Published in final edited form as:
Arch Neurol. 2011 June ; 68(6): 814–821. doi:10.1001/archneurol.2011.110.

Neuropathy in Human and Mice with PMP22 null

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Abstract

Background/Objective—Haploinsufficiency of *PMP22* causes hereditary neuropathy with liability to pressure palsies (HNPP). However, the biological functions of PMP22 in humans are largely unexplored due to the absence of patients with PMP22 null mutations.

Design, Setting and Participants—We have evaluated a 7-year-old boy with *PMP22 null*. Findings were compared with those from nerves of *Pmp22* null mice.

Results—Motor and sensory deficits in the proband were non-length dependent. Weakness was found in cranial muscles, but not in the limbs. Large fiber sensory modalities were profoundly abnormal, which started prior to the maturation of myelin. This is in line with the temporal pattern of PMP22 expression predominantly in cranial motor neurons and DRG during embryonic development, becoming undetectable in adulthood. Moreover, there were conspicuous maturation defects of myelinating Schwann cells that were more significant in motor nerve fibers than in sensory nerve fibers.

Conclusions—Taken together, these data suggest that PMP22 is important for the normal function of neurons that express PMP22 during early development, such as cranial motor neurons and spinal sensory neurons. Moreover, PMP22 deficiency differentially affects myelination between motor and sensory nerves, which may have contributed to the unique clinical phenotype in the patient with absence of PMP22.

INTRODUCTION

Peripheral myelin protein-22 (PMP22) is encoded by the *PMP22* gene within the DNA segment of human chromosome 17p11.2. PMP22 is most abundantly expressed in the myelinating Schwann cells of peripheral nerves[1], but its function is poorly understood.

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During development, the transcription of *PMP22* in motor neurons shows a rostral-caudal pattern with cranial motor nuclei first expressing the protein embryonically and spinal motor neurons expressing it after birth. In contrast, *PMP22* transiently expresses in sensory neurons of cranial nuclei and DRG at embryonic stages, but the transcripts become undetectable in young adults[2,3]. The significance of this expression difference between sensory and motor neurons is unknown.

PMP22 is clinically important. Over-expression of *PMP22* causes Charcot-Marie-Tooth disease type-1A (CMT1A), the most common heritable neuropathy afflicting 1:5000 people. Heterozygous deletion of the *PMP22* causes a different disease, HNPP, which presents with transient, focal episodes of weakness or sensory loss. HNPP nerves show focal excessive myelin folds, the tomacula[4]. *Pmp22* deficient (*Pmp22*+/−) mice recapitulate many abnormalities found in patients with HNPP, including the tomacula[5]. While those findings are important, the functional consequences of having no PMP22 have not been evaluated to date since no human patient with total absence of PMP22 has been described. We have evaluated such a patient, including his skin biopsy, and compared these results with those in *pmp22−/−* mice.

METHODS

Molecular analysis

Multiplex Ligation-dependent Probe Amplification (MLPA) assay was used to characterize the segment deleted in chromosome 17p. A MLPA kit (SALSA P033B) was used according to the manufacturer's protocol (MRC-Holland, Netherlands)[6]. This kit contains eleven probes specific for sequences present in the CMT1A/HNPP region. There were 22 control probes interspersed throughout the genome, which were used for relative quantification purposes. More detailed information on probes, genes, and sequences can be found at www.mlpa.com.

Immunohistochemistry study on human skin biopsies and mouse tissues

This technique has been described before[7]. In brief, skin biopsies were fixed in 4% paraformaldehyde for 30 minutes – 12 hours, embedded in OCT medium, and cut in vertical with 10–60μm thickness. The sections were incubated with the primary antibodies (Table 1) overnight at 4°C, followed by incubation with the secondary antibodies overnight, and mounted onto the slides on the 3rd day. The slides were examined using a Nikon laser confocal microscope (Nikon D-Eclipse C1 confocal system).

Electron microscopy

Skin biopsies and mouse tissue were fixed in 2.5% glutaraldehyde overnight, osmicated for 2 hours in 1% osmium tetroxide, dehydrated, and then embedded in epoxy resin. Tissue blocks were sectioned, trimmed and examined by electron microscopy (Zeiss EM900)[7].

Mouse genotyping

PMP22 deficient mice and genotyping—The *pmp22−/−* mouse was generated by using homologous recombination technique to inactivate the *Pmp22* gene[5]. A breeding colony is maintained in Vanderbilt University animal facility. The Animal Investigational Committee in the institution has approved the use of animals for this study. Genotypes were determined as described in previous publications[5].

RESULTS

Clinical evaluation

The proband is a 7-year and 10-month-old boy, who was first noticed to be floppy at the age of 4 months during a hospitalization for a non-related condition. He did not walk until three years of age. When he did walk he walked with an unsteady gait. Currently he requires a walker to take more than a few steps. His parents opine that he requires the walker because of unsteadiness rather than weakness. His parents first noted difficulty with him using his fingers when he began school at four years of age. His grip was weak and he began dropping objects. He currently cannot fasten buttons or close zippers but he can write. He states that he has normal sensation in his feet although he complains of occasional numbness in both hands.

On neurological examination he could ambulate with an ataxic, narrow based gait, if his father held both of his hands. With someone holding his hands for balance he could stand up both on his heels and toes very easily. Cranial nerve testing revealed bilateral facial weakness with Bell signs confirming the lower motor neuron basis for this weakness. Mild bilateral ptosis was present. He had no visible muscle atrophy in upper or lower extremities; for example, he had good bulk of extensor digitorum brevis muscles. He had very mild hammer toes but no overt pes cavus. Strength was full to confrontation in proximal and distal muscles of both upper and lower extremities. He cooperated well with his sensory examination. There was bilateral, symmetric reduction of pinprick in the fingers and in the toes. Vibration was normal in the upper limbs but in the lower limbs it was reduced to the costal margins. Joint position sense was normal in the upper limbs but in the lower limbs was reduced up to his ankles bilaterally. He had mild pseudoathetosis in both hands. Deep tendon reflexes were unobtainable.

Because of the patient's age only a limited electrodiagnostic study was performed which revealed very prolonged distal motor latencies and a slow peroneal motor conduction velocity. The only sensory nerve tested was the sural nerve and its response was unobtainable (Table 2). The proband's parents are first cousins and both have symptoms and neurophysiologic studies consistent with HNPP (Table 2)[8].

Molecular diagnosis

MLPA of the proband's DNA (Figure 1) revealed a homozygous deletion of a segment of at least 1.1Mb in chromosome 17p. This segment included all five exons of the *PMP22,* and the TEKT3 and FLJ genes flanking the *PMP22*. Both copies of the COX10 gene were spared. MLPA analysis of the proband parents' DNA revealed a heterozygous deletion of the same segment in both parents, confirming the diagnosis of HNPP.

Human skin biopsy analysis

Glabrous skin biopsies were taken from the index finger of the proband, as we have previously described[7]. Immunohistochemistry of the biopsy with antibodies against MBP and PGP9.5 revealed a profound reduction in myelinated fiber density. In the few fibers positive for MBP staining, Schwann cells were surrounding PGP9.5 positive axons but did not form well-organized internodes or compact myelin (Figure 2A and 2B). In contrast, Schwann cells from ***control*** biopsy formed regular internodes, ensheathed by compact myelin (Figure 2C) as previously reported[9]. We next performed electron microscopy. We were unable to identify any compact myelin; rather there were only Schwann cell processes loosely wrapped around a space where the axon presumably has degenerated. In many cases these Schwann cells form "onion bulb"-like structures but axons usually were absent (Figure 3). Taken together, these changes were consistent with axonal loss. Schwann cells around

degenerated axons were proliferative and unable to form myelinating internodes along regenerating axons.

An additional finding was a striking redundancy of the basal lamina with several loose layers or empty pockets of the lamina around or nearby Schwann cells (Figure 3). Similar findings were also described in a recent model of Pmp22 null mice[10]. As with our patient, mouse skin biopsies showed a few myelinated nerve fibers (Figure 3). The redundancy of basal lamina was prominent in the biopsies from the mice (Figure 3).

