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Demyelinating hereditary neuropathies in children: a morphometric and ultrastructural study

F. Guzzetta[1], J. Rodríguez[2], M. Deodato[1], A. Guzzetta[1] and G. Ferrière[3]

[1]Istituto di Neuropsichiatria Infantile, Università di Messina, Policlinico Universitario, Messina, Italy, [2]Departamento de Pediatria, Universidad de Valparaiso, Chile and [3]Unité de Neurologie du Développment, Service de Neurologie Pédiatrique, Université Catholique de Louvain, Medical School, Bruxelles, Belgium

Summary. Twenty-three cases of hereditary demyelinating neuropathies are reported, 13 with different types of hereditary motor and sensory neuropathy (HMSN) and 9 with globoid cell or meta-chromatic leucodystrophies. Ultrastructural and morpho-metric studies showed some critical pathological features emphasizing: 1) the variability of the recessive forms of HMSN; 2) the morphological distinction between HMSN type I and type III; and 3) differences between these types of HMSN and other «onion bulb» neuropathies such as those found in leucodystrophies, which account for distinct underlying mechanisms.

Key words: Child neuropathies, Demyelinating neuropathies, Hereditary neuropathies, Peripheral nerves

Introduction

There is evidence that peripheral nerve demyelination is the effect of a failure of cell communication between axon and Schwann cell (Weinberg and Spencer, 1976; Allt and Gabriel, 1982; O'Neil and Gilliatt, 1987; Lemke, 1988; Lemke and Chao, 1988). Nevertheless, the underlying biochemical mechanism, namely in neuropathies without known metabolic etiology, is still obscure. Some authors have shown that demyelination can occur without axonal degeneration either in experimental studies (Shimono et al., 1978; Dyck et al., 1981) or in hereditary human neuropathies (Dyck et al., 1979; Nordborg et al., 1984); there is also evidence that axon degeneration can secondarily produce demyelination (Dick, 1975; Raine, 1977). Recently, molecular genetic studies have given new insights into the understanding of axon-Schwann cell relationship stressing the role of peripheral myelin proteins in the pathogenesis of hereditary peripheral neuropathies (Suter and Patel, 1994).

There is a marked pathological variability among the different types of genetic demyelinating neuropathies. In the case of absence of specific metabolic or histological markers (HMSN type I and III), this variability has been used either to demonstrate clinical differences betweeen them (Ouvrier et al., 1987) or to exclude them (Nordborg et al., 1984). The latter hypothesis seems to contrast with genetic advances that have shown a heterogeneity even if we consider only type I HMSN (Defesche et al., 1990).

In the present study we analyze a series of demyelinating HMSN with onset in childhood, and compare them with other demyelinating genetic neuropathies with known metabolic etiology, in an attempt to better define the pathological characteristics of demyelination.

Offprint requests to: Prof. F. Guzzetta, Istituto di Neuropsichiatria Infantile, Policlinico Universitario, I-98125 Messina, Italy

Materials and methods

Cases reported

We summarize in Table 1 the main clinical data of 13 cases of HMSN with congenital or childhood onset. We have separated them into four groups. The first group (cases 1 and 2) consists of congenital hypomyelination polyneuropathies, extensively reported elsewhere (Guzzetta et al., 1982). In the second group (cases 3 and 4) and in the third group (cases 5, 6, 7 and 8) we consider the recessively inherited type of demyelinating HMSN, distinguished according to age of onset in early infancy or in late infancy, respectively. Both may correspond to type III HMSN as classified by Dyck (1975), with very low CV values (generally less than 10 m/s) and increased CSF proteins (generally higher than 0.50 g/l). Finally, the fourth group (cases 9 to 13) represents the dominant form of demyelinating HMSN with a small decrease of CV values and normal CSF proteins, corresponding to type I HMSN, according tothe same classification. No case showed clinical or laboratory evidence of acquired neuropathies or

Table 1. Clinical data of HSMN

FAMILY HISTORY	ONSET AND MAIN CLINICAL SIGNS	AGE OF BIOPSY
Group A		
1. Parents normal	Severe hypotonia at birth. Independent walking impossible at 30 months. MNCV under 5 m/s. CSF protein: 1.2 g/l.	28 months
2. Parents and brother normal	Severe hypotonia at birth. Independent walking impossible at 30 months. MNCV under 5 m/s. CSF protein: 0.97 g/l.	6 years
Group B		
3. Parents normal	Delay in motor development. Independent walking at 5 years. MNCV under 5 m/s. CSF protein: 0.58 g/l.	22 months
4. Parents and two brothers normal	Delay in motor development. Walked unaided at 26 months. MNCV under 5 m/s. CSF protein: 0.66 g/l.	9 years
Group C		
5. Parents and brothers normal	Progressive difficulty in walking from 6 years. MNCV under 10 m/s. CSF protein: 0.66 g/l.	14 years
6. Parents normal	Progressive difficulty in walking from 12 years. MNCV and SNCV under 10 m/s. Peripheral nerve hypertrophy	16 years
7. Parents and four siblings normal	Difficulty in walking at 7 years. Peripheral nerve hypertrophy. Slight progression. MNCV and SNCV under 10 m/s. CSF protein: 0.48 g/l	16 years
8. Parents normal	Progressive difficulty in walking from 11 years. Marked peripheral nerve hypertrophy. MNCV and SNCV under 10 m/s. CSF protein: 0.70 g/l	19 years
Group D		
9. Father and brother with polyneuropathy	Progressive difficulty in walking from 6 years. Low MNCV and SNCV but over 10 m/s. CSF protein normal	9 years
10. Mother, Grand-mother, 3 sisters and 2 cousins with polyneuropathy	Progressive difficulty in walking from 4 years. Low MNCV and SNCV, but over 10 m/s. CSF protein normal	11 years
11. Cousin of case ten	Progressive difficulty in walking from 5 years. Low MNCV and SCNV, but over 10 m/s	12 years
12. Mother and brother with polyneuropathy	Progressive difficulty in walking from 7 years. Low MNCV and SCNV, but over 10 m/s	14 years
13. Father with polyneuropathy	Progessive difficulty in walking from 10 years. Low MNCV and SCNV, but over 10 m/s	19 years

CSF: cerebrospinal fluid; MNCV: motor nerve conduction velocity; SNCV: sensory nerve conduction velocity.

Table 2. Leucodystrophies (Krabbe disease and MLD).

CASES	TYPE OF LEUCODYSTROPHY	AGE OF BIOPSY
14	Krabbe disease	12 months
15	Krabbe disease	12 months
16	Krabbe disease	18 months
17	Krabbe disease	35 months
18	Infantile MLD	2 years, 4 months
19	Infantile MLD	2 years, 7 months
20	Juvenile MLD	7 years
21	Juvenile MLD	9 years
22	Juvenile MLD	12 years

MLD: metachromatic leucodistrophy.

neuropathies associated with known metabolic diseases.

Table 2 shows the cases of gobloid cell and metachromatic leucodystrophies; the diagnosis was based on the biochemical assay of enzymatic lack and on the histological detection of specific inclusions in peripheral nerve biopsies. With regard to the MLD, two of our cases (5 and 6) can be considered as late infantile (onset before 30 months) and the other three as juvenile forms (onset between 3 and 10 years).

