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REVIEW ARTICLE

Neuropathology of Charcot-Marie-Tooth and Related Disorders

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Abstract

The peripheral nervous system (PNS), with all its branches and connections, is so complex that it is impossible to study all components at the light or electron microscopic level in any individual case; nevertheless, in certain diseases a simple nerve biopsy may suffice to arrive at a precise diagnosis. Structural changes of the PNS in neuropathies of the Charcot-Marie-Tooth (CMT) type and related disorders comprise various components of the PNS. These include peripheral motor, sensory, and autonomous neurons with their axons, Schwann cells, and myelin sheaths in the radicular and peripheral nerves as well as satellite cells in spinal and autonomous ganglia. Astrocytes, oligodendroglial cells, and microglial cells around motor neurons in the anterior horn and around sensory neurons in other areas of the spinal cord are also involved. In addition, connective tissue elements such as endoneurial, perineurial, and epineurial components including blood and lymph vessels play an important role. This review focuses on the cellular components and organelles involved, that is, myelin sheaths, axons with their microtubules and neurofilaments; nuclei, mitochondria, endoplasmic reticulum, and connective tissue including the perineurium and blood vessels. A major role is attributed to recent progress in the pathomorphology of various types of CMT1, 2, 4, CMTX, and HMNSL, based on light and electron microscopic findings, morphometry, teased fiber studies, and new immunohistochemical results such as staining of certain periaxin domains in CMT4F.

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Index Entries: Neuropathology of CMT; CMT subtypes; Schwann cells; demyelination and remyelination; axonal degeneration and regeneration; blood vessels; connective tissue; electron microscopy; fine structure.

Introduction

Compared with the large and growing number of mutations in genes and chromosomal loci known to cause a Charcot-Marie-Tooth (CMT) type of neuropathy (De Jonghe et al., 1997, 1998; Nelis et al., 1999), there is only a limited number of structural changes in peripheral nerves including small nerve fascicles in muscle and skin biopsies. The main changes consist in segmental demyelination

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and remyelination, or axonal degeneration and regeneration finally resulting in loss of nerve fibers. Distally accentuated reduction of the number of nerve fibers ("dying back") is usually involved and needs quantification using morphometric technics.

Yet the traditional basic neuropathological distinction between primarily "axonal" and primarily "demyelinating" disorders of the peripheral nervous system (PNS) needs further subdivision:

1. according to the topographical site of attack on the neuron (motor, sensory, and/or autonomous neurons);
2. in respect to the perikaryon (cell body with its nucleus),
   a. its distal peripheral,
   b. proximal peripheral,
   c. central peripheral, and central proximal processes (neurite, axon),
   d. its axonal and dendritic terminals or synapses contacting postsynaptic elements; and
3. concerning the myelin sheath with its paranode, internode, Schmidt-Lanterman incisures, and the complicated attachment zone of myelin loops to the axolemma.

In addition, differentiation is needed according to the organelle that is primarily affected: nucleus, mitochondria, endoplasmic reticulum of the perikaryon and axoplasmic reticulum, Golgi complex, pinocytotic vesicles, coated vesicles, lysosomes, peroxisomes, microtubules, intermediate filaments (neurofilaments), microfilaments (actin) and others. Furthermore, the metabolism of lipids, carbohydrates, iron, copper, sodium, potassium, and calcium ions may be affected within neurons, Schwann cells, endoneurial fibroblasts, and perineurial cells as well as endoneurial and epineurial blood vessels including endothelial and smooth muscle cells, and rarely lymph vessels. All these elements may be primarily or secondarily affected. Logically, all these structures, as far as we know, may give rise to disorders, in man or under experimental conditions, inherited or sporadic (acquired). Some of these disorders are restricted to the PNS; others affect the PNS *and* other systems to a more or less severe degree.

Examples with representative changes are illustrated (Figs. 1–5). More illustrations are presented in recent reviews, handbooks, or atlases (King, 1999; Schröder, 1999, 2001; Dyck and Thomas, 2005). The overwhelming proportion of changes are nonspecific and not pathognostic. But in certain diseases, a single nerve biopsy allows a specific diagnosis. Nevertheless, many changes are group-specific and offer ways to estimate the severity and time-course of the disease.

Changes of Myelin Sheaths

More or less, severe s*egmental demyelination* is a basic reaction of nerve fibers in all conditions primarily or secondarily affecting the myelin sheath (Fig. 1A–F). It usually starts with *paranodal demyelination* and spreads to the complete internode. The onset of demyelination at the paranode is particularly impressive in CMTX in which microvesiculation of terminal myelin loops represents one of the major changes before other pleomorphic changes at this site lead to segmental demyelination. Because of the limited space in journals, the wide spectrum of fine structural changes seen at the paranode has not yet been sufficiently covered despite a considerable number of studies on this subject (Bergoffen et al., 1993; Scherer, 1996; Sander et al., 1998; Scherer et al., 1998; Senderek et al., 1998, 1999; Hahn et al., 2001; Vital et al., 2001). A classification of developmental, pathological, and artificial changes has been provided (Schröder, 1996). It is important to know that some paranodal myelin loops are not attached to the axon causing an erroneous impression of pathological "axoglial dysjunction" (Thomas et al., 1996). The number of these "detached" loops increases in proportion to the growing thickness of the myelin sheaths forming the so-called bracelets of Nageotte, whereas the paranodal myelin attachment zone is not proportionately increasing in length (Bertram and Schröder, 1993). Its minimal length is about 2.3 μm at birth and not longer than 4–7.8 μm at 2–17 mo of age, despite an increase of the number of myelin lamellae from 40 to 125 during this period of development in human sural nerves. This needs to be considered when discussing changes of the paranode in any type of neuropathy.

The traditional classification of neuropathies of the CMT type starts with demyelinating diseases (Dyck and Thomas, 2005). The most frequent one is CMT1A or "hereditary motor and sensory neuropathy type Ia" (HMSN Ia) owing to duplication

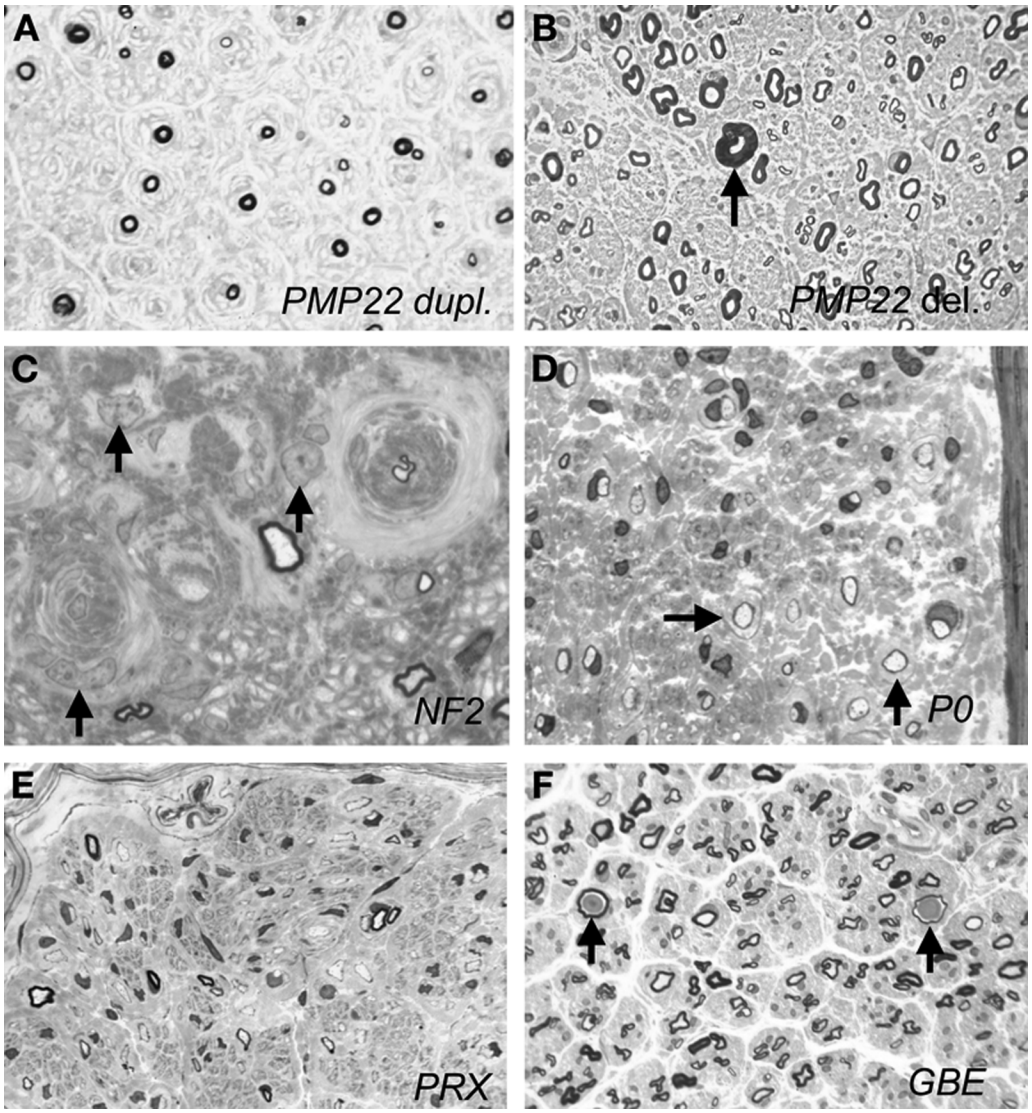

Fig. 1. Neuropathies of predominantly demyelinating type. **(A)** CMT1A owing to *PMP22* duplication showing multiple onion bulb formations. Nerve fiber density is severely reduced. Instead, the number of Schwann cells and the connective tissue is increased in amount "hypertrophic neuropathy." **(B)** Tomaculous neuropathy (neuropathy with liability to pressure palsies) owing to *PMP22* deletion showing only one tomaculous fiber (arrow). **(C)** Large onion bulb formations and abnormally shaped and large nuclei (arrows) in neurofibromatous neuropathy type 2 (NF2). **(D)** Congenital hypomyelination neuropathy (CHN) owing to *P0* mutation showing numerous hypomyelinated or demyelinated fibers and severe reduction of the number of nerve fibers. There is endoneurial edema and the number of Schwann cells is increased (dark nuclei). The light halo around thinly remyelinated fibers indicates "basal lamina onion bulb formation" (arrows). **(E)** CHN in *PRX* mutation showing similar changes as in Fig. 1D, but with some thickly remyelinated fibers. **(F)** Adult polyglucosan body (PGB) neuropathy resulting from a mutation in the branching enzyme gene *(GBE)*. The myelin sheaths around axons containing PGB (arrows) are too thin others are irregular in contour because of axonal shrinkage. PGB in Schwann cells and perineurial cells are not apparent in this plane of section.

