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Pelizaeus-Merzbacher Disease

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Abstract. Pelizaeus-Merzbacher disease (PMD) can now be defined as an X-linked recessive leukodystrophy that is caused by a mutation in the *proteolipid protein* (*PLP*) gene on chromosome Xq22. The most common mutation is gene duplication followed in frequency by missense mutations, insertions, and deletions. The clinical spectrum ranges from severe neonatal cases to relatively benign adult forms and X-linked recessive spastic paraplegia type 2. The lack of PLP is accompanied by deficits in the other myelin proteins of the central nervous system, including myelin basic protein, myelin-associated glycoprotein, and cyclic nucleotide phosphodiesterase. Surprisingly, the total absence of PLP due to gene deletion or a null allele causes a relatively benign form of PMD. Abnormal PLP is thought to impair protein trafficking and to induce apoptosis in oligodendroglia. Immunocytochemistry with specific antibodies reveals the PLP deficiency and insufficient generation of myelin sheaths with the remaining proteins. Both excessive biosynthesis of PLP, as in gene duplications, or conformational change of the protein, as in missense mutations, are detrimental to myelination. Several naturally occurring and transgenic animal models with *PLP* gene mutations or deletions have contributed to our understanding of dysmyelination in PMD and the general knowledge of myelination and myelin repair.

Key Words: Mutation; Myelin; Pelizaeus-Merzbacher disease; Proteolipid protein; X-chromosome.

INTRODUCTION

Current Definition

Pelizaeus-Merzbacher disease (PMD) was named after Pelizaeus who described the clinical features in 1885 (1) and Merzbacher who presented additional observations in 1910, including a postmortem examination (2). The paper by Pelizaeus may have been the first report of X-linked recessive transmission of a disease of the nervous system (1). Family members said that the disease ''passes through the mother but does not affect her'' (1). At first, Merzbacher did not realize that his 14 cases came from the previously reported family (2). The occurrence of the disease in 2 sisters in the same family may raise doubts about X-linked inheritance. However, one of the sisters had autopsy confirmation of the disorder (3). Merzbacher included several photographs of his patients (2). Two siblings, a brother and a sister, were young adults with limited disability. Autopsies were completed on a younger brother and sister who were severely disabled in childhood. Merzbacher reported the neuropathology of the brother who died at the age of 20 yr from pulmonary tuberculosis (2). It is not known how long the sister survived after Merzbacher's clinical description, but her brain and spinal cord were examined in 1928 after prolonged storage (3). In the autopsy cases (2, 3), the patchy myelin deficit in the centrum semiovale was identical, and this pattern of ''tigroid'' dysmyelination was later termed the ''classical'' form of PMD (4). The cerebellar white matter was less seriously affected though still tigroid. The brainstem showed only minor myelin loss. The spinal cord was available in Liebers's (3) case and revealed only minor myelin deficiency. The detailed descriptions by Pelizaeus (1) and Merzbacher (2) established that there may be considerable variability in the severity of PMD in the same family, though the mutation is likely the same. The classical tigroid type of PMD may have a more benign course but early or intrauterine onset is often followed by a more malignant progression. In these patients, central nervous system (CNS) myelin is entirely absent, and this form has been called ''connatal'' (4).

The understanding of the genetic defect in PMD was aided by progress in several contemporary research efforts. They included the discovery and isolation of proteolipid protein (PLP) (5) and the realization that it is the main CNS myelin protein and essentially absent from the peripheral nervous system (PNS). Zeman et al (6) suggested that PMD might be due to a deficit in PLP since the peripheral nervous system remained unaffected. This suggestion was proved correct when CNS proteins were extracted from PMD white matter and examined by Western blotting (7). Systematic study of lymphoblast DNA from this case established that PMD was due to a point mutation in exon 4 of the 7-exon PMD gene (8). The assignment of the *PLP* gene to the long arm of the human X-chromosome (9) provided the scientific confirmation of X-linked recessive transmission of PMD. Based on these observations, PMD must now be defined as an X-linked recessive disease of the CNS due to PLP deficiency. Numerous recent reviews have appeared (10–14) and an Internet website has been established (15). The number of publications has risen steeply and much has been

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learned about the molecular genetics of PMD. However, information on the pathogenesis and the reasons for the highly variable clinical and neuropathological phenotypes remains limited. In 1996, Seitelberger et al (13) estimated that between 1970 and 1996 only 25 pathological descriptions of PMD were published. The differential effects of *PLP* gene mutations are also highlighted by the observation that X-linked spastic paraplegia type 2 (SPG-2) is linked to the same locus as PMD (16). This disorder had been described in great detail in 1962 (17), and analysis of the *PLP* gene in this family in 1994 disclosed a mutation that was identical to that in the *rumpshaker (rsh)* mouse (18). Accordingly, PMD and SPG-2 are allelic disorders though they are listed separately in Online Mendelian Inheritance in Man (OMIM; PMD, 312080; SPG-2, 312920) (19).

The normal *PLP* gene is transcribed and translated into 2 proteins, PLP and DM20, due to alternate splicing of messenger ribonucleic acid (mRNA). DM20 lacks a segment of 35 amino acids that are part of the PLP molecule. Accordingly, PLP may be mutated while DM20 is entirely normal. The co-discoverer of PLP recently summarized the current state of knowledge of this remarkable protein (20). It has become apparent that DM20 has biological functions in the maturing CNS that are distinct from the role of the larger PLP (20).

This review focuses on the tissue changes in PMD and some related animal mutants in an effort to further the understanding of the pathogenesis.

Clinical Features

In the absence of a family history or a male-inherited neurological illness, it may be impossible to diagnose PMD by clinical examination and imaging. When Boulloche and Aicardi (21) compiled information on the clinical features of 148 patients in 19 families (1986), the PLP deficit in PMD was not yet known (21). X-linked recessive inheritance was present in all families, and the most common observations were nystagmus or nystagmoid eye movements, psychomotor deterioration, dystonia, and progressive signs of upper motor neuron disease and cerebellar dysfunction. Epilepsy is probably more frequent than reported by Boulloche and Aicardi who found it in only 4 of 148 patients (21). Where listed, head circumferences were below the fifth percentile. Diffuse atrophy of the extremities suggested motor neuron disease, and some patients had muscle biopsies (7). The tissue showed no denervation. Optic atrophy is common, and in many childhood cases there is visual impairment. An interesting ancillary observation is the retention of pupillary light reflexes despite visual impairment and optic atrophy. The interpretation was that axons in the optic nerves are sufficiently preserved to conduct the light stimulus even though myelin is essentially absent (7). Occasional case reports include stridor as a prominent symptom. Across the entire spectrum of PMD symptoms and signs, nystagmus or nystagmoid eye movements are perhaps the most consistent observations.

