Rothstein JD (2009) Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. Ann Neurol 65(Suppl 1):S3–S9

Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16:675–686

Rovelet-Lecrux A, Hannequin D, Raux G, Le Meur N, Laquerriere A, Vital A, Dumanchin C, Feuillette S, Brice A, Vercelletto M, Dubas F, Frebourg T, Campion D (2006) APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. Nat Genet 38:24–26

Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, Kawaguchi T, Tsunoda T, Watanabe M, Takeda A, Tomiyama H, Nakashima K, Hasegawa K, Obata F, Yoshikawa T, Kawakami H, Sakoda S, Yamamoto M, Hattori N, Murata M, Nakamura Y, Toda T (2009) Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet 41:1303–1307

Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med 2:864–870

Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin JF, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HA, Haines JL, Perkicak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St George-Hyslop PH (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 375:754–760

Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG, Kruger R, Federoff M, Klein C, Goate A, Perlmutter J, Bonin M, Nalls MA, Illig T, Gieger C, Houlden H, Steffens M, Okun MS, Racette BA, Cookson MR, Foote KD, Fernandez HH, Traynor BJ, Schreiber S, Arepalli S, Zonozi R, Gwinn K, Van Der Brug M, Lopez G, Chanock SJ, Schatzkin A, Park Y, Hollenbeck A, Gao J, Huang X, Wood NW, Lorenz D, Deuschl G, Chen H, Riess O, Hardy JA, Singleton AB, Gasser T (2009) Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet 41:1308–1312

Simpson JE, Ince PG, Lace G, Forster G, Shaw PJ, Matthews F, Savva G, Brayne C, Wharton SB, Function, M. R. C. C. & Ageing Neuropathology Study, G (2010) Astrocyte phenotype in relation to Alzheimer-type pathology in the ageing brain. Neurobiol Aging 31:578–590

Snowden JS, Hu Q, Rollinson S, Halliwell N, Robinson A, Davidson YS, Momeni P, Baborie A, Griffiths TD, Jaros E, Perry RH, Richardson A, Pickering-Brown SM, Neary D, Mann DM (2011) The most common type of FTLD-FUS (aFTLD-U) is associated with a distinct clinical form of frontotemporal dementia but is not related to mutations in the FUS gene. Acta Neuropathol 122:99–110

Sreedharan J, Brown RH Jr (2013) Amyotrophic lateral sclerosis: problems and prospects. Ann Neurol 74:309–316

Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance C, Rogelj B, Ackerley S, Durnall JC, Williams KL, Buratti E, Baralle F, De Belleroche J, Mitchell JD, Leigh PN, Al-Chalabi A, Miller CC, Nicholson G, Shaw CE (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319:1668–1672

Steinberg SJ, Moser AB, Raymond GV (1993) X-linked adrenoleukodystrophy. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K (eds) GeneReviews(R). University of Washington, Seattle

Timmerman V, Strickland AV, Zuchner S (2014) Genetics of charcot-marie-tooth (CMT) disease within the frame of the human genome project success. Genes (Basel) 5:13–32

kinoue@ncnp.go.jp

Vance C, Rogelj B, Hortobagyi T, De Vos KJ, Nishimura AL, Sreedharan J, Hu X, Smith B, Ruddy D, Wright P, Ganesalingam J, Williams KL, Tripathi V, Al-Saraj S, Al-Chalabi A, Leigh PN, Blair IP, Nicholson G, De Belleroche J, Gallo JM, Miller CC, Shaw CE (2009) Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science 323:1208–1211

Watts GD, Wymer J, Kovach MJ, Mehta SG, Mumm S, Darvish D, Pestronk A, Whyte MP, Kimonis VE (2004) Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet 36:377–381

Zoghbi HY, Orr HT (2000) Glutamine repeats and neurodegeneration. Annu Rev Neurosci 23:217–247

kinoue@ncnp.go.jp

Column 2

小児神経疾患におけるMR spectroscopy（MRS）の臨床応用

Proton MR spectroscopy（MRS）は，臨床用MR装置（1.5ないし3テスラ）で簡便に検査可能となりつつある．小児は頭蓋骨脂肪が少なく，水分に富み，容積も小さいことから成人に比して磁場が均一であり，シャープなスペクトルを得ることができる．MRI所見が非特異的であってもMRSを加えることにより疾患の診断，病態に迫ることが時に可能である．例えば，本誌に掲載されているCreatine transporter欠損症ではMRSでCreatine（3.0 ppm）を欠くことが診断の決め手となる．また，神経疾患の病態ごとにMRSのパターンに特徴が認められる．

1．MRSで観察されるスペクトルとその意義（図1）

1）N-acetylaspartate（NAA）［2.02 ppm］

中枢神経系，特に神経細胞内に局在しており，神経細胞・軸索機能の指標とされる．神経疾患では非特異的に低下する．

2）Creatine（Cr）［3.03 ppm］

Crはエネルギー代謝の指標とされる．神経膠細胞で比較的高濃度であり，グリオーシスでは高値を呈する．種々の病的状態において比較的安定しており，NAA，Choの相対評価の指標（分母）として使用されてきたが，今後は定量的評価が望まれる．

3）Choline（Cho）［3.22 ppm］

細胞膜，特に髄鞘代謝の指標と考えられている．そのため髄鞘が活発に作られる新生児期，髄鞘代謝の亢進する脳腫瘍，脱髄疾患では高値を呈する．

以上はT2値が長いため，long TE法でも観察される主要な3スペクトルである．

4）Lactate（Lac）［1.33 ppm］

Lacの検出は，種々の病態における嫌気性解糖の亢進を意味する．またマクロファージは嫌気性解糖を行うため，マクロファージの増加する病態（demyelination，necrosis）でも検出しうる．Lacでは，スピンースピン結合のためTEの値によってスペクトルの向きが逆転する．TE＝136 msでは下向きに，TE＝272 msでは上向きのスペクトルとして観察される．Lacの同定にこの特性を用いることがある．

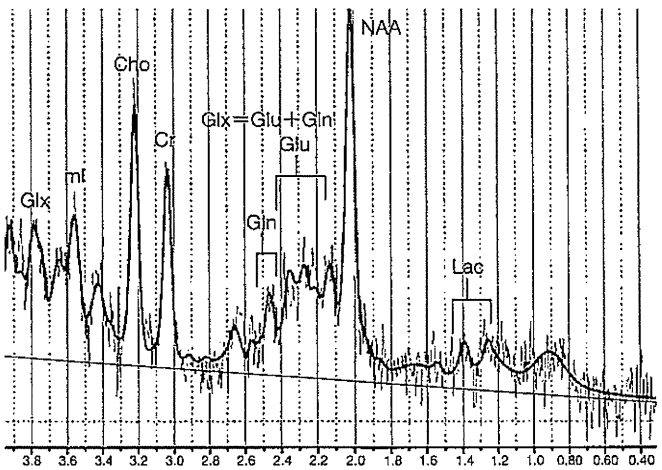

図1　1歳の正常MRS
PRESS，TR＝5000，TE＝30，NEX＝62，VOI＝15×15×15，頭頂部白質．
N-acetylaspartate（NAA：2.02 ppm），Creatine（Cr：3.03 ppm），Choline（Cho：3.22 ppm），myo-inositole（mIns：3.56 ppm），Glutamate（Glu）/glutamine（Gln）complex（Glx，2.1～2.5 ppm）を認める．1歳では成人に比べてChoは高値である．

5) myo-inositole (mIns) [3.56 ppm]

mIns は神経膠細胞にのみ存在するため，神経膠細胞の指標とされグリオーシスでは高値を呈する．細胞浸透圧調整物質の機能も有する．

6) Glutamate (Glu)/glutamine (Gln) [2.1～2.5 ppm]

Glu/Gln は 2.1～2.5 ppm に多数のピークを有し，1.5 テスラ MR 装置ではこれらの区別は困難であり，Glu と Gln をあわせて Glx と表現されることもある．Glu は興奮性神経伝達物質であり，シナプス前神経細胞からシナプス間隙に放出され，星状神経膠細胞に取り込まれ Gln に代謝 (Glu+NH3→Gln) される．Glu は神経細胞の指標，Gln は神経膠細胞の指標ともされる．

上記以外に測定可能な物質として，taurine，GABA，aspartate，glycine，glutathione，phenylalanine などがあげられる．

2. 疾患特異的な MRS 所見

MRS 所見が診断に直結しうる疾患につき記載する．

1) NAA [2.0 ppm] の著明高値

Canavan 病は NAA を分解する aspartoacylase 活性低下により NAA が蓄積し高値を呈する．Cr，Cho は白質菲薄化を反映して低値であり，mIns は高値とされる．大頭症を呈し，MRI では皮質下白質から広範な T2 高信号を呈する．

2) Cr [3.0 ppm] の欠失 (図 2)

Cr deficiency syndrome には，現在 3 種類の病型が知られている．

Creatine transporter (CRTR) 欠損症，Guanidinoacetate methyltransferase (GAMT) 欠損症，Arginine：glycine amidinotransferase (AGAT) 欠損症であり，いずれもけいれん，発達障害を呈しうる．後二者に対して，Cr の補充療法により臨床的改善，MRS での Cr スペクトル出現が観察される．**図 2** は GAMT 欠損症患児の MRS であり Cr ピークを欠いている．

3) Cho [3.2 ppm] の欠失

Cho は高アンモニア血症 (尿素サイクル異常症) で低下し，時に欠失する．Glx (glu+gln) の高値，mIns，Cho の低値を伴う．肝臓移植後の検査でスペクトルは正常化する．

以下に，MRS で通常認められないスペクトルが存在し，診断の決め手となりうる疾患について列挙する．

4) Sjögren-Larsson's syndrome (SLS) [0.9，1.3 ppm]

SLS は fatty aldehyde dehydrogenase 欠損による脂質代謝異常症で，先天性魚鱗癬，精神発達遅滞，痙性両麻痺を呈する．MRS では，0.9，1.3 ppm に 2 つの異常スペクトル (long chain fatty alcohols) を認める．

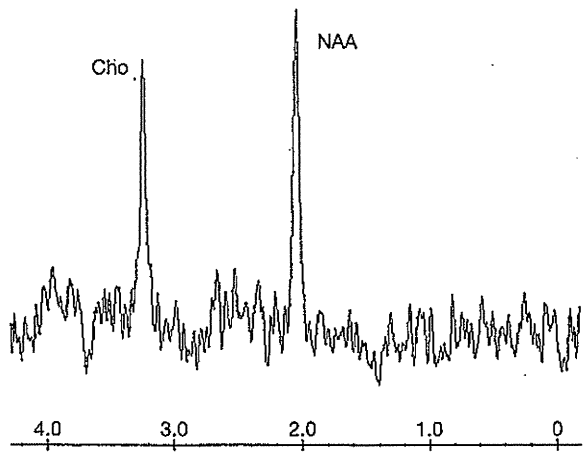

図 2 GAMT 欠損症の MRS，1 歳男児
MRS (PRESS，2000/144) では Cr (3.0 ppm) のスペクトルを認めない．
(UCSF, A. James Barkovich 先生のご厚意により掲載)