Differential effect of myelination between motor and sensory nerve fibers in *pmp22* null mice

Because of the predominance of proprioception loss in our patient, we wondered whether PMP22 might be particularly important in sensory rather than motor myelinated nerve fibers. Our previous study has demonstrated that axonal loss affects both dorsal and ventral roots equally in *pmp22−/−* mice at the ages of 10–13 months[11]. However, differential effect between sensory and motor nerves could still be present in younger mice. We analyzed both dorsal and ventral roots of three *pmp22−/−* and three wild-type animals. There were a large number of immature Schwann cells that have established 1:1 ratio with axons, but did not form myelin in *pmp22−/−* mice (Table 3 and Figure 4). The immature Schwann cells failed to produce tomacula since tomacula only can form in myelinated internodes. This defect appeared particularly prominent in ventral roots. All three mice showed abundant immature Schwann cells in ventral roots. In dorsal roots, Schwann cells were either fully differentiated or differentiated in a majority of them. These findings are consistent with our previous observation[11]. In addition, we noticed a small group of swollen axons in the roots, consistent with axonal loss shown in skin biopsies. Overall, as documented in our previous publication,[11] myelinated nerve fibers appeared significantly reduced. Taken together, the data suggest PMP22 deficiency delays maturation of Schwann cells predominantly in motor nerve fibers.

Because PMP22 expresses in spinal sensory and motor neurons during early development, we questioned whether PMP22 deficiency affects the survival of these neurons. TUNEL staining was performed in mouse spinal cords and DRG. There was no increase of apoptosis (For spinal anterior horns: 0.33±0.58 positive cells in 3 *wt* mice vs 0.33±0.58 positive cells in 3 *pmp22−/−* mice; for DRG: 2.33±2.52 vs 2.67±2.08; Supplementary Figure 1). In addition, we have examined the spinal cord and DRG with semithin sections, H&E staining and immunohistochemistry with antibodies against nuclear envelope protein laminin-B. No abnormality was detected in *pmp22−/−* mice (Supplementary Figure 2).

DISCUSSION

This 7-year-old boy is the first reported patient unable to express any PMP22. This null mutation has revealed several features that have crucial implication for PMP22 functions. First, this patient presented with a predominantly large fiber sensory loss resulting in decreased proprioception and sensory ataxia. This differs from many length-dependent inherited neuropathies that present with symmetric distal sensory loss, but with minimal sensory ataxia. Moreover, his motor deficits are also non-length dependent. While his muscles in upper and lower limbs have normal strength, weakness was found in cranial muscles. These features suggest a proximal pathology. This notion is supported by pathological changes in the spinal roots of *pmp22−/−* mice. Our previous study has also quantitatively demonstrated that axonal loss in *pmp22* null mice is more severe in roots than in distal nerves[11].

The pattern of sensory/motor deficits in the patient mirrors the expression pattern of PMP22 during development. Sensory loss and ataxia were severe, and cranial motor nerves were significantly afflicted in the patient. In contrast, functions related to spinal motor neurons were largely spared. Interestingly, PMP22 is highly expressed in rodent DRG sensory and cranial motor neurons during embryonic development and declines after birth[2,3]. However, expression of PMP22 in spinal motor neurons initiates only after P10 and persists until adulthood[12,13]. Taken together, these data suggest that transient expression of PMP22 in developing DRG and cranial motor neurons may be important for the survival of these neurons during early development. Dysfunction of these neurons would manifest early when PMP22 is deficient. We speculate that the late-expression of PMP22 in the spinal motor neurons makes these neurons less dependent on the protein during development. This notion is consistent with no spinal motor neuron loss observed in young *pmp22−/−* mice[14]. However, PMP22-null spinal motor neurons may degenerate later in life. Alternatively, predominant sensory neuropathy could just be an individual variation of phenotype. These changes in the PMP22 null patient are not seen in patients with heterozygous deletion of *PMP22*, HNPP[4], suggesting that PMP22 null produces phenotypes distinct from those in *PMP22* haploinsufficiency.

PMP22 function appears shifted after the early phase of development. Robust expression of PMP22 occurs in the PNS along with the maturation of myelin during the first 3 weeks of development[1]. Deficiency of PMP22 causes numerous tomacula in the myelinated nerve fibers of peripheral nerves. Axons encased by tomacula become constricted or deformed which may impair action potential propagation or axonal transport, leading to axonal degeneration[5,15]. We observed a profound delay of myelination that appears more severe in *pmp22−/−* motor nerves than in *pmp22−/−* sensory nerves. The second striking feature of this patient is that the motor nerve conduction was extremely slow in the range of 10 m/s. Schwann cells in spinal roots of *pmp22−/−* mice are often immature and fail to form normal compact myelin. These amyelinated nerve fibers should alter the normal saltatory conduction of action potential to continuous propagation, which would substantially reduce the conduction velocity. Finally, PMP22 deficient Schwann cells form shorter internodes, which may also slow conduction[16,17].

A previous report identified another 7-year-old boy who had a compound heterozygous mutation of *PMP22* in which there was a 1.4Mb HNPP deletion in one allele and a smaller deletion of *PMP22* exons 2 and 3 in the other[18]. The motor deficits of this child were more severe than that in our patient. It is possible that this patient is not expressing any PMP22 and that the differences between his and our patient's phenotypes simply represent phenotypic variability. Alternatively, truncated PMP22 from one of the alleles may cause "toxic" gain of function that leads to a more severe phenotype than if there was no PMP22 at all. Consistent with this hypothesis, a patient with the relatively benign *PMP22* T118M mutation[19] was much more severely affected when this was combined with the HNPP deletion on the other allele[20].

We observed a striking redundancy of the basal lamina in our patient's skin biopsy. This redundancy has been identified in *Pmp22−/−* mice. The authors demonstrated a direct interaction between Pmp22 and α6β4 integrin, a laminin receptor localized adjacent to the basal lamina. Beta-4 integrin levels are reduced in sciatic nerves of *Pmp22* deficient mice, leading the authors to hypothesize that the abnormalities in the basal lamina are the result of decreased interactions between PMP22 and α6β4 integrin[10]. One function of PMP22, therefore, may be to stabilize the linkage between the extracellular matrix and the abaxonal surface of the myelin sheath.

Recently, we have demonstrated a predisposition for *Pmp22*+/− mice to develop conduction block and focal constrictions of axons underneath tomacula that may increase axial resistance to action potential propagation[15]. Tomacula and axonal constrictions were predominantly localized at paranodes, important areas of interactions between the axon and myelin[15]. Therefore, PMP22 deficiency also appears to disrupt interactions between the adaxonal surface of myelin and the underlying axon. Taken together, these data suggest that an important role for PMP22 is to regulate myelin functions at both the abaxonal surface, where it interacts with the extracellular matrix, and at the adaxonal surface, where it interacts with the axon.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work is, in part, supported by grants from Veterans Affairs (B6243R) and NIH (R01NS066927). Work in the lab of US is also supported by the Swiss National Science Foundation and the National Center for Competence in Research (NCCR) Neural Plasticity and Repair. MMR is grateful to the Medical Research Council (MRC), the Muscular Dystrophy Campaign and the NIH for their support.