Tissue treatments

Ten-millimetre segments of the medial sural cutaneous nerve were excised at the mid-calf level under local anaesthesia. Biopsies were fixed for 2 hours at 4 °C in 2.5 per cent glutaraldehyde in Millonig buffer (pH 7.2, 520 mOsm/l), postfixed for 2 hours in 1% osmium tetroxide diluted in Millonig buffer supplemented with 0.5% (w/v) sucrose, dehydrated in alcohol, and then embedded in Epon. Identical surgical techniques and histological methods were used for control cases of the same age range pertaining to a normal series, the results of which are extensively reported elsewhere (Ferriere et al., 1985).

Transverse sections, 1 µm-thick, were stained with toluidine blue and 1,4-paraphenylenediamine for light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a Philips EM 300 electron microscope.

Endoneurial area, myelinated and unmyelinated

fibres as well as Schwann cell nuclei were measured on camera lucida drawings from micrographs with a Quantimet 720 Image Analyzer (Metal Research, Keystone, England) connected on line with a PDP 11/10 computer (Digital equipment Co., Galway, Ireland), following a technique reported elsewhere (Ferriere et al., 1985). These measurements were also used for a series of derived parameters: density (Na) and total number (Nt) of myelinated fibres (MF) and unmyelinated fibres (UF), and Schwann cell nuclei (Scn); size histograms of axons and total myelinated fibres as well as unmyelinated fibres; and regression lines for relationship of myelin thickness to axon diameter.

Results

Microscopic and ultrastructural findings

Myelinated fibres: There was no myelin at all in congenital HMSN and myelin was lacking in 25% and 50% of the fibres in the range of myelinated axons in patients with early-onset recessive HMSN. Sheaths of otherwise normal myelin, thinner than expected for the axon diameter, were usually seen in the other forms of HMSN with a major frequency in recessive ones. These findings expressing myelin regeneration were rarer in leucodystrophies.

Myelin breakdown was found in all cases with the exception of those with congenital HMSN; its frequency grossly paralleled the regeneration phenomena of myelin. There was no apparent evidence of axonal changes in any case and yet, some clusters of regenerating axons were shown in leucodystrophies and in the dominant form of HMSN.

Onion bulbs: Onion bulbs were found in every form of the considered demyelinating neuropathies. Nevertheless, differences, both in density and in quality, were evident. In congenital HMSN each large axon in the range of the normal population of myelinated fibres was naked and surrounded by several whorls of simple or double-layered basement membrane, rarely containing small Schwann cell processes. This atypical kind of onion bulb (Lyon type) was also frequently found in early-onset recessive HMSN; in these cases, however, other onion bulbs with single basement membrane whorls or, more typically, with Schwann cell processes were seen around thinly myelinated fibres (Fig. 1).

Onion bulbs in the other HMSN cases were always typical with an evident difference in density: in semithin transverse sections recessive HMSN showed onion bulbs surrounding almost every myelinated fibre, while dominant HMSN presented a lower density of onion bulbs with a lesser degree of endoneurial fibrosis (Fig. 2).

Onion bulbs in HMSN were associated with an evident increase of collagen fibres and often with a hypertrophy of the fibroblasts. In one case of recessive HMSN (case 6) fibroblast proliferation was particularly strong and intermixed with the onion-bulb structure, giving it a very special large appearance (Fig. 1).

The number of onion bulbs in leucodystrophies was very low; they were generally formed by a few concentric cytoplasmic processes of Schwann cells surrounding myelinated fibres with frequent images of myelin breakdown (Fig. 1).

Unmyelinated fibres: The population of Remak fibres in some forms of HMSN showed equivocal aspects of abnormality. In some cases of recessive HMSN, and at a higher degree in the dominant form, unmyelinated fibres were generally condensed in clusters with frequent budded bands and collagen pockets. Whorls of Schwann cell processes or basement membranes sometimes surrounded single Remak fibres or clusters of them (Fig. 3). The phenomenon was more evident in those cases in which a low total number of fibres was found.

In early onset leucodystrophy (Krabbe disease), clusters of Remak fibres encircled by a unique Schwann cell cytoplasm (looking like a foetal type of aggregation) were sometimes found (Fig. 3); more frequently, Schwann cell processes with only one or two axons were observed. There was no evidence of changes in the MLD cases.

Other findings: In MLD cases typical inclusions in Schwann cells or endoneurial macrophages (prismatic inclusions, tuffstone and vacuoles) were observed. Similarly, Krabbe's cases showed normal cellular inclusions, such as crystalloid structures with a multiangular cross-section.

Morphometric analysis (Tables 3, 4)

Considering the HMSN cases, the *endoneurial area* (EA) was within normal limits in congenital hypomyelination polyneuropathy and in one of the early-onset recessive forms. Values increased in the group of patients with dominant HMSN, and most of these were beyond the superior level of normal controls of the same age studied by the same technique. They reached the highest level in the recessive form. EA in MLD was generally within normal limits, and had the highest normal values in Krabbe disease.

The *ratio between the surface area of nerve fibres and that of the endoneurium* was very low (0.06-0.16) in all the recessive cases of HMSN, despite variable EA values. In dominant HMSN this ratio was just below or above the lower normal limit (0.21-0.40) except in one case (case 12), which showed a very low value (0.07) similar to the recessive forms.

In contrast, EA values in leucodystrophies were generally in the upper normal range, and in two patients with the juvenile form of MLD they were even above the normal range.

The *total number of myelinated fibres* (or, for congenital and early-onset recessive HMSN, «myelinated fibres», i.e. fibres naked of myelin