5）Succunate dehydrogenase（SDH）欠損症［2.4 ppm］

SHD 欠損症はミトコンドリア脳症（complex II 欠損症）の原因の一つである．白質の MRS で，乳酸のスペクトルに加え，2.4 ppm に succinate（コハク酸）のスペクトルを認める

6）メープルシロップ尿症［Maple syrup urine disease（MSUD）］［0.9～1.0 ppm］

メープルシロップ尿症は分岐鎖アミノ酸（バリン，ロイシン，イソロイシン）の代謝異常症（branched-chain α-keto acid dehydrogenase complex，E1，E2，E3 いずれかの欠損）で，重篤な脳症を呈しうる．MRS では，0.9～1.0 ppm に branched chain amino acid，keto acid を反映したピークを認める．治療により MRS 所見は正常化する．

7）ガラクトース血症（Galactocemia）［3.67，3.74 ppm］

ガラクトース血症はガラクトース代謝に関わる 3 つの酵素異常（ガラクトキナーゼ欠損など）で生じる．MRS では，Galactitol の蓄積を反映して 3.67，3.74 ppm にスペクトルを認める．治療により異常スペクトルは消失する．

8）非ケトン性高グリシン血症（Nonketotic hyperglycinemia）［3.55 ppm］

非ケトン性高グリシン血症はグリシン開裂酵素の異常で，新生児期に昏睡，てんかんを呈しうる．MRS では，グリシンの蓄積を反映して 3.55 ppm にスペクトルを認める．Short TE では mIns と重なるが，グリシンは T2 値が長いため long TE でも残存する．

3．病態に則した MRS 所見（表 1）

1）大脳白質形成不全（hypomyelination）

髄鞘構成タンパクの異常により髄鞘が形成されない疾患群である．多くの神経疾患と異なり白質の NAA は高値を示す．NAA は oligodendrocytes で aspartoacylase により分解される．大脳白質形成不全では oligodendrocytes の障害により NAA が代謝されず高値を示すと考えられる．グリオーシスを反映して mIns，Cr，Gln は高値を示し，Cho は正常ないし低下（ミエリン合成・代謝の低下）する．**図 3** は Pelizaeus-Merzbacher 病の MRS であり，NAA は高値を呈する．

2）脱髄（demyelination）

MRS では，活発な髄鞘破壊，代謝の亢進を反映して Cho 高値を呈する．加えて，二次的な神経細胞・軸索変性を反映して NAA，Glu 低値，グリオーシスを反映して mIns，Gln，Cr の高値を認める．髄鞘崩壊で生じる lipid & protein を反映して 0.9～1.3 ppm の広いピークや，Macrophage の増加を反映して Lac を認めることもある．

3）神経細胞変性（neuronal degeneration）

神経細胞変性では一次的に神経細胞が変性，消失し，それに伴い軸索，髄鞘が二次的変性をきたす．

表 1　病態ごとの MRS 所見

	NAA	Cr	Cho	mIns	Lac	
大脳白質形成不全 (hypomyelination)	↑/→	↑	→/↓	↑		
脱髄 (demyelination)	↓	↑/↓	↑	↑	検出しうる	lipid & protein を反映して 0.9～1.3 ppm の広いピーク
神経細胞変性 (neuronal degeneration)	↓	↓	→	↑	検出しうる	
白質菲薄化 (white matter rarefraction)	↓	↓	↓	↓	検出しうる	glucose 検出しうる
高アンモニア血症 (hyperammonemia)	→	→	↓	↓		Glutamine+Glutamate complex 高値
ミトコンドリア障害 (mitochondrial disorders)	↓	↓	→?	↓?	↑	Alanine 検出しうる

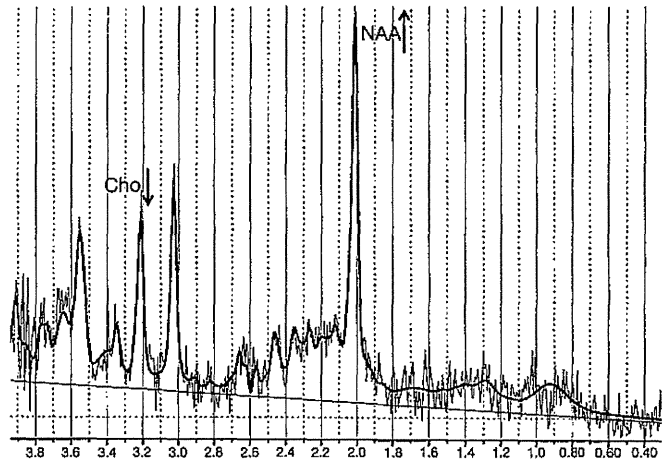

図 3　Pelizaeus-Merzbacher 病の MRS，11 歳男児

MRS（PRESS 5,000/30，白質）では NAA の高値，Cho の低値を認める．

したがって NAA の低下は皮質でより著明である．MRI で萎縮が明らかとなる前から，NAA の低下は認められる．

4）白質菲薄化（white matter rarefraction）
5）高アンモニア血症（hyperammonemia）
6）ミトコンドリア障害（mitochondrial disorders）

4）～6）の MRS パターンは表 1 を参考にされたい．

〔高梨潤一〕

1. Introduction
2. Epidemiology and genetics of PMD
3. *PLP1* gene structure and function
4. The clinical background of PMD
5. Animal models of PMD
6. Pathophysiology of PMD
7. Induced pluripotent stem cells may serve as a novel model system
8. Approaches and opportunities for the treatment of PMD
9. Cell-based therapy
10. Conclusion
11. Expert opinion

Pathophysiology and emerging therapeutic strategies in Pelizaeus–Merzbacher disease

Hitoshi Osaka[†] & Ken Inoue
[†]*Department of Pediatrics, Jichi Medical School, Shimotsuke-shi, Japan*

***Introduction*:** Pelizaeus–Merzbacher disease (PMD) is an X-linked recessive disorder caused by mutations in the proteolipid protein 1 (*PLP1*) gene, which encodes PLP1 and DM20. PMD is characterized by a defect in myelin formation associated with *PLP1* gene mutations (i.e., exonic and intronic mutations, duplication, or deletion of the entire gene). A combination of magnetic resonance imaging (MRI) and genetic testing is essential to diagnose PMD. The disease phenotype manifests due to the loss of PLP/DM20 function or the toxicity of mutant PLP/DM20 or overexpressed PLP/DM20.

***Areas covered*:** Potential approaches at the RNA level include restoring correct splicing with oligonucleotides and decreasing PLP1 expression using a transcriptional activator antagonist. Two approaches used in mouse models that have clinical potential include cholesterol supplementation and use of compounds that decrease the excessive unfolded protein response (UPR) in the endoplasmic reticulum (ER). Two types of cell-based therapy, bone marrow transplantation and stem cell engraftment, were recently employed safely in humans.

***Expert opinion*:** Elucidation of PMD pathophysiology has enabled several recent therapeutic approaches. Administration of cholesterol or curcumin in mouse models of PMD reportedly extends survival time. Stem cell therapies await evaluation of long-term effectiveness and safety in patients with PMD.

Keywords: endoplasmic reticulum (ER), hypomyelinating leukodystrophy (HLD), induced pluripotent stem cells (iPSCs), oligodendrocyte precursor cell (OPC), Pelizaeus–Merzbacher disease (PMD), umbilical cord blood transplantation (UCBT), unfolded protein response (UPR)

Expert Opinion on Orphan Drugs (2015) 3(12):1447-1458

1. Introduction

In 1885, Friedrich Pelizaeus, a German physician, issued a clinical report on a family in which multiple males manifested nystagmus and spastic quadriplegia; Ludwig Merzbacher subsequently published a pathological report on myelin deficiency in 1910. In 1957, Seitelberger established this clinical–pathological entity as Pelizaeus–Merzbacher disease (PMD).[1–3] The combination of the discovery of the proteolipid protein 1 (*PLP1*) gene as a causative factor for PMD and emerging magnetic resonance imaging (MRI) enabled the diagnosis of PMD without pathological evidence.[4,5] Initially, it was unclear why the majority of patients with suspected PMD lacked PLP1 coding mutations; genomic duplication of the entire *PLP1* gene was subsequently identified as the major cause of PMD[6,7]. In the past two decades, a growing number of clinical entities related to PMD have been recognized.[8–10] These conditions are collectively called hypomyelinating leukodystrophies (HLDs), and share common features of defect in myelin formation in the central nervous system

10.1517/21678707.2015.1106315 © 2015 Taylor & Francis e-ISSN 2167-8707

Article highlights.

- Pelizaeus–Merzbacher disease (PMD): X-linked recessive disorder caused by mutations in the proteolipid protein 1 (*PLP1*) gene.
- *Approaches for the treatment of PMD caused by PLP1 missense mutations*:
  1) Splicing restoration by exon skipping: use of morpholino antisense oligonucleotides.
  2) Altering signal transduction: screening for drugs that improve oligodendrocyte precursor cell differentiation, which is inhibited by PLP1.
  3) Attenuation of endoplasmic reticulum (ER) stress: curcumin and chloroquine to lessen ER stress.
- *Approaches for PLP1 duplication or overexpression*:
  1) Cholesterol diet: restore its deficiency produced by overexpression.
  2) Lowering PLP1 expression: the nuclear progesterone receptor antagonist lonaprisan for transgenic mice; miR-20a administration to decrease endogenous PLP1 expression due to binding to its putative binding site in the PLP 3′UTR.
- *Approaches for both PLP1 missense mutations and duplication*:
  Cell-based therapy, bone marrow transplantation and oligodendrocyte progenitor cell transplantation.

This box summarizes key points contained in the article.

(CNS).[11,12] HLDs differ from demyelinating leukodystrophies, in which myelin is initially formed and then is progressively destroyed. Therefore, clinical regression is commonly observed in demyelinating leukodystrophies (e.g., Alexander disease and adrenoleukodystrophy).