References

1. Jetten AM, Suter U. The peripheral myelin protein 22 and epithelial membrane protein family. Prog Nucleic Acid Res Mol Biol. 2000; 64:97–129. [PubMed: 10697408]
2. Parmantier E, Cabon F, Braun C, et al. Peripheral myelin protein-22 is expressed in rat and mouse brain and spinal cord motoneurons. Eur J Neurosci. 1995; 7:1080–1088. [PubMed: 7613613]
3. Parmantier E, Braun C, Thomas JL, et al. PMP-22 expression in the central nervous system of the embryonic mouse defines potential transverse segments and longitudinal columns. J Comp Neurol. 1997; 378:159–172. [PubMed: 9120057]
4. Li J, Krajewski K, Lewis RA, et al. Loss-of-function phenotype of hereditary neuropathy with liability to pressure palsies. Muscle Nerve. 2004; 29:205–210. [PubMed: 14755484]
5. Adlkofer K, Martini R, Aguzzi A, et al. Hypermyelination and demyelinating peripheral neuropathy in Pmp22-deficient mice. Nat Genet. 1995; 11:274–280. [PubMed: 7581450]
6. Schouten JP, McElgunn CJ, Waaijer R, et al. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res. 2002; 30:e57. [PubMed: 12060695]
7. Li J, Bai Y, Ghandour K, et al. Skin biopsies in myelin-related neuropathies: bringing molecular pathology to the bedside. Brain. 2005; 128:1168–1177. [PubMed: 15774502]
8. Li J, Krajewski K, Shy ME, et al. Hereditary neuropathy with liability to pressure palsy: the electrophysiology fits the name. Neurology. 2002; 58:1769–1773. [PubMed: 12084875]
9. Saporta MA, Katona I, Lewis RA, et al. Shortened internodal length of dermal myelinated nerve fibres in Charcot-Marie-Tooth disease type 1A. Brain. 2009
10. Amici SA, Dunn WA Jr, Murphy AJ, et al. Peripheral myelin protein 22 is in complex with alpha6beta4 integrin, and its absence alters the Schwann cell basal lamina. J Neurosci. 2006; 26:1179–1189. [PubMed: 16436605]
11. Sancho S, Magyar JP, Aguzzi A, et al. Distal axonopathy in peripheral nerves of PMP22-mutant mice. Brain. 1999; 122 (Pt 8):1563–1577. [PubMed: 10430839]
12. Maier M, Berger P, Nave KA, et al. Identification of the regulatory region of the peripheral myelin protein 22 (PMP22) gene that directs temporal and spatial expression in development and regeneration of peripheral nerves. Mol Cell Neurosci. 2002; 20:93–109. [PubMed: 12056842]
13. Ohsawa Y, Murakami T, Miyazaki Y, et al. Peripheral myelin protein 22 is expressed in human central nervous system. J Neurol Sci. 2006; 247:11–15. [PubMed: 16626749]

14. Nattkamper H, Halfter H, Khazaei MR, et al. Varying survival of motoneurons and activation of distinct molecular mechanism in response to altered peripheral myelin protein 22 gene dosage. J Neurochem. 2009; 110:935–946. [PubMed: 19493167]
15. Bai YH, Zhang XB, Katona I, et al. Conduction block in PMP22 deficiency. J Neurosci. 2009 Ref Type: In Press.
16. Amici SA, Dunn WA Jr, Notterpek L. Developmental abnormalities in the nerves of peripheral myelin protein 22-deficient mice. J Neurosci Res. 2007; 85:238–249. [PubMed: 17131416]
17. Court FA, Sherman DL, Pratt T, et al. Restricted growth of Schwann cells lacking Cajal bands slows conduction in myelinated nerves. Nature. 2004; 431:191–195. [PubMed: 15356632]
18. Al-Thihli K, Rudkin T, Carson N, et al. Compound heterozygous deletions of PMP22 causing severe Charcot-Marie-Tooth disease of the Dejerine-Sottas disease phenotype. Am J Med Genet A. 2008; 146A:2412–2416. [PubMed: 18698610]
19. Shy ME, Scavina MT, Clark A, et al. T118M PMP22 mutation causes partial loss of function and HNPP-like neuropathy. Ann Neurol. 2006; 59:358–364. [PubMed: 16437560]
20. Roa BB, Garcia CA, Pentao L, et al. Evidence for a recessive PMP22 point mutation in Charcot-Marie-Tooth disease type 1A. Nat Genet. 1993; 5:189–194. [PubMed: 8252046]

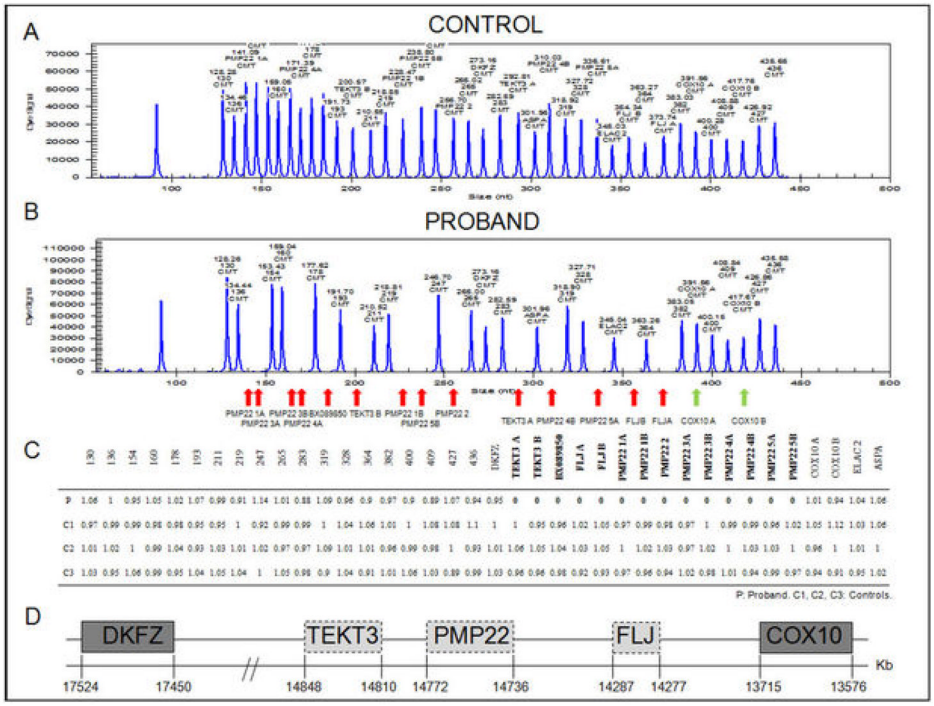
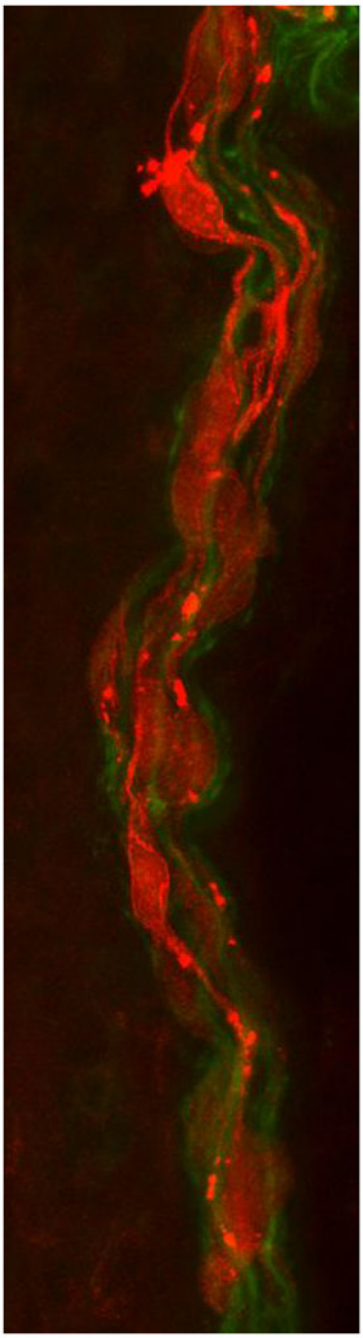
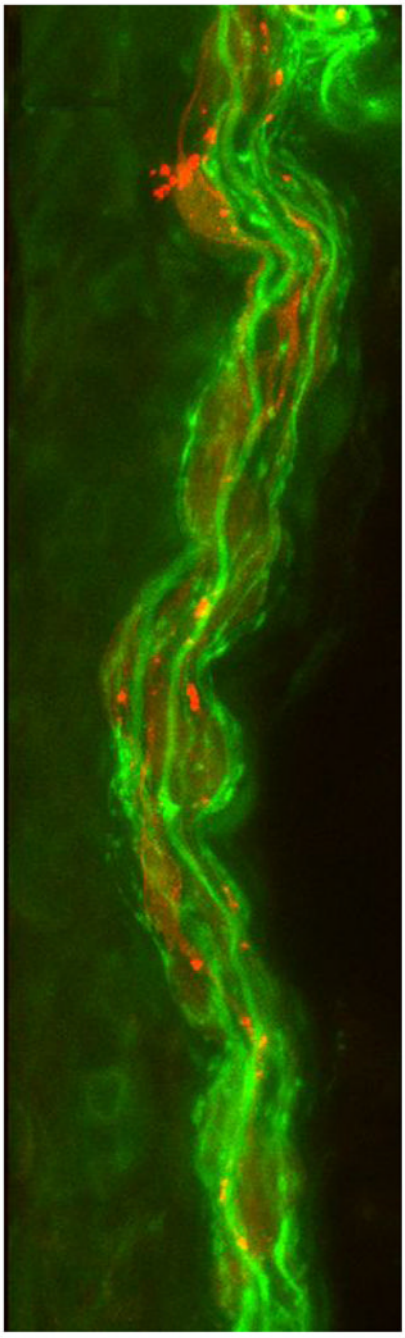