Pelizaeus-Merzbacher disease may result from one of at least 85 mutations (15), and a universal gene test on lymphocyte deoxyribonucleic acid (DNA) does not exist. Duplications are the most common cause of PMD (22) and may be detected by fluorescent in situ hybridization (FISH) of lymphocytes in interphase (23) or comparative multiplex polymerase chain reaction (PCR) (24).

Among the ancillary investigations, magnetic resonance imaging (MRI) clearly shows the failure to assemble myelin when the observations are compared with age-matched controls (25). T1-weighted images reveal the lack of high signal in the white matter that is characteristic of myelination. Cortex and white matter are homogeneous and tend to blend without demarcation. T2-weighted techniques show persistent high intensity signals where progressively lower signals should reflect myelination. Most of the abnormalities can be explained by the persistence of tissue water in the white matter, and fluid-attenuated inversion recovery and diffusion-weighted images can be used to refine the information obtained from MRI. Figure 1 shows an example of MRI in PMD. Nezu et al made a brief review of MRI in PMD (26). Magnetic resonance spectroscopy (MRS) has produced somewhat inconsistent results, including reductions (27) and elevations of N-acetylaspartate (NAA) (28). Takanashi et al critically reviewed prior MRS studies in PMD and suggested that methodological differences accounted for the discrepancies (28). Though NAA is widely viewed as a neuronal and axonal marker, it may also be present in oligodendroglia (28). Premature death of these cells in PMD may thus affect NAA concentrations and the signal may not be solely due to axonal localization. It is also possible that the nature of the *PLP* gene mutation and the inclusion of female carriers (27) have affected the overall result.

Visual, auditory, and sensory evoked responses may show variable abnormalities (29). Electroencephalograms are helpful in the diagnosis of PMD-associated seizures. Routine examination of cerebrospinal fluid is normal.

Standard cytogenetic analysis of suspected PMD is generally unrewarding (7). However, FISH and PCR confirmation of *PLP* gene duplication are becoming more readily available, and FISH has also been applied to metaphase spreads (23, 30). It is not surprising that major chromosomal rearrangements involving the Xq22 locus (31–33) caused PMD in addition to other developmental abnormalities.

Johnston and McKusick gave detailed clinical descriptions in 8 cases of SPG-2, including 6 photographs of their patients (17). Invariably, the disorder began with ''pure'' spastic leg weakness but gradually other symptoms and signs developed, including sensory loss, nystagmus, optic atrophy, dysarthria, and dementia. The

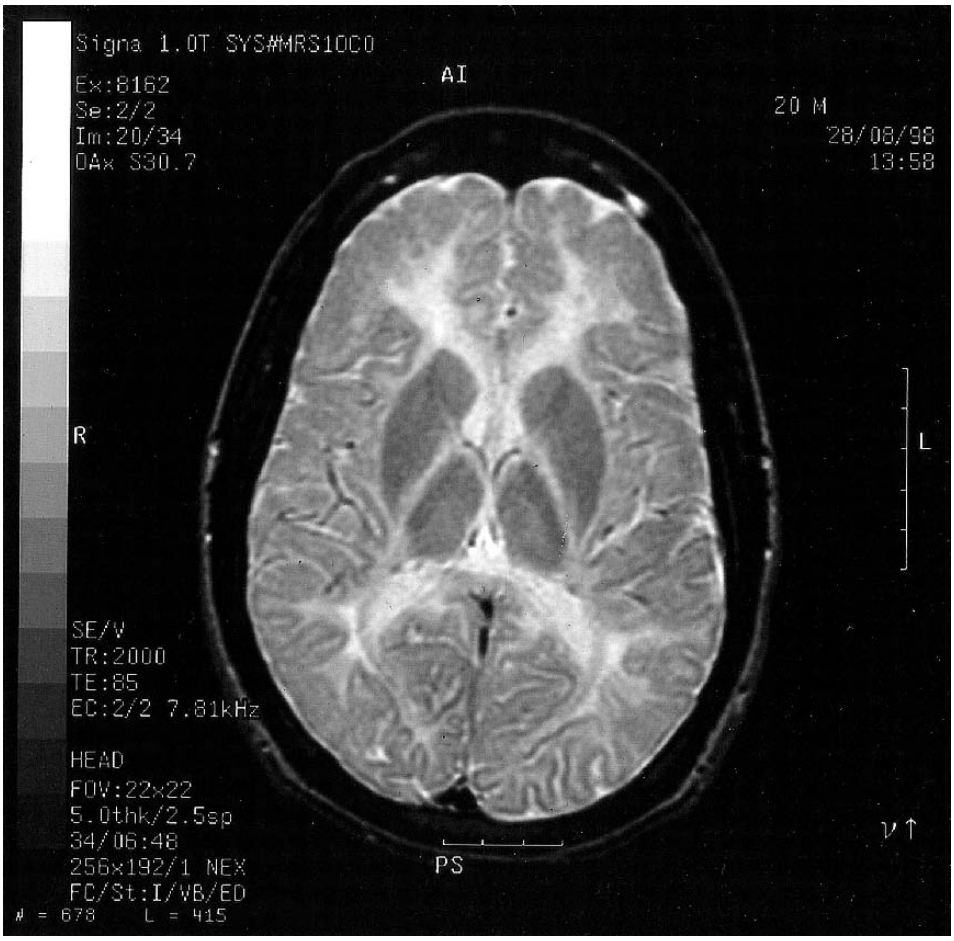

Fig. 1. Magnetic resonance image in PMD. This T2-weighted image was obtained at the age of 20 yr from the second patient in this review (Fig. 3). The abnormally high signal in the white matter and the posterior internal capsule is due to high water content. The relatively low signal intensity in the anterior internal capsule suggests the accumulation of some mature myelin. The mottled appearance of the frontal white matter may represent islands of myelination.

course was generally more benign than that in the allelic PMD, and Johnston and McKusick listed the age at the time of examination as between 4 and 61 yr (17). A summary of 11 patients with SPG-2 has also been published (16). One patient of the previously published family with SPG-2 (17) had a cranial MRI. The T2-weighted images revealed mottled high signal areas throughout the centrum semiovale (34). Hodes et al reported more diffuse dysmyelination on the MRI of another patient with SPG-2 (35).