2. Epidemiology and genetics of PMD

The prevalence of PMD in the population is estimated at 1:200,000 to 1:500,000 in the US.[13] The incidence of PMD is ~0.13/100,000 live births in Germany.[14] In Japan, PMD is estimated to occur in approximately 0.26/100,000 live male births .[15]

3. *PLP1* gene structure and function

3.1 Gene structure

Human *PLP1* contains seven exons that encode the 276-amino acid PLP1 and its alternative splicing product, DM20 (Figure 1A).[16,17] DM20 lacks the 35 amino acids derived from the latter half of *PLP1* exon 3 (exon 3B). The PLP1 coding sequence is highly conserved among species. PLP1/DM20 is the most abundant myelin protein, and the amino acid sequence of PLP is conserved among human, mouse, rat and pig.[18]

3.2 Gene expression

Expression of PLP and DM20 is spatially and temporally regulated.[19–21] Oligodendrocytes express PLP during the postnatal period and into adulthood; DM20 is preferentially expressed during the embryonic stage in many cell types. In postnatal oligodendrocytes, the ratio of PLP1 message to DM20 is ~3:1. Preferential PLP1 splicing in adult oligodendrocytes is mediated by highly conserved splicing enhancers present in intron 3.[22] For high PLP1 expression, ~200 bp upstream of the transcription start site is sufficient; however, transcriptional regulation is not fully under the control of the 5′ promoter region and intron 1 likely contains regulatory elements.[19, 63, 64] PLP1 mRNA is relatively stable and its estimated half-life in primary mixed glial culture is more than 24 h.[23,24] The developmental expression pattern of PLP1 is mainly regulated at the transcriptional level. Transcriptional factors bind to the 5′ promoter region and modulate PLP1 mRNA expression. These factors include the zinc finger protein Ying Yang 1 (YY1),[25] thyroid hormone receptor-β (THR-β), peroxisome proliferator-activated receptor (PPAR),[26] myelin transcriptional factor1 (MyT1), homeobox protein Nkx-2.2 (Nkx2.2) and sex determining region Y-box 10 (SOX10). The 3′ untranslated region (3′UTR) of the PLP transcripts contains binding sites for RNA-stabilizing factors present in oligodendrocytes.[24]

3.3 Protein structure and function

The PLP protein and its splice isoform, DM20, contain four transmembrane segments, with both N- and C-termini situated in the cytosol. Two extracellular loops are opposed at the myelin plasma membrane (Figure 1C) and contribute to homophilic binding.[27,28] Plp1 deficiency results in the loss of myelin compact laminar structure in mice and delays the central conduction time in humans.

4. The clinical background of PMD

4.1 Geno-phenotypic correlation between the *PLP1* mutation and the natural history of PMD

Disease severity differs considerably among individuals depending on the degree of CNS hypomyelination.[13] Patients with connatal PMD, the most severe form, initially manifest nystagmus and stridor soon after birth.[29] Some patients also exhibit seizures. Spasticity and dystonia of the extremities are prominent and can mask the cerebellar symptoms. These symptoms may lead physicians to misdiagnose PMD as cerebral palsy. Patients with connatal PMD never gain head control or the ability to sit. Compared with their severe motor impairment, verbal comprehension is relatively good and patients often understand simple words, whereas verbal expression is very limited. Amino acid substitutions, particularly those in evolutionarily conserved residues, usually cause this severe phenotype.[13]

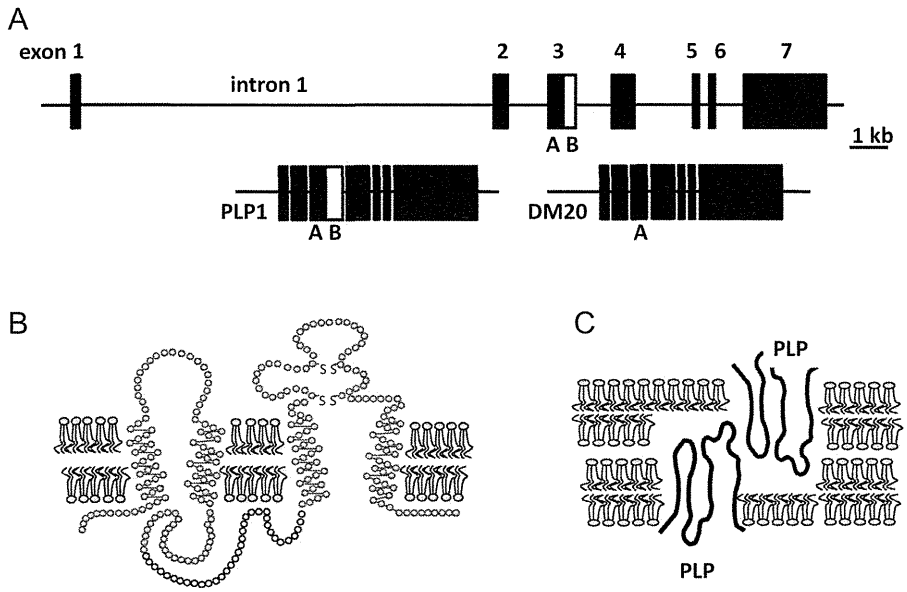

Figure 1. Structure of the myelin proteolipid protein (*PLP*)/*DM* gene and protein. *PLP* contains seven exons that encode the 276-amino acid PLP1 and its alternative splicing product, DM20 (Figure 1A, B). DM20 lacks the 35 amino acids derived from the latter half of exon 3 (exon 3B; open rectangle; Figure 1A) and part of the PLP1 intracellular loop (open circle; Figure B). PLP and its splice isoform, DM20, contain four transmembrane protein segments, with both N- and C-termini situated in the cytosol (Figure 1B) and two opposing extracellular loops in the myelin plasma membrane (Figure 1C).

In classic PMD, the most common form of the disease, nystagmus becomes apparent at a few months of age.[13,36] Hypotonia is noticed in infancy. Exaggerated deep tendon reflexes with pathological reflexes as well as occasional dystonic posture and athetosis point to the cerebral pathology. In later infancy, patients exhibit truncal ataxia and spastic quadriparesis. Patients usually gain the ability to sit, and some are able to stand and walk with support. Cerebellar dysfunction, which manifests as dysmetria and intention tremor, is also observed. Typically, *PLP1* duplication and amino acid substitutions located in less-conserved residues cause the classic form of PMD.

PLP1-specific exon 3B mutations cause the mildest form of PMD, where patients can walk with assistance.[13] Some patients are diagnosed with X-linked spastic paraplegia type 2 (SPG2), an allelic disease to PMD.[9,30] Both of these conditions represent the milder end of the clinical spectrum.

Unique PMD phenotypes are associated with PLP1 null syndrome.[31–33] Patients often lack nystagmus, and exhibit mild neurological phenotypes in the beginning. However, patients present with symptoms that are unique to this genotype, including length-dependent peripheral axonal neuropathy and spasticity, mainly in the legs. In addition, regression begins around adolescence, and patients typically become wheelchair-bound in their second decade. Patients with *PLP1* null syndrome live longer than patients with *PLP1* duplication.[34]

Cailloux et al. proposed a classification system based on the clinical severity of PMD by evaluating patients' maximal motor achievements [36](Figure 2). Patients with Form 0 never gain head control, whereas patients with Form 1 can achieve head control. Form 2 includes patients who are able to maintain a sitting position. Form 3 includes patients who can walk with support, while patients with Form 4 can walk autonomously. This last form overlaps with the clinical phenotype of SPG2, an allelic disorder of PMD.[35]

4.2 Radiographic features of PMD

During the first 2 years of life, myelination occurs and extends spatially from the spinal cord to the brain, from central to peripheral areas, and from the occipital to the frontal lobes. During this process, white matter changes from hypointense to hyperintense in T1-weighted images and from hyperintense to hypointense in T2-weighted images, compared with gray matter [36](Figure 3A–E). In patients with PMD, white matter MR images either remain isointense or become slightly hyperintense compared with gray matter in T1-weighted images; white matter is diffusely hyperintense in T2-weighted images [37](Figure 3F). In PMD, the white matter is typically less myelinated than that observed in normal neonates. Although a predominant feature of PMD is hypomyelination, white matter atrophy also reflects clinical severity in patients. Quantitative white

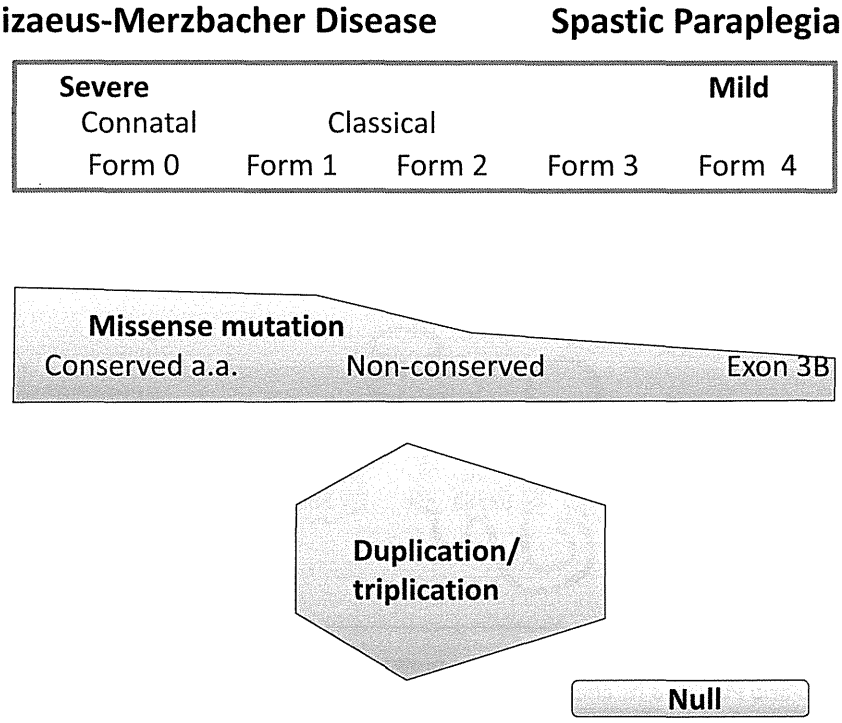

Figure 2. Genetic-phenotypic correlation among PMD and *PLP1* gene mutations. PMD and X-linked spastic paraplegia 2 are allelic disorders caused by *PLP1* mutations. Connatal PMD belongs to a clinicopathological subgroup that falls at the severe end of this disease. X-linked SPG2 represents a milder form of the disease. Clinical severity grades are as follows: Form 2, able to maintain a sitting position; Form 3, can walk with support; and Form 4, can walk autonomously.

matter volume correlates with disease severity and would be useful not only for following the natural history of the disease, but also for evaluating the effect of therapies.[38]

4.3 Diagnosis of PMD

To aid in the diagnosis of potential PMD, MRI studies are recommended in male infants with hypotonia, nystagmus, exaggerated deep tendon reflexes, and primary reflexes such as the Babinski reflex that persist for more than 6 months. Diffuse and homogeneous hypomyelination on the MRI is distinctive and characteristic of PMD.[12,13,39] Neurophysiological examinations, including evoked potential studies (e.g., auditory brain response or somatosensory evoked potentials), are helpful in the diagnosis of PMD, as they reveal conduction defects in the CNS.[29] Peripheral nerve conduction velocity measurements should be conducted to distinguish PMD from other HLDs in which both CNS and peripheral nervous system myelination are compromised.[13] Strong indicators of PMD include the absence of atrophy in the basal ganglia or cerebellum (as determined by MRI) and chromosomal aberrations other than at the PLP1 locus (particularly terminal deletion of 18q, which is 18q- syndrome) in conjunction with normal thyroid hormone levels (free T3). For a diagnosis of PMD, PLP1 dosage should be initially examined using fluorescence *in situ* hybridization, quantitative polymerase chain reaction, multiplex ligation-dependent probe amplification or array comparative genomic hybridization to determine if *PLP1* is duplicated, the most frequent cause of PMD.[13] If *PLP1* duplication is excluded, DNA sequencing that covers all exons and neighboring intronic regions is required.

