Patients lacking the major CNS myelin protein, proteolipid protein 1, develop length-dependent axonal degeneration in the absence of demyelination and inflammation

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Summary
Axonal degeneration contributes to clinical disability in the acquired demyelinating disease multiple sclerosis. Axonal degeneration occurs during acute attacks, associated with inflammation, and during the chronic progressive phase of the disease in which inflammation is not prominent. To explore the importance of interactions between oligodendrocytes and axons in the CNS, we analysed the brains of rodents and humans with a null mutation in the gene encoding the major CNS myelin protein, proteolipid protein (PLP1, previously PLP). Histological analyses of the CNS of Plp1 null mice and of autopsy material from patients with null PLP1 mutations were performed to evaluate axonal and myelin integrity. In vivo proton magnetic resonance spectroscopy (MRS) of PLP1 null patients was conducted to measure levels of N-acetyl aspartate (NAA), a marker of axonal integrity. Length-dependent axonal degeneration without demyelination was identified in the CNS of Plp1 null mice. Proton MRS of PLP1-deficient patients showed reduced NAA levels, consistent with axonal loss. Analysis of patients' brain tissue also demonstrated a length-dependent pattern of axonal loss without significant demyelination. Therefore, axonal degeneration occurs in humans as well as mice lacking the major myelin protein PLP1. This degeneration is length-dependent, similar to that found in the PNS of patients with the inherited demyelinating neuropathy, CMT1A, but is not associated with significant demyelination. Disruption of PLP1-mediated axonal-glial interactions thus probably causes this axonal degeneration. A similar mechanism may be responsible for axonal degeneration and clinical disability that occur in patients with multiple sclerosis.

Keywords: Pelizaeus–Merzbacher disease; axonal, proteolipid protein 1; DM20, N-acetyl aspartate; magnetic resonance spectroscopy

Abbreviations: MBP = myelin basic protein; MRS = magnetic resonance spectroscopy; NAA = N-acetyl-aspartate; PMD = Pelizaeus–Merzbacher disease; PLP1 = (myelin) proteolipid protein 1; VOI = volume of interest

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Introduction
Axonal degeneration has been shown recently to contribute to the pathogenesis of the acquired demyelinating disease multiple sclerosis (Trapp et al., 1998). Axonal degeneration occurs both in acute lesions during a clinical relapse, as well as in more chronic lesions and normal appearing white matter in chronic progressive disease. Importantly, the extent of axonal injury, measured by proton magnetic resonance spectroscopy (MRS) analysis of N-acetylated-aspartate (NAA) in white matter (De Stefano et al., 1998; Lee et al., 2000) or by direct analysis of multiple sclerosis tissue (Ferguson et al., 1997; Trapp et al., 1998), correlates with clinical disability, strongly suggesting that axonal injury is the cause. Understanding the mechanisms of axonal injury will thus be important for designing future treatment strategies for patients with multiple sclerosis.

Axonal degeneration is also the cause of clinical disability in the inherited demyelinating neuropathy CMT1 (Krajewski et al., 2000) caused by mutation of one of several genes encoding the myelin proteins PMP22, P0 (Marrosu et al., 1998; Chapon et al., 1999; de Jonghe et al., 1999; Frei et al., 1999; Senderek et al., 2000) or connexin 32 (Hahn et al., 1990, 1999, 2000). Although the primary physiological defect in these diseases occurs in myelinating Schwann cells, altered interactions between Schwann cells and their axons lead to disturbed axonal physiology, including decreased axonal transport and neurofilament phosphorylation, and ultimately axonal damage (de Waegh et al., 1992). Consistent with these findings, the axonal injury in CMT1 is length-dependent, worse distally than proximally, suggesting that decreased transport of a factor required for normal axonal integrity and function is, at least in part, the cause of axonal injury in this disease (Krajewski et al., 2000).