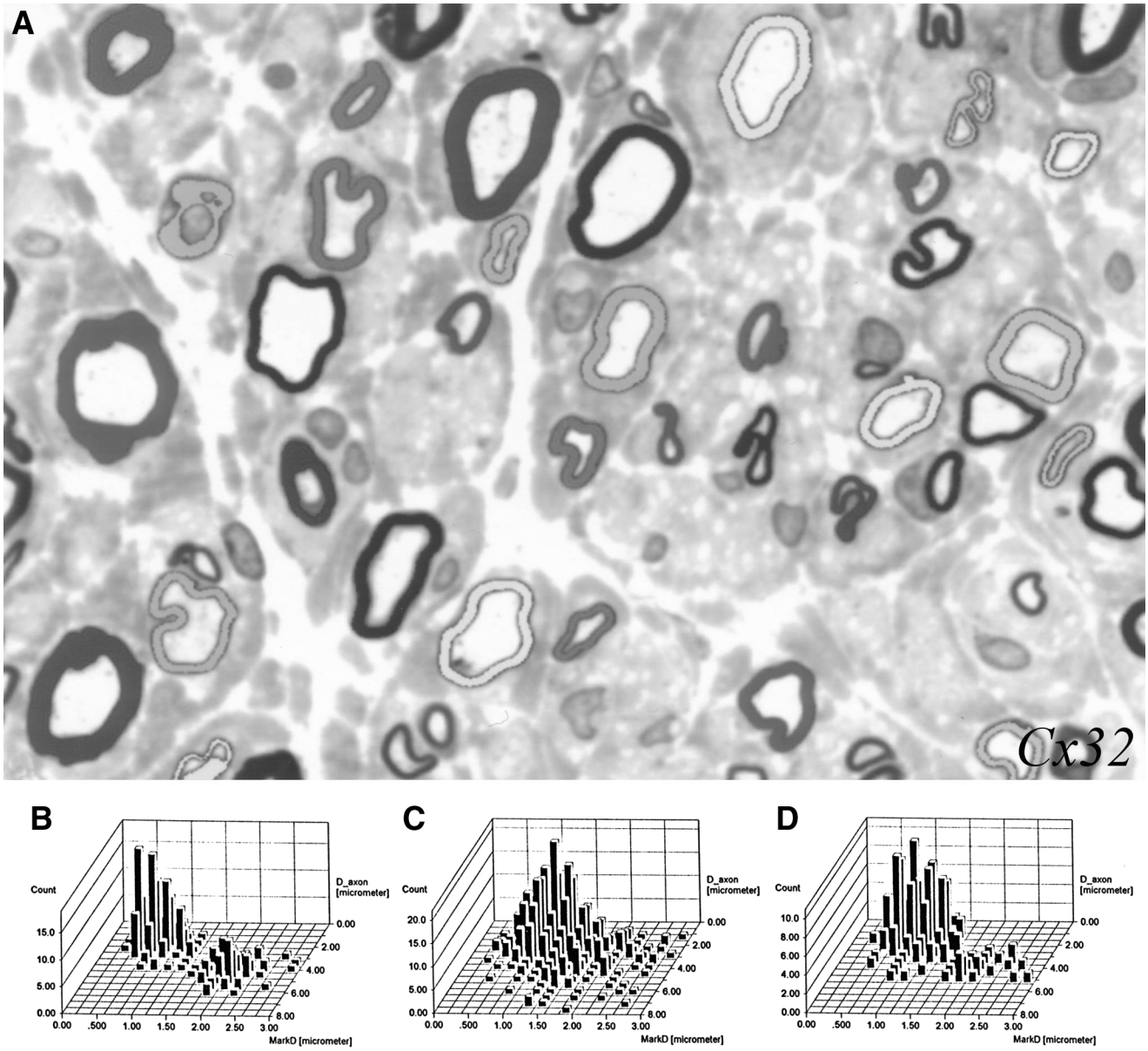

Fig. 2. Optic-electronic evaluation of a cross-sectional area of a semithin section from the sural nerve of a 15-yr-old boy **(A**,**C)** and his mother **(D)** with CMTX shown at the highest available light microscopic resolution with a planapochromat objective lens (×100) in comparison with an age-matched control (B). The number of nerve fibers is reduced and the myelin sheaths are disproportionately thin in relation to the size of their axons and are matched rather precisely by pseudocolors using the KS 300 system of Zeiss-Kontron (Oberkochen, Germany) (Schröder, 1998). The three dimensional diagrams **(B–D)** illustrate the large scatter of myelin thickness in relation to the axonal dimensions, especially in the boy's nerve. (Modified from Senderek et al., 1998.)

of *PMP22* on chromosome 17p11.2-p12 (Lupski et al., 1991; Raeymaekers et al., 1991) (Fig. 1A). This type of neuropathy is structurally characterized by *onion bulb formations* that were shown by electron microscopy to be made up of supernumerary Schwann cells concentrically arranged around demyelinated and remyelinated axons (Webster et al., 1967). "Onion bulbs" are formed by proliferation of

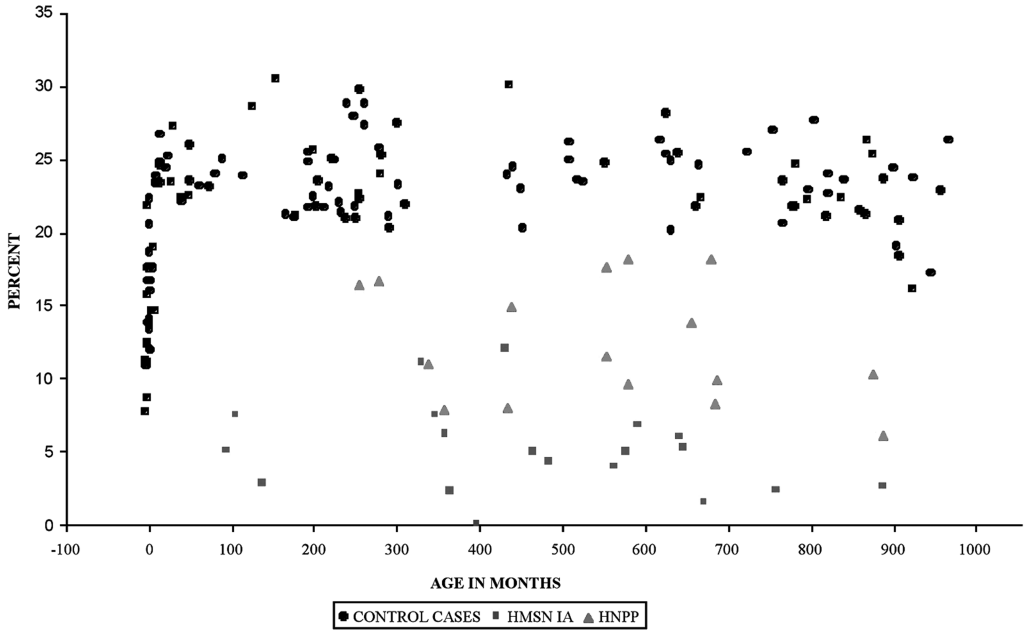

Fig. 3. Scatter diagram comparing the myelin area per endoneurial area in percent (*y*-axis) of 133 control cases (black dots), cases with *PMP22* duplication (CMT 1A; blue rectangles) and *PMP22* deletion (tomaculous neuropathy; red triangles) as evaluated by the same optic-electronic system, which was used for Fig. 2. The myelin area in CMT1A is on the average more severely reduced than in tomaculous neuropathy despite similar age distribution of the patients, which is indicated in months on the *x*-axis. This reflects the well-known differences in the clinical severity of both diseases. The blue rectangle on the *x*-axis (abscissa) indicates complete loss of myelinated nerve fibers in a case of CMT2A complicated by severe diabetes mellitus. (Modified from Thiex and Schröder, 1998.) (*See* online version for color.)

Schwann cells during de- and remyelination, whereas the basal lamina of the demyelinated nerve fiber acts as a scaffold to keep the Schwann cells in place close to the central axon and causing their semicircular or sometimes even circular arrangement. The number of Schwann cells increases by a factor of 8–14, depending on the size of the degenerating myelin sheath, when demyelination occurs, for example, in experimental allergic neuritis (Schröder, 1968b; Schröder and Krücke, 1970). This figure probably equals the number of Schwann cells increasing after segmental demyelination in hereditary neuropathies of man. The typical aspect of onion bulb formations may be mitigated if CMT1A is combined with chronic diabetic neuropathy or other conditions; this may finally lead to a severe neuronal type of neuropathy with rather complete loss of myelinated nerve fibers (Thiex and Schröder, 1998; Beckmann and Schröder, 2000). The *collagen* between the layers of Schwann cells is also increasing. The thickness of the collagen filaments and the amount of collagen tends to be directly related to the duration of the disease and the number of fibroblasts involved.

Myelin thickness and internodal length of the largest *remyelinated fibers* remains usually reduced. Thus on cross-sections the ratio between axon caliber and myelin thickness appears to be increased and, as measured in longitudinal sections or teased fiber preparations, internodal length is shortened to about 250–300 µm. These features are of diagnostic importance for identifying chronic and slowly progressive demyelinating types of neuropathy. For estimating myelin thickness it must be considered that the axon/myelin ratio changes toward thicker myelin sheaths during development as determined in sural, femoral, radial, and facial nerves reaching the adult value around puberty (15 yr of age) (Schröder et al., 1978, 1988). Thus myelin sheaths in infants and children are relatively thin. The normal range must therefore be kept in mind before

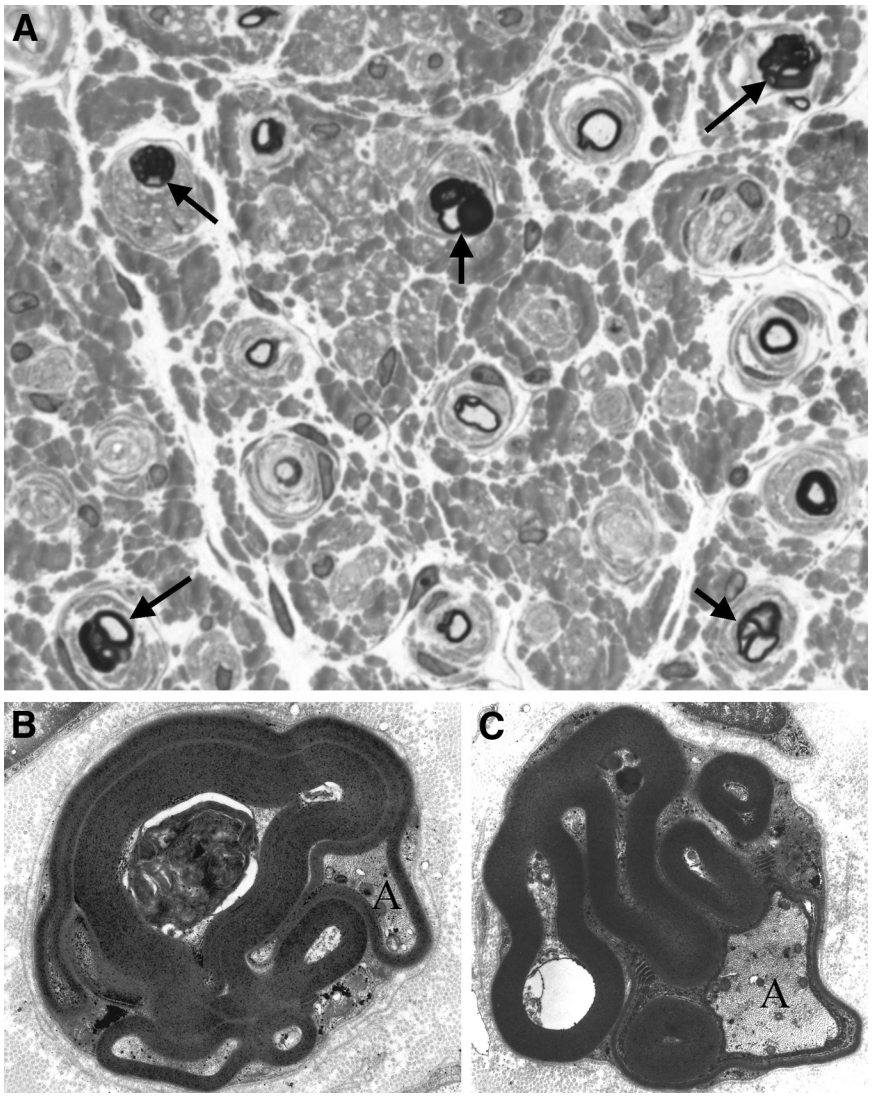

Fig. 4. Severe reduction of the number of nerve fibers, multiple onion bulb formations with demyelinated or thinly remyelinated axons in their center, and five typical, focally folded myelin sheaths (arrows in **A**; electron micrographs in **B** and **C**) in the sural nerve of a 8-yr-old boy with CMT4B. The axons (A) in B and C are thinly remyelinated despite severe "hypertrophy" of their myelin sheaths. (B and C modified from Schröder, 1999.)