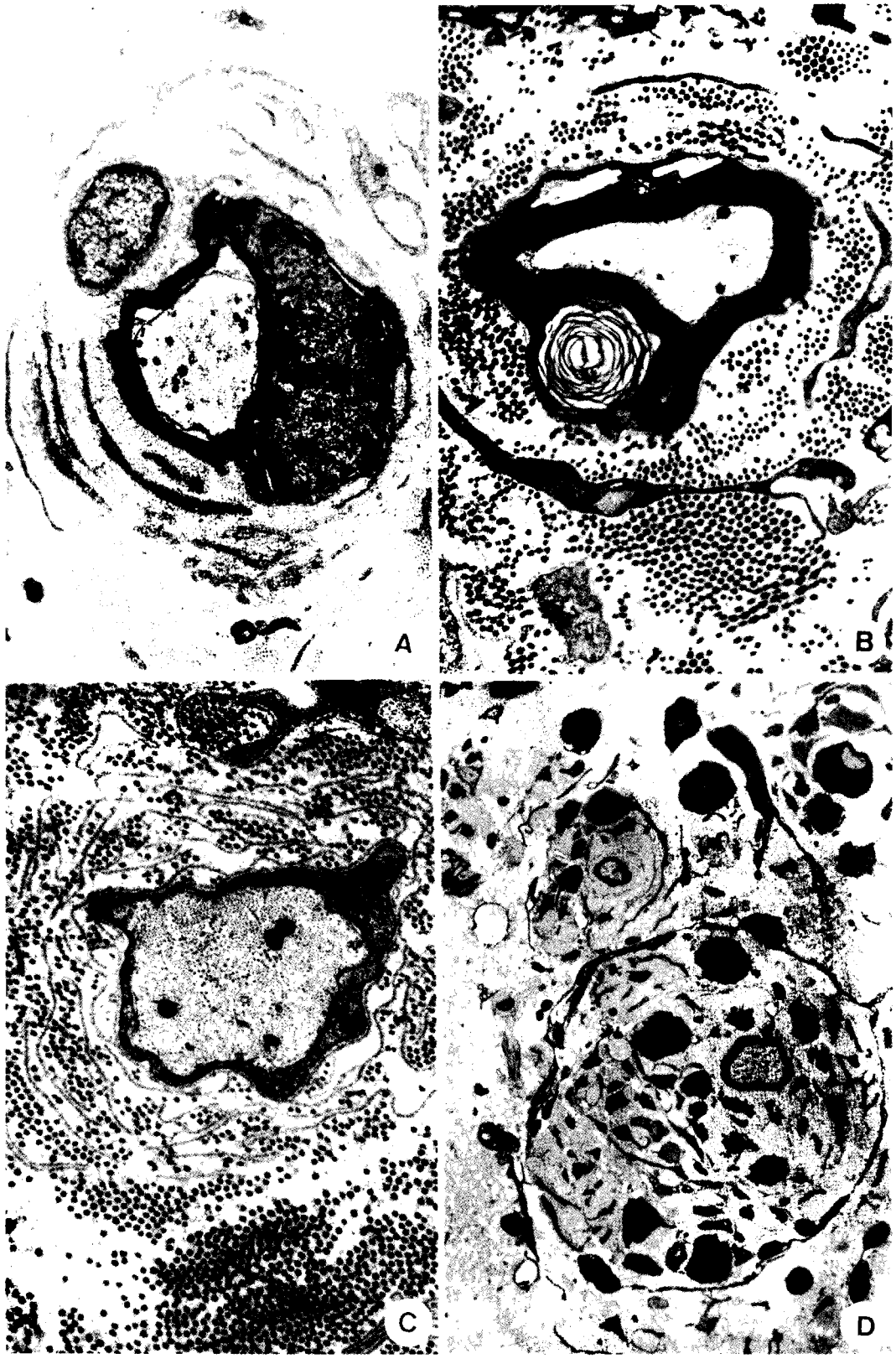


Fig. 1. Different kinds of onion-bulb (OB) formation: electron micrographs of transverse sections from sural nerve, showing:
A. Typical OB observed in case number 6 (HMSN III) with several whorls of Schwann cell processes separated by a large amount of cross-sectioned collagen fibres, and wrapping a thin myelinated axon (remyelination). x 4,000.
B. Small OB in Krabbe disease (case number 16); typical crystalloid inclusions are evident within Schwann cell cytoplasm as well as concentric lamellar material possibly originating from myelin breakdown. x 9,000.
C. Simple or double-layered basement membranes are the OB whorls generally found in congenital hypomyelination neuropathies (case number 2): the axon is totally naked of myelin. x 11,000.
D. Enormous atypical OB I observed in case number 7 (HMSN III) with impressive endoneurial fibrosis and fibroblast involvement. x 1,300

even though presumed to be myelinated according to their large axon diameter) was within the normal range, generally in the lower half, in recessive HMSN. Very low values, even below the normal range (cases 10 and 13) were found in dominant HMSN. The reduction of the total number of «myelinated fibres» was definitely marked in congenital cases and in case 4. No substantial changes were observed in leucodystrophies; however, there was a trend towards the increase with age of the myelinated fibre number and density in MLD, in contrast with what happens in normal controls.

Generally, there was a decrease in the *density of myelinated fibres* in all cases. Nevertheless, it varied, grossly showing an inverse relationship with EA values; the only exception was the congenital HMSN where low endoneurial values coexisted with a low number of «myelinated fibres».

Regression lines expressing the ratio of myelin thickness to axon diameter were not possible or not significant in congenital and early-onset recessive HMSN, due to the lack or paucity of myelin sheaths. Generally, the slope was low in the other HMSN groups, with a gradient from the lower values of recessive forms