To determine whether altered interactions between oligodendrocytes and their axons might also produce a similar length-dependent axonopathy in the CNS, we analysed the CNS of rodents and humans carrying a null mutation in the gene encoding the major CNS myelin protein, proteolipid protein 1 (PLP1), expressed predominantly in myelinating oligodendrocytes. Mutations of the PLP1 gene in man cause a spectrum of neurological disease, ranging from the severe Pelizaeus–Merzbacher disease (PMD), that typically begins during infancy with nystagmus and hypotonia and evolves into spastic quadripareisis, cognitive impairments and ataxia, to ‘pure’ spastic paraparesis, that is characterized exclusively by leg spasticity and weakness (Yoo et al., 2000; Garbern, 2001). The pathological abnormalities in PMD are described primarily as thinning to almost complete absence of myelin in the CNS, without any abnormalities of either CNS axons or of PNS myelin (Seitelberger, 1970, 1995). Axonal injury has been demonstrated previously, however, in the CNS of mice in which the Pld1 gene has been inactivated by homologous recombination (Griffiths et al., 1998), but the rostro-caudal extent of this damage has not been studied. In our studies, we find a length-dependent pattern of axonal degeneration in the CNS of both Pld1 null mice and in patients with PLP1 null mutations. Interestingly, there is little demyelination associated with this axonal degeneration. Disruption of PLP1-mediated axo-glial interactions, rather than demyelination per se, is thus the likely cause of this axonal degeneration. Disruption of axo-glial interactions may be, at least in part, responsible for the axonal degeneration and clinical disability that occur in patients with multiple sclerosis, even in the absence of overt demyelination, but perhaps evoked by immune-mediated responses against PLP1 epitopes.

Material and methods
Mice
Pld1 knockout mice were generated by standard gene targeting strategies and maintained on a C57BL/6 background. Full details of their generation, identification and major phenotypic features have been published (Klugmann et al., 1997; Griffiths et al., 1998). Pld1 knockout mice and wild-type littermates at various ages from P20 to 2 years were perfusion-fixed with a glutaraldehyde/paraformaldehyde mixture and blocks of cervical, thoracic and lumbar spinal cord prepared for resin embedding and electron microscopy as described (Anderson et al., 1998; Griffiths et al., 1998).

Patients
The patients were members of one of two families with PMD. Those identified as V-5, V-6, VI-6 and VI-7 in the pedigree reported previously by Garbern et al. (1997) were found by SSCP (single strand conformation polymorphism) and direct sequencing to have a base deletion (G1) in the second codon (but encoding the first amino acid in the mature PLP1/DM20) of PLP1, creating a frameshift and introducing a stop codon after two codons. The patient identified as III-1 by Sistermans et al. (1996) has a G to A mutation of the initiation codon of PLP1. Informed consent was obtained from the subjects or parents for the study, which was approved by the respective institutions’ human subjects research committees.

Brief clinical histories: family 1
More complete clinical information was available for patients VI-6 and VI-7 and is therefore presented first.

Subject VI-6. This individual had a normal gestation and delivery. He first sat at 9 months of age, did not walk until 4 years of age and remained clumsy. Language development was slow, and he remained in special education throughout his school years. His vision was impaired and he was noted to have strabismus at age 6 years, but nystagmus was never noted. He was able to walk until 12 years of age but had leg spasticity and hyper-reflexia. At age 22 years, he was alert, cooperative and communicative, although his speech was
very dysarthric. His upper extremities were strong and of normal tone, but he had severe contractures in all lower extremity joints and marked spastic paraplegia. He also had truncal and upper extremity ataxia. By age 25 years, he had developed spasticity, athetosis and dystonia of the upper extremities. He was incontinent of urine and totally depend-ent for most activities of daily living, but was able to follow simple commands, count fingers and distinguish colours. He died at age 34 years.

Subject VI-7. The syndrome of this patient was similar to that of subject VI-6. He learned to walk at age 3 years, but after age 8 years his gait progressively deteriorated and by age 15 years he was able to walk only with the aid of a walker. By age 17 years, he was very spastic in his legs and confined to a wheelchair. He retained full strength and was not spastic in his arms, although he was mildly ataxic in the upper extremities. He had marked circumscription and spasticity in the legs, with hyperactive leg reflexes and bilateral Babinski signs. His speech was dysarthric, but readily understood, and he was able to read and perform simple arithmetic calculations as well as follow simple commands without difficulty.

Subjects V-5 and V-6. These subjects had a similar clinical syndrome to that of subjects VI-6 and VI-7. They were described as not retarded, communicated verbally until their mid-twenties and attended special schools for the handi-capped. At ages 35 and 37 years, both were in wheelchairs with severe spasticity, contractures of all four limbs and were incontinent of urine. They were alert, cooperative, aware of their surroundings and had severe dysarthria. Overflow smiling and laughing were noted. V-5 died at age 47 years and V-6 died at age 49 years.

