs of transverse sections through the biopsy specimen at a final magnification of $\times$10 000.

Results

Clinical features of affected individuals

Pregnancy and birth were normal in all affected patients, 19 of whom were male and 20 female. The initial symptom in each was disturbance of gait. Walking began at the age of 12–15 months in 30 patients, at 18 months in six and at 24 months in one. Two patients never walked normally from the outset although they began at a normal age. The onset of disordered gait in the remaining 33 patients ranged from 5 to 10 years with a mean ($\pm$ SEM) of 6.4 $\pm$ 1.04 years. Difficulty in using the hands became evident later, between the ages of 5 and 15 years (mean 12.7 $\pm$ 1.27 years). A complaint of hearing loss was made by 25 patients, the onset being between the ages of 13 and 26 years (mean 22.7 $\pm$ 1.66 years). The 14 without subjective hearing loss ranged in age from 7 to 48 years with a mean of 23.2 $\pm$ 1.89 years. Apart from the hearing loss there were no symptoms of cranial nerve dysfunction. Bladder and bowel functions were normal.

On neurological examination in the Bulgarian subjects, the cranial nerves were normal except for the presence of horizontal nystagmus in two patients, an impaired pupillary reaction to light in two, hearing loss (see later), and muscle atrophy in the tongue in nine. There was distally accentuated lower limb weakness in all patients, which was severe in all except the younger individuals. It was usually but not consistently associated with muscle wasting. Seven patients,

aged 26, 27, 35, 38, 39, 40 and 50 years, were unable to walk. Less severe distal weakness and wasting was evident in the upper limbs. There was no tremor or ataxia. The lower limb tendon reflexes were absent in all except three individuals who had absent ankle jerks but retained knee jerks. The upper limb tendon reflexes were mildly reduced in seven subjects but were depressed or absent in the remainder. The plantar responses were flexor or unobtainable. Sensation was impaired distally in the limbs, with greater involvement of the lower limbs, in all except three subjects, aged 9, 13 and 19 years. All sensory modalities were affected. On neurological examination in the three older Italian patients, aged 15, 13 and 11 years, there was distal muscle wasting and weakness in all four limbs, which was marked in the oldest sibling. The youngest child, aged 7 years, showed mild distal weakness in the upper and lower limbs. The tendon reflexes were absent in the legs in all four siblings. Sensory examination was limited because of poor cooperation, but in all four there was distal loss of pain sensation in the legs.

Skeletal deformities were frequent. Out of the 39 patients, pes cavus was present in 19 and clawing of the toes in 14 and of the fingers in 17. Scoliosis was observed in eight patients.

Nerve conduction studies

Motor nerve conduction velocity was severely and diffusely reduced. In the ulnar nerve the mean value was 9.6 ± 1.39 (SEM) m/s with increased distal motor latency (23.2 ± 1.86 ms). The corresponding values for the median nerve were 13.2 ± 1.76 m/s and 14.8 ± 1.77 ms. Lower limb motor conduction was not assessed in the Italian patients and could only be recorded in the youngest Bulgarian subject, aged 9 years. The values for this subject for peroneal and tibial motor nerve conduction velocity were 20.4 and 18.0 m/s with distal latencies of 7.0 and 8.1 ms, respectively. The latency of the evoked response in the facial nerve was also increased, with a mean (± SEM) value of 14.9 ± 0.68 ms (normal value is <4 ms). The same was true for the axillary nerve on recording from the deltoid (15.6 ± 0.72 ms) and from the musculocutaneous nerve on recording from biceps brachii (18.9 ± 0.74 ms) (normal value is <3.5 ms for both nerves). Median, ulnar and sural sensory potentials were unobtainable in all Bulgarian subjects. In an Italian patient aged 11 years median sensory conduction velocity was 8 m/s (finger–wrist) and 14 m/s (wrist–elbow).

Auditory function

Hearing was impaired in 15 out of the 19 individuals tested. On audiometry the loss was greater in the 1500–3000 and >4000 Hz bands than in the <1000 Hz range. The subjects with normal audiometric findings were aged 9, 20, 26 and 39 years. The hearing loss was of sensorineural type in all except 1 of the Bulgarian patients, in whom there was an additional conduction defect. In the Italian patients, the youngest patient, aged 7 years, had a pure sensorineural loss. The other three, aged 11, 13 and 14 years, showed a mixed pattern bilaterally, the conduction component being indicated by the loss of the stapedius reflex.