5. Animal models of PMD

A number of PMD animal models have served as critical tools in understanding disease mechanisms and in developing potential therapies for PMD.[40–42] Major molecular and cellular mechanisms underlying PMD were uncovered in studies using these animal models.[43] Both spontaneous and genetically modified mutants were established in a variety of animal species, including dog, rabbit, rat and, most importantly, mouse.

Spontaneous mutants mostly harbor point mutations within coding exons or splicing junctions, leading to amino acid substitutions or exonic rearrangement. Of these, *jimpy* (*jp*) and *myelin synthesis deficit* (*msd*) are well-studied models

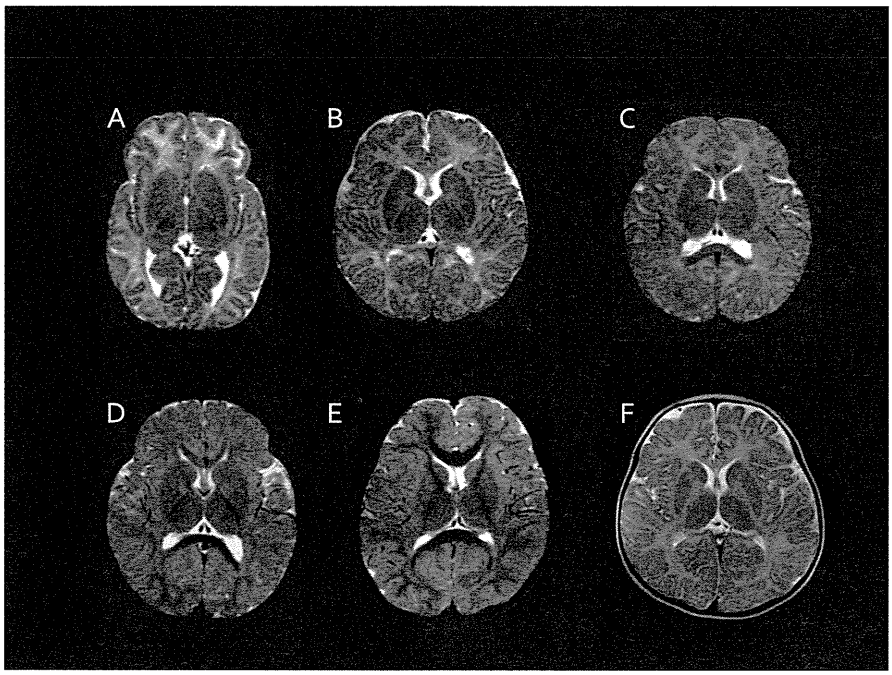

Figure 3. The myelination process over the first 2 years as visualized with a T2 magnetic resonance imaging sequence. Panels show normal myelinating processes at 0, 3, 6, 12 and 19 months of age (**A–E**). A patient with Pelizaeus–Merzbacher disease shows no progression of myelination at 3 years of age (F; *PLP1* duplication). (Courtesy of Noriko Aida, Kanagawa Children's Medical Center.)

of severe PMD.[42,44] These mice have severe neurological manifestations that lead to premature death. Dramatically reduced myelin sheath volume and fewer mature oligodendrocytes are observed in the brains of these models, which likely result from the increased rate of oligodendrocytic apoptotic cell death. In contrast, the *rumpshaker* (*rsh*) mouse, *shaking* (*sh*) pup and *paralytic tremor* (*pt*) rabbit serve as models of a milder form of PMD and SPG2.[40,45,46] These animals display a less-severe phenotype and survive into adulthood. Milder hypomyelination and a reasonable number of oligodendrocytes are observed in the brains of these model animals. Some of these mutants (*jp*, *msd* and *rsh*) mirror mutations in human PMD/SPG2 patients with corresponding clinical severities, and therefore serve as models to study the molecular and cellular basis of phenotypic variations.

Transgenic overexpression and knockout animals are examples of genetically modified animals.[47–51] Transgenic mice mimic PMD caused by a duplication mutation. Transgenic mice harboring either Plp1 cDNA or Dm20 were initially reported; however, genomic transgenic mice were subsequently established using cosmid clones. These mice express both Plp1 and Dm20 under a native *Plp1* promoter, but the transgenes are expressed from autosomal chromosomes wherein the transgenes are integrated. A genomic duplication at the *Plp1* locus on the X chromosome was recently generated using recombineering technology in mice, revealing X-linked inheritance.[41] The phenotype of these overexpressing mice varies depending on the gene dosage. In each line, heterozygous mice (i.e., low copy number) have a milder phenotype than do homozygous mice (i.e., high copy number), suggesting a gene dosage effect. Conversely, a mild phenotype characterized by abundant myelin and an almost normal number of oligodendrocytes is observed in *Plp1* null mice. These mice also mimic the phenotype of patients with *PLP1* deletions or null mutations.

6. Pathophysiology of PMD

PLP1 is synthesized in the ER and transported through the Golgi complex (Figure 4).[52,53] During this vesicular transport, PLP1 associates with cholesterol and forms myelin rafts enriched in cholesterol and galactosylceramide; these myelin rafts are recruited to the plasma membrane to form myelin. PLP1 is unique in that it is abundantly expressed in maturating oligodendrocytes; more than half of the entire protein generated in mature oligodendrocytes is PLP1. Therefore, it is not surprising that both qualitative and quantitative changes in the *PLP1* gene cause marked ER stress in addition to oligodendrocyte and neuronal cell death in PMD patients.[54]

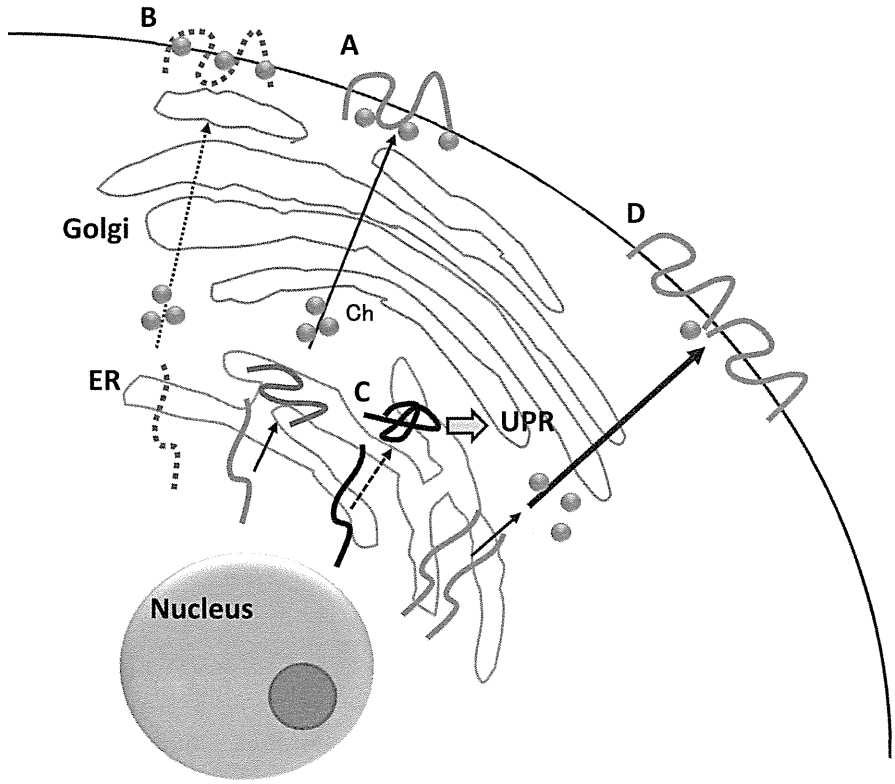

Figure 4. Normal and abnormal trafficking of PLP1. PLP1 is synthesized in the endoplasmic reticulum (ER) and transported through the Golgi complex (Figure 4A). PLP1 associates with cholesterol and forms myelin rafts enriched in cholesterol (Ch), which are recruited to the plasma membrane to form myelin. Modification of PLP1 at Golgi apparatus, where myelin lipid and protein components assemble, is essential for normal trafficking to the membrane. If *PLP1* is deleted, PLP1 function is lost (Figure 4B). When *PLP1* is mutated via an amino acid substitution, misfolded PLP1 is trapped in the ER, and therefore does not reach the membrane, resulting in loss of function at the cell membrane. Moreover, misfolded PLP1 elicits the apoptotic unfolded protein response (UPR; Figure 4C). PLP1 overexpression may impair cholesterol trafficking from the ER and Golgi to the plasma membrane (Figure 4D).

