# Myelin dysfunction/degradation in the central nervous system: why are myelin sheaths susceptible to damage?

**M. Bradl**

Max-Planck-Institute for Neurobiology, Department of Neuroimmunology, Martinsried, Federal Republic of Germany

**Summary.** In the central nervous system, myelin sheaths are produced to electrically insulate axons and to increase the velocity of axonal conduction. They are highly complex structures, which are often destructed in neurological disorders. One possible reason for the vulnerability of myelin sheaths to damage became apparent from analyses of animals with altered amounts of otherwise normal myelin components: Due to limited redundance in function between different myelin proteins, dysfunction or loss of one protein may cause loss of function and instability of the entire myelin sheath.

## Introduction

Destruction of myelin sheaths in the central nervous system (CNS) is a common theme in many neurological disorders, with a wide range of underlying causes. For example, demyelination can result from CNS inflammation, where it is caused by T cell/macrophage infiltration, especially when this is associated with myelin-specific autoantibody responses (Linington et al., 1988), or by pro-inflammatory mediators such as cytokines (Probert et al., 1995; Corbin et al., 1996), complement (Dietzschold et al., 1995) and free radicals (Brett and Rumsby, 1993; Bagasra et al., 1995). Alternatively, myelin destruction can be triggered by viral infections (Rodriguez, 1985; Rodriguez et al., 1996; Fleming et al., 1993). Finally, it can be caused by defects of genes encoding structural or regulatory myelin proteins (Duncan, 1995). But why are myelin sheaths so extremely vulnerable?

In the CNS, myelin sheaths are produced by oligodendrocytes to electrically insulate axons and thus increase the velocity of axonal conduction. They are comprised of multiple layers of oligodendrocytic plasma membrane, and contain many proteins highly specific to CNS myelin (Fig. 1).

The important contribution of individual myelin components to myelin stability and function is immediately evident from a broad spectrum of natural mutations resulting in unstable myelin and corresponding behavioral abnormalities (for review see Duncan, 1995). However, usage of such mutants to deduce the function of individual myelin components is limited by one impor-

W. Poewe et al. (eds.), *Advances in Research on Neurodegeneration*

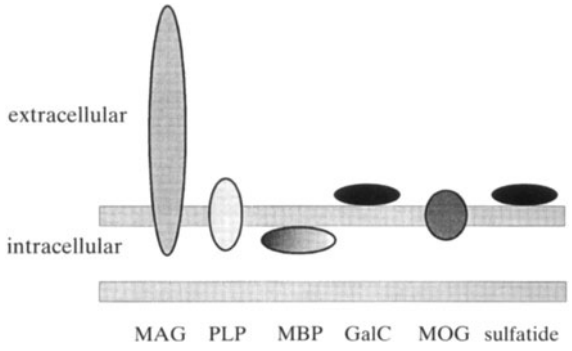


**Fig. 1.** Myelin components in the mammalian CNS

tant draw-back: mutated proteins could either perform new functions (gain-of-function phenotypes), could be unable to perform some of their functions (loss-of-function phenotypes) or could interfere with the proper performance of other components of the myelin sheath.

One way to circumvent such problems is to analyze animals with altered amounts of otherwise normal myelin components. Occasionally, such animals are natural mutants (for example mice carrying the mutation *shiverer*, see below). More often, however, they had been genetically manipulated — either by increasing the gene dosage with introduced transgenes, or by targeted disruption ("knock-out") of relevant gene loci.

What did we learn from such animals about the function of individual myelin constituents?

## Myelin basic protein (MBP)

Reduction, or even complete absence of MBP is seen in mice carrying the autosomal recessive mutations shiverer (shi) or shiverer myelin deficient ($shi^{mld}$), respectively. In shiverer mice, a large portion of the MBP gene is deleted, leading to a complete absence of functional MBP products (Roach et al., 1983; Roach et al., 1985; Kimura et al., 1985). In $shi^{mld}$, the entire MBP gene is duplicated, and some of its exons are inverted, resulting in antisense RNA for MBP transcripts and, eventually, in a significant reduction of total MBP production (Popko et al., 1987, 1988). MBP deficient animals can be easily recognized by a characteristic "shivering" which is most evident when they initiate voluntary movements. The CNS of these mutants is characterized by apparently normally differentiated oligodendrocytes, and a profound reduction in the amount of myelin. Only few, disproportionally thin myelin sheaths with an atypical ultrastructure can be detected. These sheaths lack a major dense line — the intracellular adhesion zone of the myelin membrane (Roach et al., 1983). Shi and $shi^{mld}$ are natural mutants of the mouse fancy. Their phenotype was faithfully reproduced in transgenic animals upon MBP reduction by antisense technology (Katsuki et al., 1988), and corrected