Neuropathology and Neurochemistry

Figures 2 and 3 illustrate the neuropathological spectrum of PMD. The photographs in Figure 2 are from a previously described case of childhood PMD (7) in which total PLP deficiency was due to a point mutation in exon 4 of the *PLP* gene (8). The patient died at the age of 18 yr and the brain weighed 830 g. A coronal slice (Fig. 2A) showed incomplete demarcation of gray and white matter, ventricular dilatation, and poorly developed internal capsule and corpus callosum. Routine myelin stains of brain and spinal cord revealed no myelin (Fig. 2B) while spinal roots (Fig. 2B, C) and cranial nerves were normally myelinated (Fig. 2D). Immunocytochemistry with antisera to myelin basic protein (MBP), myelin-associated glycoprotein (MAG), and cyclic nucleotide phosphodiesterase (CNP) revealed small clusters of myelin-like profiles (Fig. 2E) that were connected by thin processes to the perikarya of oligodendroglia-like cells (Fig. 2F–H). Axons in the myelin-deficient white matter were abundant (Fig. 2I). Double-label immunofluorescence of MAG and MBP supported the colocalization of these proteins and the finding may be interpreted as an abortive effort at myelination (Fig. 2J, K).

The patient whose tissues are illustrated in Figure 3 had a much more benign course. Signs of CNS dysfunction did not occur until he was in grammar school when a learning disability was manifest. He became ataxic at

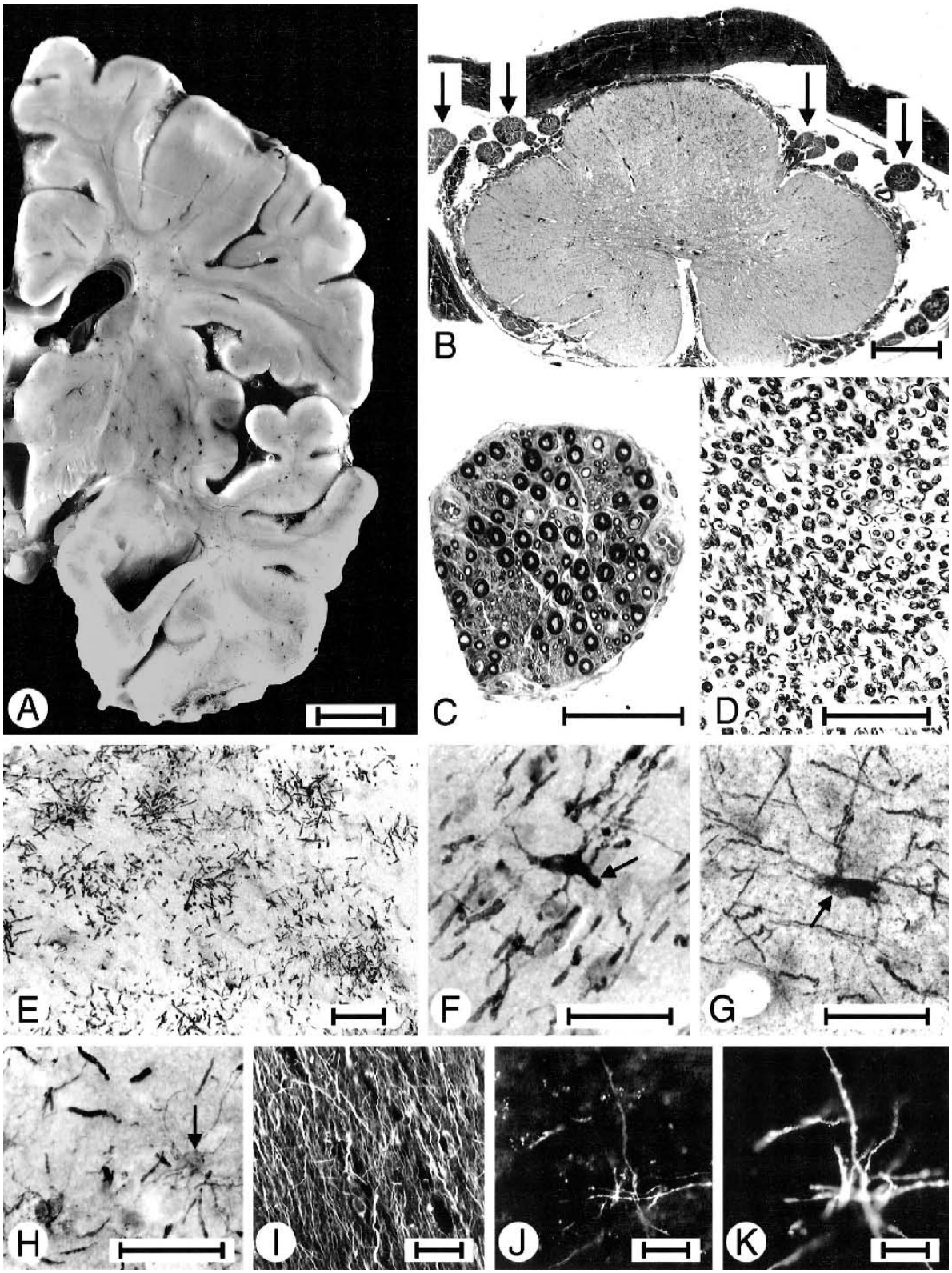

Fig. 2. Pelizaeus-Merzbacher disease in an 18-yr-old man with a point mutation in exon 4 of the *PLP* gene (case 1). A: Gross specimen. The coronal slice reveals poorly developed white matter, thin corpus callosum and internal capsule, and hydrocephalus. Gray and white matter are not sharply demarcated. B: Transverse section of the thoracic spinal cord, Luxol fast blue-periodic acid-Schiff. The technique for myelin shows no reaction product in the parenchyma of the spinal cord whereas the dorsal (arrows) and anterior spinal roots are well stained. C: Dorsal spinal root, epoxy resin, toluidine blue. This transverse section shows a dense population of large and small myelinated fibers. D: Oculomotor nerve, Luxol fast blue-periodic acid-Schiff stain for myelin. The

the age of 12 yr and died at the age of 22 yr after a slowly progressive course that included absence-like seizures. There was no family history of a similar disease, and molecular confirmation was not obtained during life. However, PCR of lymphocyte DNA of his surviving mother and sister revealed *PLP* gene duplications. His brain weighed 1,194 g. Coronal slices (Fig. 3A) showed atrophy of the corpus callosum, asymmetrical lack of myelin in the internal capsules, and a gelatinous retracted white matter of the centrum semiovale. Routine myelin stains detected islands of myelinated fibers in the affected cerebral white matter and almost normal myelination in brainstem and cerebellum. The spinal cord was not available for examination. Immunocytochemistry with anti-PLP yielded vigorous reaction product in myelin islands in the subcortical white matter (Fig. 3B, C). There was only a modest and focal reduction in the staining intensity of myelinated fibers in the brainstem (Fig. 3D). Myelination in the cerebellum appeared normal (Fig. 3E). Similar results were obtained with anti-MBP (Fig. 3F–H). Figure 3I shows reduced axonal density in the affected subcortical white matter. Invariably, the myelin-like profiles in the islands were associated with an axon, as shown by the double-label immunofluorescent images of MBP and non-phosphorylated neurofilament protein (Fig. 3J, K). However, many naked axons were present in the same area (Fig. 3K).