diagnosing *hypomyelination* (*see* Developmental Disorders of the Myelin Sheath section). Following *axonal degeneration and regeneration* the myelin sheaths of the largest nerve fibers do not reach their regular thickness or a normal axon/myelin ratio, respectively (Schröder, 1972); they remain thinner so that an increased axon/myelin ratio does not necessarily indicate develomental delay or preceding segmental demyelination and remyelination. This is particularly important to realize when isolated nerve fibers have degenerated and regenerated without apparent formation of typical clusters, i.e., bundles of regenerated fibers of uneven size indicating overshooting regeneration ("hyperneurotization" of bands of Büngner) (Schröder, 1968a). Myelin sheaths may also become disproportionately thin when axons increase in diameter as seen in polyglucosan body neuropathy (Fig. 1F), or giant axonal neuropathy (Asbury et al., 1972) (Fig. 5L).

Progressive demyelination is a common feature in "congenital hypomyelination neuropathy" (CHN) (Kennedy, 1977; Guzzetta et al., 1982). CHN is clinically characterized by a Dejerine Sottas syndrome (DSS) with severely reduced conduction velocity (few m/s) and structurally characterized by demyelinated axons, disproportionately thin myelin sheaths following remyelination, and onion bulb formations of a special type, i.e., "basal lamina onion bulbs" Fig. 1D,E). In these, the circularly oriented Schwann cells processes have degenerated, whereas their basal laminae are persisting. Progressive CHN may be caused by point mutations in the following genes: *PMP22* (Simonati et al., 1999), myelin protein zero *(MPZ; P0)* (Warner et al., 1996) (Fig. 1D), early growth response gene *(EGR2)* (Warner et al., 1998; Timmerman et al., 1999), periaxin *(PRX)* (CMT4F; Fig. 1E) (Boerkoel et al., 2001; Guilbot et al., 2001; Takashima et al., 2002), *N*-myc downstream-regulated gene 1 *(NDRG1)* (HMSN Lom) (Baethmann et al., 1998; Kalaydjieva et al., 1998; Merlini et al., 1998; King et al., 1999; Chandler et al., 2000; Colomer et al., 2000; Kalaydjieva et al., 2000), and *KIAA1985* (CMT4C) (Senderek et al., 2003b). Distinguishing features between these different forms of CHN were elaborated for the first time by *immunohistochemical technics* for staining subunits of periaxin (Takashima et al., 2002) allowing a specific morphological diagnosis. Immunohistochemical diagnosis has thus far not been achieved in other hereditary neuropathies except for amyloidosis (Linke, 1982). In CMT4C, thin extended Schwann cell processes were described as a distinguishing feature (Kessali et al., 1997; Gabreels-Festen et al., 1999; Senderek et al., 2003b), although this does not appear to be specific; clinically thoracic scoliosis appears to be a more characteristic feature in *KIAA1985* mutations.

It is of interest that onion bulb formation in autoimmune disorders such as Guillain Barré syndrome and chronic inflammatory demyelinating neuropathy (CIDP) do not result in basal lamina onion bulb formations so typical for CHN. Schwann cells in early manifesting, genetically determined neuropathies are obviously more liable to die and to waste away, forming empty basal lamina onion bulbs than in disorders in which the myelin sheath is attacked by immunological pathomechanisms leading, for example, to chronic inflammatory demyelinating neuropathy or multifocal hypertrophic neuropathy with palpably enlarged nerves owing to increased numbers of Schwann cells, fibroblasts, and collagen (Webster et al., 1967).

Cell "viability" is even more obvious in neoplastic disorders such as neurofibromatosis type 2, in which onion bulb formations appear to be more cellular and larger in size showing prominent nuclei with irregular shape (Fig. 1C) (Sperfeld et al., 2002).

The myelin sheath may also be disproportionately thick in relation to the size of the axon. The pathomechanism underlying this type of *disproportionately increased myelin thickness* or myelin "hypertrophy" is not definitely clarified, but can best be explained by a model of concentric slippage of the spiral of the myelin lamella around the axon following reduction of the axonal perimeter. It is seen more or less frequently in nearly all types of neuropathy because of axonal atrophy and may be owing to primary disorders of neurons or axons, or may follow secondarily to segmental demyelination in a series of proximal segments (Fig. 1A). On the other hand, axonal atrophy may cause *secondary segmental demyelination*, which can only be verified morphometrically using statistical analysis of teased fiber preparations as exemplified in uremic neuropathy and Friedreich's disease (Dyck et al., 1971; Thomas et al., 1971). Because this needs laborious verification of an increased number of demyelinated or remyelinated segments on very long teased nerve fibers in primarily axonal types of neuropathy, this method did not gain widespread application. Nevertheless, secondary segmental demyelination must always be considered when, on cross-section, disproportionately thin myelin sheaths are seen in a primarily neuronal or axonal type of neuropathy.

"Hypertrophy" of myelin sheaths is also seen in "tomaculous neuropathy" or "hereditary neuropathy with liability to pressure palsies" (Behse et al., 1972; Madrid and Bradley, 1975; Jacobs and Gregory, 1991; Malandrini et al., 1992; Thomas et al., 1994; Mancardi et al., 1995; Tachi et al., 1997). It is usually caused by *PMP22* deletion (Chance et al., 1993) and tends to be less severe than CMT1A caused by *PMP22* duplication (Fig. 3). Typical tomaculous fibers (Fig. 1B) are the result of a pathomechanism of intussusception or invagination of a hypertrophic myelin sheath by a double, complete ring of additional myelin around itself thus causing three complete layers of myelin sheaths on cross-sections (Madrid and Bradley, 1975). This results in focal

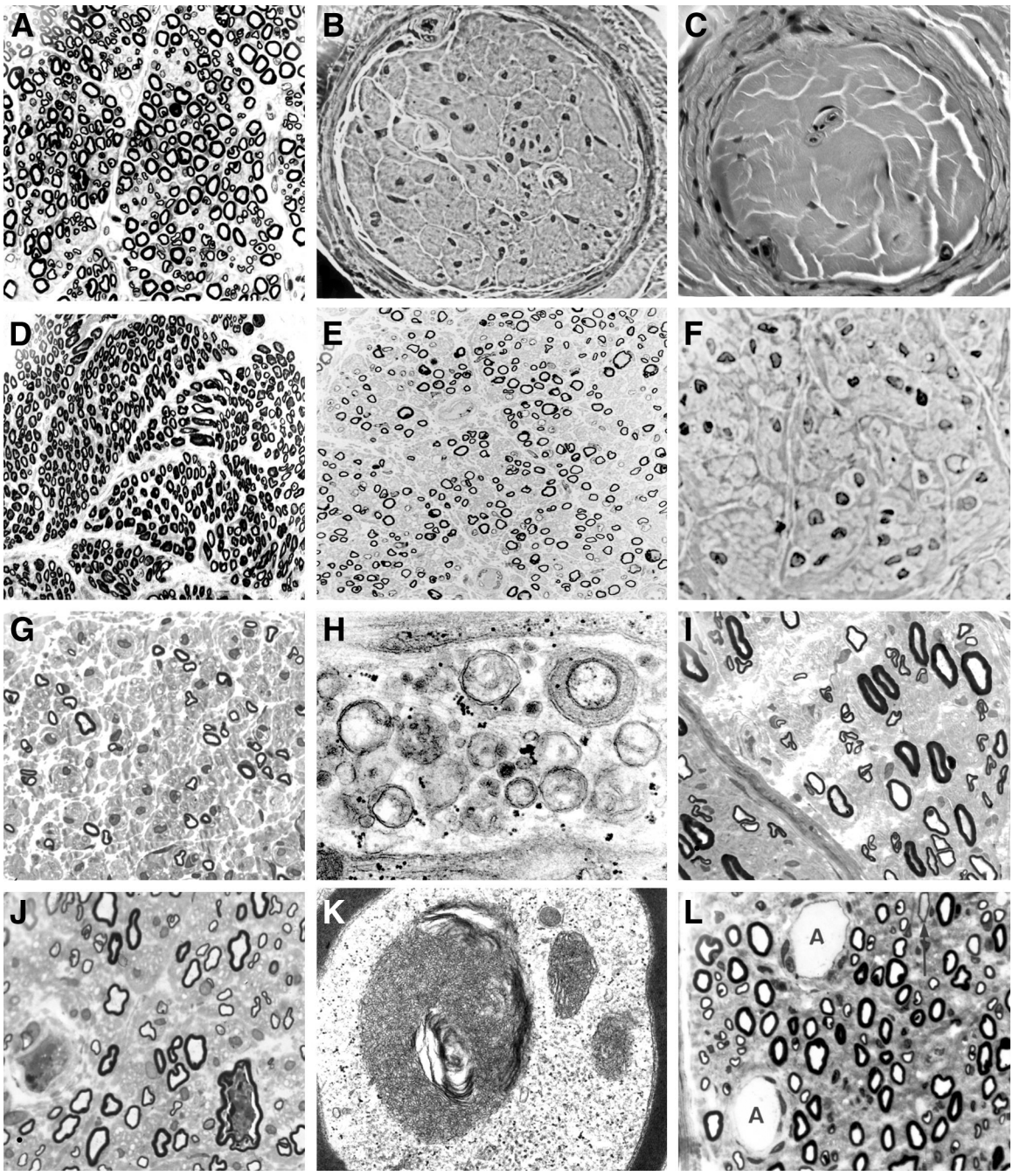

Fig. 5. Representative axonal types neuropathy in comparison with a normal control **(A)**. **(B)** Complete aplasia of myelinated nerve fibers in a 14-yr-old girl without any evidence of preceding degeneration of myelinated nerve fibers and with corresponding hypoplasia of this nerve fascicle (most unmyelinated fibers are preserved) (modified from Schröder et al., 1993a). **(C)** Complete degeneration of myelinated and unmyelinated nerve fibers including their Schwann cells in the sural nerve of a case with Tangier disease resulting from a mutation in the *ABC1* gene (case V:2 in Züchner et al., 2003). There are only several fibroblasts and a single blood vessel left over within this sclerotic nerve fascicle, which is filled with collagen (fibrosis). The perineurium is remarkably well

tomaculous (sausage-like) thickening of the myelin sheath at the paranode or at other, circumscribed portions of the internode, best visualized on longitudinal sections or in teased fiber preparations. Tomaculous fibers are not absolutely specific; they may also occur in *PMP22* duplication (CMT1A) or other conditions, although a single typical tomaculous fiber can already lead to the correct diagnosis of a *PMP22* deletion (Thiex and Schröder, 1998). Tomacula must be distinguished from the most frequent traumatic (artificial) damage of myelin sheaths consisting in severe distortion of the myelin sheath, usually in a larger number of adjacent fibers; the focal accumulation of clumsy myelin sheaths usually allows distinction between artefacts and real tomaculous fibers. This traumatic lesion leads to the most frequent misinterpretation of a nerve biopsy.