**Table 1.** Function of proteins and lipids in CNS myelin, as revealed by transgenic overexpression or genetic ablation

| Component | Alteration | Principal observation | Conclusion |
|---|---|---|---|
| CNP<br>Gravel et al. (1996) | overex | temporally accelerated MBP and PLP expression<br>lack of myelin compaction | CNP regulator of oligodendrocyte maturation |
| GalC and sulfatides<br>Bosio et al. (1996)<br>Coetzee et al. (1996)<br>Dupree et al. (1998) | cgt-k.o. | myelin compact, but instable, insulator function of myelin sheaths lost, abnormal nodes (Dupree et al., 1998) | GalC/sulfatides important for myelin stability and node of Ranvier formation |
| MAG<br>Montag et al. (1994)<br>Li et al. (1994) | MAG-k.o. | delayed onset of myelination; some axons with two or more sheaths (Montag et al. 1994)<br>reduced content of oligocyte cytoplasm at inner aspects of most myelin sheaths (Montag et al., 1994; Li et al., 1994) | L-MAG necessary for glial-axonal interactions; participation in recognition between oligodendrocyte processes and axons |
| MBP<br>Katsuki et al. (1988)<br>Shine et al. (1992) | MBP-red<br>shi<br>$shi^{mld}$ | Reduction in CNS myelin<br>presence of well differentiated oligodendrocytes myelin sheaths lack major dense line (Shine et al., 1992) | MBP necessary for compaction of myelin |
| PLP<br>Boison and Stoffel (1994)<br>Boison et al. (1995) | k.o. | reduced myelin stability | PLP needed for stabilization of compacted myelin |
| PLP<br>Readhead et al. (1994)<br>Kagawa et al. (1994) | overex | overexpression by factor >2: oligodendrocytes apoptotic and developmentally arrested<br>profound lack of myelin sheaths<br>Overexpression by factor <2: myelin degeneration (Kagawa et al., 1994) | PLP regulator of oligodendrocyte differentiation/survival; needed for maintaining myelin sheaths |
| DM-20<br>Mastronardi et al. (1993)<br>Simons-Johnson et al. (1995) | overex | reduced myelin content in CNS, disrupted myelin lamellae late onset demyelination with lymphocytic infiltrates | DM-20 needed for maintaining myelin sheaths |

upon introduction of wildtype MBP sequences (Readhead et al., 1987; Kimura et al., 1989), indicating first, that the amount of MBP available to oligodendrocytes is a limiting factor for CNS myelin assembly, and secondly, that the presence of MBP is crucial for the compaction of myelin (Table 1).

Since lack of MBP had such profound consequences for myelination in the CNS, it was interesting to see, whether complete absence of proteolipid

protein (PLP) would also be deleterious to assembly or maintenance of intact myelin sheaths.

## Proteolipid protein (PLP)

It was known for a long time that the mouse mutation jimpy (jp) and the corresponding rat mutation myelin deficient (md) affect the PLP gene, leading to abnormally folded and rapidly degraded PLP (Nave et al., 1986; Boison and Stoffel, 1989). Animals carrying these mutations display abnormal numbers of apoptotic oligodendrocytes, associated with activated microglia cells and astrocytes. The CNS of these mutants is severely dysmyelinated, and the few remaining myelin sheaths do not contain immunoreactive PLP (for review see Nave, 1995). Since jp mice and md rats had such severe changes in their phenotype, it was quite surprising to see that oligodendrocytes from PLP knock-out mice were viable and able to ensheath axons of all calibers with compacted myelin (Klugmann et al., 1997; Boison and Stoffel, 1994), and that PLP deficiency even rescued the lethal phenotype of shiverer mutants (Stoffel et al., 1997). It needed careful analyses by electron microscopy to reveal a lack of firm intermembrane bonding and a tendency of CNS $PLP^{-/-}$ myelin to losen up (Rosenbluth et al., 1996). These experiments clearly demonstrated that PLP is needed to ensure proper physical stability of myelin sheaths. But there was more to be learned about the function of PLP in the CNS. Transgenic mice (Readhead et al., 1994; Kagawa et al., 1994) and rats (own, unpublished results) overexpressing PLP were produced. These animals have two different phenotypes, depending on the degree of overexpression.