Figure 1. PMP22, but not COX10, is absent in both alleles of the proband

MLPA revealed absent peaks (red arrows) in the proband (A), corresponding to all five exons of PMP22 as determined in the control sample (B). Peaks corresponding to TEKT3, BX089850 and FLJ were also absent on the proband, but COX10 was retained in both alleles (green arrows). (C) Table showing the ratios of the analyzed peaks (relative peak areas) compared to the normal controls. (D) Schematic of the segment of chromosome 17p deleted in the proband.





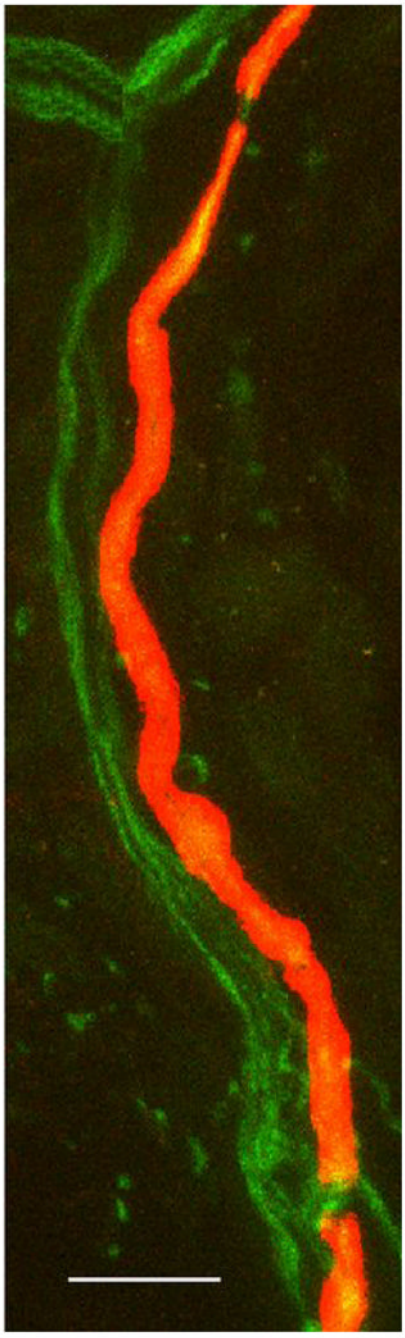
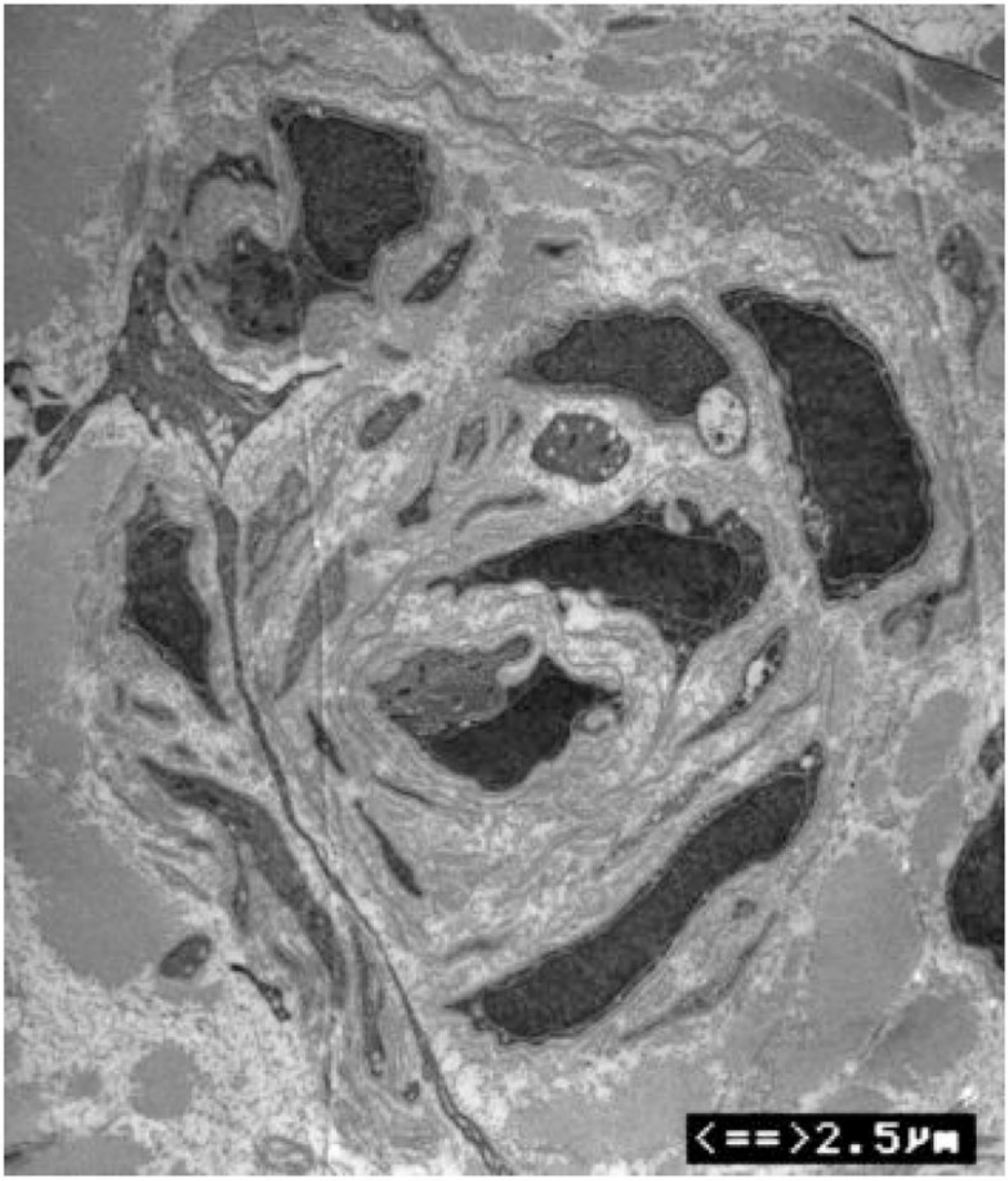
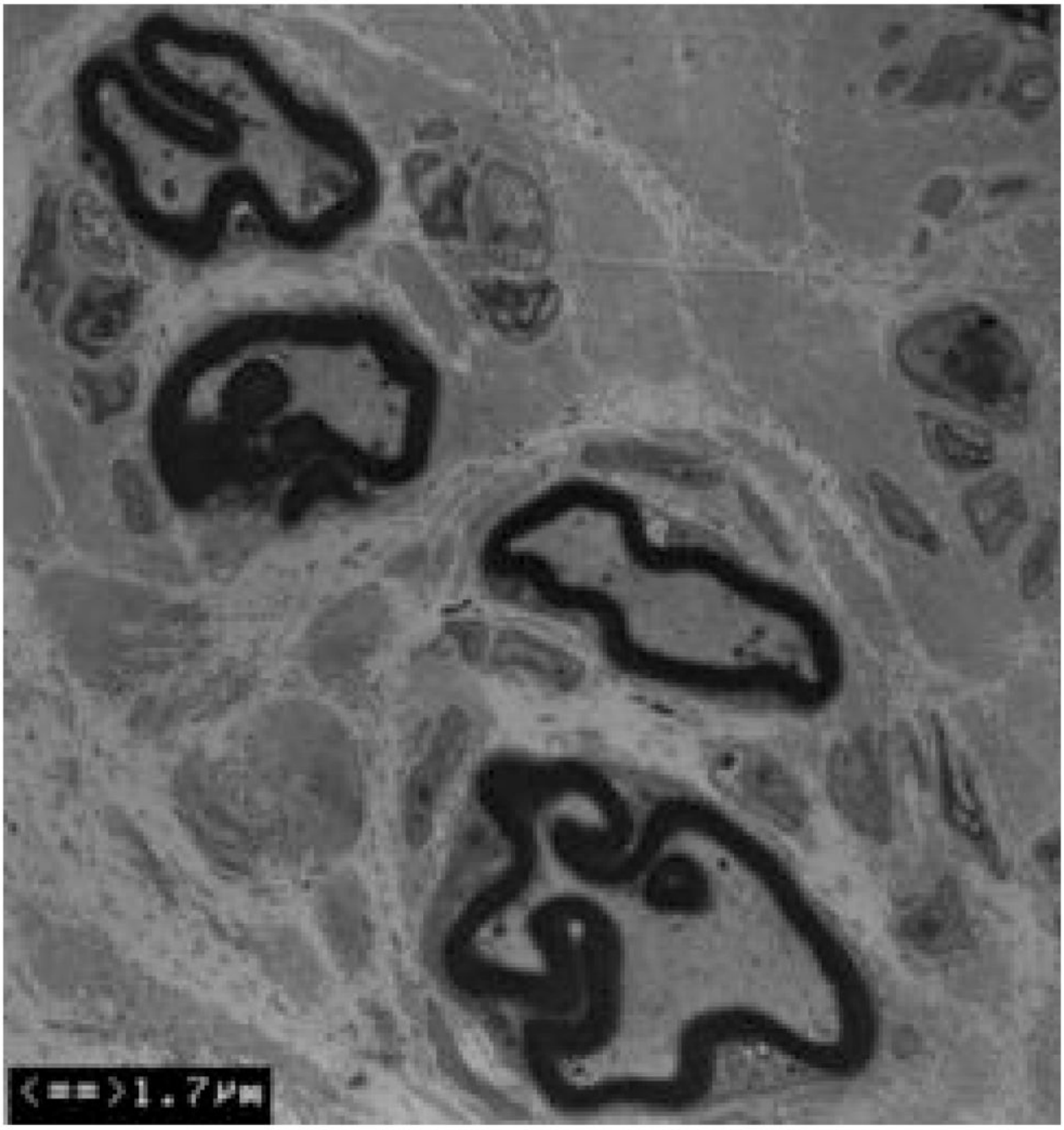
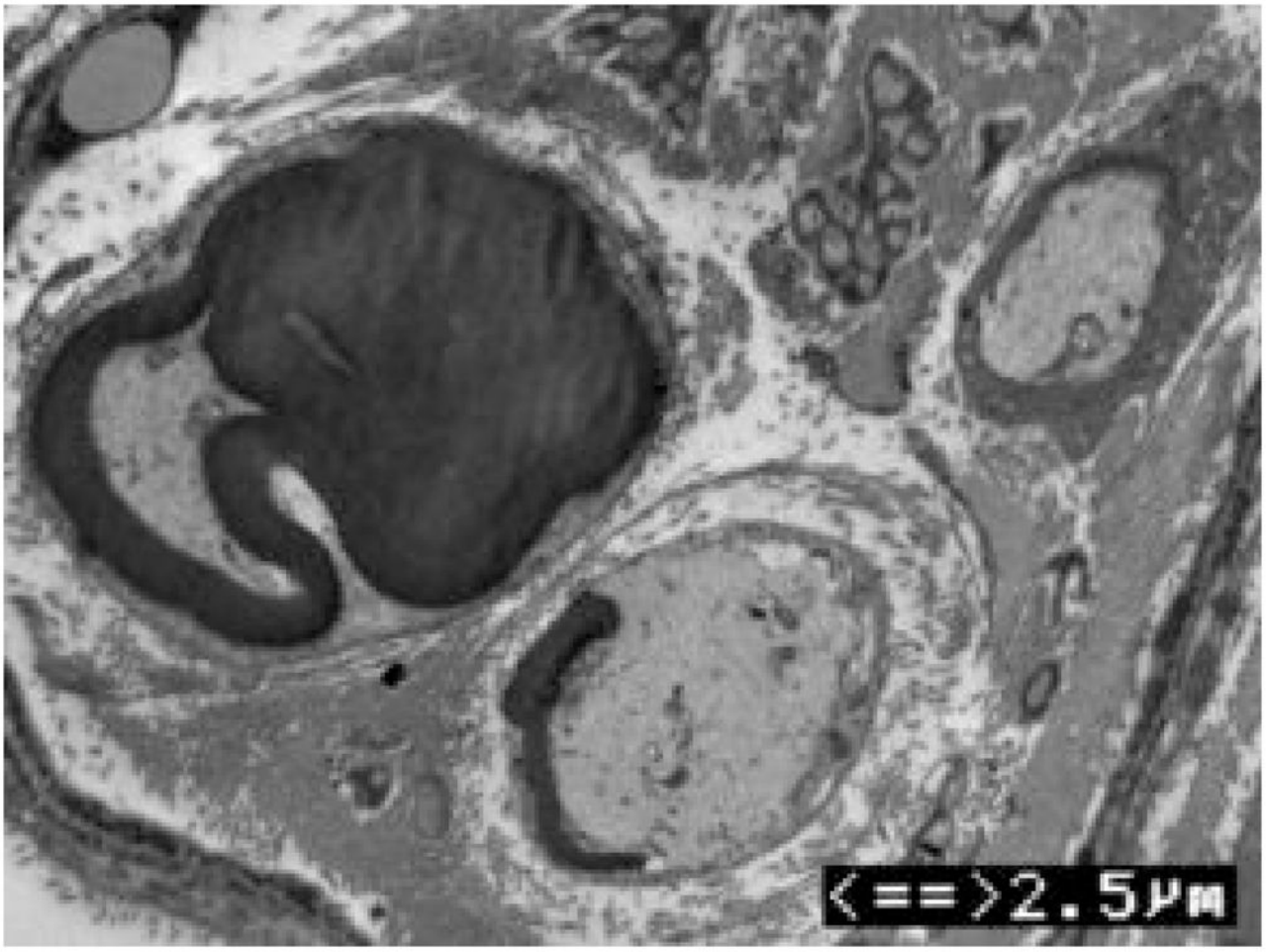


Figure 2. Abnormal internodes in skin biopsies from the patient with PMP22 null

(A&B) Immunohistochemistry using MBP (red) and PGP9.5 (green) antibodies reveals multiple MBP-positive Schwann cells (red) surrounding axons (green) without forming internodes in dermal myelinated fibers in the patient's skin sample. In A, red channel gain was artificially increased to highlight MBP positive Schwann cells. In B, the same image as A with the green channel gain increased to highlight PGP9.5 positive axons. (C) A control skin biopsy discloses normal internode of dermal myelinated fibers flanked by MBP negative regions corresponding to nodes of Ranvier. Scale bar = 10 um.