Tomaculous fibers must also be distinguished from "focally folded myelin" originally described in "globular neuropathy" (Dayan et al., 1968), in "congenital hypo- and hypermyelination neuropathy" (Vallat et al., 1987), and in an autosomal-recessive motor and sensory neuropathy with "excessive myelin outfolding" (Ohnishi et al., 1989) (Fig. 4). Autosomal-recessive inheritance was also noted in other families (Gabreels-Festen et al., 1990; Sabatelli et al., 1994; Quattrone et al., 1996; Gambardella et al., 1998) initially linked to chromosome 11q23 (Salih et al., 2000). Focally folded myelin has, meanwhile, been attributed to mutations in the myotubularin-related protein-2 gene on chromosome 11q22 (CMT4B) (Bolino et al., 1996, 2000; Previtali et al., 2003). We noted extensive myelin folds also in a 57-yr-old man with a mitochondrial myopathy (Fig. 163 in Schröder, 2001). Extensive myelin folds may also occur in vasculitis and some other conditions although not in the typical form of intussusception as seen in tomaculous fibers or as excessive as in CMT4B (Fig. 4).

Concerning genotype–phenotype correlation, molecular genetically defined cases are frequently associated with different clinical and histopathological phenotypes. For example, in P0 mutations, the resulting phenotype may be HMSN I, DSS, or CHN (Hayasaka et al., 1993a, 1993b; Tachi et al., 1994; Yoshikawa et al., 1994; Ikegami et al., 1996; Warner et al., 1996; Hattori et al., 2003). In this context, it should be mentioned that delineation of DSS vs CHN is presently not clear because the molecular genetic basis of the original DSS cases is not defined whereas, for example, the original family with HMSN Ib has later been attributed to a mutation in

Fig. 5. *(Continued)* preserved although moderately thickened (fibrotic). **(D)** Riley–Day syndrome with preservation of large and many medium-sized or small myelinated nerve fibers but rather complete absence of unmyelinated fibers. Thus the density of myelinated fibers appears to be increased compared with the control **(A)** (modified from Schröder, 1999). **(E)** Absence (aplasia) of large myelinated fibers with epilepsy, deafness, and mental retardation in a 2.5-yr-old girl without any evidence of preceding degeneration (modified from Müller et al., 2000). **(F)** Complete absence of myelinated and unmyelinated nerve fibers owing to Wallerian degeneration after implantation of an inefficient nerve graft, 8 cm in length, 6 mo after surgery in a dog. Compared with Fig. 5C, most of the Schwann cells appear to be preserved in bands of Büngner (Schröder and Seiffert, 1972) although many of their nuclei are condensed and darkly stained because of inactivity. Myelin degradation products have been removed after this period of time. **(G)** In CMT2A, resulting from *mitofusin2* mutation in a 7-yr-old boy, there is severe loss of nerve fibers with little evidence of regeneration (Schröder et al., 2005). **(H)** Electron micrograph of the same case as in Fig.5G. The mitochondria are focally accumulated in an unmyelinated axon, showing various changes, possibly including inefficient fusion although this cannot be concluded from such a static image. **(I)** Intermediate, axonal and demyelinating type of neuropathy in a 54-year-old woman with CMT1F owing to a mutation in the *NEFL* gene (modified from Züchner et al., 2004b). The number of nerve fibers is reduced. Clusters of small regenerated myelinated fibers are never the less apparent in addition to thinly remyelinated fibers, some of which show incipient onion bulb formation (as seen on electron micrographs). No giant axons (cf. Fig. 5L below) with increased numbers of neurofilaments were seen in this case. **(J)** Severe neuropathy in neuroaxonal dystrophy in a 3-yr-old girl. Dystrophic nerve fibers are apparent, although the characteristic axonal changes with complex membranous structures and splits can only be identified on electron micrographs (**K**). (Modified from Schröder, 1999.) **(L)** Giant axonal neuropathy in an 11-yr-old girl. A small number of nerve fibers only are affected. The enlarged axons **(A)** are demyelinated or thinly remyelinated.

the P0 gene (Bird et al., 1997). As outlined earlier, CHN may be caused by at least six different genes.

Developmental Disorders of the Myelin Sheath

These consist of apparently extremely rare conditions with complete absence of myelin only in the peripheral nervous system (PNS; Palix and Coignet, 1978; Charnas et al., 1988), or in the PNS and central nervous system (CNS; Schröder and Bohl, 1978) (Fig. 5B). Disproportionately thin myelin sheaths without any or very little evidence of progression have been reported, in addition to the conditions mentioned earlier and also in systemic disorders with presumably anabolic disturbance of myelination (Schröder, 1982a) such as Leigh's syndrome (Goebel et al., 1986; Peiffer et al., 1988; Jacobs et al., 1990), Cockayne syndrome (Moosa and Dubowitz, 1970; Ohnishi et al., 1987; Schröder, 2001), and muscle phosphoglycerate kinase deficiency (Schröder et al., 1996). It is not settled whether disproportionately thin myelin sheath, such as seen in certain cases with *Cx32* mutation (CMTX; Fig. 2), are caused by a developmental disturbance or early demyelination and remyelination.

There is a *great variety of other fine structural changes of myelin sheaths*. Although recommended, the complete spectrum of myelin changes is only rarely evaluated because a large number of electron micrographs at different magnifications are needed (cf., Table 3 in Senderek et al., 2000). Which of these fine structural changes of the myelin sheath are associated with which and how many mutations is not yet settled. For example, dissociation of terminal myelin loops from the axon and loss of transverse bands was defined as the initial change in periaxin neuropathy (CMT4F) (Takashima et al., 2002). Yet the paranodium is the site of initial changes in other demyelinating neuropathies such as diphtheric neuropathy (Webster et al., 1961), although exactly similar changes have not been documented at the electron microscopic level in diphtheritic and other neuropathies.

Changes of Axons

Neuropathies of "axonal" or "neuronal" type (Fig. 5A–L) are 3.2-fold more frequent in the present author's data bank of 8052 sural nerve biopsies, collected over 40 yr, than those of a "demyelinating" type (4500:1406). Of these, 578 were classified as "mixed axonal and demyelinating" in type. The other cases were not classified using this simplifying scheme.

The distinction between an axonal and a neuronal type of neuropathy may be arbitrary. But we consider neuropathies of *axonal* vs neuronal type when there is evidence of regeneration, i.e., when there are clusters of regenerated or regenerating fibers; these clusters indicate persisting viability of the cell body (perikaryon of the neuron). When there is loss of nerve fibers with no evidence of regeneration, a *neuronal* type of neuropathy is assumed which initially does not exclude more proximal viability of the axon and neuron. However, in a chronic type of neuropathy of several years duration at least some regenerating sprouts forming clusters within their original basal lamina or band of Büngner should have reached the distal (sural) site of the nerve biopsy if the proximal axon and neuron have persisted. The preponderance of axonal types of neuropathy is presumably owing to the extraordinary length of the axon. An axon depends on the integrity of its perikaryon as well as on adequate blood supply for nutrition, and a proper endoneurial milieu along its length.

Developmental disturbances of peripheral neurons are seen in several rare conditions. The most severe form includes total absence of myelinated fibers as already mentioned when describing disorders of myelination. A selective involvement of certain neuronal systems is apparent in the following diseases, which need to be ascertained using morphometry. One of these conditions is characterized by autosomal-recessive *deficiency of large myelinated nerve fibers* in the sural nerve (Sabatelli et al., 1998) with a corresponding deficiency of large neurons in spinal ganglia and in the spinal cord (Müller et al., 2000) (Fig. 5E). The condition is nearly nonprogressive, and is associated with epilepsy, deafness, and mental retardation. The underlying molecular genetic background has not yet been analyzed. A hereditary sensory neuropathy with selective *reduction of small myelinated nerve* fibers has also been described (Donaghy et al., 1987). This condition may be difficult to differentiate from cases with nonhereditary "small fiber neuropathy," which is increasingly studied using skin biopsies. Rather complete *absence of unmyelinated fibers* is seen in hereditary sensory autonomic neuropathy type III (HSAN III; Riley–Day

syndrome; Fig. 5D); some unmyelinated axons may still develop and survive in this condition when studied electron microscopically (Schröder, 1999).

Inherited (nontoxic, ischemic, inflammatory, or traumatic) neuropathies of the axonal type primarily tend to involve the most distal portions of a nerve fiber ("dying back" type of neuropathy). The prototype of an axonal type of neuropathy is *neuroaxonal dystrophy*, which also affects the CNS (Fig. 5J,K) (Wolfe et al., 1995). Other disorders affecting different organelles in the perikaryon and axon can cause a neuropathy of the axonal or neuronal type. An interesting example for a neuropathy caused by a primary disorder of *neurofilaments*, one of the main components of the axoplasmic cytoskeleton, is CMT2E (Fig. 5I), which is allelic to CMT1F showing a more demyelinating type of neuropathy. Both phenotypes are caused by mutations in the *NEFL* gene (Jordanova et al., 2003; Züchner et al., 2004b). There is phenotypic variation of axonal changes in CMT2E. In some cases, typical giant axons characteristic for giant axonal neuropathy may occur (Fabrizi et al., 2004). Yet giant axonal neuropathy (Asbury et al., 1972; Sabatelli et al., 1992) is a disorder affecting other intermediate filaments as well and is caused by mutations in the gene gigaxonin *(GAN1)* (Bomont et al., 2000). In addition to axons, Schwann cells, fibroblasts, cardiac muscle fibers, and hairs are characteristically involved (Timmerman et al., 2000) so that a diagnosis can be achieved by scanning electron microscopy of a single "curly" hair (cf. Fig. 191E in Schröder, 2001).

Microtubules were also considered as a primary site of CMT2A resulting from mutations in *KIF1B* (Zhao et al., 2001); however, this could not be confirmed by others (Bissar-Tadmouri et al., 2004). Microtubules are always involved when axons degenerate as a result of any lesion causing distal (Wallerian type) degeneration. Yet such impressive changes of tubular structures as seen in experimental taxol neuropathy (Röyttä and Raine, 1986) have not yet been recorded in human nerves.