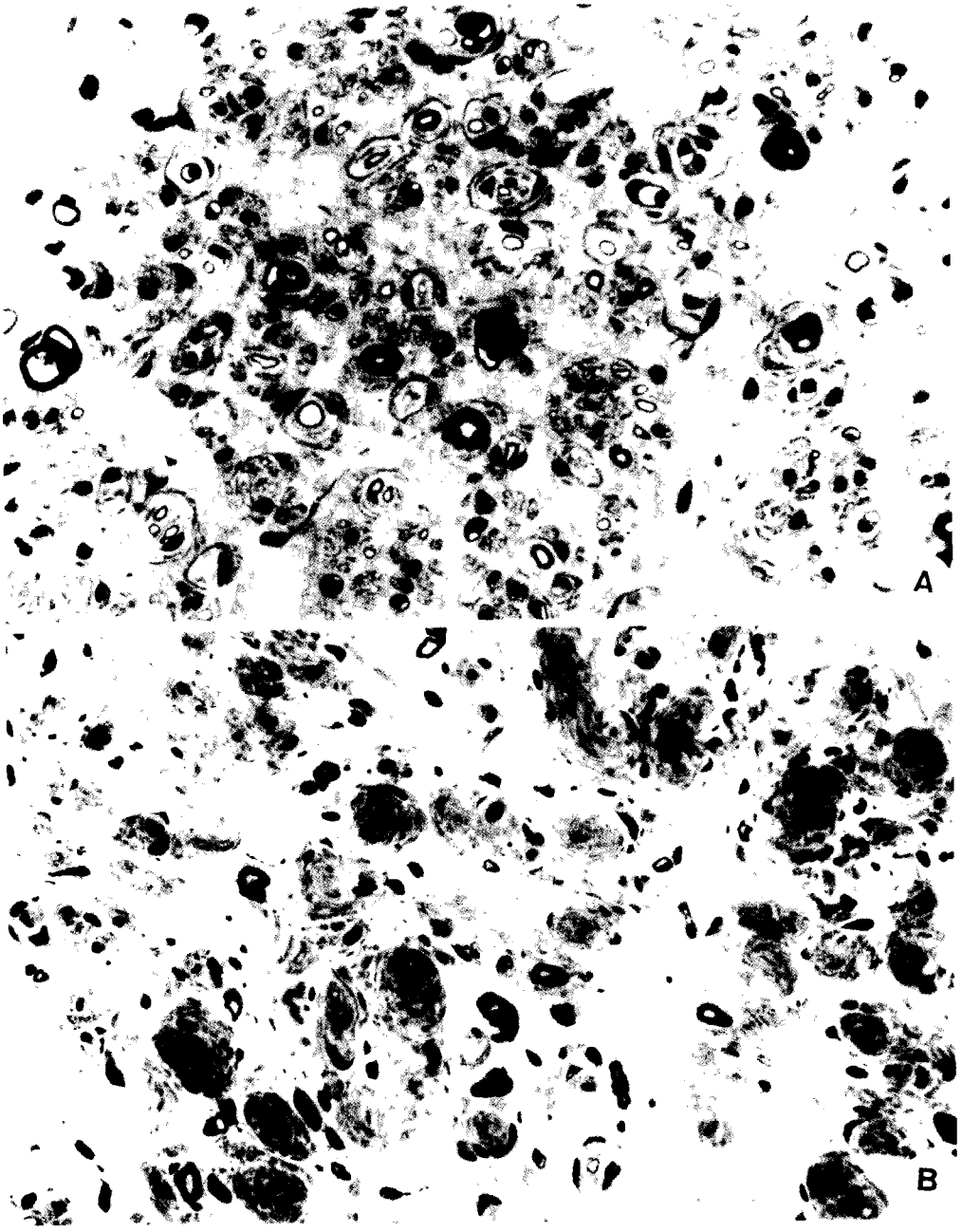


Fig. 2. Semithin transverse sections from sural nerves of case number 12 (**A**, HMSN I) and case number 7 (**B**, HMSN III); in the latter there is a major endoneurial fibrosis and almost every myelinated fibre is surrounded by OB formations. x 800

to the higher values in dominant ones. A slight decrease in the slope was generally found in leucodystrophies (Fig. 4).

The *histogram of the distribution of myelinated fibre diameters* (Fig. 5) lost its bimodality in three out of four cases with Krabbe disease, as well as in the juvenile form of MLD.

In HMSN the bimodality was questionable, due to the lack of most larger fibres; when present, the second peak of both axon and whole fibre diameters was very small. Furthermore, the analysis of the diameter means of the axons showed evident greater values in the recessive form than in the dominant one, in which the percentage of axons larger than 3.5 μm is reduced (Table 5).

As regards the *unmyelinated fibres*, in four out of six cases of type III HMSN the *total number* was higher compared to normal controls of the same age, and both HMSN and leucodystrophies were within the normal range in all the other patients. The *density of unmyelinated fibres* was always lower than control cases, with the exception of three out of 4 cases of congenital and early-onset HMSN type III, which presented values within the normal range. A mild reduction of density, in most cases near the inferior limit of the normal range, was also observed in the leucodystrophies. The histogram of diameter distribution showed a general predominance of fibres smaller than 1 μm, especially in congenital HMSN and in the leucodystrophies.

The *total number of Schwann cell nuclei* was definitely increased in recessive HMSN, and was

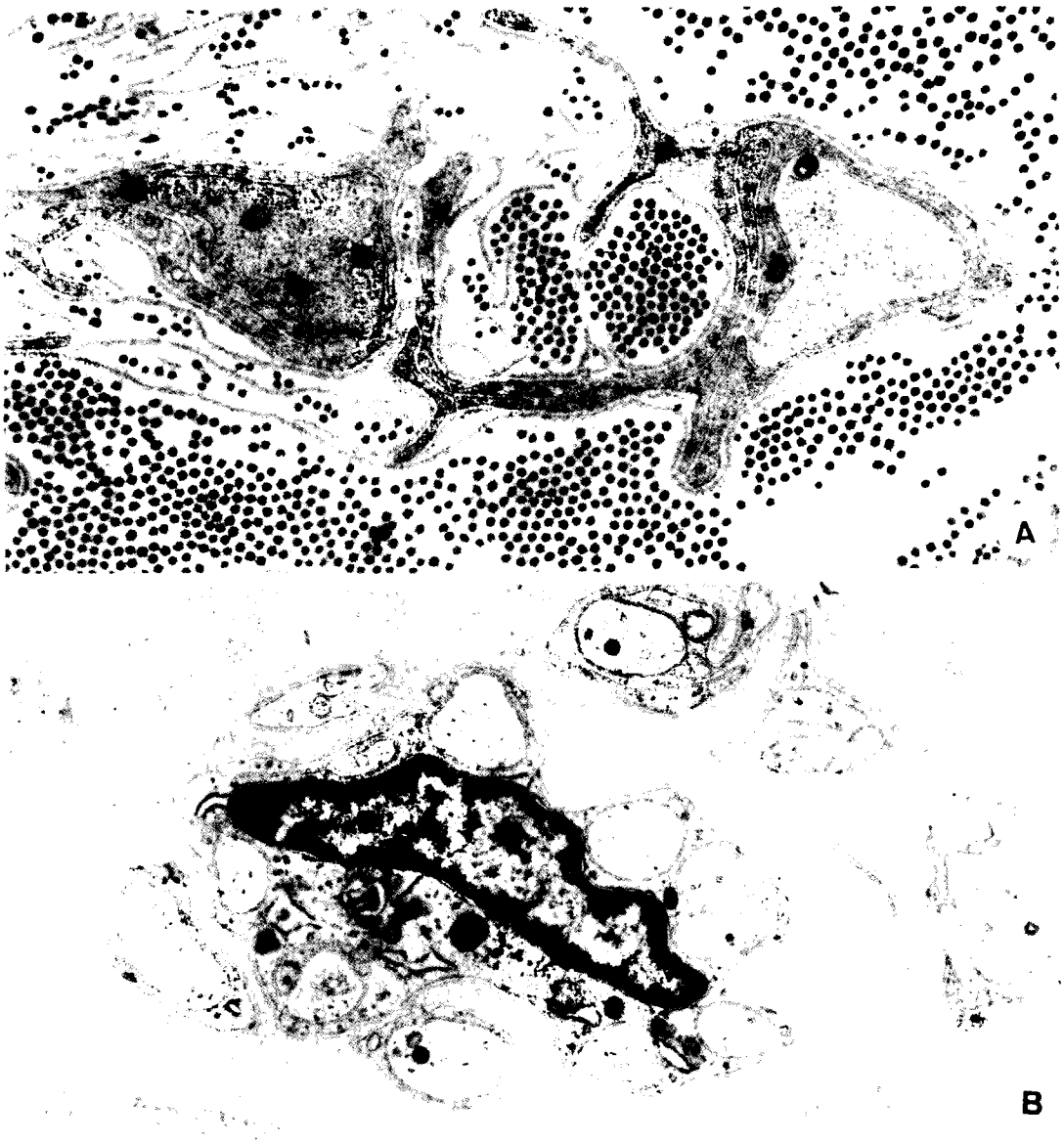


Fig. 3. Electron micrographs of transverse sections from sural nerve, showing unmyelinated fibres. In **A** (case number 11, x 11,000) one can observe several anomalous Schwann cell processes (budded bands, collagen pockets) associated with redundancy of basement membranes. In **B** (case number 15, x 7,100) a large bundle of unmyelinated axon is shown encircled by a unique Schwann cell; its endoplasmic reticulum appears dilated in some places with electron-lucent substance intermixed with granular material.

Table 3. Total endoneurial area and fibre area.