An attempt was made to record BAEPs in 19 patients. For the 15 Bulgarian patients they were undetectable in 11. The mean values ± SEM for the individuals in whom recordings were obtained, combining the right and left sides, were as follows: wave I latency, 2.16 ± 0.92 ms (normal 1.56 ms); wave III latency, 4.80 ± 1.22 ms (normal 3.46 ms); wave V latency, 7.55 ± 1.40 ms (normal 4.94 ms); I–V interpeak latency, 5.39 ± 0.38 ms (normal 4.15 ms). In the four Italian siblings the mean values for latency (± SEM), again combining the right and left sides, were as follows: wave I, 2.47 ± 1.0 ms [normal 1.6 ± 0.09 (SD) ms]; wave III, 5.35 ± 1.39 ms (3.8 ± 0.15 ms); wave V, 8.02 ± 1.43 ms (5.6 ± 0.8 ms); wave I–V latency, 5.48 ± 1.25 ms (4.0 ± 0.21). All these values were increased in the patients. The detailed results are given in Table 1A and B.

Nerve biopsy findings

Sural nerve biopsy specimens were obtained from four individuals, aged 11, 17, 28 and 32 years. All four specimens showed a severe depletion in the myelinated nerve fibre population, even in the youngest patient (Fig. 1). Fibre density was 1192, 1310, 878 and 302/mm^2, respectively. The normal range is 7500–10 000/mm^2 (Jacobs and Love, 1985). The surviving fibres were of small size (Fig. 2). Relative myelin thickness was assessed by the *g* ratio, i.e. axon diameter/total fibre diameter. The proportion of fibres with a *g* ratio >0.7 was increased in all four patients, being 35.4, 35.6, 24.7 and 33.3%, due to loss of the thickly myelinated fibre population. For normal subjects the mean value is 7.3 ± 0.53% (Thomas *et al.*, 1997*a*). The proportion with a *g* ratio <0.4 was 1.33, 3.32, 13.14 and 0% (normal 11.0 ± 1.4%). There was therefore no indication of significant hypermyelination. The density of unmyelinated axons was 77 856, 33 632, 35 664 and 120 756/mm^2, respectively. The range for control subjects is 28 000–33 000 (Llewelyn *et al.*, 1991). It is not possible to state the relative proportions of surviving unmyelinated axons and non-myelinated regenerating sprouts for the patients studied here. The increased densities observed in the HMSNL patients are probably partly attributable to the latter and partly to the severe loss of myelinated nerve fibres.

No active myelinated fibre degeneration or demyelination was detected, but myelin debris was occasionally observed in Schwann cells (Fig. 3), indicating recent demyelination or fibre degeneration.

Hypertrophic changes were present in the biopsy specimens from the three younger subjects. These specimens displayed multiple ‘onion bulbs’; they were poorly developed except in the youngest individual (Figs 4 and 5). They consisted of concentrically proliferated Schwann cells around central