Expressing proteolipids above a certain threshold (i.e. expressing at least double the amount than normal) resulted in the death of mature, and a developmental arrest of remaining immature oligodendrocytes. Consequently, the CNS of these animals is severely dysmyelinated. Death of mature oligodendrocytes is almost certainly consequence of retention and accumulation of incorrectly folded PLP proteins in the endoplasmic reticulum — a condition also described in mice and rats carrying PLP missense mutations (Gow et al., 1994). However, the developmental arrest of immature, premyelinating oligodendrocytes strongly suggests that PLP has an important role in cellular differentiation as well, possibly by forming ion channels. This speculation is supported by a high similarity of proteolipids to pore-forming proteins (Yan et al., 1993; Kitagawa et al., 1993), although formal proof for it is lacking to date.

Important information was also gained from mice and rats with lower levels of PLP overexpression (i.e. overexpressing PLP by factor $<2$). At first glance, these animals seemed entirely healthy and were fully competent to produce normal myelin sheaths. However, these sheaths were extremely susceptible to degeneration and/or frank demyelination (Readhead et al., 1994; Kagawa et al., 1994, own unpublished results), indicating that a correct

stoichiometric ratio of PLP in the myelin membrane is crucial for maintaining myelin sheaths.

## Myelin-associated glycoprotein (MAG)

PLP and MBP are major myelin proteins, and so it is just natural that they had been analyzed first. Other proteins were to follow soon, though. Mice were produced with a targeted deletion of the MAG gene. These animals were viable (Li et al., 1994; Montag et al., 1994), but showed a delay in CNS myelin formation (Montag et al., 1994). Moreover, their CNS myelin was abnormal: cytoplasmic collars of oligodendrocytes usually present in mature sheaths were either completely missing, or significantly reduced in numbers, and many axons were surrounded by 2 or more sheaths. Subsequently it could be shown, that MAG is also an inhibitory compontent for axonal growth in peripheral (Schäfer et al., 1996), but not central myelin (Bartsch et al., 1995). These data indicated that MAG plays a critical role in glial-axonal interactions. But there were two more points to consider. First, aged $MAG^{-/-}$ mutants displayed evidence of dying-back oligodendrogliopathy (Lassmann et al., 1997) in the CNS, and of myelin degeneration in the PNS (Fruttiger et al., 1995). Secondly, MAG exists in 2 distinct isoforms, as larger L-MAG and as the shorter splice variant S-MAG. To analyze the contribution of individual MAG isoforms to CNS and PNS pathology, a novel set of "knock-out" mice had to be produced, which carried dysfunctional L-MAG, and normal S-MAG. $L\text{-}MAG^{-/-}$ were viable, and displayed most of the CNS, but none of the PNS abnormalities (Fujita et al., 1998). Thus, L-MAG is responsible for the integrity of myelin sheaths in the CNS. Interestingly, a profund decrease in L-MAG and myelin malformations (Fujita et al., 1990; Bö et al., 1995) is also seen in mice carrying the mutation quaking (qk), which affects a protein possibly involved in RNA processing and signal transduction (Ebersole et al., 1996).

## Galactocerebroside (Gal-C) and sulfatides

All myelin components described so far were proteins. Also lipid components of the myelin sheath could be manipulated, by indirect means. This was achieved by genetically disrupting UDP-galactose ceramide galactosyltransferase (cgt), a key enzyme for the synthesis of Gal-C and sulfatides. Accordingly, these glycoplipids are completely absent in $cgt^{-/-}$ mice (Coetzee et al., 1996; Bosio et al., 1996). Although affected animals exhibit a severe tremor that is associated with hind limb paralysis, most of their myelin sheaths are remarkably intact. Nevertheless, axonal conduction velocity was significantly decreased, due to a defect in the formation of nodes of Ranvier in the CNS (Dupree et al., 1998). Thus, Gal-C and sulfatides are important in ensuring proper glial-axonal interactions.