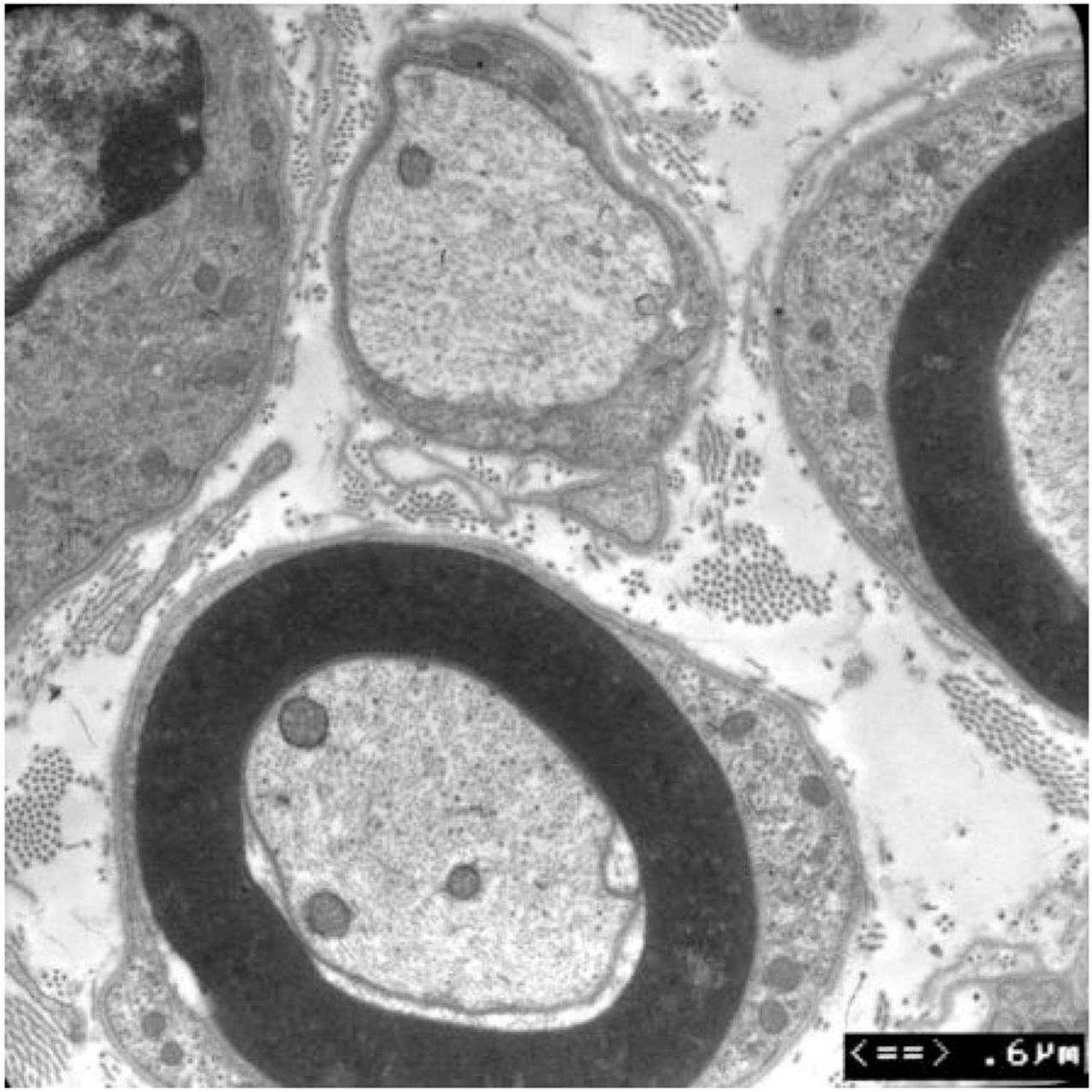
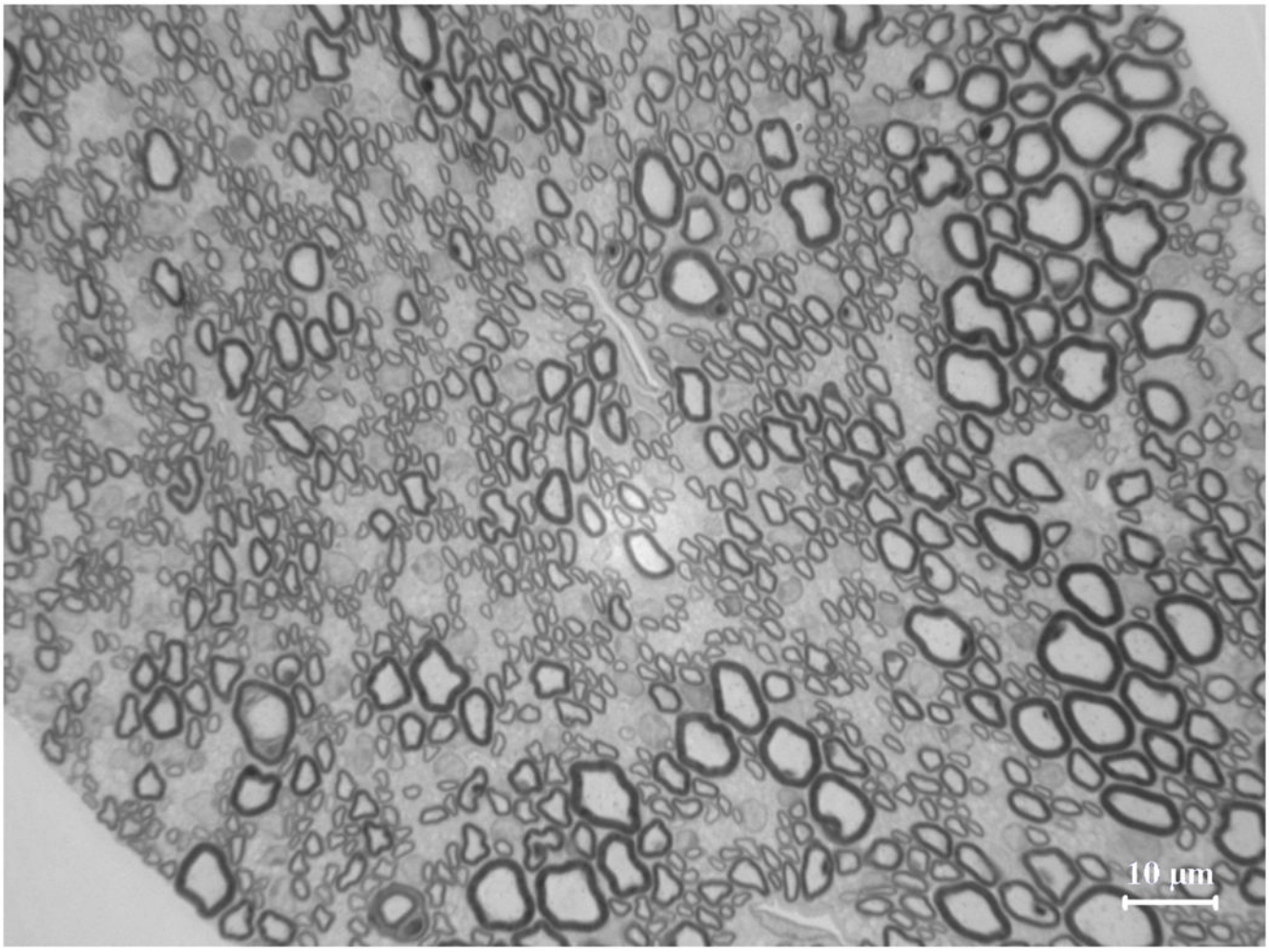
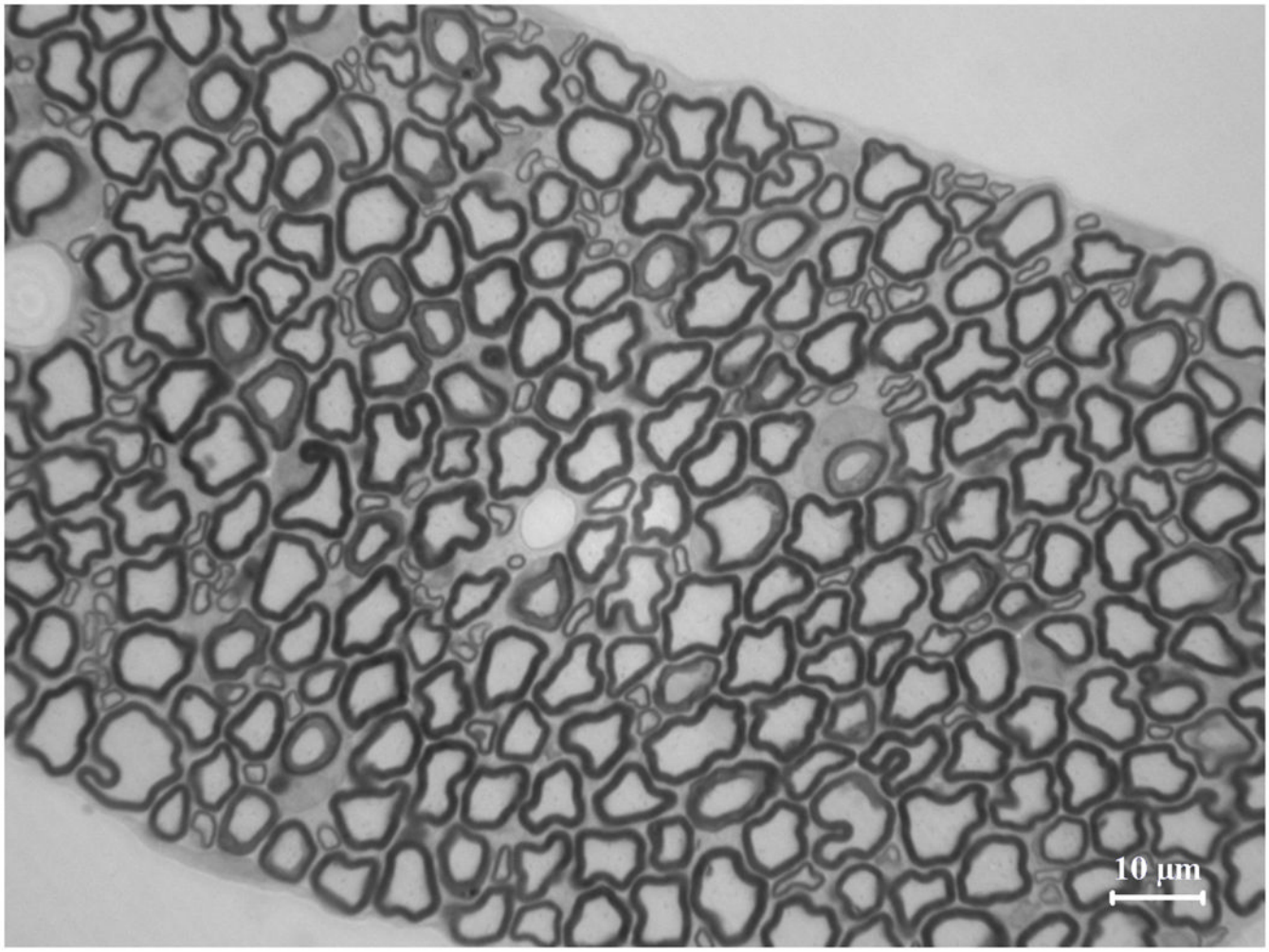