When PLP1 is mutated due to an amino acid substitution, the protein is unable to fold properly and becomes misfolded. Such aberrant proteins cannot traffic to the Golgi apparatus, and consequently are trapped in the ER. The large amount of abnormally accumulated misfolded PLP1 protein induces ER stress.[55–57] In response, cells activate an intracellular signal transduction pathway collectively known as the unfolded protein response (UPR). The UPR increases the ER protein-folding capacity, reduces global protein synthesis and enhances ER-associated degradation to maintain ER homeostasis for survival. However, if the burden overwhelms the UPR capacity, the apoptotic cell death pathway is activated. Three ER transmembrane sensor proteins initiate the UPR signaling pathways: inositol-requiring enzyme 1, protein kinase RNA-like endoplasmic reticulum kinase and activating transcription factor 6. These pathways work together to regulate genes in either an adaptive or an apoptotic manner.[58,59] The high PLP1 expression level eventually overloads the maturating oligodendrocytes and leads to a rapid increase in ER stress, UPR activation and oligodendrocyte cell death. Activation of the UPR due to *PLP1* mutations has been demonstrated in cultured cells, model animals and, most recently, in oligodendrocytes derived from induced pluripotent stem cells (iPSCs) generated from PMD patient cells carrying missense mutations. The degree of UPR activation appears to relate to PMD disease severity; mutations associated with connatal PMD induce greater ER stress, while those associated with SPG2 result in subtler changes.[55] Activation of the apoptotic branch of the UPR is thought to correlate with oligodendrocytes cell death in PMD. However, evidence exists that depleted levels of C/EBP homology protein, caspase 12, or activating transcription factor 4, which function in the UPR apoptotic branch, do not rescue the phenotype of *PLP1* mutant mice.[60] This suggests the possibility that other

pathomechanisms may also contribute to disease manifestation.

Pathomechanisms underlying *PLP1* duplication have not been completely elucidated. Why just twice the amount of normal PLP1 (if each allele expresses a normal amount of mRNA and protein) results in rapid oligodendrocyte cell death and severe dysmyelination is still a mystery. However, recent studies have made significant progress in elucidating the nature of excessive PLP1 using cell culture systems and *PLP1* transgenic mouse models. Unlike point mutations, PLP1 overexpression does not activate the UPR, but leads to an altered distribution of PLP1 within the cells. Instead of incorporating into the myelin plasma membrane or myelin sheath, excessive PLP1, accompanied by cholesterol, accumulates in the cytoplasmic endosomes/lysosomes.[61,62] The association of PLP1 with cholesterol appears to be critical. In mice that lack cholesterol synthesis in oligodendrocytes, hypomyelination is observed due to low levels of myelin protein gene expression, including PLP1.[63] In addition, a recent preclinical study demonstrated that dietary supplementation of high-dose cholesterol ameliorated the hypomyelinating phenotype observed in *PLP1* transgenic mice. These data further support the importance of cholesterol metabolism in the pathology of *PLP1* duplication.[63]

In addition to lysosomal accumulation, it was shown that overexpressed *Plp1/PLP1* can be inserted into mitochondria in mice and in humans, which may induce metabolic dysfunction in oligodendrocytes.[64] Contribution of the microglia-mediated inflammatory response is also likely a major player in the pathophysiology of PMD, and represents another potential target for therapy.[65,66]

7. Induced pluripotent stem cells may serve as a novel model system

Recently, two groups in Japan succeeded in establishing iPSCs from patients with PMD. Numasawa and colleagues established iPSCs from two PMD patients carrying a missense mutation, and differentiated them into oligodendrocytes *in vitro*.[67,68] In oligodendrocytes derived from iPSCs harvested from PMD patients, mutant PLP1 proteins were mislocalized to the ER. In addition, an association was observed between an increased susceptibility to ER stress and an increased number of apoptotic oligodendrocytes. Moreover, quantitative electron microscopic analysis of the number of myelin lamellae per myelinated fiber demonstrated the presence of drastically reduced myelin formation accompanied by abnormal ER morphology. Thus, this study demonstrated that ER stress was involved in pathogenic dysmyelination of oligodendrocytes in PMD patients that carried the *PLP1* missense mutation.

8. Approaches and opportunities for the treatment of PMD

8.1 Missense mutation

8.1.1 Splicing restoration

Exon skipping with oligonucleotides has been applied to correct missplicing in several genetic diseases, such as congenital glycosylation disorders and neurofibromatosis types 1 and 2.[69] In PMD, Regis et al. found a single point mutation in the latter part of exon 3, c. 436 C > G. This mutation not only causes an amino acid substitution within PLP1-specific peptides, but also generates putative exonic enhancer elements, which may lead to a perturbed splicing pattern and loss of the major PLP1 transcript (but not DM20). They further designed a morpholino antisense oligonucleotide to mask these novel splicing regulatory motifs, which resulted in recovering the PLP1 transcript from murine oligodendroglial Oli-neu cells transfected with the *PLP1* minigene harboring this mutation.[70] This strategy could be applied to the development of therapeutic strategies targeting mutations that modify splicing regulatory elements.[70]

8.1.2 Altering signal transduction

Miyamoto et al. designed a retrovirus-mediated PLP1 expression vector, PLP1-IRES-ZsGreen, for transfection into primary oligodendrocyte precursor cells (OPCs) harvested from embryonic day 15 rat cerebral cortices. They showed that expression of PLP1 markedly inhibited OPC differentiation in a concentration-dependent manner. They also established a high-throughput screening (HTS) system to identify small chemical compounds that improved differentiation of OPCs.[71] Using this assay, they detected a subset of signaling molecule inhibitors, including the extracellular signal-regulated kinase (ERK) inhibitor U0126 and the mitogen-activated protein (MAP) kinase 1/2 (MAPKK or MEK1/2) inhibitor PD98058, that significantly improved OPC differentiation. OPC differentiation was also improved by inhibiting ERK activity. ERK inhibition improved PLP1-induced dysmyelination in co-cultured OPCs and dorsal root ganglion neurons. Thus, ERK inhibition helped to improve defective OPC differentiation induced by PLP1 expression, suggesting that molecules belonging to the MEK1/2-ERK signaling pathway may be novel PMD therapeutic targets. As discussed later, there is no available functional assay suitable for large-scale drug screening; therefore, visualization of cell phenotypes in their system was valuable.[72]

8.1.3 Attenuation of ER stress

Curcumin, a dietary compound derived from the curry spice turmeric, has been tested in several rodent models of genetic diseases caused by misfolded proteins, including cystic fibrosis, Charcot-Marie-Tooth neuropathy type 1 and retinitis pigmentosa.[73–76] Yu and colleagues determined whether curcumin could rescue the lethal phenotype of a PMD mouse model, *msd*.[77] Curcumin was orally administered

to *msd* mice from postnatal day 3. Oral curcumin treatment extended survival by 25% and reduced oligodendrocytic apoptosis. Conversely, no apparent improvements in motor function, neurological phenotype or myelin formation were observed. However, molecular mechanisms underlying this curative effect remain undetermined.

Morimura et al. reported that an antimalarial drug, chloroquine, can decrease the amount of an ER-accumulated mutant PLP1 containing an alanine-243 to valine (A243 V) substitution, which causes severe PMD in humans.[78] Chloroquine attenuated the translation of mutant PLP1 by increasing the phosphorylation of eukaryotic initiation factor 2α (eIF-2α) and decreased ER stress in HeLa cells. Chloroquine also attenuated ER stress in primary oligodendrocytes obtained from *msd* mice carrying the same A243 V substitution. In the spinal cords of *msd* mice, chloroquine inhibited ER stress and upregulated the expression of marker genes of mature oligodendrocytes. These findings suggest that ER stress attenuators, such as chloroquine, may represent a potential treatment for PMD.

Pharmacological chaperones have been recently extensively investigated for their potential to refold misfolded proteins.[79,80] If pharmacological chaperones can aid in the correct folding of PLP1, they may potentially ameliorate the ER stress observed in PMD due to missense *PLP1* mutations.

8.2 Duplication or overexpression

8.2.1 Cholesterol

PLP1 binds cholesterol, which is a major constituent of membrane lipid raft microdomains.[81] Cholesterol accessibility is the rate-limiting factor in CNS myelin synthesis. The finding that PLP1 and cholesterol accumulate in late endosomes and lysosomes in *Plp1*-transgenic oligodendrocytes has raised the possibility that the reduction of cholesterol in the myelin membrane may play a role in the pathophysiology of *PLP1* duplication.[61,82] Surprisingly, introduction of a cholesterol-enriched diet in Plp1 transgenic mice resulted in restoration of oligodendrocyte number and reduction of intracellular PLP1 accumulation, which was accompanied by improved myelination. Therefore, a cholesterol-enriched diet may have potential as a viable therapeutic intervention for PMD.[63]

8.2.2 Attenuating PLP1 expression

The nuclear progesterone receptor, ligand-activated zinc-finger protein, is a widely expressed transcription factor that is present in oligodendrocytes.[83] This receptor is a member of a much larger steroid receptor superfamily that binds to palindromic response elements in the promoter regions of steroid-responsive genes.[66] Progesterone and its receptor are synthesized and expressed in neurons and glial cells.[48,65,67–71] *In vivo*, daily progesterone injections upregulate Plp1 mRNA in rat sciatic nerve, indicating that progesterone receptor binding sites may be present in the *PLP1* promoter.[72–74,79] The nuclear progesterone receptor antagonist lonaprisan was administered to *Plp1* transgenic mice for 10 weeks, and then the clinical phenotypes were assessed. Although Plp1 mRNA levels were increased 1.8-fold in PMD model mice compared with wild-type controls, daily lonaprisan treatment reduced overexpression at the RNA level to approximately 1.5-fold, which significantly improved the poor motor phenotype observed in the PMD model mice.[84]

In the developing nervous system, a small subset of short RNA molecules is important in orchestrating the rapid switch from OPCs to myelin-forming oligodendrocytes and in regulating the synthesis of myelin structural proteins.[85] Wang et al. examined the miRNA that controls PLP abundance using the oligodendroglial cell line Oli-neu and in enhanced green fluorescent protein-positive oligodendrocytes *ex vivo*.[86] They identified 145 miRNAs that were expressed during oligodendrocyte cell lineage progression. They attempted to identify miRNAs that controlled PLP expression; therefore, they selected miRNAs characterized by lower expression in differentiated vs. undifferentiated Oli-neu cells and with one or more binding site(s) in the PLP 3′UTR. When the PLP 3′UTR was fused to the luciferase gene, reporter activity was reduced, suggesting that it negatively regulated message stability or translation. Such suppression was relieved by knockdown of miR-20a. Overexpression of miR-20a decreased the expression of endogenous PLP in primary oligodendrocytes and of the reporter gene. Deletion or mutation of the putative binding site for miR-20a in the PLP 3′UTR abrogated such effects, indicating that miR-20a, a component of the cluster that controls oligodendrocyte cell number, regulates PLP gene expression through its 3′UTR.[86]

Introduction of a small RNA molecule that negatively regulates PLP1 mRNA expression, such as miR-20a, might be a viable approach for gene therapy in PMD. Evidence exists demonstrating that an AAV9-delivered short hairpin RNA effectively reduced mutant superoxide dismutase 1 (SOD1) expression and increased survival time in a mouse model of amyotrophic lateral sclerosis.[87]

9. Cell-based therapy

9.1 Bone marrow transplantation

Umbilical cord blood transplantation (UCBT) has been successfully applied in several inherited metabolic diseases, including lysosomal and peroxisomal storage disorders.[88] The effect appears to be mediated by enzyme replacement from engrafted cells. UCBT is most commonly administered in adrenoleukodystrophy, where its mechanism of efficacy is unclear.[89]

Wishnew and colleagues recently reported on the clinical course of two young boys with PMD, the first known patients to receive UCBT as a therapy.[90] One PMD patient with

PLP1 duplication received UCBT at 9 months; he has been followed up for 7 years. The second PMD patient with a splicing mutation, c.697-54_711delinsTTTATTT, received UCBT at 25 months and was followed up for 1 year. After UCBT, both patients showed some myelination revealed by MRI. Due to the small number of patients and the lack of negative controls for comparison, it is difficult to assess the effectiveness of this treatment. However, PMD is a disorder of myelin structural protein and a potential mechanism for efficacy is lacking.