## Why are myelin sheaths vulnerable?

As pointed out above, myelin sheaths are highly complex, almost crystalline arrangements of lipids, glycolipids and proteins. The balance of these individual elements seems so delicately tuned, that not only overt mutations, but even slightest increases or decreases in the amount of otherwise normal components perturb the entire structure (Scherer, 1997). Contrary to other systems, where dysfunction or loss of one protein could be compensated for by the action of others (Erickson, 1993; Kelso, 1994), myelin components do not seem to have such a "back-up". Interfering with the function of one indivual component — for whatever reasons — may cause a loss of function of the whole structure, making myelin sheaths to prime targets in many different neurological disorders.

## References

Bagasra O, Michaels FH, Zheng YM, Bobroski LE, Spitsin SV, Fu ZF, Tawadros R, Koprowski H (1995) Activation of the inducible form of nitric oxide synthase in the brains of patients with multiple sclerosis. Proc Natl Acad Sci USA 92: 12041–12045

Bartsch U, Bandtlow CE, Schnell L, Bartsch S, Spillmann AA, Rubin BP, Hillenbrand R, Montag D, Schwab ME, and Schachner M (1995) Lack of evidence that the myelin-associated glycoprotein (MAG) is a major inhibitor of axonal regeneration in the CNS. Neuron 15: 1375–1382

Boison D, Stoffel W (1989) Myelin deficient rat: A point mutation in exon III (A > C, Thr75 > Pro) of the myelin proteolipid protein causes dysmyelination and oligodendrocyte death. EMBO J 8: 3295–3302

Boison D, Stoffel W (1994) Disruption of the compacted myelin sheath of axons of the central nervous system in proteolipid protein-deficient mice. Proc Natl Acad Sci USA 91: 11709–11713

Boison D, Büssow H, D'Urso D, Müller HW, Stoffel W (1995) Adhesive properties of proteolipid protein are responsible for the compaction of CNS myelin sheaths. J Neurosci 15: 5502–5513

Bosio A, Binczek E, Stoffel W (1996) Functional breakdown of the lipid bilayer of the myelin membrane in central and peripheral nervous system by disrupted galactocerebroside synthesis. Proc Natl Acad Sci USA 93: 13280–13285

Bö L, Quarles RH, Fujita N, Bartoszewicz Z, Sato S, Trapp BD (1995) Endocytic depletion of L-MAG from CNS myelin in quaking mice. J Cell Biol 131: 1811–1820

Brett R, Rumsby MG (1993) Evidence of free radical damage in the central nervous system of guinea pigs at the prolonged acute and early relapse stages of chronic relapsing experimental allergic encephalomyelitis. Neurochem Int 23: 35–44

Coetzee T, Fujita N, Dupree J, Shi R, Blight A, Szuzuki K, Suzuki K, Popko B (1996) Myelination in the absence of galactocerebroside and sulfatide: Normal structure with abnormal function and regional instability. Cell 86: 209–219

Corbin JG, Kelly D, Rath EM, Baerwald KD, Suzuki K, Popko B (1996) Targeted CNS expression of interferon-y in transgenic mice leads to hypomyelination, reactive gliosis, and abnormal cerebellar development. Mol Cell Neurosci 7: 354–370

Dietzschold B, Schwaeble W, Schäfer MK-H, Hooper DC, Zehng YM, Petry F, Sheng H, Fink T, Loos M, Koprowski H, Weihe E (1995) Expression of C1q, a subcomponent of the rat complement system, is dramatically enhanced in brains with either Borna disease or experimental allergic encephalomyelitis. J Neurol Sci 130: 11–16

Duncan ID (1995) Inherited disorders of myelination of the central nervous system. In: Kettenmann H, Ransom B (eds) Neuroglia. Oxford University Press, Oxford, U.K., pp. 990–1009

Dupree JL, Coetzee T, Blight A, Suzuki K, Popko B (1998) Myelin galactolipids are essential for proper node of Ranvier formation in the CNS. J Neurosci 18: 1642–1649

Ebersole TA, Chen Q, Justice MJ, Artzt K (1996) The quaking gene product necessary in embryogenesis and myelination combines features of RNA binding and signal transduction proteins. Nat Genet 12: 260–265

Erickson HP (1993) Gene knockouts of c-src, transforming growth factor b1, and tenascin suggest superfluous, nonfunctional expression of proteins. J Cell Biol 120: 1079–1081