Other examples of axonal neuropathies are those caused by *mitochondrial disorders*. These may be the result of either mutations in the mitochondrial DNA such as NARP, MELAS, MERRF, and KSS (Schröder and Sommer, 1991; Schröder, 1993; Zanssen et al., 1998), or mutations in the nuclear DNA such as MNGIE (Bardosi et al., 1987; Papadimitriou et al., 1998; Nishino et al., 2001), and the mitofusin 2 gene *(MFN2)* in CMT2A (Züchner et al., 2004a) (Fig. 5G,H).

The fine structural changes of *mitochondria* owing to *MFN2* mutations in CMT2A are of special interest because CMT2A includes about 20% of all axonal CMT neuropathies and because mitochondria are of major importance concerning energy supply. The neuropathy is characterized by severe axonal loss and progressive axonal degeneration. There are large bands of Büngner but very little evidence of axonal regeneration. Aggregates of abnormal mitochondria were seen at paranodal protrusions of the axon and at sites of axonal degeneration. These mitochondrial abnormalities consisted of swelling, shrinkage, and irregularities of the outer and inner membranes of mitochondria (Schröder et al., 2005). Defective mitochondrial fusion was suggested because the longest mitochondria in CMT2A measured only 1.35 µm on electron micrographs of longitudinally oriented myelinated axons, whereas in CMTX cases, which were used as (pathological) controls, a maximal length of 5.31 µm was measured. No paracrystalline or homogeneous globular inclusions were detectable such as those seen in muscle fibers of cases with mtDNA mutations. Some paracrystalline inclusions in mitochondria of Schwann cells of unmyelinated fibers were present; yet these are nonspecific and occur in a large number of conditions. Tilting the goniometer stage of the electron microscope revealed changing directions of the "paracrystalline lines" suggesting a granular rather than a membranous composition of the mitochondrial inclusions in addition to an amorphous component (Schröder, 1993).

In dominantly inherited neuropathies with optic atrophy of the Vizioli type (HMSN VI), abnormal cristae were seen in Schwann cells of myelinated fibers and rare, abnormal mitochondria with needle-like inclusions (presumably of hydroxyapatite) in axons (Sommer and Schröder, 1989b; Schröder and Sommer, 1991; Schröder, 1993). The Vizioli type of neuropathy is likely to be caused by mitofusin 2 mutations as in CMT2A (Züchner, personal communication). Perikarya of motor neurons in the spinal cord, or of sensory neurons in spinal ganglia have not been studied so far in respect to mitochondrial changes, although this would be of major interest.

In Leber's hereditary optic neuropathy (LHON), which is known to be caused by mutations in the mtDNA, paracrystalline inclusions were noted in

Schwann cells of unmyelinated fibers in which they have already been seen in various neuropathies (Lyon and Evrard, 1970) including Refsum's disease (Fardeau and Engel, 1969) and mitochondrial myopathy of undefined cause (Yiannikas et al., 1983). In Schwann cells of myelinated fibers, closely opposed mitochondrial cristae with paracrystalline material were an interesting finding not seen in controls (Sommer and Schröder, 1989b). It would be worthwhile to study DNA in archival material from these cases to classify these disorders with molecular genetic techniques as has been done in other cases (Thiex and Schröder, 1998; Senderek et al., 1998, 1999, 2000, 2003a; Schröder et al., 1999b; Züchner et al., 2003).

Intermediate (Axonal and Demyelinating) Forms of Neuropathy

An increasing number of neuropathies are neither typically demyelinating (conduction velocities <45 m/s) nor typically axonal (conduction velocities ranging from normal to 25 m/s). These are designated as "intermediate" and occur following mutations, for example, in *Cx32* (Rouger et al., 1997; Senderek et al., 1999a), *NEFL* (Züchner et al., 2004b) (Fig. 5I) and *GDAP1* (Senderek et al., 2003a) and other dominant intermediate forms of CMT (DI-CMT) such as those caused by mutations of the pleckstrin homology domain of dynamin 2 *(DNM2)* (Züchner et al., 2005). Nerve biopsies from the latter type of neuropathy are not available as long as no biopsies, previously performed and stored in archives of certain laboratories, have been attributed to such a mutation. Biopsies in cases with *GDAP1* mutations revealed evidence of axonal degeneration and regeneration as well as segmental demyelination and remyelination with a moderate degree of onion bulb formation (Senderek et al., 2003a). In P0 mutations, a predominantly axonal type of neuropathy may be encountered (Senderek et al., 1999), although P0 is the major component of peripheral myelin sheaths.

Endoneurial Connective Tissue Changes

The endoneurial connective tissue is regularly, although seemingly always secondarily, involved in all types of peripheral neuropathy. These neuropathies are made up of various forms of (hereditary) *amyloidosis* that can be delineated and differentiated immunohistochemically and electron microscopically by their characteristic fibrillar endoneurial deposits (for references *see* Sommer and Schröder, 1989a).

The endoneurial *collagen* is particularly increased in chronic "hypertrophic" types of neuropathy where an interaction between proliferated Schwann cells and fibroblasts may lead to a severe increase of the endoneurial collagen. As mentioned earlier, collagen fibers are, in chronic cases, a major component of onion bulb formations. The increase of collagen may be most prominent in multifocal hypertrophic neuropathy (Webster et al., 1967). As illustrated in Fig. 5C, collagen may persist and may be the major endoneurial component after all parenchymal elements (axons and Schwann cells) have disappeared.

Degenerating endoneurial *fibroblasts* are a frequent phenomenon in a large spectrum of peripheral neuropathies, including inherited neuropathies. They are particularly frequent in vasculitic neuropathy (Grehl and Schröder, 1991).

Neuropathy as a result of *neurofibromatosis type 2* (Fig. 1C; v. s.) may be included in this group of diseases, although Schwann cells, which are considered as parenchymal cells, appear to be more severely affected in this disease than *fibroblasts* which, aside from blood vessels, are the main cellular component of the connective tissue. Quiescent or active endoneurial *macrophages* may also be considered as a component of the connective tissue. They play a major role in various storage diseases or in removing debris from degenerating nerve fibers. Macrophages with large vacuoles occur in chronic hereditary neuropathies and are nonspecifically indicating chronicity. *Matrix metalloproteinases* as a primary site of a genetic disorder have thus far not been delineated as a cause of hereditary peripheral neuropathy, although they form an important component in peripheral nerves.

Perineurial Changes

A rare, presumably heterogeneous and autosomal-recessive, "complicated" type of neuropathy is associated with mental retardation and cataract. The

hallmark are perineurial alterations consisting of unusual, abnormally proliferated, indented, and extended perineurial cells in the endoneurium (Schröder et al., 1999a; Thomas et al., 2000; Wolfe et al., 2000). These are unlike those seen in "minifascicles" and perineurioma (Schröder, 1999).

Similar perineurial cell alterations were not noted in Marinesco-Sjögren syndrome, which is also characterized by mental retardation and cataracts but additionally by short stature. It has recently been attributed to mutations in *SIL1* (Senderek et al., 2005) and shows a nonprogressive or very slowly progressive neuropathy of a mixed, axonal, and demyelinating type (Serratrice et al., 1973; Alexianu et al., 1983); this is supported by muscle biopsies revealing some degree of neurogenic atrophy (Zimmer et al., 1992). Yet the impressive and seemingly specific fine structural changes involving sarcolemmal nuclei (Schröder, 1982b; Goto et al., 1990; Sewry et al., 1988) were not seen in peripheral nerves.

Vascular Changes

Certain hereditary diseases are associated with generalized vascular changes. These diseases may be diagnosed by a peripheral nerve biopsy because of characteristic alterations in endothelial, smooth muscle, or perivascular cells. Such diseases include neuraminidase A and B (Sandhoff's disease) (Schröder, 2001), CADASIL (Schröder et al., 1995; Schröder et al., 2005), and ferritinopathy (Schröder, 2005), which has previously been described as "granular nuclear inclusion body disease" (Schröder et al., 1985).

Hereditary Multisystem (Metabolic) Diseases With Characteristic Changes Affecting Peripheral Nerves More or Less Severely

As already mentioned, the most severe neuropathy in our whole series of 8052 sural nerve biopsies was neuronal in type with complete loss of axons as well as Schwann cells in a case of *Tangier disease* (Fig. 5C). This disease is caused by a mutation in the ATPase binding cassette transporter gene 1 *(ABC1)* gene (Züchner et al., 2003). There were only a few surviving blood vessels and some macrophages with characteristic lipid storage products in the endoneurium, whereas the perineurium and the epineurium were rather well preserved. Usually, following Wallerian degeneration and definite loss of axons, Schwann cells tend to survive in bands of Büngner despite lack of reinnervation (Fig. 5F). Other cases were less severely affected showing characteristic endoneurial macrophages and Schwann cells with lipid inclusions that are not membrane-bound (Gibbels et al., 1985). When the lipids are extracted during the embedding procedure these inclusions may be misinterpreted as "membrane-bound vacuoles."

Fabry's disease, Sandhoff's disease (neuraminidase A and B deficiency), gangliosidosis types 1 and 2, Niemann Pick's disease, and various forms of mucopolysaccharidosis (Chen et al., 1999) should also be mentioned because of characteristic fine structural changes allowing a specific diagnosis (for illustrations *see* Schröder, 2001). Among the latter, Sanfilippo's disease may be associated with a severe form of peripheral neuropathy. In addition, *polyglucosan body disease (branching enzyme deficiency; glycogenosis type IV)* owing to mutations in the branching enzyme gene may cause a peripheral neuropathy showing impressive and specific inclusions (polyglucosan bodies) in axons (Fig. 1F), Schwann cells, and perineurial cells (Busard et al., 1990; Schröder et al., 1993b; Ziemssen et al., 2000), eventually also in epineurial smooth muscle cells. In *peroxisomal diseases* bilaminar or trilaminar inclusions in Schwann cells and other cells may allow a group-specific fine structural diagnosis (Schröder et al., 2004). These diseases, however, are not restricted to the PNS, although PNS symptoms may predominate.

In conclusion, although most pathological changes in peripheral nerves are nonspecific, a large number of those listed allow a more or less specific fine structural diagnosis. In many cases, it remains a difficult neuropathological task to distinguish *pathognostic* from *nonspecific* and *normal structures* in developing, adult, and aging peripheral nerves.

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References

Alexianu M. D., Christodoresc C., Vasilescu A., et al. (1983) Sensorimotor neuropathy in a patient with Marinesco-Sjögren syndrome. *Eur. Neurol*. **22,** 222–226.

Asbury A. K., Gale M. K., Cox S. C., Baringer J. R., and Berg B. O. (1972) Giant axonal neuropathy—a unique case with segmental neurofilamentous masses. *Acta Neuropathol. (Berl.)* **20,** 237–247.

Baethmann M., Göhlich-Ratmann G., Schröder J. M., Kalaydjieva L., and Voit T. (1998) HMSNL in a 13-year-old Bulgarian girl. *Neuromusc. Disord*. **8,** 90–94.