	TEA (1)	MFA (2)	UFA (3)	(2+3)/1
Congenital HMSN				
Case 1	0.20	5,900	6,600	0.06
Case 2	0.32	9,900	8,100	0.06
Early-onset HMSN type III				
Case 3	0.72	66,900	31,400	0.14
Case 4	0.39	45,100	5,600	0.13
Typical HMSN type III				
Case 5	1.21	11,900	19,800	0.11
Case 6	2.05	68,200	33,300	0.05
Case 7	1.42	61,600	25,700	0.06
Case 8	1.16	162,300	25,000	0.16
HMSN type I				
Case 9	0.76	181,000	8,900	0.25
Case 10	0.34	87,800	5,500	0.21
Case 11	0.78	173,000	7,500	0.23
Case 12	1.21	80,200	8,600	0.07
Case 13	0.36	135,000	12,200	0.40
Krabbe disease				
Case 14	0.67	194,600	9,600	0.30
Case 15	0.51	207,000	9,000	0.42
Case 16	0.78	227,500	9,000	0.30
Case 17	0.42	204,800	10,400	0.44
MLD				
Case 19	0.50	203,000	7,600	0.42
Case 20	0.34	152,000	6,700	0.46
Case 21	0.52	208,000	11,600	0.42
Case 22	0.86	368,900	11,900	0.45
Controls (5 cases: 28 m - 17 y, 4 m)				
(ranges*)	0.16-0.52	71,000-210,700	5,000-17,200	0.30-0.60

*: variations not related to age; MFA: area of myelinated fibres; TEA: total endoneurial area; UFA: area of unmyelinated fibres.

Table 4. Number (Nt) and density (Na) of myelinated fibres, Remak fibres, and Schwann cell nuclei.

	MFNt	MFNa	UFNt	UFNa	ScnNt	ScnNa
Congenital HMSN						
Case 1	"700" (+30)	"3,480" (+100)	12,500	65,000	680	3,400
Case 2	"700" (+30)	"2,180" (+100)	16,700	52,000	1,300	4,100
HMSN type III						
Case 3	"2,800" (+2,300)	"3,900" (+3,200)	51,500	72,000	2,700	3,800
Case 4	"500" (+2,000)	"300" (+5,200)	12,300	31,600	1,200	3,200
Case 5	4,800	3,900	24,700	20,400	3,500	4,200
Case 6	3,900	1,900	42,200	20,600	5,900	2,900
Case 7	5,100	3,600	30,500	21,800	5,400	3,800
Case 8	5,100	4,400	66,600	40,200	3,200	2,700
HMSN type I						
Case 9	3,900	5,100	11,600	15,400	1,100	1,500
Case 10	1,900	5,600	5,200	15,200	500	1,600
Case 11	3,600	4,600	18,200	23,400	600	750
Case 12	2,700	2,200	24,700	20,400	1,900	2,300
Case 13	1,400	3,800	10,400	28,600	400	1,100
Krabbe disease						
Case 14	5,200	7,200	28,200	42,400	2,500	3,700
Case 15	4,400	7,800	19,900	39,200	1,800	3,500
Case 16	3,900	5,000	29,700	38,000	1,500	1,900
Case 17	2,600	6,200	18,000	42,400	650	1,500
MLD						
Case 18	4,300	6,900	28,200	45,200	450	700
Case 19	2,600	5,200	20,200	40,400	950	1,900
Case 20	2,000	5,700	7,400	22,400	400	1,100
Case 21	3,700	7,100	22,500	43,400	1,700	3,400
Case 22	3,400	7,900	16,200	37,600	1,200	2,800
CONTROLS (28m - 17y, 4m)						
(ranges)	2,300-6,100	10,300-26,200*	8,500-29,700*	44,100-111,600*	450-1,200*	1,900-2,900*

*: inversely proportional to age; MF: myelinated fibres; Scn: Schwann cell nuclei; UF: unmyelinated fibres.

generally normal in the other patients. In contrast, *density* was decreased in type I HMSN and in some cases of leucodystrophy.

Discussion

Variability of the recessive form of HMSN

As we have pointed out (Guzzetta et al., 1982), morphometric and ultrastructural findings in recessive forms of HMSN are rather variable. Variability concerning onion bulb formation has already been emphasized (Joosten et al., 1974a,b). Even in the same subtype, e.g. the congenital form of HMSN, there is evidence of different features: focally-folden myelin sheaths (Lutschig et al., 1985; Vital et al., 1987; Gabreels-Festen et al., 1990); hypomyelinating neuropathies with Lyon-type onion bulbs (Lyon, 1969; Anderson et al., 1973; Karch and Urich, 1975; Kennedy et al., 1977; Moss et al., 1979; Guzzetta et al., 1982; Ono et al., 1982; Tachi et al., 1984; Harati and Butler, 1985); absence of onion bulbs in rapid lethal forms (Palix and Coignet, 1978; Hakamada et al., 1983; Pleasure et al., 1986; Seitz et al., 1986; Charnas et al., 1988; Sahenk et al., 1991; Boylon et al., 1992); and cases with prevailing axonal changes (Guzzetta and Ferriere, 1985).

In our sample, the main differences were: 1) the Nt of MF, quite reduced in congenital HMSN and within the range of normal controls in the other groups; 2) the morphology of onion bulbs, with predominance of the Lyon type (basement membrane onion bulb) in the congenital forms, classical aspect in HMSN type III and a mixture of the two types in early onset HMSN type III; 3) marked elevation of Scn Nt in HMSN type III compared to the other groups; and 4) marked increase of EA in HMSN type III, within normal limits in congenital forms, and intermediate values in early-onset HMSN type III. Changes of UF population observed by some authors in HMSN type III (Narazaki et al., 1986) were

Table 5. Axons of myelinated fibres (diameter means and percentage of the largest axons) in HMSN type I and III.

	AXON DIAMETER		% OF AXONS WITH DIAMETER > 3.5μm
	M	sigma	
HMSN type III			
Case 5	3.2	1.0	23%
Case 6	2.8	1.2	18%
Case 7	3.0	1.9	20%
Case 8	3.2	2.2	29%
HMSN type I			
Case 9	2.3	1.2	10%
Case 10	1.9	1.5	8%
Case 11	2.5	1.2	10%
Case 12	2.2	1.5	5%
Case 13	2.1	1.7	12%

not confirmed.

All these findings can possibly account for an array of increasing severity of the same disease, characterized by the incapacity of Schwann cells to form or to maintain myelin (Guzzetta et al., 1982). The most severe form is the congenital one; its defectual rather than degenerative character can be assumed because of the total lack of myelin and myelin breakdown. This primary absence of myelin formation can in turn account for the loss of «myelinated fibres». In contrast, a very active demyelination/remyelination in HMSN type III, associated with a greater number of Schwann cells and larger endoneurial areas due to increase of collagen fibres and to the elevated number of onion bulbs, fit well with the classical HMSN type III, as has been reported elsewhere (Weller, 1967; Dyck and Gomez, 1968; Joosten et al., 1974a,b; Dyck, 1975). Nevertheless, a pathogenetic distinction between congenital and later-onset recessive HMSN is hard to affirm only on the base of pathological findings. However, due to the rather distinctive characteristic of the neuropathological data in congenital forms and partially in early-onset HMSN type III, we will avoid considering them in further comparisons between the groups.