Fleming JO, Wang FI, Trousdale MD, Hinton DR, Stohlman SA (1993) Interaction of immune and central nervous systems: Contribution of anti-viral Thy-1$^+$ cells to demyelination induced by coronavirus JHM. Reg Immunol 5: 37–43

Fruttiger M, Montag D, Schachner M, Martini R (1995) Crucial role for the myelin-associated glycoprotein in the maintenance of axon-myelin integrity. Eur J Neurosci 7: 511–515

Fujita N, Sato S, Ishigura H, Inuzuka T, Baba H, Kurihara T, Takahashi Y, Miyatake T (1990) The large isoform of myelin-associated glycoprotein is scarcely expressed in the quaking mouse brain. J Neurochem 55: 1056–1059

Fujita N, Kemper A, Dupree J, Nakayasu H, Bartsch U, Schachner M, Maeda N, Suzuki K, Suzuki K, Popko B (1998) The cytoplasmic domain of the large myelin-associated glycoprotein isoform is needed for proper CNS but not peripheral nervous system myelination. J Neurosci 18: 1970–1978

Gow A, Friedrich VL, Lazzarini RA (1994) Many naturally occurring mutations of myelin proteolipid protein impair its intracellular transport. J Neurosci Res 37: 574–583

Gravel M, Peterson J, Yong VW, Kottis V, Trapp BD, Braun PE (1996) Overexpression of 2′,3′-cyclic nucleotide 3′-phosphodiesterase in transgenic mice alters oligodendrocyte development and produces aberrant myelination. Mol Cell Neurosci 7: 453–466

Johnson RS, Roder JC, Riordan JR (1995) Over-expression of the DM-20 myelin proteolipid causes central nervous system demyelination in transgenic mice. J Neurochem 64: 967–976

Kagawa T, Ikenaka K, Inoue Y, Kuriyama S, Tsujii T, Nakao J, Nakajima K, Aruga J, Okano H, Mikoshiba K (1994) Glial cell degeneration and hypomyelination caused by overexpression of myelin proteolipid protein gene. Neuron 13: 427–442

Katsuki M, Sato M, Kimura M, Yokoyama M, Kobayashi K, Nomura T (1988) Conversion of normal behavior to shiverer by myelin basic protein antisense cDNA in transgenic mice. Science 241: 593–393

Kelso A (1994) The enigma of cytokine redundancy. Immunol Cell Biol 72: 97–101

Kimura M, Inoko H, Katsuki M, Ando A, Sato T, Hirose T, Takashima H, Inayama S, Okano H, Takamatsu K, Mikoshiba K, Tsukuda Y, Watanabe I (1985) Molecular genetic analysis of myelin deficient mice: Shiverer mutant mice show deletion in gene(s) coding for myelin basic protein (MBP). J Neurochem 44: 692–696

Kimura M, Sato M, Akatsuka A, Nozawa-Kimura S, Takahashi R, Yokoyama N, Nomura T, Katsuki M (1989) Restoration of myelin formation by a single type of myelin basic protein (MBP) in transgenic shiverer mice. Proc Natl Acad Sci USA 86: 5661–5665

Kitagawa K, Sinoway MP, Yang C, Gould RM, Colman DR (1993) A proteolipid protein gene family: Expression in sharks and rays and possible evolution from an ancestral gene encoding a pore-forming polympeptide. Neuron 11: 433–448

Klugmann M, Schwab MH, Pühlhofer A, Schneider A, Zimmermann F, Griffiths IR, Nave K-A (1997) Assembly of CNS myelin in the absence of proteolipid protein. Neuron 18: 59–70

Lassmann H, Bartsch U, Montag D, Schachner M (1997) Dying back oligodendrogliopathy: A late sequel of myelin-associated glycoprotein deficiency. Glia 19: 104–110

Li C, Tropak MB, Gerlai R, Clapoff S, Abramow-Newerly W, Trapp BD, Peterson A, Roder J (1994) Myelination in the absence of myelin-associated glycoprotein. Nature 369: 747–750

Linington C, Bradl M, Lassmann H, Brunner C, Vass K (1988) Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. Am J Pathol 130: 443–454

Mastronardi FG, Ackerley CA, Arsenault L, Roots BI, Moscarello MA (1993) Demyelination in a transgenic mouse: A model for multiple sclerosis. J Neurosci Res 36: 315–324