Bardosi A., Creutzfeldt W., DiMauro S., et al. (1987) Myo-, neuro-, gastrointestinal encephalopathy (MNGIE syndrome) due to partial deficiency of cytochrome-c-oxidase. A new mitochondrial multisystem disorder. *Acta Neuropathol. (Berl.)* **74,** 248–258.

Beckmann A. and Schröder J. M. (2000) Screening for Charcot-Marie-Tooth type 1A and hereditary neuropathy with liability to pressure palsy in archival nerve biopsy samples by direct-double-differential PCR. *Acta Neuropathol*. **100,** 459–463.

Behse F., Buchthal F., Carlsen F., and Knappeis G. G. (1972) Hereditary neuropathy with liability to pressure palsies. Electrophysiological and histopathological aspects. *Brain* **95,** 777–794.

Bergoffen J., Scherer S. S., Wang S., et al. (1993) Connexin mutations in X-linked Charcot-Marie-Tooth disease. *Science* **262,** 2039–2042.

Bertram M. and Schröder J. M. (1993) Developmental changes at the node and paranode in human sural nerves: morphometric and fine-structural evaluation. *Cell Tissue Res*. **273,** 499–509.

Bird T. D., Kraft G. H., Lipe H. P., Kenney K. L., and Sumi S. M. (1997) Clinical and pathological phenotype of the original family with Charcot-Marie-Tooth type 1B: a 20-year study. *Ann. Neurol.* **41,** 463–469.

Bissar-Tadmouri N., Nelis E., Züchner S., et al. (2004) Absence of KIF1B mutation in a large Turkish CMT2A family suggests involvement of a second gene. *Neurology* **62,** 1522–1525.

Boerkoel C. F., Takashima H., Stankiewicz P., et al. (2001) Periaxin mutations cause recessive Dejerine-Sottas neuropathy. *Am. J. Hum. Genet*. **68,** 325–333.

Bolino A., Brancolini V., Bono F., et al. (1996) Localization of a gene responsible for autosomal recessive demyelinating neuropathy with focally folded myelin sheaths to chromosome 11q23 by homozygosity mapping and haplotype sharing. *Hum. Mol. Genet*. **5,** 1051–1054.

Bolino A., Muglia M., Conforti F. L., et al. (2000) Charcot-Marie-Tooth type 4B is caused by mutations in the gene encoding myotubularin-related protein-2. *Nat. Genet*. **25,** 17–19.

Bomont P., Cavalier L., Blondeau F., et al. (2000) The gene encoding gigaxonin, a new member of the cytoskeletal BTB/kelch repeat family, is mutated in giant axonal neuropathy. *Nat. Genet*. **26,** 370–374.

Busard H., Gabreels-Festen A., Van t'Hof M., Renier W., and Gabreels F. (1990) Polyglucosan bodies in sural nerve biopsies. *Acta Neuropathol. (Berl.)* **80,** 554–557.

Chance P. F., Alderson M. K., Leppig K. A., et al. (1993) DNA deletion associated with hereditary neuropathy with liability to pressure palsies. *Cell* **72,** 143–151.

Chandler D., Angelicheva D., Heather L., et al. (2000) Hereditary motor and sensory neuropathy - lom (HMSNL): refined genetic mapping in romani (Gypsy) families from several european countries. *Neuromuscul. Disord*. **10,** 584–591.

Charnas L., Trapp B., and Griffin J. (1988) Congenital absence of peripheral myelin: abnormal Schwann cell development causes lethal arthrogryposis multiplex congenita. *Neurology*. **38,** 966–974.

Chen C. S., Patterson M. C., Wheatley C. L., O'Brien J. F., and Pagano R. E. (1999) Broad screening test for sphingolipid-storage diseases. *Lancet* **354,** 901–905 (*see* comments).

Colomer J., Iturriaga C., Kalaydjieva L., Angelicheva D., King R. H., and Thomas P. K. (2000) Hereditary motor and sensory neuropathy-Lom (HMSNL) in a spanish family: clinical, electrophysiological, pathological and genetic studies.*Neuromuscul Disord*. **10,** 578–583.

Dayan A. D., Graveson G. S., Robinson P. K., and Woodhouse M. A. (1968) Globular neuropathy. A disorder of axons and Schwann cells. *J. Neurol. Neurosurg. Psychiatry* **31,** 552–560.

De Jonghe P., Timmerman V., Nelis E., Martin J. J., and Van Broeckhoven C. (1997) Charcot-Marie-Tooth

Disease and related peripheral neuropathies. *J. Periph. Nerv. Syst*. **2,** 370–387.

De Jonghe P., Timmerman V., De Visser M., et al. (1998) Classification and diagnostic guidelines for Charcot-Marie-Tooth type 2 (CMT2-HMSNII) and distal hereditary motor neuropathy (distal HMN-spinal CMT). 2nd Workshop of the European CMT-Consortium, 53rd ENCM International Workshop, 26–28 September 1997, Naarden, Niederlande. *J. Periph. Nerv. Syst*. **3,** 283–307.

Donaghy M., Hakin R. N., Bamford J. M., et al. (1987) Hereditary sensory neuropathy with neurotrophic keratitis. Description of an autosomal recessive disorder with a selective reduction of small myelinated nerve fibres and a discussion of the classification of the hereditary sensory neuropathies. *Brain* **110,** 563–583.

Dyck P. J., Johnson W. J., Lambert E. H., and O'Brien P. C. (1971) Segmental demyelination secondary to axonal degeneration in uremic neuropathy. *Mayo Clin. Proc*. **46,** 400–431.

Dyck, P. J. and Thomas P. K. (2005) Peripheral Neuropathy (4th ed.). Philadelphia, London, Toronto: Saunders.

Fabrizi G. M., Cavallaro T., Angiari C., et al. (2004) Giant axon and neurofilament accumulation in Charcot-Marie-Tooth disease type 2E. *Neurology* **62,** 1429–1431.

Fardeau M. and Engel W. K. (1969) Ultrastructural study of a peripheral nerve biopsy in Refsum's disease. *J. Neuropathol. Exp. Neurol*. **28,** 278–294.

Gabreels-Festen A. A., Joosten E. M., Gabreels F. J., Stegeman D. F., Vos A. J., and Busch H. F. (1990) Congenital demyelinating motor and sensory neuropathy with focally folded myelin sheaths. *Brain* **113,** 1629–1643.

Gabreels-Festen A., van Beersum S., Eshuis L., et al. (1999) Study on the gene and phenotypic characterisation of autosomal recessive demyelinating motor and sensory neuropathy (Charcot-Marie-Tooth disease) with a gene locus on chromosome 5q23-q33. *J. Neurol. Neurosurg. Psychiatry* **66,** 569–574.

Gambardella A., Bolino A., Muglia M., et al. (1998) Genetic heterogeneity in autosomal recessive hereditary motor and sensory neuropathy with focally folded myelin sheaths (CMT4B). *Neurology* **50,** 799–801.

Gibbels E., Schaefer H. E., Runne U., Schröder J. M., Haupt W. F., and Assmann G. (1985) Severe polyneuropathy in Tangier disease mimicking syringomyelia or leprosy. Clinical, biochemical, electrophysiological, and morphological evaluation, including electron microscopy of nerve, muscle, and skin biopsies. *J. Neurol*. **232,** 283–294.

Goebel H. H., Bardosi A., Friede R. L., Kohlschutter A., Albani M., and Siemes H. (1986) Sural nerve biopsy studies in Leigh's subacute necrotizing encephalomyelopathy. *Muscle Nerve* **9,** 165–173.

Goto Y., Komiyama A., Tanabe Y., Katafuchi Y., Ohtaki E., and Nonaka I. (1990) Myopathy in Marinesco-Sjogren syndrome: an ultrastructural study. *Acta Neuropathol. (Berl.)* **80,** 123–128.

Grehl H. and Schröder J. M. (1991) Significance of degenerating endoneurial cells in peripheral neuropathy. *Acta Neuropathol. (Berl.)* **81,** 680–685.

Guilbot A., Williams A., Ravise N., et al. (2001) A mutation in periaxin is responsible for CMT4F, an autosomal recessive form of Charcot-Marie-Tooth disease. *Hum. Mol. Genet*. **10,** 415–421.

Guzzetta F., Ferriere G., and Lyon G. (1982) Congenital hypomyelination polyneuropathy. Pathological findings compared with polyneuropathies starting later in life. *Brain* **105,** 395–416.

Hahn A. F., Ainsworth P. J., Bolton C. F., Bilbao J. M., and Vallat J. M. (2001) Pathological findings in the x-linked form of Charcot-Marie-Tooth disease: a morphometric and ultrastructural analysis. *Acta Neuropathol*. **101,** 129–139.

Hattori N., Yamamoto M., Yoshihara T., et al. (2003) Demyelinating and axonal features of Charcot-Marie-Tooth disease with mutations of myelin-related proteins (PMP22, MPZ and Cx32): a clinicopathological study of 205 Japanese patients. *Brain* **126,** 134–151.

Hayasaka K., Himoro M., Sato W., et al. (1993a) Charcot-Marie-Tooth neuropathy type 1B is associated with mutations of the myelin P0 gene *Nat. Genet*. **5,** 31–34 (*see* comments).

Hayasaka K., Himoro M., Sawaishi Y., et al. (1993b) De novo mutation of the myelin P0 gene in Dejerine-Sottas disease (hereditary motor and sensory neuropathy type III). *Nat. Genet*. **5,** 266–268.

Ikegami T., Nicholson G., Ikeda H., et al. (1996) A novel homozygous mutation of the myelin P0 gene producing Dejerine-Sottas disease (hereditary motor and sensory neuropathy type III). *Biochem. Biophys. Res. Commun*. **222,** 107–110.

Jacobs J. M. and Gregory R. (1991) Uncompacted lamellae as a feature of tomaculous neuropathy. *Acta Neuropathol. (Berl.)* **83,** 87–91.

Jacobs J. M., Harding B. N., Lake B. D., Payan J., and Wilson J. (1990) Peripheral neuropathy in Leigh's disease. *Brain* **113,** 447–462.

Jordanova A., De Jonghe P., Boerkoel C. F., et al. (2003) Mutations in the neurofilament light chain gene (NEFL) cause early onset severe Charcot-Marie-Tooth disease. *Brain* **126,** 590–597.

Kalaydjieva L., Gresham D., Gooding R., et al. (2000) N-myc downstream-regulated gene 1 is mutated in hereditary motor and sensory neuropathy-Lom. *Am. J. Hum. Genet*. **67,** 47–58.

Kalaydjieva L., Nikolova A., Turnev I., et al (1998) Hereditary motor and sensory neuropathy—Lom, a novel demyelinating neuropathy associated with deafness in gypsies. Clinical, electrophysiological and nerve biopsy findings. *Brain* **121,** 399–408.

Kennedy W. R., Sung J. H., and Berry J. F. (1977) A case of congenital hypomyelinisation neuropathy. Clinical, morphological and chemical studies. *Arch. Neurol. (Chic.)* **34,** 337–345.

Kessali M., Zemmouri R., Guilbot A., et al. (1997) A clinical, electrophysiologic, neuropathologic, and genetic study of two large Algerian families with an autosomal recessive demyelinating form of Charcot-Marie-Tooth disease. *Neurology* **48,** 867–873.