Table 1 *Results for brainstem auditory evoked potentials (ms)*

Age (years)	Side	Latencies			Interpeak latencies		
		Wave I	Wave III	Wave V	I–III	III–V	I–V
(A) Bulgarian families							
9	R	2.32	4.84	8.10	2.50	3.26	5.76
	L	2.14	4.58	7.66	2.44	3.08	5.52
13	R	2.20	5.26	7.76	3.86	2.48	5.54
	L	2.66	4.98	7.70	2.34	2.72	5.06
29	R	1.98	4.46	7.38	2.48	2.92	5.40
	L	1.52	–	7.38	–	–	5.86
25	R	2.32	4.74	7.16	2.42	2.42	4.84
	L	2.14	4.72	7.24	2.56	2.54	5.10
Carriers							
33	R	1.54	3.56	5.46	2.02	1.90	3.92
	L	1.56	3.54	5.24	1.98	1.70	3.68
57	R	1.56	3.46	4.94	1.90	1.48	3.38
	L	1.60	3.60	5.10	2.00	1.48	3.48
32	R	1.60	3.60	5.60	2.00	2.00	4.00
	L	1.60	3.66	5.70	2.06	2.04	4.10
49	R	1.78	3.66	5.76	1.88	2.10	3.98
	L	1.86	3.82	5.60	1.94	1.88	3.82
19	R	1.54	3.50	5.18	1.96	1.68	3.64
	L	1.58	3.42	5.10	1.84	1.66	3.50
24	R	1.64	3.48	5.46	1.84	1.98	3.82
	L	1.62	3.36	5.18	1.74	1.82	3.56
31	R	1.70	3.94	5.72	2.22	1.88	4.02
	L	1.74	4.08	5.88	2.34	1.72	4.06
Control values		1.56	3.46	4.94	2.20	1.95	4.15
(B) Italian family							
14	R	2.96	7.04	8.84			5.88
	L	–	–	8.44			–
13	R	3.06	–	7.76			4.70
	L	2.42	–	7.38			4.96
11	R	3.72	–	8.00			5.46
	L	2.20	4.56	8.20			6.00
7	R	2.10	5.08	7.90			5.80
	L	2.04	4.72	7.62			5.58
Control values (± SD)		1.6 ± 0.09	3.8 ± 0.15	5.6 ± 0.18			4.0 ± 0.21

R = right ear; L = left ear; – = no detectable potential.

myelinated axons or Schwann cell processes associated with unmyelinated axons. The Schwann cell laminae of the onion bulbs were frequently associated with unmyelinated axons. Particularly in the biopsy from the 28-year-old patient, many of the onion bulbs appeared to be regressing, with no central axon and a limited number of concentrically arrayed Schwann cell layers (Fig. 6). In the oldest patient definite onion bulbs were no longer evident. Groups of Schwann cells were present without an obvious concentric arrangement (Fig. 7). These were associated with unmyelinated axons or, infrequently, small myelinated axons.

No abnormal axonal or Schwann cell inclusions were encountered. All four biopsy specimens showed extensive endoneurial collagenization (Fig. 6). In the three older patients, many of the endoneurial blood vessels were surrounded by multiple layers of reduplicated basal lamina. The perineurium showed no noteworthy abnormalities.

Carriers

Twelve obligate carriers were examined. All were asymptomatic neurologically and none showed muscle wasting, weakness or sensory loss, including both parents from the Italian family. Of the Bulgarian carriers, one showed depressed knee jerks, absent ankle jerks and extensor plantar responses, and another had absent ankle jerks. Two others were recorded as having depressed lower limb tendon reflexes; their plantar responses were flexor. Motor and sensory conduction was performed on 10 of these individuals, including the Italian parents and the Bulgarian man with

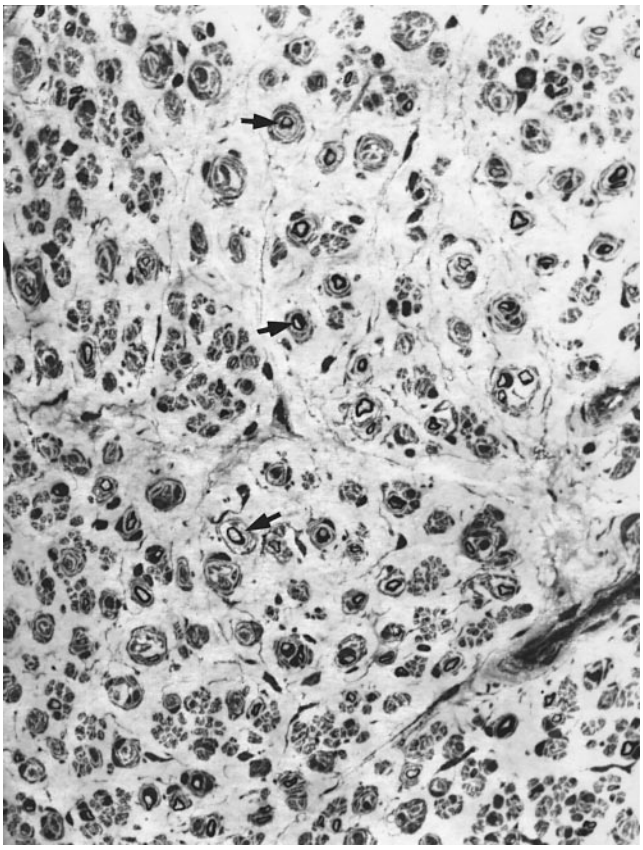

Fig. 1 Transverse semithin section of sural nerve from a male with HMSNL aged 11 years showing severe loss of myelinated axons and multiple onion bulbs (arrowed). Thionin and acridine orange, ×400.