Montag D, Giese KP, Bartsch U, Martini R, Lang Y, Bluethmann H, Karthigasan J, Kirschner DA, Wintergerst E, Nave K-A, Zielasek J, Toyka KV, Lipp H-P, Schachner M (1994) Mice deficient for the myelin-associated glycoprotein show subtle abnormalities in myelin. Neuron 13: 229–246

Nave K-A (1995) Neurological mouse mutants: a molecular-genetic analysis of myelin proteins. In: Kettenmann H, Ransom B (eds) Neuroglia. Oxford University Press, Oxford, U.K., pp. 571–586

Nave K-A, Lai C, Bloom FE, Milner RJ (1986) Jimpy mutant mouse: A 74-base deletion in the mRNA for myelin proteolipid protein (PLP) and evidence for a primary defect in RNA splicing. Proc Natl Acad Sci USA 83: 9264–9268

Popko B, Puckett C, Lai E, Shine HD, Readhead C, Takahashi N, Hunt III SW, Sidman R, Hood LE (1987) Myelin deficient mice: Expression of myelin basic protein and generation of mice with varying levels of myelin. Cell 48: 713–721

Popko B, Puckett C, Hood LE (1988) A novel mutation in myelin-deficient mice results in unstable myelin basic protein gene transcripts. Neuron 1: 221–225

Probert L, Akassoglou K, Pasparakis M, Kontogeorgos G, Kollias G (1995) Spontaneous inflammatory demyelinating disease in transgenic mice showing central nervous system-specific expression of tumor necrosis factor a. Proc Natl Acad Sci USA 92: 11294–11298

Readhead C, Popko B, Takahashi N, Shine HD, Saavedra RA, Sidman RL, Hood L (1987) Expression of a myelin basic protein gene in transgenic shiverer mice: correction of the dysmyelinating phenotype. Cell 48: 703–712

Readhead C, Schneider A, Griffiths I, Nave K-A (1994) Premature arrest of myelin formation in transgenic mice with increased proteolipid protein gene dosage. Neuron 12: 583–595

Roach A, Boylan K, Horvath S, Prusiner SB, Hood LE (1983) Characterization of cloned cDNA representing rat myelin basic protein: Absence of expression in brain of shiverer mutant mice. Cell 34: 799–806

Roach A, Takahashi N, Pravtcheva D, Ruddle F, Hood L (1985) Chromosomal mapping of mouse myelin basic protein gene and structure and transcription of the partially deleted gene in shiverer mutant mice. Cell 42: 149–155

Rodriguez M (1985) Virus-induced demyelination in mice: "Dying-back" of oligodendrocytes. Mayo Clin Proc 60: 433–438

Rodriguez M, Pavelko KD, Njenga MK, Logan WC, Wettstein PJ (1996) The balance between persistent virus infection and immune cells determines demyelination. J Immunol 157: 5699–5709

Rosenbluth J, Stoffel W, Schiff R (1996) Myelin structure in proteolipid protein (PLP)-null mouse spinal cord. J Comp Neurol 371: 336–344

Schäfer M, Fruttiger M, Montag D, Schachner M, Martini R (1996) Disruption of the gene for the myelin-associated glycoprotein improves axonal regrowth along myelin in C57BL/Wld$^s$ mice. Neuron 16: 1107–1113

Scherer S (1997) Molecular genetics of demyelination: New wrinkles on an old membrane. Neuron 18: 13–16

Shine HD, Readhead C, Popko B, Hood L, Sidman RL (1992) Morphometric analysis of normal, mutant, and transgenic CNS: Correlation of myelin basic protein expression to myelinogenesis. J Neurochem 58: 342–349

Stoffel W, Boison D, Büssow H (1997) Functional analysis in vivo of the double mutant mouse deficient in both proteolipid protein (PLP) and myelin basic protein (MBP) in the central nervous system. Cell Tiss Res 289: 195–206

Yan Y, Lagenaur C, Narayanan V (1993) Molecular cloning of M6: Identification of a PLP/DM20 gene family. Neuron 11: 423–431

Author's address: Dr. Monika Bradl, Max-Planck-Institut für Neurobiologie, Abteilung Neuroimmunologie, D-82152 Martinsried, Federal Republic of Germany