King R. H. M. (1999) Atlas of Peripheral Nerve Disorders. London, Sydney, Auckland: Arnold, 217 pp.

King R. H. M., Tournev I., Colomer J., Merlini L., Kalaydjieva L., and Thomas P. K. (1999) Ultrastructural changes in peripheral nerve in hereditary motor and sensory neuropathy—Lom. *Neuropathol. Appl. Neurobiol*. **25,** 306–312.

Linke R. P. (1982) Immunohistochemical identification and cross reactions of amyloid fibril proteins in senile heart and amyloid in familial polyneuropathy. Lack of reactivity with cerebral amyloid in Alzheimer's disease. *Clin. Neuropathol*. **1,** 172–182.

Lupski J. R., de Oca-Luna R. M., Slaugenhaupt S., et al. (1991) DNA duplication associated with Charcot-Marie-Tooth disease type 1A. *Cell* **66,** 219–232.

Lyon G. and Evrard P. (1970) Sur la présence d'inclusions cristalline dans les cellules d Schwann dans diverses neuropathies périphériques. *C.R. Des. Sci. (Paris) D. Sci. Nat. D*. **272,** 1000–1002.

Madrid R. and Bradley W. G. (1975) The pathology of neuropathies with focal thickening of the myelin sheath (tomaculous neuropathy). Studies on the formation of the abnormal myelin sheath. *J. Neurol. Sci*. **25,** 415–448.

Malandrini A., Guazzi G. C., and Federico A. (1992) Sensory-motor chronic neuropathy in two siblings: atypical presentation of tomaculous neuropathy. *Clin. Neuropathol*. **11,** 318–322.

Mancardi G.L., Mandich P., Nassani S., et al. (1995) Progressive sensory-motor polyneuropathy with tomaculous changes is associated to 17p11.2 deletion. *J. Neurol. Sci*. **131,** 30–34.

Merlini L., Villanova M., Sabatelli P., et al. (1998) Hereditary motor and sensory neuropathy Lom type in an Italian Gypsy family. *Neuromuscul. Disord*. **8,** 182–185.

Moosa A. and Dubowitz V. (1970) Peripheral neuropathy in Cockayne's syndrome. *Arch. Dis. Child* **45,** 674–677.

Müller H. D., Mugler M., Ramaekers V. T., and Schröder J. M. (2000) Hereditary motor and sensory neuropathy with absence of large myelinated fibers due to absence of large neurons in dorsal root ganglia and anterior horns, clinically associated with deafness, mental retardation, and epilepsy (HMSN-ADM). *J. Periph. Nerv. Syst*. **5,** 1–11.

Nelis E., Timmerman V., De Jonghe P., Van Broeckhoven C., and Rautenstrauss B. (1999) Molecular genetics and biology of inherited peripheral neuropathies: a fast-moving field. *Neurogenetics* **2,** 137–148.

Nishino I., Spinazzola A., and Hirano M. (2001) MNGIE: from nuclear DNA to mitochondrial DNA. *Neuromuscul. Disord*. **11,** 7–10.

Ohnishi A., Mitsudome A., and Murai Y. (1987) Primary segmental demyelination in the sural nerve in Cockayne's syndrome. *Muscle Nerve* **10,** 163–167.

Ohnishi A., Murai Y., Ikeda M., Fujita T., Furuya H., and Kuroiwa Y. (1989) Autosomal recessive motor and sensory neuropathy with excessive myelin outfolding. *Muscle Nerve* **12,** 568–575.

Palix C. and Coignet J. (1978) Un cas de polyneuropathie péripherique neo-natale par amyélinisation. *Pediatrie* **33,** 201–207.

Papadimitriou A., Comi G. P., Hadjigeorgiou G. M., et al. (1998) Partial depletion and multiple deletions of muscle mtDNA in familial MNGIE syndrome. *Neurology* **51,** 1086–1092.

Peiffer J., Kustermann-Kuhn B., Mortier W., et al. (1988) Mitochondrial myopathies with necrotizing encephalopathy of the Leigh type. *Pathol. Res. Pract*. **183,** 706–716.

Previtali S. C., Zerega B., Sherman D. L., et al. (2003) Myotubularin-related 2 protein phosphatase and neurofilament light chain protein, both mutated in CMT neuropathies, interact in peripheral nerve. *Hum. Mol. Genet*. **12**, 1713–1723.

Quattrone A., Gambardella A., Bono F., et al. (1996) Autosomal recessive hereditary motor and sensory neuropathy with focally folded myelin

sheaths: clinical, electrophysiologic, and genetic aspects of a large family. *Neurology* **46,** 1318–1324.
Raeymaekers P., Timmerman V., Nelis E., et al. (1991) Duplication in chromosome 17p11.2 in Charcot-Marie-Tooth neuropathy type 1a (CMT 1a). The HMSN Collaborative Research Group. *Neuromuscul. Disord*. **1,** 93–97.
Rouger H., LeGuern E., Birouk N., et al. (1997) Charcot-Marie-Tooth disease with intermediate motor nerve conduction velocities: characterization of 14 Cx32 mutations in 35 families. *Hum. Mutat*. **10,** 443–452.
Röyttä M. and Raine C. S. (1986) Taxol-induced neuropathy: chronic effects of local injection. *J. Neurocytol*. **15,** 483–496.
Sabatelli M., Bertini E., Servidei S., Fernandez E., Magi S., and Tonali P. (1992) Giant axonal neuropathy: report on a case with focal fiber loss. *Acta Neuropathol. (Berl.)* **83,** 543–546.
Sabatelli M., Mignogna T., Lippi G., et al. (1994) Autosomal recessive hypermyelinating neuropathy. *Acta Neuropathol. (Berl.)* **87,** 337–342.
Sabatelli M., Mignogna T., Lippi G., et al. (1998) Hereditary motor and sensory neuropathy with deafness, mental retardation, and absence of sensory large myelinated fibers: confirmation of a new entity. *Am. J. Med. Genet*. **75,** 309–313.
Salih M. A., Maisonobe T., Kabiraj M., et al. (2000) Autosomal recessive hereditary neuropathy with focally folded myelin sheaths and linked to chromosome 11q23: a distinct and homogeneous entity *Neuromuscul. Disord*. **10,** 10–15.
Sander S., Nicholson G. A., Ouvrier R. A., McLeod J. G., and Pollard J. D. (1998) Charcot-Marie-Tooth disease: histopathological features of the peripheral myelin protein (PMP22) duplication (CMT1A) and connexin32 mutations (CMTX1). *Muscle Nerve* **21,** 217–225.
Scherer S. S. (1996) Molecular specializations at nodes and paranodes in peripheral nerve. *Microsc. Res. Tech*. **34,** 452–461.
Scherer S. S., Xu Y. T., Nelles E., Fischbeck K., Willecke K., and Bone L. J. (1998) Connexin32-null mice develop demyelinating peripheral neuropathy. *Glia* **24,** 8–20.
Schröder J. M. (1968a) Die Hyperneurotisation Büngnerscher Bänder bei der experimentellen Isoniazid-Neuropathie: Phasenkontrast- und elektronenmikroskopische Untersuchungen. *Virch. Arch. Abt. B Zellpath*. **1,** 131–156.
Schröder J. M. (1968b) Überzählige Schwannzellen bei der Remyelinisation regenerierter und segmental demyelinisierter Axone im peripheren Nerven. *Verh. Dtsch. Ges. Pathol*. **52,** 222–228.
Schröder J. M. (1972) Altered ratio between axon diameter and myelin sheath thickness in regenerated nerve fibers. *Brain Res*. **45,** 49–65.
Schröder J. M. (1982a) Feinstrukturell-morphometrische Analyse anaboler Myelinisationsstörungen im peripheren Nerven. *Verh. Dtsch. Ges. Pathol*. **66,** 272–275.
Schröder J. M. (1982b) Pathologie der Muskulatur. Berlin, Heidelberg, New York: Springer pp. 813.
Schröder J. M. (1993) Neuropathy associated with mitochondrial disorders. *Brain Pathol*. **3,** 177–190.
Schröder J. M. (1996) Developmental and pathological changes at the node and paranode in human sural nerves. *Microsc. Res. Tech*. **34,** 422–435.
Schröder J. M. (1998) Recommendations for the examination of peripheral nerve biopsies. *Virchows Arch*. **432,** 199–205.
Schröder J. M. (1999) Pathologie peripherer Nerven. *In* Spezielle pathologische Anatomie. Vol. **XIII/8**. G. Seifert, (ed.), Berlin, Heidelberg, New York: Springer, pp. 862.
Schröder J. M. (2001) Pathology of peripheral nerves. An Atlas of Structural and Molecular Pathological Changes. Berlin, Heidelberg, New York: Springer, 380 pp.
Schröder J. M. (2005) Ferritinopathy: diagnosis by muscle or nerve biopsy, with a note on other nuclear inclusion body diseases. *Acta Neuropathol. (Berl.)* **109,** 109–114.
Schröder J. and Bohl J. (1978) Altered ratio between axon caliber and myelin thickness in sural nerves of children In: Canal, N. (ed.) Peripheral Neuropathies, Amsterdam: Elsevier, pp. 49–62.
Schröder J. M. and Krücke W. (1970) Zur Feinstruktur der experimentell-allergischen Neuritis beim Kaninchen. *Acta Neuropathol. (Berl.)* **14,** 261–283.
Schröder J. M. and Seiffert K. E. (1972) Untersuchungen zur homologen Nerventransplantation. Morphologische Ergebnisse. *Zentralbl. Neurochir*. **33,** 103–118.
Schröder J. M. and Sommer C. (1991) Mitochondrial abnormalities in human sural nerves: fine structural evaluation of cases with mitochondrial myopathy, hereditary and non-hereditary neuropathies, and review of the literature. *Acta Neuropathol. (Berl.)* **82,** 471–482.
Schröder J. M., Bohl J., and Brodda K. (1978) Changes of the ratio between myelin thickness and axon diameter in the human developing sural nerve. *Acta Neuropathol (Berl.)* **43,** 169–178.

Schröder J. M., Bohl J., and von Bardeleben U. (1988) Changes of the ratio between myelin thickness and axon diameter in human developing sural, femoral, ulnar, facial, and trochlear nerves. *Acta Neuropathol. (Berl.)* **76,** 471–483.

Schröder J. M., Krämer K. G., and Hopf H. C. (1985) Granular nuclear inclusion body disease: fine structure of tibial muscle and sural nerve. *Muscle Nerve* **8,** 52–59.

Schröder J. M., Sellhaus B., and Jörg J. (1995) Identification of the characteristic vascular changes in a sural nerve biopsy of a case with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). *Acta Neuropathol. (Berl.).***89,** 116–121.

Schröder J. M., Senderek J., Bergmann C., and Hermanns B. (1999b) Identification of DNA mutations in archival sural nerve biopsies confirmed by blood samples in HMSN X: occasional association with Becker's muscular dystrophy and color blindness. *J. Neurol. Sci*. **166,** 166, 167.

Schröder J. M., Dodel R., Weis J., Stefanidis I., and Reichmann H. (1996) Mitochondrial changes in muscle phosphoglycerate kinase deficiency. *Clin. Neuropathol*. **15,** 34–40.