Family 2
Subject III-1. This individual was described by Sistermans et al. (1996) at 6 years of age, and at that time had a similar phenotype to V-5 and V-6 above. Since the original report, he has deteriorated from being able to walk with a walker to being wheelchair dependent.

Histology
Various blocks of CNS embedded in paraffin or preserved in formalin were available from the autopsies of patients V-5, V-6 and VI-6. Wax sections were stained with conventional histochemical stains and by Lapham staining (Lapham et al., 1964) or immunostained by the peroxidase–antiperoxidase (ICN, Basingstoke, UK or Sigma, St Louis, USA) technique for phosphorylated and non-phosphorylated neurofilaments, using antibodies SMI-31 and SMI-32 (Affiniti Research Products Ltd, Exeter, UK), respectively, to demonstrate axons (Griffiths et al., 1998). Astrocytes were immunostained for glial fibrillar acidic protein (GFAP; from Dako Inc., Cambridgeshire, UK) and myelin was identified with antibodies to myelin basic protein (MBP; gift from N. P. Groome, Oxford University, UK) and PLP1 (gift from N. P. Groome).

Cryosections prepared from the formalin-preserved tissue, or wax sections, were double immunostained for neurofilaments and MBP. Tissue preserved in formalin was fixed in glutaraldehyde and processed routinely for resin embedding and electron microscopy.

 Neuroimaging and proton MRS
MRI/MRS scans on patient VI-7 from Garbern et al. (1997) were performed on a 1.5 T GE Signa Scanner (Horizon Software 5.7). Spectroscopic images were obtained using a multi-slice spin-echo sequence (Duyn et al., 1993), with TR = 2300 ms, TE = 280 ms, and 15 mm thick oblique slices, 2.5 mm interslice gap. We used $32 \times 32$ circular phase encoding, a $24 \times 24$ field of view, with a voxel size of $\sim 0.8$ mm. $T_1$-weighted images (TR = 400 ms, TE = 20 ms) were obtained from slices identical to those used for the MRS study. Quantitation of cerebral metabolites was performed as described using the CSX and IMAX programs of Soher et al. (1996).

Single voxel analysis of patient III-1 was performed at 1.5 T on a Siemens Magnetom scanner. Volumes of interest (VOIs) were selected in the left parietal white matter (4.5 ml), the mid-parietal cortex (10 ml) and the basal ganglia on the left (4.5 ml). In each VOI, a fully relaxed, short echo time STEAM (stimulated echo acquisition mode) spectrum (TR/TE/mixing time = 6000/20/10 ms, 64 accumulations) was obtained. Metabolite concentrations were calculated using LCModel (Provencher, 1993; Pouwels and Frahm, 1998) and expressed as mmol/l VOI. Data were compared with values obtained in age-matched controls (age range 10–18 years) (Pouwels et al., 1999).

Spectroscopic comparisons of metabolite concentrations between white or grey matter VOIs of the patients and control subjects were analysed using the Mann–Whitney U-test.

Results
Length-dependent CNS axonal degeneration in Plp1-deficient mice
To examine the spatial pattern of CNS axonal degeneration in mice lacking PLP1/DM20, we analysed the histology of the fasciculus gracilis, a well-defined ascending, sensory fibre tract located in the dorsal columns of the spinal cord, at both 8 and 18 months of age. At 8 months of age, when the animals are clinically normal, occasional degenerating fibres were detected in the cervical region of the fasciculus gracilis but not more caudally in the thoracic region (Fig. 1A and B). By 18 months, however, when the animals demonstrate gait ataxia and weakness, especially of the hind limbs, axonal degeneration was pronounced in the cervical segments, obviously evident in the thoracic region, but was not observed in the lumbar dorsal columns (Fig. 1C and D). Intact axons at both time points were well myelinated, and there was no histological evidence of demyelination, either by light or...
electron microscopic observation, as we have reported previously (Yool et al., 2001). There were some attenuated myelin sheaths around some swollen axons; however, in non-swollen regions, the sheaths were of normal thickness. The absence of PLP1/DM20 expression thus produces a length-dependent axonal degeneration within the fasciculus gracilis, in the absence of overt demyelination, and which correlates with the development of neurological signs in these mice. Proton MRS analysis of PLP1-deficient CNS reveals reduced NAA concentrations in the white matter

To extend these studies to humans, we first analysed the NAA concentration in the brains of two unrelated individuals, each carrying a null mutation of the PLP1 gene, by proton MRS. NAA concentration, measured by proton MRS, has been used extensively to analyse axonal physiology in vivo, and decreased levels of NAA in white matter of patients with multiple sclerosis correlate both with pathological measurements of axonal injury and clinical disability (Grossman et al., 1992; De Stefano et al., 1997, 1998).