9.2 Stem cell therapy: oligodendrocyte progenitor cell transplantation therapy

A phase 1 study was performed to evaluate the safety of and to detect evidence for myelin formation after human CNS stem cell (HuCNS-SC) therapy.[91] Patients received an injection of allogeneic HuCNS-SCs into the white matter of the frontal lobe following 9 months of immunosuppression. There were no serious side effects. Minimal gains in neurological function were observed in three subjects. These phase 1 findings indicate a favorable safety profile for HuCNS-SCs in subjects with PMD. To assess myelination, MRI and MR diffusion tensor imaging were employed to quantify differences in the magnitude and direction of water motion. The acquired images suggested the presence of durable cell engraftment and donor-derived myelin in the small areas of white mater surrounding the injection sites.

9.3 iPS-based therapy

With advances in iPS technology, reprogrammed somatic cells can be pursued as a cell source for use in replacing patient cells, as well as a model for use in recapitulating disease mechanisms *in vitro*.[92] In 2015, a clinical trial began to determine if the epithelial sheet derived from iPSCs harvested from a patient could repair damaged retinas in patients with age-related macular degeneration.[93,94] Using a patient's own induced cells minimizes immune rejection and myeloablative chemotherapy; ethical issues are also curtailed. However, in congenital genetic diseases such as PMD, the mutated gene should be edited before autologous transplantation. Additionally, it is unclear whether injected stem cells can ameliorate myelination defects in all areas of the CNS.

10. Conclusion

Considerable progress regarding PMD has been made over the past two decades. Subsequent to the discovery of *PLP1* as a genetic cause of this disease, distinct pathophysiological mechanisms attributable to *PLP1* were revealed, including loss-of-function, gain-of-toxicity due to missense mutations, and the effects of copy number overexpression. In PMD mouse models, several compounds used for treatment have generated encouraging results; moreover, stem cell/bone marrow transplantation has been introduced as the first cell-based therapy. However, evidence is lacking regarding treatments that are effective in ameliorating the PMD phenotype in patients. Future therapy should be aimed to lessen the various brain pathologies and lengthen the survival of PMD patients.

A single approach may not be sufficient and combination therapy may be necessary to combat this devastating disorder.

11. Expert opinion

One of the major focuses of current and future research on PMD is the development of new therapies. The efficacy of three drugs has been tested using mouse models. Treatment with cholesterol or lonaprisan was effective in *Plp1* transgenic mice.[63,84] Curcumin treatment resulted in prolonged survival times in a model mouse harboring a *Plp1* missense mutation [77]; curcumin treatment also improved the motor phenotype of the transgenic mice.[95] These candidate chemicals are waiting to be tested in clinical studies.

In addition to conventional pharmaceutical strategies, gene therapy and neural stem cell (NSC) transplantation are particular interests. For PLP1 null mutations, delivery of PLP1 via gene therapy is a compelling option. Small RNA molecules that function to decrease *PLP1* mRNA expression may be effective in cases of *PLP1* duplication. The adeno-associated viral vector (AAV) appears to be an optimal vehicle for such gene delivery into the CNS.[96] The PLP1 open reading frame is 834 bp, which is smaller than the upper limit required for AAV insertion (i.e., ~4.3 kb). AAV predominantly persists in episomes, and thus the risk of cancer occurrence is low. Thereby, it is ideal for gene therapy in pediatric patients.

AAV exhibits low immunogenicity and continues to be expressed long term (>10 year) in non-dividing post-mitotic neuronal cells.[82] AAV recognizes cell surface glycans, which are abundant in neuronal and glial cells. There are 11 naturally occurring AAV serotypes, and more than 100 variants of AAV.[97] Each serotype exhibits preferential tropism for neurons, oligodendrocytes, astrocytes or ependymal cells. Because of this, it is necessary to select available variants or engineer a viral capsid to induce PLP1 expression in a cell-type-specific manner.

Direct viral injections into the brain parenchyma result in a high level of transgene expression, which leads to remarkable improvement in patients with aromatic L-amino acid decarboxylase (AADC) deficiency.[96,98] Contrary to AADC supplementation, which only requires local expression in the basal ganglia, PLP1 is expressed in all oligodendrocytes present in CNS white matter. Therefore, systematic delivery of AAV is required to elicit therapeutic effects in individuals. Recent studies showed that AAV injection into the cerebrospinal fluid via intraventricular or intrathecal delivery

efficiently penetrated ependymal cells and brain parenchyma in mice and large animals. Further preclinical studies are warranted that focus on the development of systematic AAV delivery and high-efficiency serotype selection with specificity for oligodendrocytes.

Much attention has also been paid to cell transplantation therapy using NSCs.[99] The major challenges involved in NSC therapy include the choice of stem cells to be used and the appropriate transplantation method, along with validation of the safety and survival of grafted cells. Fetal-tissue-derived allogenic NSCs have been under phase I clinical study in congenital neurological diseases.[99] These studies of infantile neuronal ceroid lipofuscinosis and PMD showed that allogenic NSCs are safe. Furthermore, examination of the brains from three patients who died due to underlying disease in a study of ceroid lipofuscinosis showed evidence of engraftment, migration and long-term survival of the grafted cells. Other stem cells, including iPS-derived NSCs, adult tissue-derived stem cells and different types of ES-derived NSCs, have been tested in preclinical transplantation studies for their remyelination efficacy in model animals.

The benefits of stem cell therapy should outweigh the known risks of surgery and vigorous immune ablation therapy. Furthermore, it is unlikely that transplanted neighboring stem cells fully compensate for structural abnormalities in the PLP1/DM20 protein through the CNS myelin. Although NSCs have potential for use in therapy, they have not yet produced significant results for PMD treatment and require more basic and translational study.

To translate gene and cell-based therapies successfully into the clinic, collaboration among scientists and clinicians is essential. Moreover, earlier initiation of such therapies may result in better prognosis before marked progression of oligodendrocyte cell death occurs. To achieve optimal results in clinical trials, diagnostic program systems must be established and PMD patients must be added to the registry during the early stages of the disease.

Acknowledgments

We thank Kenji Kurosawa, Noriko Aida and Hiroko Shimbo (Kanagawa Children's Medical Center) for their PLP1 analysis and MRI studies.

Declaration of interest

This work was supported in part by grants from the Japan Agency for Medical Research and Development, AMED (Practical Research Project for Rare/Intractable Diseases,15ek0109016 to H.O., and K.I.), Health Labour Sciences Research Grant (H27-Nanchitou-ippan-020, H.O., and K.I.), and Grant-in-Aid for Scientific Research (Kiban (B): 15H04878 to H.O. and K.I.).

Bibliography

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

1. Griffiths I, Klugmann M, Anderson T, et al. Current concepts of PLP and its role in the nervous system. Microsc Res Tech. 1998;41(5):344–358.
2. Seitelberger F. Neuropathology and genetics of Pelizaeus–Merzbacher disease. Brain Pathol. 1995;5(3):267–273.

** **The first paper to describe these aspects of Pelizaeus–Merzbacher disease.**

3. Seitelberger F. Histochemistry & classification of Pelizaeus–Merzbacher disease. Wien Z Nervenheilkd Grenzgeb. 1957;14 (1):74–83.
4. Trofatter JA, Dlouhy SR, DeMyer W, et al. Pelizaeus–Merzbacher disease: tight linkage to proteolipid protein gene exon variant. Proc Natl Acad Sci USA. 1989;86 (23):9427–9430.

** **This paper found the causative gene for Pelizaeus–Merzbacher disease to be the proteolipid protein gene.**

5. Hudson LD, Puckett C, Berndt J, et al. Mutation of the proteolipid protein gene PLP in a human X chromosome-linked myelin disorder. Proc Natl Acad Sci USA. 1989;86(20):8128–8131.

** **This paper found the causative gene for Pelizaeus–Merzbacher disease to be the proteolipid protein gene.**

6. Inoue K, Osaka H, Sugiyama N, et al. A duplicated PLP gene causing Pelizaeus–Merzbacher disease detected by comparative multiplex PCR. Am J Hum Genet. 1996;59(1):32–39.
7. Inoue K, Osaka H, Imaizumi K, et al. Proteolipid protein gene duplications causing Pelizaeus–Merzbacher disease: molecular mechanism and phenotypic manifestations. Ann Neurol. 1999;45 (5):624–632.
8. Inoue K. PLP1-related inherited dysmyelinating disorders: Pelizaeus–Merzbacher disease and spastic paraplegia type 2. Neurogenetics. 2005;6(1): 1–16.
9. Hodes ME, Pratt VM, S.R. Dlouhy. Genetics of Pelizaeus–Merzbacher disease. Dev Neurosci. 1993;15(6):383–394.
10. Hobson GM, Garbern JY. Pelizaeus–Merzbacher disease, Pelizaeus–Merzbacher-like disease 1, and related hypomyelinating disorders. Semin Neurol. 2012;32(1):62–67.

* **Practical reviews of PMD.**

11. Boespflug-Tanguy O. Inborn errors of brain myelin formation. Handb Clin Neurol. 2013;113:1581–1592.
12. Pouwels PJ, Vanderver A, Bernard G, et al. Hypomyelinating leukodystrophies: translational research progress and prospects. Ann Neurol. 2014;76(1):5–19.
13. Hobson GM, Kamholz J. PLP1-related disorders. In: Pagon RA, et al. editors. GeneReviews(R). [Internet]. Seattle (WA): University of Washington; 1993–2015. 1999 Jun 15 [updated 2013 Feb 28].
14. Heim P, Claussen M, Hoffmann B, et al. Leukodystrophy incidence in Germany. Am J Med Genet. 1997;71(4):475–478.
15. Numata Y, Gotoh L, Iwaki A, et al. Epidemiological, clinical, and genetic landscapes of hypomyelinating leukodystrophies. J Neurol. 2014;261(4):752–758.
16. Kitagawa K, Sinoway MP, Yang C, et al. A proteolipid protein gene family: expression in sharks and rays and possible evolution

from an ancestral gene encoding a pore-forming polypeptide. Neuron. 1993;11 (3):433–448.