The outstanding difference between these 2 cases was the retention of some PLP in the second patient. In the literature, the term ''tigroid'' was based on the appearance of the white matter on traditional myelin stains that react mostly with the lipids of the sheath. With the advent of immunocytochemistry for myelin proteins, the sharp distinction between tigroid (classic) and non-tigroid (connatal) cases has become less certain.

In recent years, axonal loss in demyelinating disease has attracted attention. In case 1 of this review, there was no obvious axonal loss although all white matter axons were very thin and delicate (7) (Fig. 2I). Also, the white matter showed numerous bipolar neurons that were interpreted as defective neuronal migration during development (7). In case 2 there was frank axonal loss in the white matter of the centrum semiovale.

In the first case, oligodendroglia were totally absent. In the second case, oligodendroglia were present where myelination was normal, such as in the brainstem. They were also present in the islands of myelin in the centrum semiovale.

In PMD, stains for the glial fibrillary acidic protein show an abundance of astrocytes in the myelin-deficient white matter. It is uncertain that disease progression causes superimposed astrogliosis. The dysmyelinated tissue reveals no excess of microglia and macrophages are uncommon.

Definitive ultrastructural observations in the CNS of PMD are not available. The reasons are the lack of an accurate definition of the disease at the time of the examination and the unavoidable autolytic changes in human postmortem tissue. In retrospect, only 1 electron microscopic report of a brain biopsy in PMD is acceptable (36). The 6-yr-old boy came from a family with convincing X-linked recessive transmission, and the biopsy tissue contained no cerebrosides or sulfatides (36). The sections revealed no compact myelin. A few circular loops of a double membrane surrounded some axons and oligodendroglia were fewer than normal. In addition, these cells contained an excess of cytoplasmic dense bodies and a poorly developed endoplasmic reticulum. Condensation of nuclear chromatin strongly resembled apoptosis that has since been reported in animal equivalents of PMD (see below). In a later biopsy case reported from the same laboratory (37), X-linked inheritance was also quite convincing but myelination was much better. In this case, the normal enzymatic activity of CNP in the sample militated against PMD. The patient may represent the tigroid variety of PMD with less serious dysmyelination, and retention of CNP activity in the white matter may only reflect the better state of myelin. The published electron micrographs show regular periodicity of myelin, implying the presence of adequate amounts of PLP to generate a normal intra-period line (38, 39).

Cranial nerves, spinal roots (Fig. 2B, D), and peripheral nerves in PMD are generally normally myelinated, and clinical neuropathy is absent. The most abundant PLP counterpart in the PNS is P_0, although small amounts of PLP and DM-20 are also present in the PNS, as is messenger ribonucleic acid (mRNA) of PLP (40–42). Garbern et al found convincing electrophysiological and morphological evidence of peripheral neuropathy in PMD patients with total absence of PLP due to a deletion of a

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transverse section of this cranial nerve shows a normal density of myelinated fibers. E: Cerebral white matter, immunocytochemistry with anti-MBP. The immunocytochemical stain shows several small islands of reactive myelin-like fibers. F–H: Cerebral white matter, immunocytochemistry with anti-MBP (F), anti-MAG (G), and anti-CNP (H). Centrally located cells (arrows) probably represent oligodendroglia that extend delicate processes toward short myelin sheath-like profiles. I: Cerebral white matter, immunofluorescence with anti-phosphorylated neurofilament protein. The myelin-deficient white matter contains densely packed thin axons. J–K: Cerebral white matter, double-label confocal immunofluorescence with antibodies to MAG (J) and MBP (K). The images show colocalization of reaction product in a cluster of myelin-like sheaths. Scale bars: A = 1 cm; B = 1 mm; C–E = 100 μm; F–H = 50 μm; I–K = 50 μm.