extensor plantar responses. Normal findings were obtained in all, except for the failure to obtain a sural sensory action potential in one Bulgarian man who was entirely normal on neurological examination. BAEPs were obtained in nine individuals, again including the Italian parents, in all of whom values were normal

Discussion

The clinical picture of HMSNL is characterized by a neuropathy that consistently begins with gait disorder in the first decade of life, followed by upper limb involvement in the second decade. Motor involvement is greater than sensory, and both predominate distally in the limbs. All sensory modalities are affected. In conformity with the early onset, foot deformity is frequent, being present in 49% of the individuals included in the present series. Scoliosis was evident in 21%. Motor disability from weakness can be incapacitating, but ataxia and acrodystrophic changes were not encountered. Sensorineural deafness was present in most of the older subjects in the present study, and usually developed in the third decade but occasionally the second. Autonomic dysfunction was not observed apart from a sluggish pupillary response to light in two subjects.

Hearing loss occurs occasionally in patients with either HMSN I or HMSN II (Harding and Thomas, 1980*b*; Koussef *et al*., 1982; Raglan *et al*., 1987) and this can sometimes be the presenting feature (Hamiel *et al*., 1993). Neurootological investigation has indicated that this is the result of VIII nerve dysfunction. Deafness has also been reported in families with presumed autosomal recessive inheritance (Bouldin *et al.*, 1980; Kessali *et al.*, 1997) although, as pointed out by Kessali *et al.* (1997), autosomal recessive deafness is common and

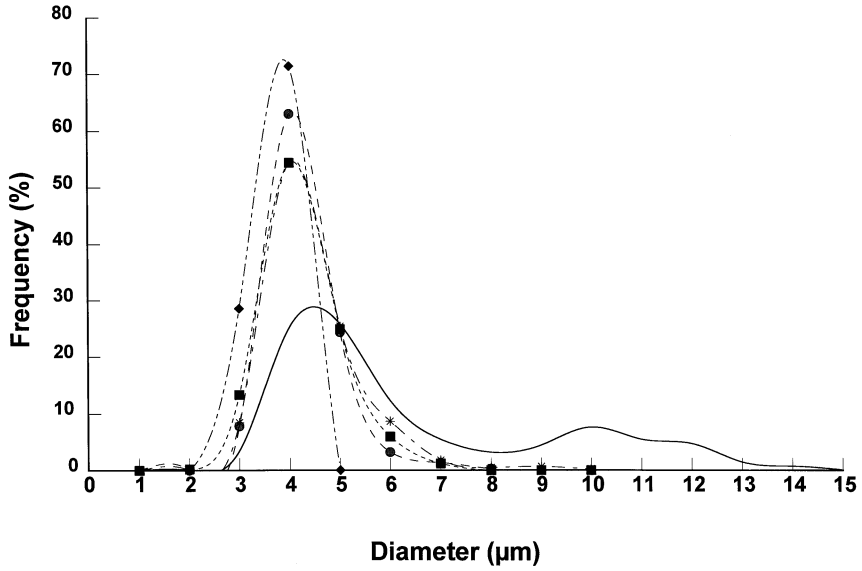

Fig. 2 Size–frequency distribution for myelinated nerve fibres in the sural nerve of patients with HMSNL aged 11 years (stars), 17 years (closed squares), 28 years (closed circles) and 32 years (closed diamonds) and for a control subject aged 29 years (continuous line). The large fibre peak has been lost in these patients.