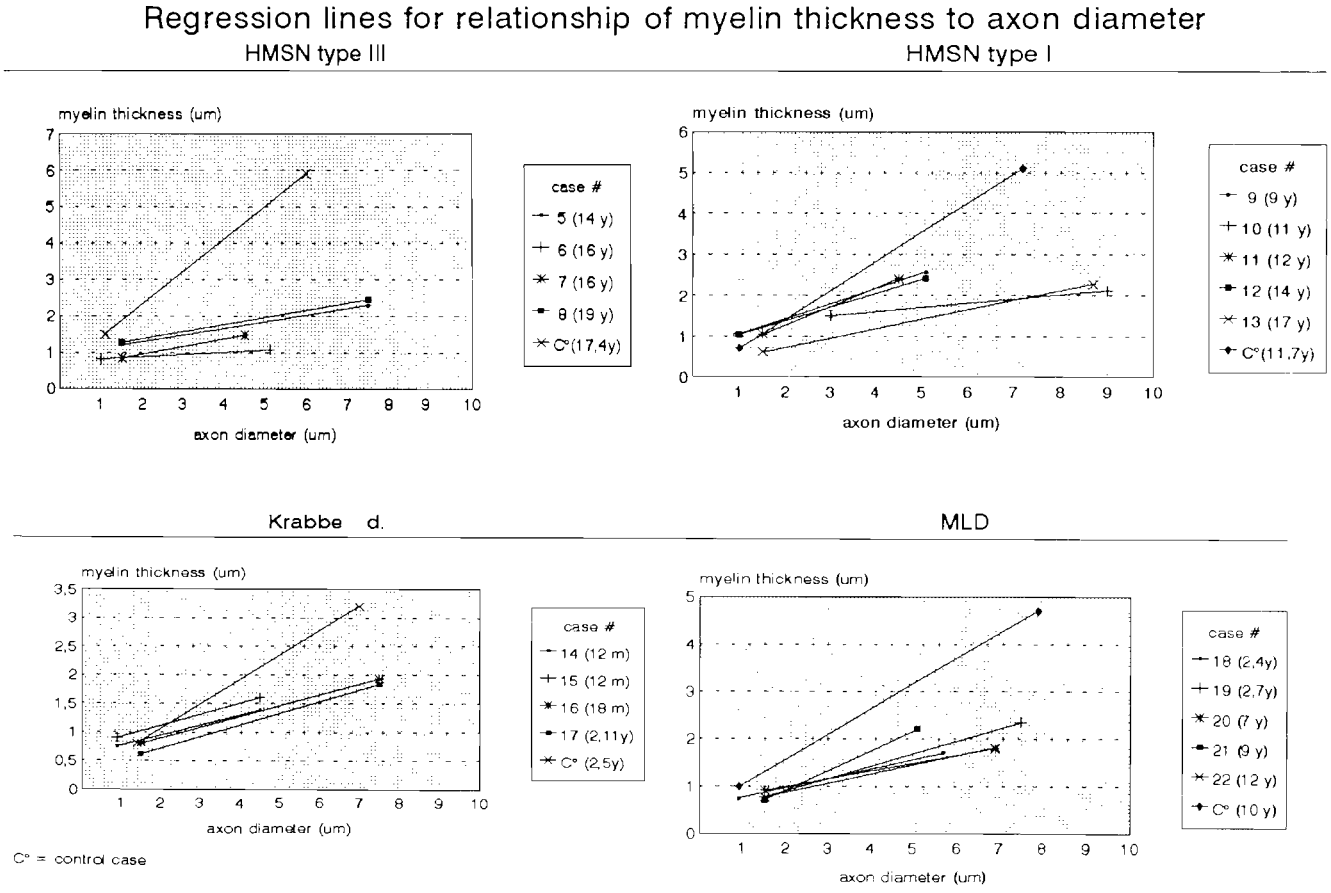


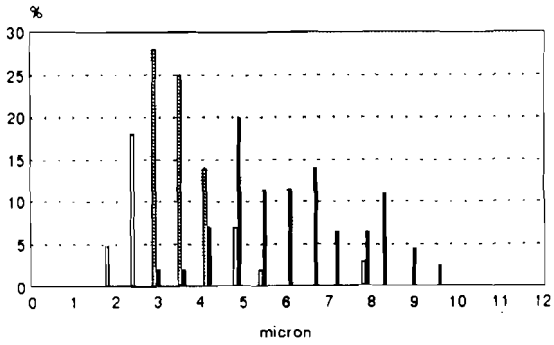
Fig. 4. Regression lines for relationship of myelin thickness to axon diameter.

Fig. 5. Size distribution of MF diameter.

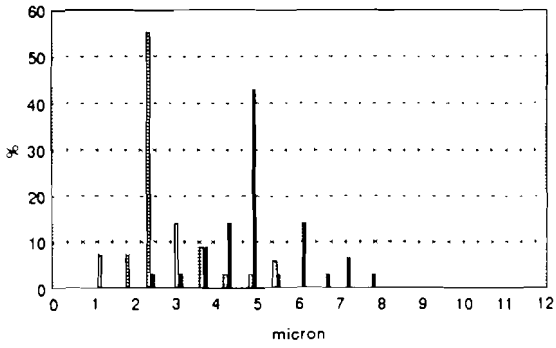
Size distribution of MF diameter

HMSN type III

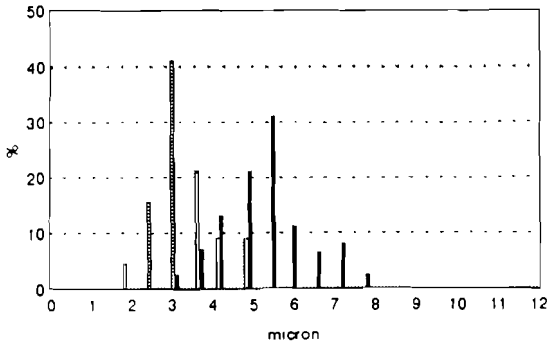
case 5 (14 y)



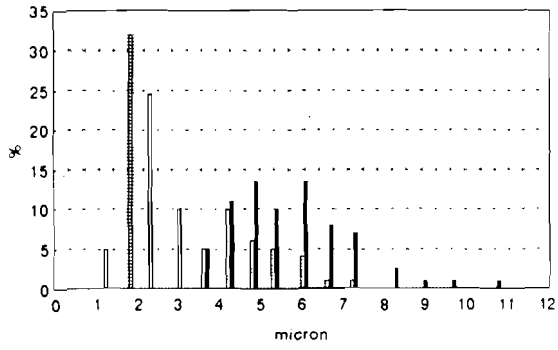
case 6 (16 y)



case 7 (16 y)

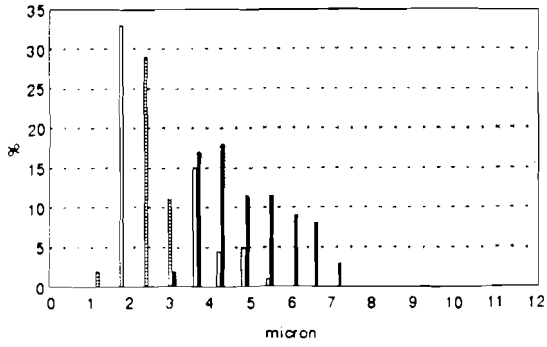


case 8 (19 y)