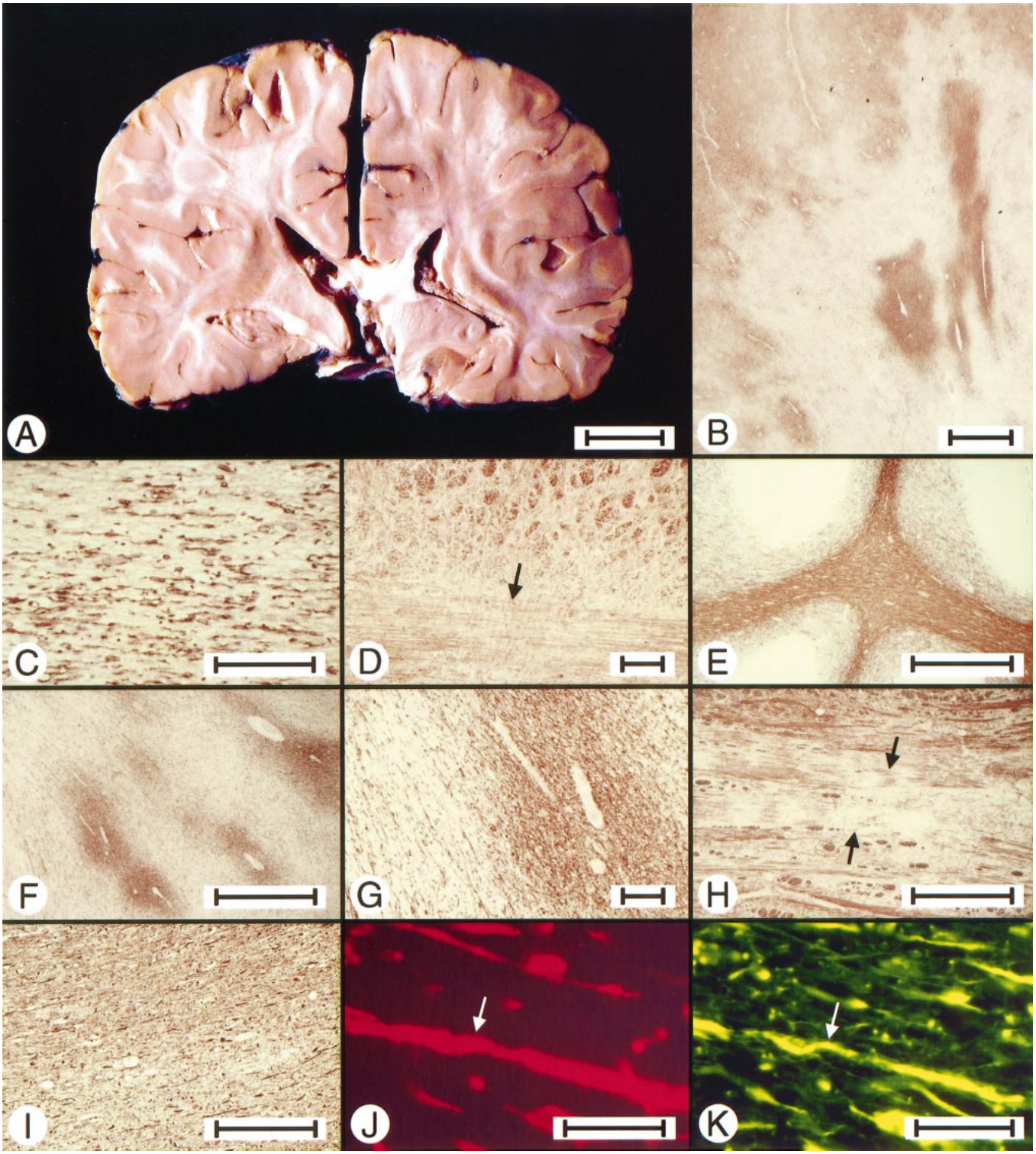

Fig. 3. Pelizaeus-Merzbacher disease in a 22-yr-old man with a *PLP* gene duplication (case 2). A: Gross specimen. The coronal slice shows deficient white matter development with thin internal capsules and corpus callosum. The subcortical white matter appears retracted. B: Cerebral white matter, immunocytochemistry with anti-PLP. A few small islands of myelinated fibers show reaction product. C: Cerebral white matter, immunocytochemistry with anti-PLP. Immunochemically reactive fibers are spaced at low density near a blood vessel. D: Basis pontis, immunocytochemistry with anti-PLP. A small focus of deficient myelination affects a bundle of transverse pontine fibers (arrow). E: Cerebellum, immunocytochemistry with anti-PLP. The white matter of the cerebellar folia appears normal. F: Cerebral white matter, immunocytochemistry with MBP. Islands of immunoreactive myelin sheaths are present. G: Cerebral white matter, immunocytochemistry with anti-MBP. A cluster of myelinated fibers surrounds a blood vessel. H: Pons, immunocytochemistry with anti-MBP. A short segment of a transverse fiber bundle shows

single base in the 5′-untranslated region of the gene (43). Peripheral nerves showed severe loss of myelin, endoneurial fibrosis, axonal loss, and Wallerian degeneration. This study also concluded that PLP is normally present in the compact myelin sheaths rather than in Schwann cell cytoplasm only, as had been observed before (40). The mutation reported by Garbern et al (43) is not identical with a complete *PLP* gene deletion (44), which causes a relatively mild PMD phenotype with some evidence of peripheral neuropathy (43).

Information on the neuropathology of SPG-2 is limited. Bond et al (45) illustrated modest lack of myelin in the centrum semiovale but severe loss in the spinal cord. At the level of the spinal cord, the case strongly resembled PMD of early onset. It was also very different from the tigroid variety in which brainstem and spinal cord are often well myelinated.

Early neurochemical investigations of PMD focused on brain lipids. Seitelberger (4) summarized the neurochemical studies prior to 1970. Later studies recognized the specificity of cerebrosides and sulfatides for the myelin sheath, and 1- and 2-dimensional thin layer chromatograms of lipids showed an utter lack of these galactolipids (7, 10, 46, 47). Reduction or total absence of cerebrosides and sulfatides are non-specific and can be expected in all conditions with deficient myelination. Also, the diagnosis of PMD in earlier cases was not always definite due to the absence of convincing X-linked transmission or adequate morphological characterization. In a familial tigroid case of PMD, brain cholesterol and phospholipids were normal whereas lipid galactose was greatly reduced (48). Although the tissue had been fixed in formaldehyde prior to extraction, the presence of some cerebroside is consistent with the much better myelination in tigroid cases.

New methods and the availability of specific antisera led to the identification of the PLP deficit in PMD (7). Whereas PLP was totally absent from the brain of this patient with early-onset PMD, MBP, MAG, and CNP were also greatly reduced. It was a first indication that the deficit in PLP also caused severe reductions of the other major myelin proteins. A similar process has since been confirmed in several animal models of X-linked PLP deficiency (see below). Interestingly, this lack of other myelin proteins is not invariable. Garbern et al found normal MBP intensity on Western blots of their PMD cases that were caused by a PLP null allele (43). Proteolipid protein was totally absent. As expected from the lack of lipid, the water content of PMD white matter is elevated (7), correlating well with the high white matter signal on T2-weighted MRI scans. No neurochemical analysis of SPG-2 has been reported.

Molecular Genetics

Pelizaeus-Merzbacher disease affects hemizygous males. However, beginning with Merzbacher (2), several authors have reported PMD in girls and women. The relative rate of occurrence exceeds that of Duchenne muscular dystrophy, and non-random inactivation of the X-chromosome may not account for the number of females. It is also unlikely that an X0 karyotype, such as in Turner's syndrome, or twinning are responsible. It is of interest that a single female jimpy (*jp*) mouse, the murine equivalent of PMD (49), contributed to the concept of gene dose and random X-inactivation (50). Hudson (14) summarized the reports of female PMD cases and attempted a correlation with the nature of the mutation. It has become apparent that female patients are more common in families with milder forms of PMD or SPG-2. Inoue et al reported 2 remarkable, unrelated, female patients with *PLP* gene duplications (51). Their severe early PMD phenotype gradually improved over a period of several years. Functional restoration was attributed to remyelination by oligodendrocytes in which the healthy X-chromosome was not inactivated. Animal experiments support the concept that in female heterozygotes with mutated *PLP* genes, a mosaic pattern of normal and deficient myelination occurs (see below).