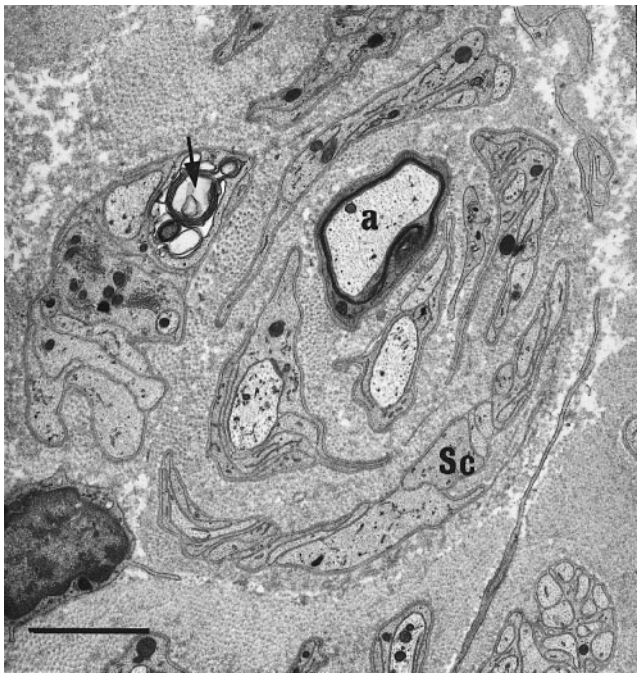

Fig. 3 Male aged 17 years. Electron micrograph of transverse section through small onion bulb consisting of concentrically arranged Schwann cell processes (Sc) surrounding a central axon (a). Its thin myelin suggests that it is in the process of remyelination. Myelin debris (arrow) is present in an adjacent Schwann cell process. Bar = 2 μm.

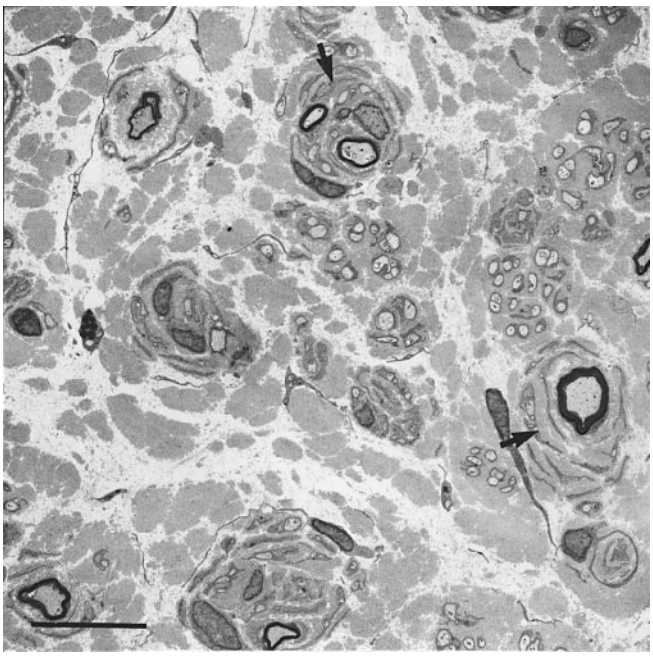

Fig. 4 Low-magnification electron micrograph of a transverse section through the sural nerve of a patient aged 11 years showing several onion bulbs (arrows) consisting of concentrically proliferated Schwann cell processes and associated axons. Bar = 5 μm.

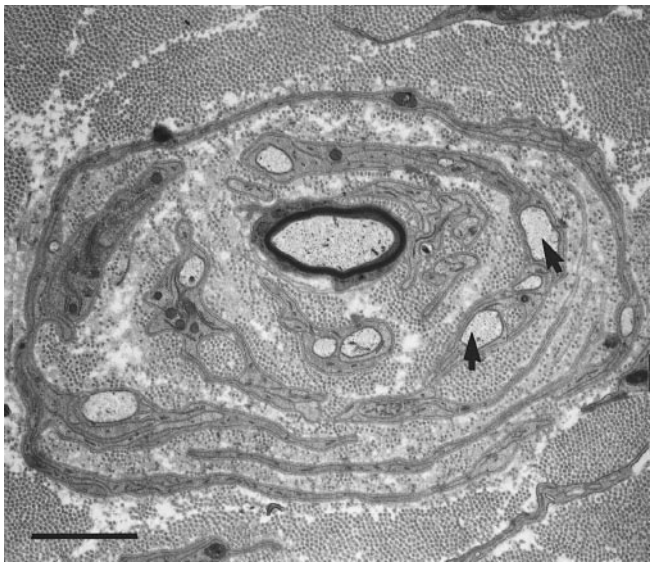

Fig. 5 Higher magnification electron micrograph from the specimen shown in Fig. 4. A central thinly myelinated axon is surrounded by a concentric array of Schwann cell processes with which are associated unmyelinated axons (arrows). Bar = 5 μm.