Schröder J. M., Heide G., Ramaekers V., and Mortier W. (1993a) Subtotal aplasia of myelinated nerve fibers in the sural nerve. *Neuropediatrics* **24,** 286–291.

Schröder J. M., Rollnik J. D., Schubert M., and Dengler R. (1999a) Demyelinating sensorimotor neuropathy with congenital cataract, mental retardation, and unique, dysplastic perineurial cells within the endoneurium. *Acta Neuropathol*. **98,** 421–426.

Schröder J. M., Hackel V., Wanders R. J., Goehlich-Ratmann G., and Voit T. (2004) Optico-cochleodentate degeneration associated with severe peripheral neuropathy and caused by peroxisomal D-bifunctional protein deficiency. *Acta Neuropathol. (Berl.)* **108,** 154–167.

Schröder J. M., May R., Shin Y. S., Sigmund M., and Nase-Hüppmeier S. (1993b) Juvenile hereditary polyglucosan body disease with complete branching enzyme deficiency (type IV glycogenosis). *Acta Neuropathol. (Berl.)* **85,** 419–430.

Schröder J. M., Verhoeven K., Van Hul M., De Jonghe P., and Timmerman V. (2005) Alterations of axonal mitochondria in CMT2A due to mitofusin 2 mutation: caparison to cases with mitochondrial DNA mutation. *J. Periph. Nervous Syst*. **10,** 84–85.

Schröder J. M., Züchner, S., Dichgans, M., et al. (2005) Peripheral nerve and skeletal muscle involvement in CADASIL. *Acta Neuropathol. (Berl.)* **110,** 587–599.

Senderek J., Bergmann C., Quasthoff S., Ramaekers V. T., and Schröder J. M. (1998) X-linked dominant Charcot-Marie-Tooth disease: nerve biopsies allow morphological evaluation and detection of connexin32 mutations (Arg15Trp, Arg22Gln). *Acta Neuropathol. (Berl.)* **95,** 443–449.

Senderek J., Bergmann C., Ramaekers V. T., et al. (2003a) Mutations in the ganglioside-induced differentiation-associated protein-1 (GDAP1) gene in intermediate type autosomal recessive Charcot-Marie-Tooth neuropathy. *Brain* **126,** 642–649.

Senderek J., Bergmann C., Stendel C., et al. (2003b) Mutations in a gene encoding a novel SH3/TPR domain protein cause autosomal recessive Charcot-Marie-Tooth Type 4C Neuropathy. *Am. J. Hum. Genet*. **73,** 1106–1119.

Senderek J., Hermanns B., Bergmann C., et al. (1999) X-Linked dominant Charcot-Marie-Tooth neuropathy: clinical, electrophysiological, and morphological phenotype in four families with different connexin32 mutations. *J. Neurol. Sci*. **167,** 90–101.

Senderek J., Hermanns B., Lehmann U., et al. (2000) Charcot-Marie-Tooth neuropathy type 2 and P0 point mutations: two novel amino acid substitutions (Asp61Gly; Tyr119Cys) and a possible "hotspot" on Thr124Met. *Brain Pathol*. **10,** 235–248.

Senderek J., Krieger M., Stendel C., et al. (2005) Mutations in *SIL1* cause Marinesco-Sjögren syndrome, a cerebellar ataxia with cataract and myopathy. Nat Gene. **37,** 1312–1314.

Serratrice G., Gastaut J. L., and Dubois-Gambarelli D. (1973) Amyotrophie neurogéne périphérique au cours du syndrome de Marinesco-Sjögren. *Rev. Neurol. (Paris)* **128,** 432–441.

Sewry C. A., Voit T., and Dubowitz V. (1988) Myopathy with unique ultrastructural feature in Marinesco-Sjögren syndrome. *Ann. Neurol*. **24,** 576–580.

Simonati A., Fabrizi G. M., Pasquinelli A., et al. (1999) Congenital hypomyelination neuropathy with Ser72Leu substitution in PMP22. *Neuromuscul. Disord*. **9,** 257–261.

Sommer C. and Schröder J. M. (1989a) Amyloid neuropathy: immunocytochemical localization of intra- and extracellular immunoglobulin light chains. *Acta Neuropathol. (Berl.)* **79,** 190–199.

Sommer C. and Schröder J. M. (1989b) Hereditary motor and sensory neuropathy with optic atrophy. Ultrastructural and morphometric observations on nerve fibers, mitochondria, and dense-cored vesicles. *Arch. Neurol*. **46,** 973–977.

Sperfeld A. D., Hein C., Schröder J. M., Ludolph A. C., and Hanemann C. O. (2002) Occurrence and characterization of peripheral nerve involvement in neurofibromatosis type 2. *Brain* **125,** 996–1004.

Tachi N., Kasai K., Chiba S., Naganuma M., Uyemura K., and Hayasaka K. (1994) Expression of P0 protein in sural nerve of a patient with hereditary motor and sensory neuropathy type III. *J. Neurol. Sci.* **124,** 67–70.

Tachi N., Kozuka N., Ohya K., Chiba S., and Sasaki K. (1997) Tomaculous neuropathy in Charcot-Marie-Tooth disease with myelin protein zero gene mutation. *J. Neurol. Sci.* **153,** 106–109.

Takashima H., Boerkoel C. F., De Jonghe P., et al. (2002) Periaxin mutations cause a broad spectrum of demyelinating neuropathies. *Ann. Neurol.* **51,** 709–715.

Thiex R. and Schröder J. M. (1998) PMP-22 gene duplications and deletions identified in archival, paraffin-embedded sural nerve biopsy specimens: correlation to structural changes. *Acta Neuropathol.* **96,** 13–21.

Thomas P. K., King R. H. M., Workman J. M., and Schröder J. M. (2000) Hypertrophic perineurial dysplasia in multifocal and generalized peripheral neuropathies. *Neuropathol. Appl. Neurobiol.* **26,** 536–543.

Thomas F. P., Lebo R. V., Rosoklija G., et al. (1994) Tomaculous neuropathy in chromosome 1 Charcot-Marie-Tooth syndrome. *Acta Neuropathol. (Berl.)* **87,** 91–97.

Thomas P. K., Beamish N. G., Small J. R., et al. (1996) Paranodal structure in diabetic sensory polyneuropathy. *Acta Neuropathol. (Berl.)* **92,** 614–620.

Thomas P. K., Hollinrake K., Lascelles R. G., et al. (1971) The polyneuropathy of chronic renal failure. *Brain* **94,** 761–780.

Timmerman V., Jonghe P. De, and Van Broeckhoven C. (2000) Of giant axons and curly hair (In Process Citation). *Nat. Genet.* **26,** 254, 255.

Timmerman V., Jonghe P. De., Ceuterick C., et al. (1999) Novel missense mutation in the early growth response 2 gene associated with Dejerine-Sottas syndrome phenotype. *Neurology* **52,** 1827–1832.

Vallat J. M., Gil R., Leboutet M. J., Hugon J., and Moulies D. (1987) Congenital hypo- and hypermyelination neuropathy. Two cases. *Acta Neuropathol. (Berl.)* **74,** 197–201.

Vital A., Ferrer X., Lagueny A., et al. (2001) Histopathological features of X-linked Charcot-Marie-Tooth disease in 8 patients from 6 families with different connexin32 mutations. *J. Periph. Nerv. Syst.* **6,** 79–84.

Warner L. E., Hilz M. J., Appel S. H.,et al. (1996) Clinical phenotypes of different MPZ (P0) mutations may include Charcot- Marie-Tooth type 1B, Dejerine-Sottas, and congenital hypomyelination. *Neuron* **17,** 451–460.

Warner L. E., Mancias P., Butler I. J., et al. (1998) Mutations in the early growth response 2 (EGR2) gene are associated with hereditary myelinopathies. *Nat. Genet.* **18,**382–384.

Webster H. D., Schröder J., Asbury A., and Adams R. (1967) The role of Schwann cells in the formation of "onion bulbs" found in chronic neuropathies. *J. Neuropathol. Exp. Neurol.* **26,** 276–299.

Webster H. D., Spiro D., Waksman B., and Adams R. D. (1961) Phase and electron microscopic studies of experimental demyelination. II. Schwann cell changes in guinea pig sciatic nerves during experimental diphtheritic neuritis. *J. Neuropath. Exp. Neurol.* 5–34.

Wolfe G. I., Brower R. D., and Burns D. K. (2000) Perineurial cell hyperplasia in early-onset polyneuropathy with multiple cranial neuropathies. *Neuromuscul. Disord.* **10,** 503–506.

Wolfe D. E., Schindler D., and Desnick R. J. (1995) Neuroaxonal dystrophy in infantile alpha-N-acetylgalactosaminidase deficiency. *J. Neurol. Sci.* **132,** 44–56.

Yiannikas C., McLeod J. G., and Walsh J. C. (1983) Peripheral neuropathy associated with polycythemia vera. *Neurology* **33,** 139–143.

Yoshikawa H., Nishimura T., Nakatsuji Y., et al. (1994) Elevated expression of messenger RNA for peripheral myelin protein 22 in biopsied peripheral nerves of patients with Charcot-Marie-Tooth disease type 1A. *Ann. Neurol.* **35,** 445–450 (see comments).

Zanssen S., Molnar M., Buse G., and Schröder J. M. (1998) Mitochondrial cytochrome b-gene deletion in Kearns Sayre syndrome associated with a mild type of peripheral neuropathy. *Clin. Neuropathol.* **17,** 291–296.

Zhao C., Takita J., Tanaka Y., et al. (2001) Charcot-Marie-Tooth disease type 2A caused by mutation in a microtubule motor KIF1Bbeta. *Cell* **105,** 587–597.

Ziemssen F., Sindern E., Schröder J. M., et al. (2000) Novel missense mutations in the glycogen-branching enzyme gene in adult polyglucosan body disease. *Ann. Neurol.* **47,** 536–540.

Zimmer C., Gosztonyi G., Cervós-Navarro J., von Moers A., and Schröder J. M. (1992) Neuropathy with lysosomal changes in Marinesco-Sjögren syndrome:

fine structural findings in skeletal muscle and conjunctiva. *Neuropediatrics* **23,** 329–335.

Züchner S., Sperfeld A. D., Senderek J., Sellhaus B., Hanemann C. O., and Schröder J. M. (2003) A novel nonsense mutation in the ABC1 gene causes a severe syringomyelia-like phenotype of Tangier disease. *Brain* **126,** 920 –927.

Züchner S., Mersiyanova I. V., Muglia M., et al. (2004a) Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat. Genet*. **36,** 449–451.

Züchner S., Vorgerd J. M., Sindern E., and Schröder J. M. (2004b) The novel neurofilament light (NEFL) mutation Glu397Lys is associated with a clinically and morphologically heterogeneous type of Charcot-Marie-Tooth neuropathy. *Neuromuscul. Disord*. **14,** 147–157.

Züchner S., Noureddine M., Kennerson M., et al. (2005) Mutations in the pleckstrin homology domain of dynamin 2 cause dominant intermediate Charcot-Marie-Tooth disease. *Nat. Genet*. **37,** 289–294.