The mutations in PMD are highly variable and include—in declining order of frequency—duplications, point mutations, insertions, and deletions. Point mutations also affect splice sites and non-coding regions of the gene (52). While duplications are thought to account for over 60% of the mutations, additional heterogeneity is introduced by the variability of the breakpoints (23, 53). While the intrachromosomal duplications occur most commonly in tandem, insertions at a greater distance, including the short arm of the X-chromosome, have also been observed (54).

In a systematic study of 82 patients with sporadic clinically diagnosed PMD, Mimault et al discovered *PLP* mutations in 63 (77%) of which 39 (62%) were duplications (55). The others (24 patients, 38%) were point mutations. This large case material also permitted the conclusion that *PLP* gene duplications most frequently

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modest myelin deficiency (arrows). I: Immunocytochemistry with anti-phosphorylated neurofilament protein. The overall axonal density is reduced. This image should be compared with Figure 2I. J–K: Double-label immunofluorescence with anti-MBP (J) and anti-phosphorylated neurofilament antibody (K). All MBP-reactive fibers show colocalization with axons (arrows). However, several thin axons do not have matching myelin sheaths. Scale bars: A = 1 cm; B = 1 mm; C, D = 100 μm; E, F = 1 mm; G–I = 100 μm; J, K = 25 μm.

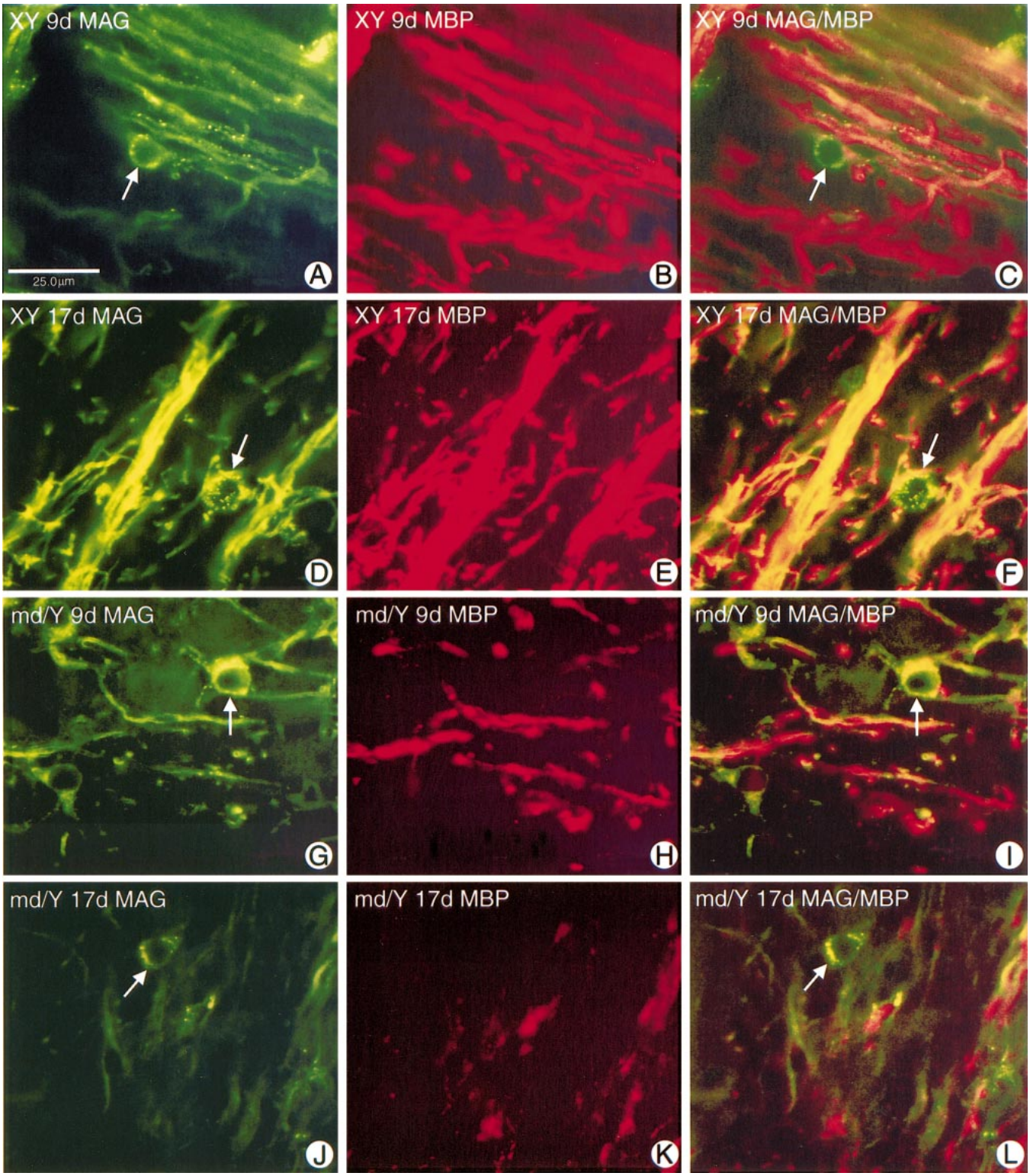

Fig. 4. Differential distribution of MAG and MBP in the developing normal rat and *md* CNS. Double-label confocal immunofluorescent images were obtained from normal male (XY, panels A–F) and *md* littermates (md/Y, panels G–L) with antisera to MAG and MBP. MAG immunofluorescence in the left panel was revealed by fluorescein isothiocyanate, MBP in the center panel by Quantum Red. The right panel represents overlaid images. At 9 days, vigorous MAG immunoreactivity exists in the oligodendroglia of the normal and *md* littermates (arrows). However, at this age, myelin sheaths are already fewer and thinner in the *md* littermate (G–I). The differential distribution of MAG and MBP is normal in unaffected and *md* animals: Perikarya of oligodendroglia show no MBP immunofluorescence whereas MAG appears in the perikarya (arrows), delicate processes, and myelin sheaths. Reaction product for MAG in perikarya appears ''packaged.'' At 17 days, myelin sheaths in the normal male are

arose through meiosis in the germ cells of the grandfather rather than the grandmother. In contrast, point mutations arose de novo in the germ cells of the patients' mothers, their grandfathers, or their grandmothers. The male bias in *PLP* gene duplication was attributed to unequal sister chromatid exchange. Many investigators have observed that the nature of the mutation relates to the severity of the clinical phenotype. The most benign course of PMD may be attributed to *PLP* gene deletion or a null mutation, whereas most missense mutations convey a serious disease with neonatal or intrauterine onset. Based on extensive case material and the available literature, Inoue et al (53), Cailloux et al (56), and Woodward and Malcolm (57) have offered diagrams of the clinical spectrum in correlation with the nature of the mutation. Hodes et al collected clinical and imaging data on several patients with non-identical mutations at the same codon of the *PLP* gene (58). The clinical phenotype varied with the nature of the exchanged amino acid, adding to the enormous heterogeneity of *PLP* mutations. Despite the rapid progress in molecular techniques, mutations in the *PLP* gene have not been identified in approximately 23% of patients with otherwise typical PMD (55).