In one of the affected male patients (VI-7) who has a PLP1 null mutation caused by a deletion of the fourth nucleotide of the PLP1 coding region, NAA levels in white matter regions were reduced relative to aged-matched controls, with an average white matter NAA concentration of $5.61 \pm 1.91$ mM compared with $11.64 \pm 1.14$ mM in controls ($P < 0.01$; Fig. 2 and Table 1). The cortical grey matter NAA levels were not significantly reduced in this individual. Studies of the patient’s clinically normal mother, however, showed significantly low levels of NAA, $9.75 \pm 1.72$ mM, in the white matter regions ($P < 0.05$), but not in the grey matter. In a second affected male patient (individual III-1 in Sistermans et al., 1996) who has a PLP1 null mutation caused by a missense mutation at the initiation codon of the PLP1 coding region, there was also reduced NAA in the white matter (Table 2). Taken together, these data demonstrate that null mutations of the PLP1 gene are sufficient to cause significant

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**Fig. 1** Age- and length-dependent degeneration of the dorsal column in the Plp1-deficient mouse. Resin sections of the dorsal columns from Plp1 null mice at 8 months (A and B) and 18 months (C and D). The cervical fasciculus gracilis is shown in A and C, and the thoracic cord in B and D. At 8 months, only an occasional degenerate fibre (arrow) is present in the cervical region while the thoracic cord is normal. By 18 months, marked degeneration (arrows indicate some representative degenerating axons) is observed in the cervical area and to a lesser extent in the thoracic segment. Scale bar = 50 μm.
Axonal dysfunction in human brain, as has been shown previously in the brain of the *Plp1* knockout mouse model. Furthermore, the normal levels of choline in both subjects suggest that active demyelination is not part of this process.

**Neuropathological observations**

To confirm and extend the findings obtained by proton MRS, we have examined post-mortem CNS tissue from three affected male relatives of individual VI-7, aged 34, 47 and 49 years at their deaths, all of whom carry the G1 deletion and in whom PLP1 expression has been shown directly to be absent by Western blot and immunohistochemical analysis (Garbern *et al.*., 1997). The general microscopic findings were similar for all three subjects. In the cerebrum, there was diffuse and extensive loss of myelin, with relative preservation of subcortical U fibres. A ‘tigroid’ pattern of myelin staining was not observed. MBP immunostaining revealed generally well-myelinated tissue with the suggestion of some diffuse reduction in cerebrum. In general, luxol fast blue staining was less intense than was MBP immunostaining. (Studies in the *Plp1* null mice showed that despite large amounts of myelin, staining with luxol fast blue was relatively poor, suggesting that the content of PLP/DM20 in myelin might influence the intensity of this histochemical stain; unpublished observations.)

Electron microscopic analysis of brain material demonstrated that all identifiable axons were surrounded by myelin sheaths of appropriate thickness and that no naked or thinly sheathed axons were observed. Individual fibres undergoing Wallerian degeneration, however, were present in the subcortical white matter (Fig. 3A) and the optic tract. Occasional axonal swellings, similar to those found in the knockout mice, were also identified (Fig. 3B and C). The density of myelinated axons also appeared reduced in much of the cerebral white matter, while astrocyte processes, as judged by both GFAP immunostaining and electron microscopy, were markedly increased. These findings thus directly...
Table 1 Absolute measurements of metabolites in a PLP1-deficient male and his mother from proton MRS imaging with values in mM/l in VOI

<table>
<thead>
<tr>
<th>Subject</th>
<th>White matter</th>
<th></th>
<th>Grey matter</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAA (mM) ± SD</td>
<td>Creatine (mM) ± SD</td>
<td>Choline (mM) ± SD</td>
<td>NAA (mM) ± SD</td>
</tr>
<tr>
<td>Affected male VI-7 del G1</td>
<td>5.61 ± 1.91</td>
<td>5.79 ± 1.79</td>
<td>2.03 ± 0.41</td>
<td>7.97 ± 3.64</td>
</tr>
<tr>
<td>Heterozygous del G1</td>
<td>9.75 ± 1.72</td>
<td>6.76 ± 1.62</td>
<td>1.95 ± 0.51</td>
<td>11.02 ± 1.65</td>
</tr>
<tr>
<td>Controls</td>
<td>11.64 ± 1.14</td>
<td>5.32 ± 1.18</td>
<td>2.15 ± 0.33</td>
<td>11.50 ± 1.33</td>
</tr>
</tbody>
</table>