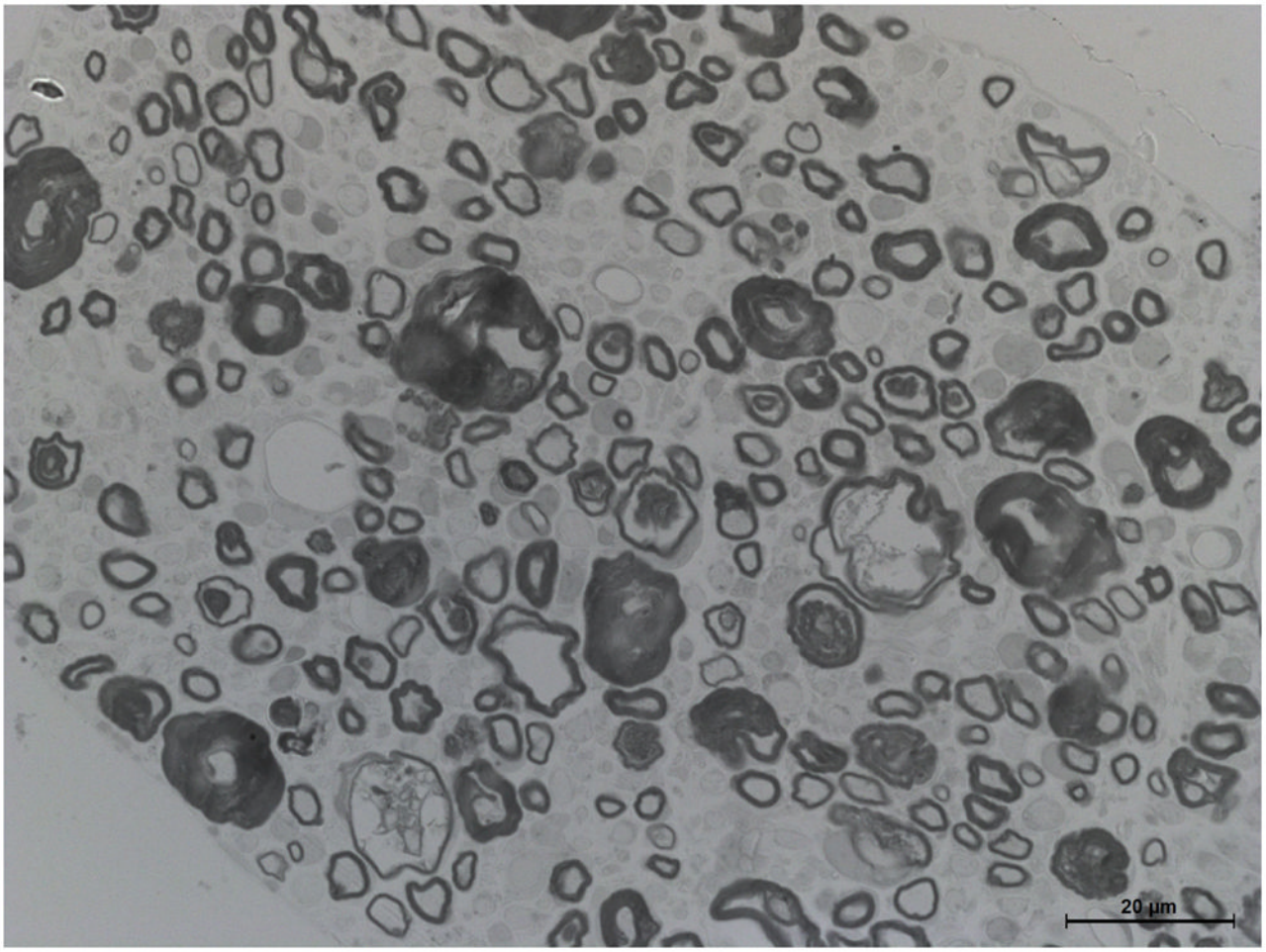
Figure 3. Axonal loss and basal lamina redundancy in skin biopsies from the patient with PMP22 null

(A) Electron microscopy of the patient's skin biopsy reveals Schwann cell processes loosely wrapping around an area where the axon appears to have degenerated. The overall appearance resembles an onion bulb. (B) The area circled by asterisks in A is enlarged to reveal redundant basal lamina of the Schwann cells (arrows). (C) A representative skin biopsy from a healthy control demonstrates the compacted myelin with normal thickness. (D) Skin biopsy of a *pmp22−/−* mouse reveals tomaculous formation (asterisk). Tomacula could not be identified in the patient's dermal nerves since myelinated nerve fibers are degenerated. Two axons on the right side fail to form compact myelin. E. EM of *pmp22−/−* mouse sciatic nerve reveals a number of relatively large axons devoid of compact myelin, as exemplified by the axon on the top. In contrast, an axon in the bottom has a comparable

diameter to the one on the top but its myelin is well formed. The excessive basal membrane redundancy is readily identifiable (arrow).