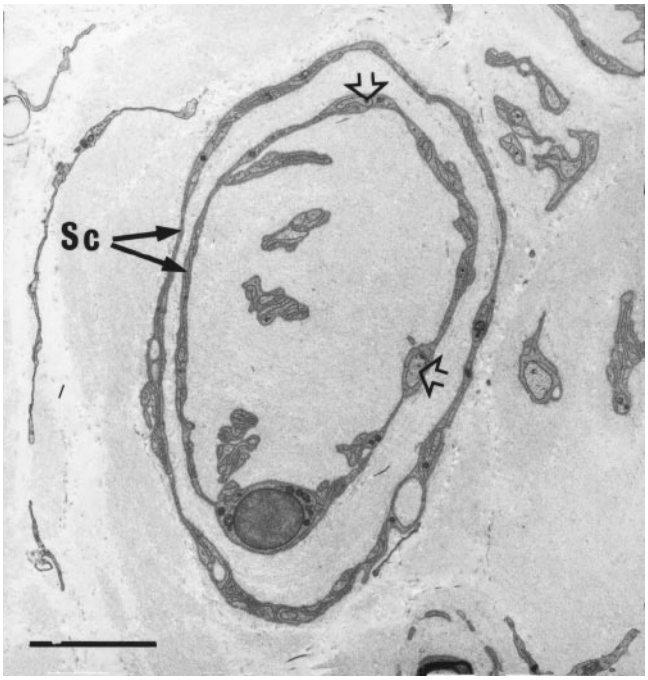

Fig. 6 Female aged 28 years. Electron micrograph through a regressing onion bulb from which the central axon has been lost. Two circular layers of Schwann cell processes (Sc) are present, associated with small unmyelinated axons (open arrows). Bar = 5 μm.

the possibility of a chance association arises. It is of particular interest that a demyelinating neuropathy of autosomal recessive inheritance with associated deafness has been described in an Indian family in South Africa (Cornell *et al.*, 1984).

Deafness has been documented in more complex disorders of autosomal recessive inheritance: in combination with optic atrophy and a distal sensorimotor neuropathy (Rosenberg and Chutorian, 1967) or with optic atrophy and distal amyotrophy (Iwashita *et al.*, 1970). Hearing loss also occurs in an X-linked

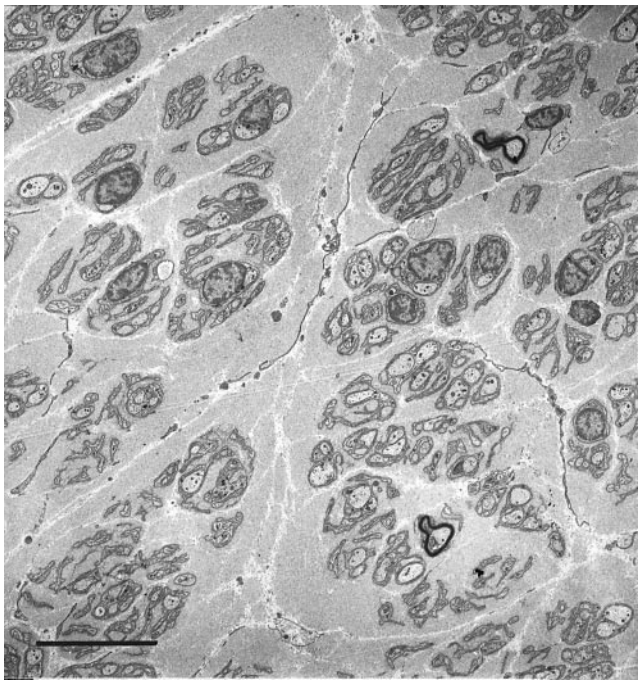

Fig. 7 Survey electron micrograph of transverse section through sural nerve biopsy from a male aged 32 years. Groups of Schwann cells are present associated with numerous unmyelinated axons. Only two myelinated axons are evident in this field. There is extensive endoneurial collagenization. Bar = 10 μm.

neuropathy accompanied by mental retardation (Cowchock *et al.*, 1985; Fischbeck *et al.*, 1986), mapped to the pericentromeric region of the chromosome.