Proteolipid Protein Deficiency in Spontaneous Animal Mutants and Transgenic Mice

Since the description of the *jp* mouse in 1954 (49), several other animal mutants with X-linked PLP deficiency have been discovered. They include the myelin synthesis-deficient *jp* mouse (*jp*^{msd}), jp-4J, myelin-deficient (*md*) rat, the shaking (*sh*) pup, the rabbit with paralytic tremor (*pt*), and the *rsh* mouse. The extraordinary conservation of PLP through evolution has made all of these mutants excellent models for the study of PMD even though natural *PLP* gene duplications in animals have not been reported. Griffiths et al (59) and Campagnoni and Skoff (60) have summarized recent investigations of the molecular biology of PLP and point out that PLP (59) and MBP (60) may have functions beyond assembly of myelin. Comparative immunocytochemistry with antisera to PLP, MBP, MAG, and CNP showed very similar clusters of abortive myelin sheaths in PMD, the *jp* mouse, and the *md* rat (61). In the animal tissues, transferrin immunocytochemistry was used to reveal the paucity of oligodendroglia (61). Human autopsy tissue is generally not suitable to reveal oligodendroglial cytoplasm. The *rsh* mouse (62) is of special interest because the single point mutation of isoleucine→threonine at position 186 of the PLP molecule causes a minor PLP deficit in the affected mice and is compatible with a normal lifespan (63). The benign nature of this mutation is apparent also in humans with SPG-2 in whom an identical amino acid substitution has occurred (18).

Extensive light and ultrastructural studies in the animal mutants point to premature death of oligodendroglia in the more severe forms of PLP deficiency. Ultimately, numerical deficiency in oligodendroglia matches the lack of myelinated fibers. In comparison with wild-type animals, the PLP-mutated animals show many more apoptotic oligodendroglia when DNA fragments in nuclei are end-labeled with biotinylated deoxyuridinediphosphate (64, 65). At the ultrastructural level, condensed chromatin accumulates beneath the nuclear membrane (65). The animal mutants also allowed confirmation of mosaicism of myelination in female heterozygous carriers in *jp* mice, *sh* pups, and *md* rats (66–68).

Certain transgenic models are relevant to *PLP* gene duplication, deletion, or null alleles in human PMD. When transgenes were introduced into murine autosomes (69, 70), homozygous and hemizygous animals were generated. Their myelin deficiency correlated well with the *PLP* gene dose. Homozygotes were severely affected and died within 2 months from convulsions (69), whereas hemizygotes with much lower *PLP* gene expression did not become ill until 10 to 18 months of age. In addition to demyelination, axonal degeneration and frank Wallerian degeneration occurred (70). Selective large overexpression of DM20 also causes myelin deficiency (71). Knockout mice with total deletion of the *PLP* gene have a normal life span and generate compact albeit ultrastructurally abnormal CNS myelin (72). Similar to the benign human equivalent of *PLP* gene deletion (44) or null allele (43), the animals develop widespread axonal lesions of the brain. The PLP knockout model provides strong support that the pathogenesis of PMD is related more specifically to abnormal PLP than to its total absence.

Pathogenesis

In the pathogenesis of PMD, the biosynthesis of all CNS myelin proteins, the topology of PLP, and protein ''trafficking'' must be considered. There is general agreement that PLP and DM20 are strongly hydrophobic transmembrane proteins with amino and carboxy terminals located in the cytosol. They are synthesized in the

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more abundant and give strong immunoreactivity with antisera to MAG and MBP (D–F). In contrast, the *md* littermate now shows fewer oligodendroglia, with sparse MAG immunofluorescence (arrows) and only a few, thin, and disorganized myelin sheaths. Scale bar in part A = 25 μm and applies to all parts.