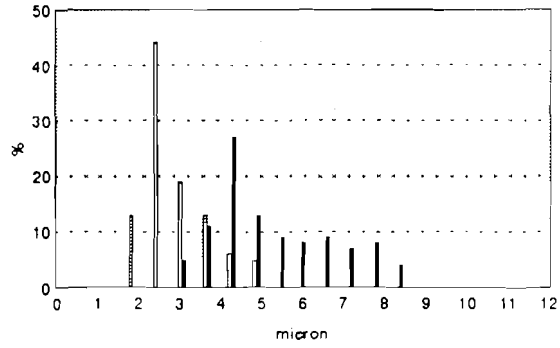


HMSN type I

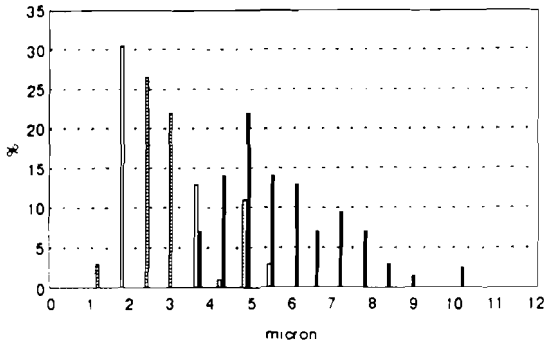
case 9 (9 y)



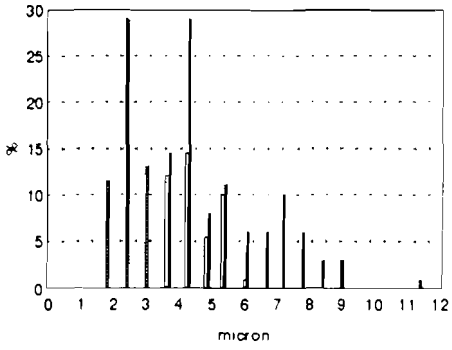
case 11 (12 y)

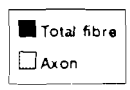


case 12 (14 y)



case 13 (17 y)

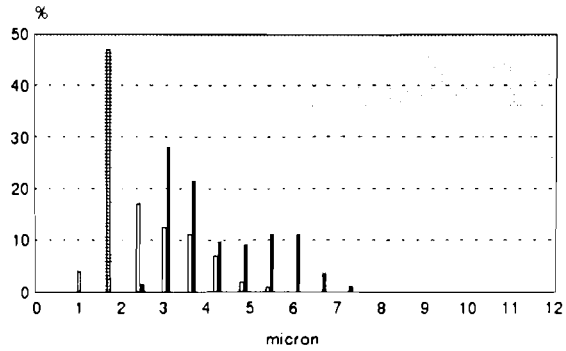




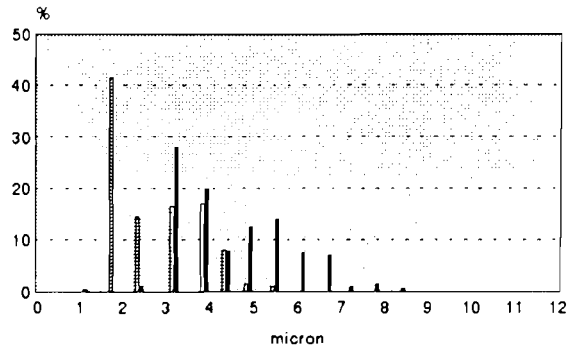
Size distribution of MF diameter

Krabbe dis.

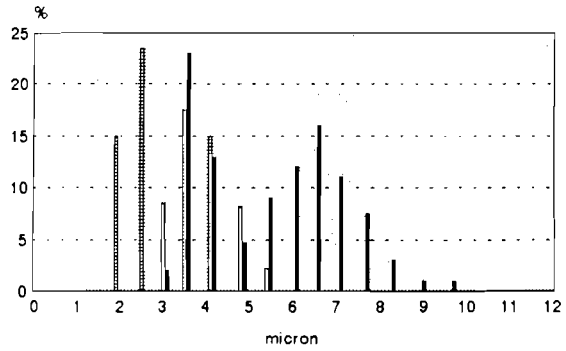
case 14 (12 m)



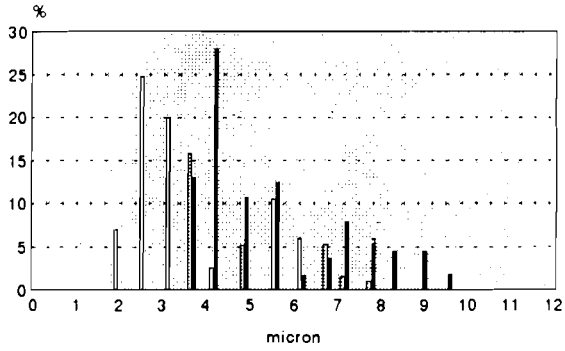
case 15 (12 m)



case 16 (18 m)

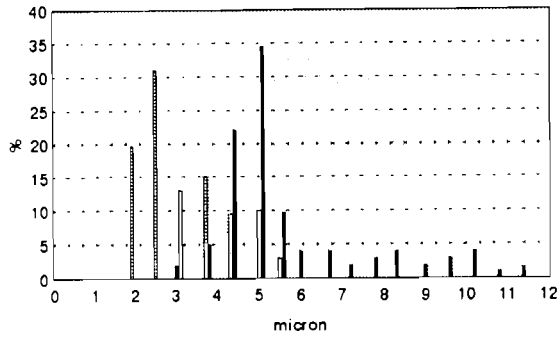


case 17 (2 y, 11 m)

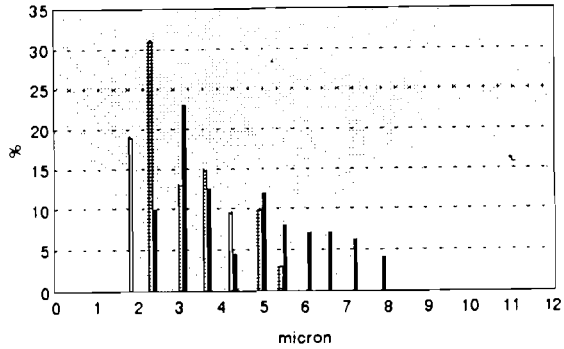


MLD

case 21 (7 y)

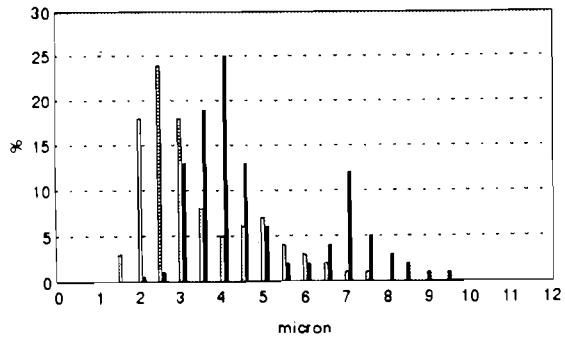


case 22 (9 y)