In all our HMSNL patients with hearing loss, this was of sensorineurial type, although in one Bulgarian patient and two Italian patients there was an additional conductive component; in the latter this was evidenced by loss of the stapedial reflex. Loss of this reflex could be explained either by fixation of the stapes or by a conduction abnormality affecting the acousticofacial reflex.

The results of BAEP recordings that we obtained require comment. The generators for the different components of the human BAEP are presumed to be in the distal eighth nerve for wave I, the proximal eighth nerve for wave II, the lower pons for wave III, the mid or upper pons for wave IV, and upper pons or inferior colliculus for wave V (Chiappa, 1990). Observations on BAEPs in HMSN have been recorded in a number of studies. Satya-Murti *et al.* (1979) reported the results for BAEPs in two brothers with HMSN without deafness or abnormalities on conventional audiometric testing but who showed increased wave I–III interpeak latencies. Peaks IV and V were not clearly identifiable. The classification of the neuropathy is uncertain. Motor nerve conduction velocity was modestly reduced in one brother but was within normal limits in the other. Scaioli *et al.* (1992) examined 31 HMSN I patients and 11 with HMSN II. The HMSN II patients showed no consistent increase in absolute latencies and only minor abnormalities in I–III interpeak latencies. For the HMSN I patients, wave I latency was increased unilaterally or bilaterally in nine patients and I–III interpeak latency was increased in one; two patients had borderline wave III–V latencies. It was considered that the salient abnormality was an increase in wave I latency, consistent with eighth nerve demyelination, as was concluded by Nicholson and Corbett (1996) in 17 patients with HMSN Ia.

In the eight patients with HMSNL for which BAEP recordings could be obtained, not only was the latency of wave I increased but the same was true for the interpeak latencies. This suggests that the central auditory pathways in the brainstem may also be affected, although observations on larger numbers of patients are needed. It is also of interest that in X-linked HMSN related to connexin 32 mutations, although wave I BAEP latency is normal, wave I–V interpeak latency is increased, here suggesting involvement that is confined to the central auditory pathways (Nicholson and Corbett, 1996). In a single patient from a family with autosomal dominant HMSN, of a type not defined, who had hearing loss, the major defect involved the central auditory pathways (Musiek *et al.*, 1982).

The severe reduction in motor nerve conduction velocity in HMSNL demonstrates that the neuropathy is demyelinating in nature. This affected the nerves to both distal and proximal muscles. Demyelination was confirmed by the nerve biopsy findings. It was accompanied by a profound loss of axons even in the youngest subject biopsied, aged 11 years. The neurological disability can be attributed to axonal loss as conduction block is not a feature of hereditary demyelinating neuropathies (Lewis and Sumner, 1982), apart from hereditary neuropathy with liability to pressure palsies.

Demyelination can be primary, related to a disturbance affecting Schwann cell function, or secondary to axonal atrophy. It is presumably of the primary type in HMSNL as no indication of hypermyelination, reflecting axonal atrophy, was detected. Nevertheless, it will be important to study nerve pathology at earlier stages of the disorder. In patients with HMSN Ia resulting from a chromosome 17p11.2 duplication, hypermyelination is evident in younger patients (Gabreëls-Festen *et al.*, 1995), this possibly being a gene dosage effect since such patients possess an extra copy of the gene for PMP22. In patients with point mutations of this gene nerve biopsies show hypomyelination (Gabreëls-Festen *et al.*, 1995), which appears to be the situation in older patients with a chromosome 17p11.2 duplication (Thomas *et al.*, 1997*a*).