Concentrations of voxels of the indicated compounds calculated from the spectroscopic imaging from a minimum of 12 different exclusively white matter voxels (columns 1–3) or cerebral cortex (columns 4–6) from the cerebrum of each subject. Note the significantly reduced NAA levels in the PLP1 null subject. The \( P \) values are indicated in parentheses and calculated using the Mann–Whitney \( U \)-test. Note the highly significant reduction of NAA in the white matter of the affected male.

Table 2 Single voxel proton MRS study of patient III-1

<table>
<thead>
<tr>
<th>Subject</th>
<th>Parieto-occipital cortex</th>
<th>Parieto-occipital white matter</th>
<th>Basal ganglia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAA</td>
<td>Cr</td>
<td>Cho</td>
</tr>
<tr>
<td>III-1 (age 11 years)</td>
<td>7.5</td>
<td>6.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Controls (ages 10–18 years)</td>
<td>9.0 ± 0.8</td>
<td>6.5 ± 0.5</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

Three volumes of interest were quantitated for the indicated metabolites measured in mM/l in the VOI. Note the reduction of NAA in the parieto-occipital white matter by >2 SDs from the corresponding value for age-matched control subjects. The elevation of myoinositol in the white matter may indicate gliosis. NAA = N-acetyl aspartate; Cr = creatine; Cho = choline; MI = myo-inositol. The difference in white matter NAA concentrations between patient and age-matched controls is statistically significant \( P < 0.05 \).
demonstrate axonal degeneration, as suggested by the proton MRS studies of living patients.

As the distal portions of long spinal tracts were the most severely affected in the Plp1-deficient mouse, we looked for similar length-dependent defects in the patients. Patchy axonal loss was evident in the medullary pyramids, both by immunostaining for neurofilaments and by silver impregnation (not shown). However, more distally, axonal degeneration was prominent in the crossed and uncrossed corticospinal spinal tracts, the major descending motor pathways, at the cervical level (Fig. 4B) where the abnormalities were confined to the lateral regions that supply the legs (see Fig. 4A) (Brazis et al., 1996). Additionally, there was degeneration in the fasciculus gracilis of the cervical (Fig. 4D), but not the lumbar cord (Fig. 4E). Appropriate stains demonstrated the absence of both axons and myelin in the degenerated areas (Fig. 4C). These data are consistent with length-dependent degeneration, without significant demyelination, of these long motor and sensory axonal tracts, as shown previously in Plp1 null mice.

Discussion

In this report, we present evidence that the absence of expression of the myelin protein, PLP1/DM20, produces axonal degeneration in the CNS. The simplest explanation for
the axonal degeneration we have observed is disruption of oligodendrocyte–axonal interactions. Although we can only speculate as to a mechanism, since the causal defect in the humans and mice is mutation of the PLP1 gene, and since PLP1 is essentially expressed normally only by oligodendrocytes and not in neurones, there must be interaction(s) between glia and axons, that must in some way be dependent upon PLP1, and that prevents axonal degeneration in healthy animals and humans. Moreover, the patterns of pathological change in the long ascending and descending tracts are most consistent with length-dependent degeneration, in agreement with the clinical observations that the lower extremities are more severely affected in patients with the PLP1 null syndrome. Our findings confirm that the absence of PLP1
causes axonal damage in humans as well as in rodents. This axonal degeneration occurs in spite of normal axonal ensheathment by oligodendrocytes and in the absence of overt demyelination. Any reduction in myelin staining could be accounted for by a reduced number of fibres rather than demyelination or dysmyelination of intact axons. PLP1/DM20-mediated interactions between myelinating oligodendroglia and their axons thus play a vital role in maintaining normal axonal function in the CNS.