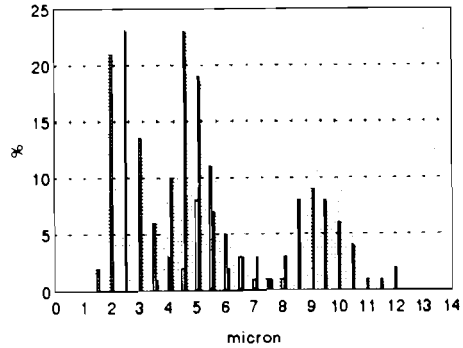


Control cases

age: 7 months



age: 10 years



Type I HMSN vs type III HMSN

In our cases of autosomal-dominant HMSN type I we found a Nt of MF in the lower range of normal controls or even below. Furthermore, density was always reduced, as previously reported (Dyck et al., 1974; Ouvrier et al., 1987; Gabreels-Festen et al., 1992). There was also evidence of a loss of the largest myelinated fibres, as remarked on by Dyck et al. (1974), van Weerden et al., (1982), and Ouvrier et al. (1987). These data and the ultrastructural findings that show some aspects of fibre clusters accounting for an axon regeneration seem to stress the primary involvement of axons in HSMN type I, as already reported (Dyck et al., 1974; Nukada et al., 1983); however, recent knowledge on molecular regulation in axon-Schwann cell interaction (de Waeg et al., 1992) can explain both myelin and axon changes in HMSN type I as due to an abnormal expression of the PMP-22 gene by Schwann cells (Suter and Patel, 1994).

On the other hand, HMSN type III showed strictly normal values of the Nt of MF, as others have reported (Gabreels-Festen et al., 1990); the reduced density is evidently linked to the major increase of endoneurial area. There is also evidence of larger axonal diameters. Thus, ultrastructural findings, suggesting the most active and predominant process of demyelination/re-myelination, account for a specific primary failure of Schwann cells in maintaining myelin.

This, at least, relative predominance of myelin defect in HMSN type III in relation to type I can also be inferred by the comparison of the morphometric data concerning the Schwann cell nuclei; they were markedly elevated only in HMSN type III.

The Nt and Na of UF are both reduced in HMSN type I. This appears to be in contrast with previous results (Dyck et al., 1970; Ouvrier et al., 1987). However, an involvement of the Remak fibres has generally been shown both in ultrastructural and some morphometric findings, such as the increase in number of small fibres (Low et al., 1978; Ouvrier et al., 1987).

Leucodystrophies vs HMSN

The cases of leucodystrophy studied presented ultrastructural and morphometric findings generally similar to those observed in previous studies on neuropathy in Krabbe disease (Sourander and Ollson, 1968; Bishoff and Ulrich, 1969; Schlaepfer and Prensky, 1972; Joosten et al., 1974a,b; Martin et al., 1974) or in MLD (Dayan, 1967; De Silva and Pearce, 1973; Martin and Joris, 1973; Meier and Bishoff, 1976; Percy et al., 1977; Thomas et al., 1977; Luijten et al., 1978; Martin et al., 1982; Bardosi et al., 1987). No nerve hypertrophy, relative loss of larger myelinated fibres, demyelination with significant low slope of the regression line of the ratio of myelin thickness to axon diameter, or small onion bulb formation were evident. Typical inclusions in Schwann cells and macrophages were also found.

The values of endoneurial area were variable according to different reports; only a few authors have shown a major endoneurial fibrosis both in Krabbe disease (Martin et al., 1974) and in adult MLD (Percy et al., 1977; Thomas et al., 1977). Our findings locate the EA values in leucodystrophies, namely in Krabbe disease, in the higher normal values of controls of the same age. This probably accounts for the relative decrease in the density of myelinated and unmyelinated fibres. Even though the total number seems within the normal range, there are histological signs of axonal degeneration suggesting a loss of fibres, as others have inferred in Krabbe disease (Schlaepfer and Prensky, 1972; Martin et al., 1974) or in MLD (Joosten et al., 1974a,b; Thomas et al., 1977). In addition, in the juvenile form of MLD the population of myelinated fibres seems less involved by axonal and myelin changes.

The comparison with recessive HMSN patients shows that demyelination also plays a predominant role in leucodystrophies; however, its degree seems definitely lower, and some qualitative differences are observable as well. The smaller number and dimension of onion bulbs account for a less marked abnormality of Schwann cells; as does the number of the Schwann cell nuclei within the normal range. Furthermore, there is no evidence of endoneurial fibrosis in contrast with that which is usually seen in hypertrophic forms of HMSN. These findings are probably at the base of the normal values of the ratio between fibre surface and total endoneurial area. They are also consistent with the data regarding the remodelling of the myelinated fibre population whose impairment is definitely lower than that found in HMSN. A possible explanation of this relatively slower activity of demyelinating processes in leucodystrophies could be an impaired mechanism of re-forming myelin in normal myelin turnover (Friede, 1989). A major defect in the anabolic phase of myelin metabolism (dysmyelination) is indirectly confirmed by the absence of relationship between myelin debris and lipid storage in Schwann cells both in Krabbe disease (Sourander and Olsson, 1968; Suzuki and Grover, 1970; Martin et al., 1974) and in MLD (Dayan, 1967; Martin and Joris, 1973; Luijten et al., 1978; Martin et al., 1982), and the presence of inclusions even before the beginning of myelination in foetal life (Leroy et al., 1973; Meier and Bishoff, 1976). The storage products, thus, do not seem to come from the degradation of myelin.

There is some evidence that the biochemical pathogenesis of demyelination in Krabbe disease is the effect of an inhibition in galactose incorporation into cerebroside and sulphatide linked to accumulation of toxic psychosine (Svennerholm et al., 1980; Ida et al., 1990); a not well-defined toxic effect of the lipid storage is also evoked for MLD (Friede, 1989; Thomas, 1993). Furthermore, the signs of axonal degeneration confirm the more diffuse cellular injury involving both Schwann cells and neurons. On the contrary, a more

severe abnormality of Schwann cells, or failure of Schwann cell-axon communication, to form and maintain myelin could be at the base of HMSN pathology.

Conclusions

Although a reliable classification of the HMSN can be based only on biochemical and molecular genetic criteria, genetic advances, even though impressive (Vance, 1991), do not yet allow a consistent clinical location of different forms of HMSN. The great genetic heterogeneity in the better studied form (HMSN type I) leaves many open questions about the type of mutation (duplication, point mutation), its location (chromosome 17, chromosome 1 and the X chromosome) and the mechanisms of gene expression. Basic and clinical research need to be combined. Thus, accurate studies that try to define pathological identities can still be useful. Our study shows that a distinction between type III and type I HMSN based on the type of hereditary transmission and on a few main clinical aspects (onset in infancy or early childhood, discriminant NCV level of 10 m/s, increased values of CSF proteins) allows us to collect some critical histopathological, ultrastructural and morphometric findings.

Furthermore, a rather distinctive character of demyelination stands out in HMSN type III compared to other «onion-bulb» neuropathies, accounting for different underlying mechanisms.

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