In the oldest patient in the present study to have a nerve biopsy, aged 32 years, regression of the onion bulbs had taken place. The hypertrophic changes were most prominent in the youngest patient, who was aged 11 years, and present but less obvious in those aged 17 and 28 years. Onion bulb regression is likely to be the result of progressive axonal loss. Experimentally, Schwann cells that are deprived of axonal contact atrophy and ultimately disappear (Weinberg and Spencer, 1978). Regression of onion bulbs is observed in patients with HMSN Ia with severe axonal loss (Thomas

et al., 1997*a*) or if there is superimposed axonal loss for some other reason such as diabetes (Thomas *et al.*, 1997*b*). The explanation for axonal loss in demyelinating neuropathies is unknown, but it is a question of considerable importance. Loss of trophic support from Schwann cells is one possibility (Friedman *et al.*, 1996).

The reduplication of the basal lamina around endoneurial microvessels seen in the present patients is of interest. This is presumably a secondary manifestation. It is also observed in HMSN Ia (Bradley *et al.*, 1990).

Six parental couples who were parents of HMSNL patients were examined in detail in the present study, and were shown to have normal nerve conduction and BAEPs, which supports autosomal recessive inheritance. The explanation for the reflex abnormalities found on examination in two Bulgarian carriers is uncertain as in neither carrier were there accompanying abnormalities of nerve conduction. They were not investigated further.

Genetic analysis of HMSNL indicated autosomal recessive inheritance, and the gene responsible was mapped to a narrow interval on chromosome 8q24 (Kalaydjieva *et al.*, 1996). No myelin genes are known to be located at this site. It will be important to discover whether a mutation in a gene for a novel myelin protein is involved. Alternatively, as myelination is determined by particular sets of axons (Aguayo *et al.*, 1976; Weinberg and Spencer, 1976) the disorder could involve a disruption of axon–Schwann cell signalling. Linkage to approximately the same region on chromosome 8 was reported by Ionasescu *et al.* (1995) in a black American family with a dominantly inherited neuropathy described as having Dejerine–Sottas neuropathy. This suggests that allelic mutants at the same locus could be responsible for different demyelinating phenotypes with either a recessive or a dominant mode of inheritance.

All HMSNL patients detected to date are of gypsy origin. The gypsies in the area of Lom, where the disease was originally identified, arose from a small colony that originated in Macedonia and settled in Lom in 1886. They belonged to the Djambazi, an ethnonym meaning ‘trading in horses’. The largest numbers of this particular gypsy group still live in Macedonia and Serbia and are believed to have migrated there in the 17th century, from the neighbouring Wallachian kingdom (present day Romania) (Marushiakova and Popov, 1993). The exodus of gypsies from India has been dated to around 1000 AD, probably as small nomadic bands travelling west (Kenrick and Paxton, 1972). During the 13th and 14th centuries, a mass settlement in the Balkans took place (Marushiakova and Popov, 1993) where the vast majority of European gypsies remained concentrated until the end of the 17th century. Currently there are several million gypsies living in the Balkan countries and the largest numbers of HMSNL patients will probably be found there. However, it is already clear that the disorder has spread through gypsy migrations and also occurs in gypsy groups across Europe (A. Urtizberea and T. Tunon, personal communications). Haplotype and linkage disequilibrium analyses suggest that HMSNL is caused by a single mutation that arose before the divergence of gypsy groups (Kalaydjieva *et al.*, 1996). A preliminary estimate of the age of the mutation (Kalaydjieva *et al*., 1996) indicates that it may have originated prior to the migration out of India. At present there are no proper epidemiological data as to the prevalence of HMSNL in gypsy communities, but preliminary evidence suggests that the numbers are in excess of those of any autosomal recessive HMSN so far described.

In conclusion, we have described the clinical, electrophysiological and nerve biopsy findings for a novel demyelinating neuropathy, HMSNL, in gypsies. It has an early age of onset, is regularly associated with deafness and frequently leads to incapacity by middle life. This is reflected in the severe loss of myelinated axons evident in nerve biopsies even at young ages, which probably leads to the regression of the hypertrophic changes observed initially. The biopsy from the patient aged 32 years no longer resembled that of a demyelinating neuropathy. HMSNL appears to be widespread in gypsy communities but further epidemiological data are required.

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