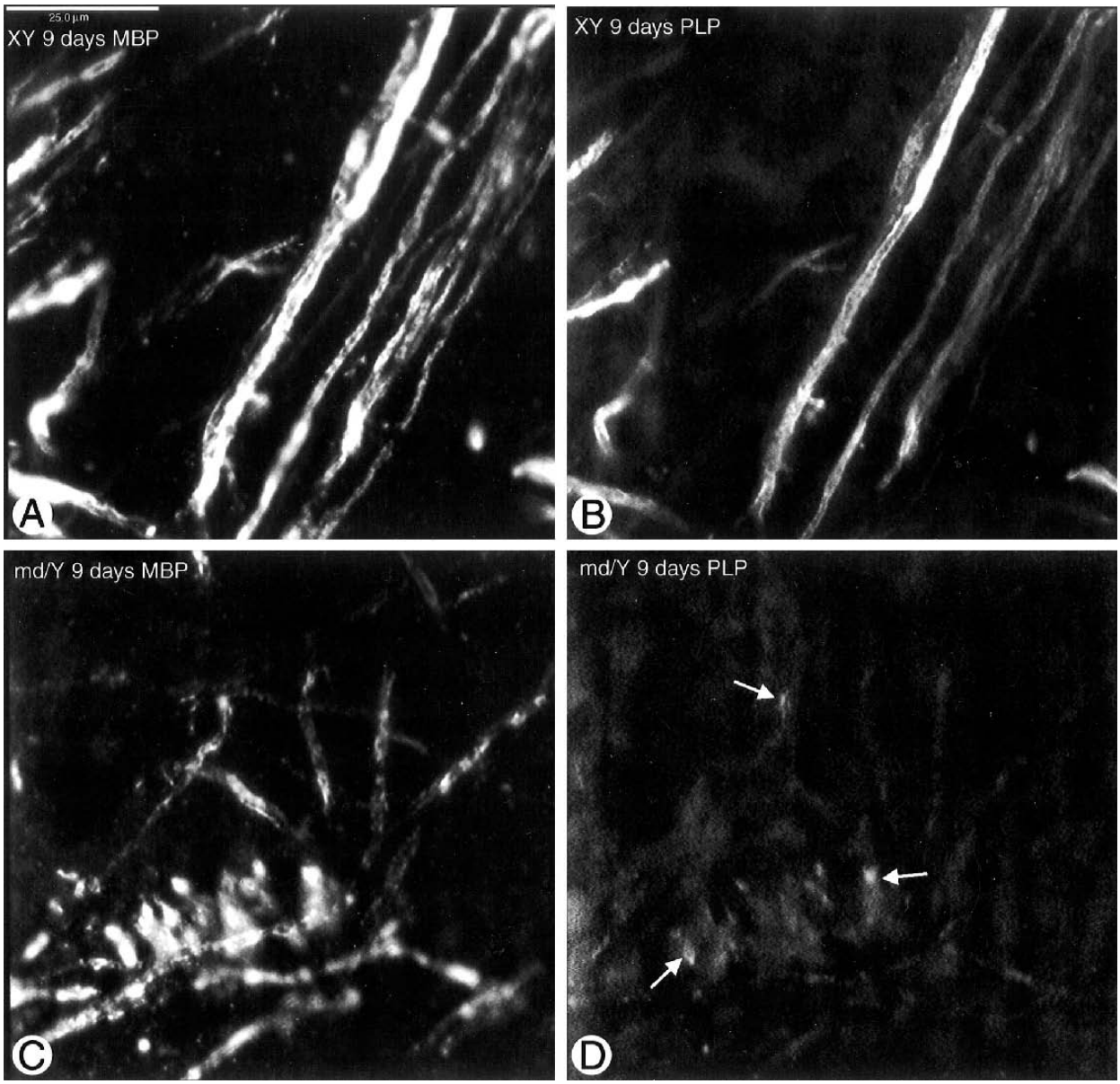

Fig. 5. Myelination in normal and *md* littermates. Double-label confocal immunofluorescent images were obtained from 9-day-old normal (A, B) and *md* littermates (C, D) with antisera to MBP (A, C) and PLP (B, D). In the normal male littermate, myelin sheaths react strongly with antisera to MBP (A, fluorescein isothiocyanate) and PLP (B, Quantum Red). Myelin sheaths in the *md* littermate contain MBP (C, fluorescein isothiocyanate) but are essentially devoid of PLP (D, Quantum Red). Granular reaction product in D (arrows) may represent traces of PLP. Scale bar in A = 25 µm and applies to all parts.

endoplasmic reticulum and then transferred to the oligodendroglial plasma membrane. The 4 membrane spanning domains are commonly termed A, B, C, and D while extracellular loops are designated A–B and C–D. The single cytoplasmic loop under this concept is named B–C and it contains the PLP-specific segment. Several authors have published cartoons of this arrangement with recent examples in refs. (59) and (60). Point mutations occur mainly in the membrane domains and the extracellular loops. While normal myelin requires all protein and lipid components, the biosynthesis of PLP, MBP, MAG, and CNP is not synchronous. Proteolipid protein is a relative latecomer in myelination (10). In cultured oligodendrocytes from rat brain at defined neonatal ages, PLP also arrives after MBP and MAG (73), but all proteins are first synthesized in the perikaryon and then transported into delicate cell processes and abortive myelin sheaths. This straightforward mechanism in cultured cells does not necessarily apply to the actively myelinating animal. After the discovery of MBP mRNA in isolated myelin (74), an entirely new concept of myelin sheath assembly developed: MBP is made at the site of its incorporation,

whereas PLP, MAG, and CNP are translated in the oligodendroglial perikaryon and exported through cellular processes to ''join'' MBP (75, 76). Myelin basic protein is nevertheless detectable in the perikaryon by immunocytochemical methods applied to sections of immature brain (77, 78). However, the persistence of immunoreactivity of MBP, MAG, and CNP in the perikarya of oligodendroglia-like cells in PMD of a young adult human (Fig. 2F–H) is distinctly abnormal. The differential localization of myelin proteins in the developing brain and in PLP mutant animals is readily apparent when double-label immunofluorescence is used. Figure 4 illustrates the localization of MAG and MBP in the neonatal *md* rat and a normal male littermate. It is evident that MAG is detectable in the perikaryon and in early myelin sheaths. The punctate reaction product for MAG suggests that the protein is ''packaged'' before being exported to the myelin sheath (Fig. 4A, D, G, J). In contrast, MBP reaction product is present only in the myelin sheath. At 9 days, myelinating cells in both littermates are not different, though myelin sheaths in the mutant appear less robust. At 17 days when the *md* males show serious CNS dysfunction, MAG reaction product in the myelinating cells is less distinct, and the myelin sheaths are thinner, shorter, and disorganized. The normal littermate continues the formation of robust myelin sheaths that exhibit intense MAG and MBP reaction product. Figure 5 shows double-label immunofluorescence of MBP and PLP at the age of 9 days. Only traces of PLP reaction product are present in the *md* littermate, whereas MBP is still abundant (Fig. 5C).

After a series of investigations with cultured cells that were transfected with wild-type and mutant PLP-complementary DNA, Gow et al developed an attractive hypothesis on the pathogenesis of PMD (79–81). Their experiments support the concept that missense mutations cause conformational changes, ''misfoldings,'' that prevent proper processing of PLP and DM20 after biosynthesis in the rough endoplasmic reticulum and delivery to the plasma membrane. The accumulation of the mutated protein sets into motion an apoptotic cascade with typical nuclear morphology and the appearance of DNA fragments on electropherograms (80). In some mutants, such as the *rsh* mouse and the matching isoleucine→threonine mutation in human SPG-2 at position 186, DM20, but not PLP, may be processed correctly. The resulting clinical and morphological phenotypes are more benign (79). The hypothesis is less compelling for the majority of PMD patients who have *PLP* gene duplications. The excessive biosynthesis of PLP and DM20 in the rough endoplasmic reticulum may have similar deleterious effects on oligodendroglia, and therapy may be directed at reducing the biosynthesis of these proteins (80) in an effort to achieve the benign phenotype of an absent or silent *PLP* gene (43, 44).

Conclusions

Pelizaeus-Merzbacher is a relatively rare disease accounting for only 6.5% of all leukodystrophies (82). As analytical techniques have improved and are more readily available, PMD may be confirmed in a greater number of patients with dysmyelination. Despite the rarity of PMD, the PLP mutations in humans and animals, and PLP deficiencies in transgenic mice, have generated enormous interest. The reason is their importance for our understanding of myelination and myelin repair. In the pathogenesis of PMD, oligodendroglial death by apoptosis is thought to be the cause for the companion deficiency of MBP, MAG, and CNP. However, the most intriguing observation is that the formation of compact myelin can proceed while PLP and DM20 are absent.

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