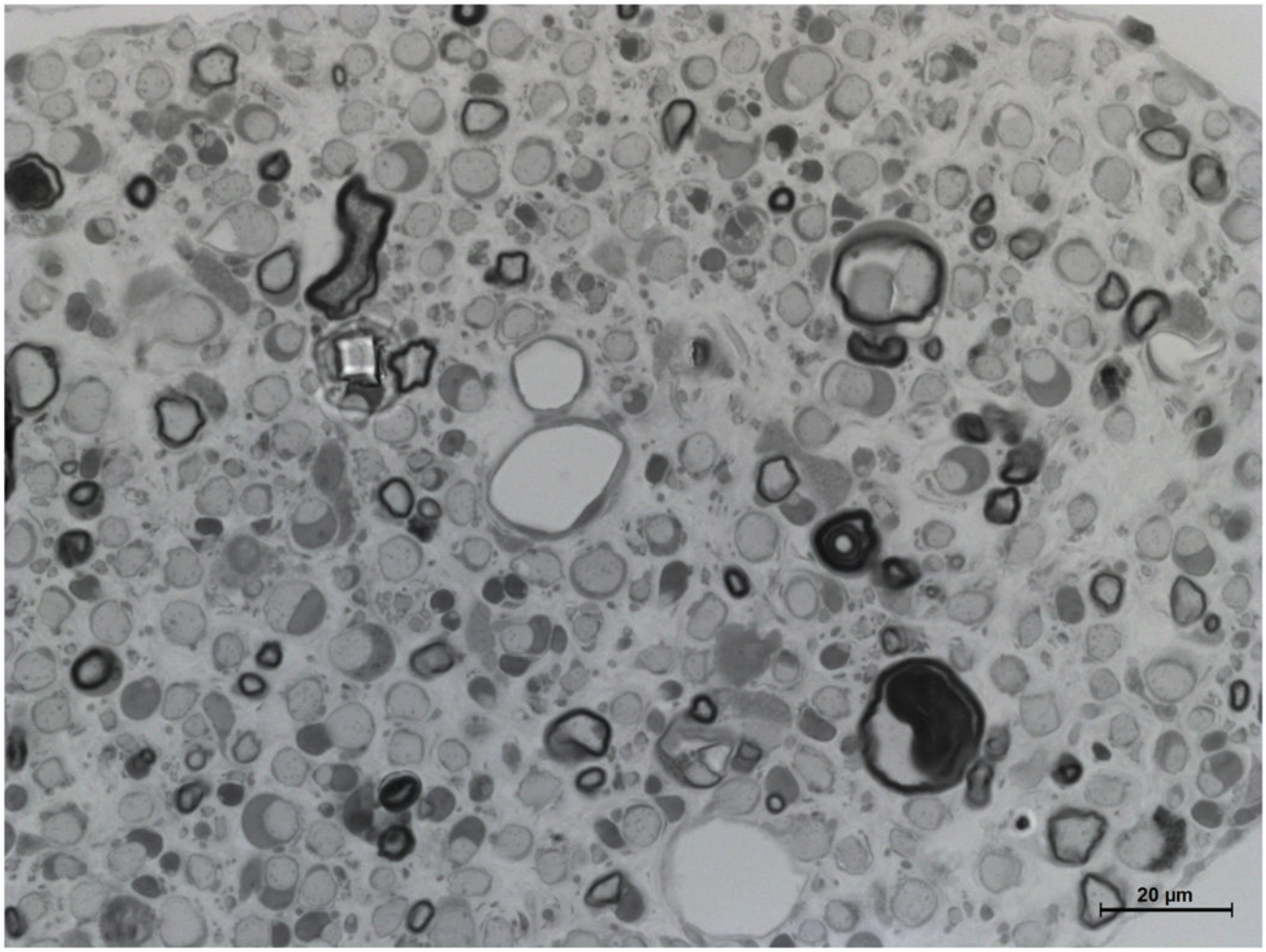


Figure 4. Delayed maturation of myelinating Schwann cells in spinal roots of *pmp22−/−* mice

A. Spinal roots were dissected from a 4-week-old mouse and processed for semithin section studies. A dorsal root was visualized under oil lens and showed numerous myelinated nerve fibers with variable diameters. B. In contrast, diameters of myelinated nerve fibers are relatively uniform in the ventral roots. Thus, Schwann cells are well differentiated to myelinate the axons by this age. However, when *pmp22−/−* ventral roots were examined at 4-month-old age, numerous myelinating Schwann cells still remained at an immature stage after establishing 1:1 relationship with axons (arrows in D). Since most myelinating Schwann cells failed to form myelin, tomacula were few in the ventral root (arrowhead in D). Overall axonal density appeared comparable to the wild-type ventral root. C. In contrast, most myelinating Schwann cells have formed myelin in the dorsal roots by the age of 4 months, and tomacula were common (arrowheads). Axonal density of myelinated nerve fibers appeared reduced. Some of the axons were swollen (arrows), suggesting axonal degeneration.

Table 1

Primary antibody list

Antibody	Source	Species raised	Specific antigen	Type	Titers	Reference
MBP	Ultraclone	Mouse	purified MBP from human brain	Monoclonal IgG1	1:800	Elfman et al., 1986
PGP9.5	AbD seroTec	Rabbit	Human PGP9.5 from brain	Polyclonal IgG	1:1000	Xue, L. et al. 2000
LaminB	Santa Biotech. SC-6217	Goat	C-terminus of Lamin B1 of mouse origin	Polyclonal IgG	1:100	Ueda et al., 2004
PERP	Abcam, ab3945	Rabbit	amino acids 37–50 of mouse PERP	Polyclonal IgG	1:50	Attardi et al., 2000

Table 2

Neurophysiologic findings in the proband and his parents.

	Father	Mother	Proband
Median DML (ms)	6.0	5.7	11.2
Ulnar DML (ms)	3.8	4.6	NP
Peroneal DML (ms)	7.1	8.0	5.8
Tibial DML (ms)	7.0	6.7	10.7
Ulnar CV BE (m/s)	45.7	44.4	NP
Ulnar CV AE (m/s)	36.7	20.2	NP
Peroneal bellow knee (m/s)	39.0	37.0	12.6
Peroneal above knee (m/s)	40.0	40.6	NP

DML: Distal motor latency CV: Conduction velocity NP: Not performed

Table 3

Pathological changes in *pmp22−/−* spinal roots.

Mouse No	Age	Sex	Slide No	Root	Axonal Swelling	Amyelinated axons
1	4m	F	18	*VR*	*2+*	*2+*
			19	*VR*	*2+*	*2+*
			16	*VR*	*2+*	*2+*
			15	*VR*	*0*	*3+*
			17	*VR*	*1+*	*3+*
			11	DR	2+	2+
			8	DR	2+	1+
			7	DR	2+	1+
			9	DR	2+	1+
			10	DR	2+	0
2	4m	F	D5	*VR*	*2+*	*3+*
			C4	*VR*	*1+*	*3+*
			C5	*VR*	*1+*	*3+*
			D2	*VR*	*3+*	*2+*
			C3	*VR*	*2+*	*3+*
			B2	DR	1+	0
			B3	DR	2+	1+
			B6	DR	2+	1+
3	2.5m	F	C6	*VR*	*2+*	*3+*
			D2	*VR*	*1+*	*1+*
			D5	*VR*	*1+*	*1+*
			33	DR	1+	0
			B5	DR	1+	0
			B2	DR	2+	0
			B1	DR	2+	0
			A6	DR	2+	0

Arch Neurol. Author manuscript; available in PMC 2013 July 15.

m=months VR = Ventral roots DR = Dorsal roots

0 = none of the pathological change; 1+ = The pathological change was in <5 myelinated fibers; 2+ = The pathological change was in >5 fibers and readily detectable in all examined fields; 3+ = The pathological change were numerous in any examined field.