17. Yan Y, Lagenaur C, Narayanan V. Molecular cloning of M6: identification of a PLP/DM20 gene family. Neuron. 1993;11(3):423–431.
18. Eng LF, Chao FC, Gerstl B, et al. The maturation of human white matter myelin. Fractionation of the myelin membrane proteins. Biochemistry. 1968;7(12):4455–4465.
19. Wight PA, Dobretsova A. Where, when and how much: regulation of myelin proteolipid protein gene expression. Cellular Mol Life Sci CMLS. 2004;61(7–8):810–821.
20. Fulton D, Paez PM, Campagnoni AT. The multiple roles of myelin protein genes during the development of the oligodendrocyte. ASN Neuro. 2010;2(1): e00027.
21. Nave KA, Milner RJ. Proteolipid proteins: structure and genetic expression in normal and myelin-deficient mutant mice. Crit Rev Neurobiol. 1989;5(1):65–91.
22. Taube JR, Sperle K, Banser L, et al. PMD patient mutations reveal a long-distance intronic interaction that regulates PLP1/DM20 alternative splicing. Hum Mol Genet. 2014;23(20):5464–5478.
23. Cook JL, Irias-Donaghey S, Deininger PL. Regulation of rodent myelin proteolipid protein gene expression. Neurosci Lett. 1992;137(1):56–60.
24. Mallon BS, Macklin WB. Overexpression of the 3′-untranslated region of myelin proteolipid protein mRNA leads to reduced expression of endogenous proteolipid mRNA. Neurochem Res. 2002;27 (11):1349–1360.
25. Kim JG, Hudson LD. Novel member of the zinc finger superfamily: A C2-HC finger that recognizes a glia-specific gene. Mol Cell Biol. 1992;12(12):5632–5639.
26. Bogazzi F, Hudson LD, Nikodem VM. A novel heterodimerization partner for thyroid hormone receptor. Peroxisome proliferator-activated receptor. J Biol Chem. 1994;269(16):11683–11686.
27. Stoffel W, Subkowski T, Jander S. Topology of proteolipid protein in the myelin membrane of central nervous system. A study using antipeptide antibodies. Biol Chem Hoppe Seyler. 1989;370 (2):165–176.

* **This paper predicted a secondary structure of PLP1.**

28. Popot JL, Pham Dinh D, Dautigny A. Major myelin proteolipid: the 4-alpha-helix topology. J Membr Biol. 1991;120 (3):233–246.

* **This paper also predicted a secondary structure of PLP1.**

29. Boulloche J, Aicardi J. Pelizaeus–Merzbacher disease: clinical and nosological study. J Child Neurol. 1986;1 (3):233–239.
30. Kobayashi H, Gauthier-Barichard F, Mimault C, et al. Molecular genetics of familial spastic paraplegia: a multitude of responsible genes. J Neurol Sci. 1996;137 (2):131–138.
31. Raskind WH, Prukop T, Nientiedt T, et al. Complete deletion of the proteolipid protein gene (PLP) in a family with X-linked Pelizaeus–Merzbacher disease. Am J Hum Genet. 1991;49(6):1355–1360.
32. Garbern JY, Cambi F, Tang XM, et al. Proteolipid protein is necessary in peripheral as well as central myelin. Neuron. 1997;19(1):205–218.
33. Garbern JY, Yool DA, Moore GJ, et al. Patients lacking the major CNS myelin protein, proteolipid protein 1, develop length-dependent axonal degeneration in the absence of demyelination and inflammation. Brain J Neurol. 2002;125(Pt 3):551–561.
34. Ellis D, Malcolm S. Proteolipid protein gene dosage effect in Pelizaeus–Merzbacher disease. Nat Genet. 1994;6 (4):333–334.

* **The first paper that reported PLP1 duplication.**

35. Cailloux F, Gauthier-Barichard F, Mimault C, et al. Genotype-phenotype correlation in inherited brain myelination defects due to proteolipid protein gene mutations. Clinical European Network on Brain Dysmyelinating Disease. Eur J Hum Genet. 2000;8(11):837–845.

* **This paper classified the disease severity and described the genotype-phenotype correlation.**

36. Schiffmann R, van der Knaap MS. Invited article: an MRI-based approach to the diagnosis of white matter disorders. Neurology. 2009;72(8):750–759.
37. Nezu A, Kimura S, Takeshita S, et al. An MRI and MRS study of Pelizaeus–Merzbacher disease. Pediatr Neurol. 1998;18(4):334–337.
38. Laukka JJ, Stanley JA, Garbern JY, et al. Neuroradiologic correlates of clinical disability and progression in the X-linked leukodystrophy Pelizaeus–Merzbacher disease. J Neurol Sci. 2013;335(1–2):75–81.
39. Steenweg ME, Vanderver A, Blaser S, et al. Magnetic resonance imaging pattern recognition in hypomyelinating disorders. Brain. 2010;133(10):2971–2982.
40. Griffiths IR, Scott I, McCulloch MC, et al. Rumpshaker mouse: a new X-linked mutation affecting myelination: evidence for a defect in PLP expression. J Neurocytol. 1990;19(2):273–283.
41. Clark K, Sakowski L, Sperle K, et al. Gait abnormalities and progressive myelin degeneration in a new murine model of Pelizaeus–Merzbacher disease with tandem genomic duplication. J Neurosci Off J Soc Neurosci. 2013;33 (29):11788–11799.

* **This paper described a genetic mouse model that had characteristics similar to those observed in humans with PLP1 duplication.**

42. Gencic S, Hudson LD. Conservative amino acid substitution in the myelin proteolipid protein of jimpy^{msd} mice. J Neurosci Off J Soc Neurosci. 1990;10 (1):117–124.
43. Duncan ID. The PLP mutants from mouse to man. J Neurol Sci. 2005;228 (2):204–205.
44. Dautigny A, Mattei MG, Morello D, et al. The structural gene coding for myelin-associated proteolipid protein is mutated in jimpy mice. Nature. 1986;321(6073):867–869.

** **The first description of a model mouse of PMD.**

45. Nadon NL, Duncan ID. Molecular analysis of glial cell development in the canine 'shaking pup' mutant. Dev Neurosci. 1996;18(3):174–184.
46. Tosic M, et al. Paralytic tremor (pt) rabbit: a sex-linked mutation affecting proteolipid protein-gene expression. Brain Res. 1993;625(2):307–312.
47. Kagawa T, Ikenaka K, Inoue Y, et al. Glial cell degeneration and hypomyelination caused by overexpression of myelin proteolipid protein gene. Neuron. 1994;13 (2):427–442.

48. Readhead C, Schneider A, Griffiths I, et al. Premature arrest of myelin formation in transgenic mice with increased proteolipid protein gene dosage. Neuron. 1994;12(3):583–595.

49. Boison D, Stoffel W. Disruption of the compacted myelin sheath of axons of the central nervous system in proteolipid protein-deficient mice. Proc Natl Acad Sci U S A. 1994;91(24):11709–11713.

50. Griffiths I, Klugmann M, Anderson T, et al. Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. Science. 1998;280 (5369):1610–1613.

51. Klugmann M, Schwab MH, Puhlhofer A, et al. Assembly of CNS myelin in the absence of proteolipid protein. Neuron. 1997;18(1):59–70.

52. Simons M, Kramer EM, Thiele C, et al. Assembly of myelin by association of proteolipid protein with cholesterol- and galactosylceramide-rich membrane domains. J Cell Biol. 2000;151 (1):143–154.

53. Garbern JY. Pelizaeus–Merzbacher disease: Genetic and cellular pathogenesis. Cellular Mol Life Sci CMLS. 2007;64(1):50–65.

54. Sima AA, Pierson CR, Woltjer RL, et al. Neuronal loss in Pelizaeus–Merzbacher disease differs in various mutations of the proteolipid protein 1. Acta Neuropathol. 2009;118(4):531–539.

55. Southwood CM, Garbern J, Jiang W, et al. The unfolded protein response modulates disease severity in Pelizaeus–Merzbacher disease. Neuron. 2002;36 (4):585–596.

** **A clear description of the ER stress response in PMD.**

56. Gow A, Sharma R. The unfolded protein response in protein aggregating diseases. Neuromolecular Med. 2003;4(1–2):73–94.

57. Roboti P, Swanton E, High S. Differences in endoplasmic-reticulum quality control determine the cellular response to disease-associated mutants of proteolipid protein. J Cell Sci. 2009;122(Pt 21):3942–3953.

58. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011;334 (6059):1081–1086.

59. Cao SS, Kaufman RJ. Unfolded protein response. Curr Biol. 2012;22(16):R622–6.

60. Sharma R, Gow A. Minimal role for caspase 12 in the unfolded protein response in oligodendrocytes in vivo. J Neurochem. 2007;101(4):889–897.

61. Simons M, Kramer EM, Macchi P, et al. Overexpression of the myelin proteolipid protein leads to accumulation of cholesterol and proteolipid protein in endosomes/lysosomes: implications for Pelizaeus–Merzbacher disease. J Cell Biol. 2002;157(2):327–336.

62. Karim SA, Barrie JA, McCulloch MC, et al. PLP/DM20 expression and turnover in a transgenic mouse model of Pelizaeus–Merzbacher disease. Glia. 2010;58 (14):1727–1738.

63. Saher G, Rudolphi F, Corthals K, et al. Therapy of Pelizaeus–Merzbacher disease in mice by feeding a cholesterol-enriched diet. Nat Med. 2012;18(7):1130–1135.

* **Cholesterol therapy for a PMD mouse model.**

64. Appikatla S, Bessert D, Lee I, et al. Insertion of proteolipid protein into oligodendrocyte mitochondria regulates extracellular pH and adenosine triphosphate. Glia. 2014;62(3):356–373.

65. Huttemann M, Zhang Z, Mullins C, et al. Different proteolipid protein mutants exhibit unique metabolic defects. ASN Neuro. 2009;1(3): e00014.

66. Tatar CL, Appikatla S, Bessert DA, et al. Increased Plp1 gene expression leads to massive microglial cell activation and inflammation throughout the brain. ASN Neuro. 2010;2(4):e00043.