Our MRS studies in PLP1-deficient patients demonstrate decreased NAA levels, suggesting the presence of diffuse axonal degeneration throughout the brain. Consistent with these findings, Bonavita et al. (2001) have also found decreased NAA/creatinine (Cr) ratios in a group of nine patients with various PLP1 mutations, including eight patients with point mutations (six with W144stop; two with K150N, that is mislabelled G150T) and one patient with a PLP1 gene duplication, suggesting that a variety of PLP1 mutations can cause axonal degeneration. In this study by Bonavita et al., however, the NAA/Cr ratios were calculated by averaging the values for brain regions among all patients, so that the measured decreases in NAA/Cr are the average regional decreases from nine individuals with three different PLP1 mutations. Since each of these three mutations probably alters PLP1 structure and oligodendrocyte physiology differently, the cellular and/or molecular basis for this NAA decrease cannot be determined from these data. In addition, there is no direct pathological confirmation of axonal injury in any of the nine families. Our data thus provide the first correlation of both MRS and neuropathology for well-characterized, specific PLP1 mutations, and suggest further that altered interactions between oligodendrocytes and axons due to the absence of PLP1 expression are the basis for this axonal degeneration.

Although PLP1/DM20 is expressed predominantly in oligodendrocytes, recent studies in the mouse have identified isoforms of PLP1 and DM20, termed somal-restricted (srPlp1 and srDm20), which are expressed in subpopulations of neurones, such as hippocampus, Purkinje cells and the olfactory system (Bongarzone et al., 1999). These neuronal subpopulations, however, are not affected in mice with null Plp1 mutations, and the somal-restricted forms of PLP1/DM20 are not expressed in the ascending and descending tracts that are affected in Plp1-deficient mice. In addition, the somal-restricted isoforms of Plp1 have not been identified in human brain. It is therefore unlikely that the length-dependent axonal degeneration is caused by the absence of somal-restricted Plp1 expression in specific neuronal subpopulations. Indeed, in ongoing studies (Edgar et al., 2001), we have transplanted oligodendrocytes from Plp1 knockout mice into the CNS of shiverer mice and reproduced the axonal changes in the transplanted region, thus demonstrating unequivocally that the axonopathy is due to absence of PLP1/DM20 in the overlying oligodendrocyte.

The cellular and molecular mechanisms underlying length-dependent axonal degeneration are not known. One possibility is that transport of a critical compound or organelle, such as a growth factor or mitochondria, to the distal axon is disrupted, making it more susceptible to damage. Consistent with this hypothesis, the CNS axonal cytoskeleton is modified in shiverer mice (Brady et al., 1999), which do not express the major myelin protein, MBP. In addition, we have observed accumulations of mitochondria in PLP1-deficient mice and humans.

Our data have important implications for the pathogenesis and treatment of multiple sclerosis. Several causes of axonal degeneration in multiple sclerosis are possible, such as direct immune-mediated destruction of axons, the secretion of cytotoxic cytokines and/or the loss of secreted or contact glial factors. Although the aetiology of multiple sclerosis is not known, there is evidence for immune responses to PLP1 in multiple sclerosis patients (Sun et al., 1991; Trotter et al., 1991; Pelfrey et al., 1994; Correale et al., 1995; Sellebjerg et al., 1995), and PLP1 and PLP1-derived peptides can induce experimental autoimmune encephalitis, an animal model of multiple sclerosis (Endoh et al., 1986; Trotter et al., 1987). Recently, Onuki et al. (2001) have shown that myelin oligodendrocyte glycoprotein-induced demyelination in a murine model of multiple sclerosis is associated with axonal degeneration. Thus, disruption of specific PLP1-mediated axonal–glial interactions may occur in multiple sclerosis, and could therefore be responsible for the ongoing axonal degeneration in patients with chronic progressive multiple sclerosis, even in the absence of persistent inflammation or demyelination. In addition, this mechanism could also account for the axonal dysfunction demonstrated by proton MRS in normal appearing white matter in multiple sclerosis brain. Efforts to modulate oligodendrocyte function in vivo are thus clearly warranted, even without myelin repair, and could significantly alter the clinical course of this chronic, debilitating disease, as well as that of PMD. The PLP1-deficient mouse may thus be a useful model for testing therapies aimed at ameliorating axonal degeneration in patients with myelin diseases, such as PMD and multiple sclerosis.

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References

Anderson TJ, Schneider A, Barrie JA, Klugmann M, McCulloch MC, Kirkham D, et al. Late-onset neurodegeneration in mice with 

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