* **The first description of massive microglial activation in a PMD mouse model.**

67. Numasawa-Kuroiwa Y, Okada Y, Shibata S, et al. Involvement of ER stress in dysmyelination of Pelizaeus–Merzbacher Disease with PLP1 missense mutations shown by iPSC-derived oligodendrocytes. Stem Cell Rep. 2014;2(5):648–661.

* **The first paper on iPS-derived oligodendrocytes from a patient with PMD.**

68. Shimojima K, Inoue T, Imai Y, et al. Reduced PLP1 expression in induced pluripotent stem cells derived from a Pelizaeus–Merzbacher disease patient with a partial PLP1 duplication. J Hum Genet. 2012;57(9):580–586.

69. Siva K, Covello G, Denti MA. Exon-skipping antisense oligonucleotides to correct missplicing in neurogenetic diseases. Nucleic Acid Ther. 2014;24(1):69–86.

70. Regis S, Corsolini F, Grossi S, et al. Restoration of the normal splicing pattern of the PLP1 gene by means of an antisense oligonucleotide directed against an exonic mutation. PLoS One. 2013;8(9):e73633.

71. Miyamoto Y, Torii T, Tanoue A, et al. Pelizaeus–Merzbacher disease-associated proteolipid protein 1 inhibits oligodendrocyte precursor cell differentiation via extracellular-signal regulated kinase signaling. Biochem Biophys Res Commun. 2012;424 (2):262–268.

72. Torii T, Miyamoto Y, Yamauchi J, et al. Pelizaeus–Merzbacher disease: cellular pathogenesis and pharmacologic therapy. Pediatr Int Off J Jpn Pediatr Soc. 2014;56 (5):659–666.

73. Egan ME, Pearson M, Weiner SA et al. Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. Science. 2004;304(5670):600–602.

74. Khajavi M, Inoue K, Wiszniewski W et al. Curcumin treatment abrogates endoplasmic reticulum retention and aggregation-induced apoptosis associated with neuropathy-causing myelin protein zero-truncating mutants. Am J Hum Genet. 2005;77(5):841–850.

75. Khajavi M, Shiga K, Wiszniewski W et al. Oral curcumin mitigates the clinical and neuropathologic phenotype of the Trembler-J mouse: a potential therapy for inherited neuropathy. Am J Hum Genet. 2007;81(3):438–453.

76. Vasireddy V, Chavali VR, Joseph VT, et al. Rescue of photoreceptor degeneration by curcumin in transgenic rats with P23H rhodopsin mutation. PLoS One. 2011;6(6):e21193.

77. Yu LH, Morimura T, Numata Y, et al. Effect of curcumin in a mouse model of Pelizaeus–Merzbacher disease. Mol Genet Metab. 2012;106(1):108–114.

78. Morimura T, Numata Y, Nakamura S, et al. Attenuation of endoplasmic reticulum stress in Pelizaeus–Merzbacher disease by an anti-malaria drug, chloroquine. Exp Biol Med. 2014;239(4):489–501.

79. Wang YJ, Di XJ, Mu TW. Using pharmacological chaperones to restore proteostasis. Pharmacol Res. 2014;83:3–9.

80. Leidenheimer NJ, Ryder KG. Pharmacological chaperoning: a primer on mechanism and pharmacology. Pharmacol Res. 2014;83:10–19.

81. Werner HB, Krämer-Albers EM, Strenzke N, et al. A critical role for the cholesterol-associated proteolipids PLP and M6B in

myelination of the central nervous system. Glia. 2013;61(4):567–586.

82. Kramer-Albers EM, Gehrig-Burger K, Thiele C, et al. Perturbed interactions of mutant proteolipid protein/DM20 with cholesterol and lipid rafts in oligodendroglia: implications for dysmyelination in spastic paraplegia. J Neurosci Off J Soc Neurosci. 2006;26(45):11743–11752.

83. Jung-Testas I, Do Thi A, Koenig H, et al. Progesterone as a neurosteroid: synthesis and actions in rat glial cells. J Steroid Biochem Mol Biol. 1999;69(1–6):97–107.

84. Prukop T, Epplen DB, Nientiedt T, et al. Progesterone antagonist therapy in a Pelizaeus–Merzbacher mouse model. Am J Hum Genet. 2014;94(4):533–546.

85. Nave KA. Oligodendrocytes and the "micro brake" of progenitor cell proliferation. Neuron. 2010;65(5):577–579.

86. Wang E, Cambi F. MicroRNA expression in mouse oligodendrocytes and regulation of proteolipid protein gene expression. J Neurosci Res. 2012;90(9):1701–1712.

87. Foust KD, Salazar DL, Likhite S, et al. Therapeutic AAV9-mediated suppression of mutant SOD1 slows disease progression and extends survival in models of inherited ALS. Mol Ther. 2013;21 (12):2148–2159.

88. Prasad VK, Mendizabal A, Parikh SH, et al. Unrelated donor umbilical cord blood transplantation for inherited metabolic disorders in 159 pediatric patients from a single center: influence of cellular composition of the graft on transplantation outcomes. Blood. 2008;112 (7):2979–2989.

89. Beam D, Poe MD, Provenzale, JM et al. Outcomes of unrelated umbilical cord blood transplantation for X-linked adrenoleukodystrophy. Biol Blood Marrow Transplant. 2007;13(6):665–674.

90. Wishnew J, Page K, Wood S, et al. Umbilical cord blood transplantation to treat Pelizaeus-Merzbacher disease in 2 young boys. Pediatrics. 2014;134(5): e1451–7.

91. Gupta N, Henry RG, Strober J, et al. Neural stem cell engraftment and myelination in the human brain. Sci Transl Med. 2012;4(155):155ra137.

* **The first stem cell therapy attempted on patients with PMD.**

92. Okano H, Yamanaka S. iPS cell technologies: significance and applications to CNS regeneration and disease. Mol Brain. 2014;7:22.

93. Osakada F, Hirami Y, Takahashi M. Stem cell biology and cell transplantation therapy in the retina. Biotechnol Genet Eng Rev. 2010;26:297–334.

94. Maeda T, Lee MJ, Palczewska G, et al. Retinal pigmented epithelial cells obtained from human induced pluripotent stem cells possess functional visual cycle enzymes in vitro and in vivo. J Biol Chem. 2013;288(48):34484–34493.

95. Epplen DB, Prukop T, Nientiedt T, et al. Curcumin therapy in a Plp1 transgenic mouse model of Pelizaeus–Merzbacher disease. Ann Clin Transl Neurol. 2015;2 (8):787–796.

96. Ojala DS, Amara DP, Schaffer DV. Adeno-associated virus vectors and neurological gene therapy. Neuroscientist. 2015;21(1):84–98.

97. Wu Z, Asokan A, Samulski RJ. Adeno-associated virus serotypes: vector toolkit for human gene therapy. Mol Ther. 2006;14(3):316–327.

98. Hwu WL, Muramatsu S, Tseng SH, et al. Gene therapy for aromatic L-amino acid decarboxylase deficiency. Sci Transl Med. 2012;4(134):134ra61.

99. Casarosa S, Bozzi Y, Conti L. Neural stem cells: ready for therapeutic applications? Mol Cell Ther. 2014;2:31.

Affiliation

Hitoshi Osaka[†1] & Ken Inoue[2]

[†]Author for correspondence

[1]Department of Pediatrics, Jichi Medical School, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi 329-0498, Japan

Tel: (81)-285-58-7366;

Fax: (81)-285-44-6123;

E-mail: hosaka@jichi.ac.jp

[2]Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

ELSEVIER

Brain & Development 38 (2016) 581–584

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Case Report

A splicing mutation of proteolipid protein 1 in Pelizaeus-Merzbacher disease

Taku Omata [a], Jun-ichi Nagai [b], Hiroko Shimbo [c], Shiro Koizume [c,d,e], Yohei Miyagi [e], Kenji Kurosawa [d], Sumimasa Yamashita [c], Hitoshi Osaka [c,e,f,*], Ken Inoue [g]

[a] *Division of Child Neurology, Chiba Children's Hospital, Chiba, Japan*
[b] *Laboratory Medicine, Clinical Research Institute, Kanagawa Children's Medical Center, Yokohama 232-855, Japan*
[c] *Division of Neurology, Clinical Research Institute, Kanagawa Children's Medical Center, Yokohama 232-855, Japan*
[d] *Division of Genetics, Clinical Research Institute, Kanagawa Children's Medical Center, Yokohama 232-855, Japan*
[e] *Molecular Pathology and Genetics Division, Kanagawa Cancer Center Research Institute, Yokohama 241-0815, Japan*
[f] *Department of Pediatrics, Jichi Medical School, Shimono, Japan*
[g] *Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Japan*

Received 29 August 2015; received in revised form 2 December 2015; accepted 3 December 2015

Abstract

A patient with an unusually mild form of Pelizaeus-Merzbacher disease was studied. Clinically, mild developmental delay with acquisition of assisted walking at 16 months and mild spastic tetraplegia were evident, but no nystagmus, cerebellar, or extrapyramidal signs were present. *PLP1* mutation analysis revealed a nucleotide substitution adjacent to the acceptor site of intron 3, NM_000533.4:c.454-9T>G. Expression analysis using the patient's leukocytes demonstrated an additional abnormal transcript including the last 118 bp of intron 3. *In silico* prediction analysis suggested the reduction of wild-type acceptor activity, which presumably evokes the cryptic splicing variant. Putative cryptic transcript results in premature termination, which may explain the mild clinical phenotype observed in this patient.

Keywords: *PLP1*; *DM20*; Hypomyelinating leukodystrophy; Intronic mutation

1. Introduction

Pelizaeus-Merzbacher disease (PMD) is a rare X-linked recessive disorder caused by mutations of the *PLP1* gene, which encodes a major myelin membrane protein in the central nervous system [1]. Consequently, PMD is characterized by arrest of myelination that clinically results in psychomotor developmental delay and various neurological symptoms including nystagmus, spastic tetraplegia, dystonia, and cerebellar and extrapyramidal symptoms. One characteristic feature of PMD is the wide spectrum of clinical severity. Patients with the most severe form show essentially no achievement in developmental milestones, while those at the mildest end attain unsupported walking with well-preserved intellectuality.

Different *PLP1* mutations give rise to PMD [1]. Genomic duplication is the most common mutation, accounting for 60–70% of patients, while exonic or

* Corresponding author at: Department of Pediatrics, Jichi Medical School, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi 329-0498, Japan. Tel.: +81 285 58 7366; fax: +81 285 44 6123.
E-mail address: hosaka@jichi.ac.jp (H. Osaka).

http://dx.doi.org/10.1016/j.braindev.2015.12.002
0387-7604/