診断を進める上で遺伝カウンセリングは不可欠である。先天性大脳白質形成不全症は遺伝的異質性が高いため、遺伝カウンセリングは慎重に行う。ペリツェウス・メルツバッハ病を例にとると、X連鎖劣性遺伝であることのほかに、発症メカニズムが重複（タンデム、転座挿入）や点変異、ナル変異など多岐にわたり複雑であることがわかる。また、遺伝医療の専門家（臨床遺伝専門医および認定遺伝カウンセラー）も加わったチーム医療を考慮する必要がある。

資料４

PLP1 遺伝子関連先天性大脳白質形成不全症（Pelizaeus-Merzbacher 病、２型痙性対麻痺）の遺伝カウンセリングガイドライン

平成 25 年 12 月　改訂版

厚生労働科学研究費補助金　難治性疾患等克服研究事業（難治性疾患克服研究事業）

「先天性大脳白質形成不全症の診断と治療を目指した研究」

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はじめに

遺伝カウンセリングとは、疾患の特性、遺伝様式、疾患がもつ意味を患者とその家族に伝え、理解を促し、医学的かつ個人的な決断が下せるように援助するプロセスを意味する。疾患がもつ意味は、それぞれの個人により捉え方が異なり、また、文化や宗教の影響も受ける。わが国においては、臨床遺伝専門医および認定遺伝カウンセラーが専門職として遺伝カウンセリングに関わっている。中枢神経ミエリンを構成する主要タンパクの一つであるプロテオリピドプロテインをコードする *PLP1* 遺伝子の異常は、先天性大脳白質形成不全症の代表である Pelizaeus-Merzbacher 病（PMD）から 2 型痙性対麻痺（SPG2）まで、臨床的に幅広い臨床像をもたらす。これらは、*PLP1* 遺伝子が X 染色体上にあることから、X 連鎖劣性遺伝形式をとる。Pelizaeus-Merzbacher 病も含め先天性大脳白質形成不全症の多くは遺伝性疾患に分類され、多くの場合診断の確定は遺伝学的検査による。発端者の診断が家系内の保因者の存在を示唆することもあり、遺伝カウンセリングが診療の一環として不可欠である。本稿では、Pelizaeus-Merzbacher 病および 2 型痙性対麻痺を含めた *PLP1* 関連先天性大脳白質形成不全症の遺伝カウンセリングを進める際に役立つと思われる情報をガイドラインとしてまとめた。

１．疾患の概要

PLP 1 遺伝子関連の中枢神経系ミエリン形成異常症は、ペリツェウス-メルツバッハー病（Pelizaeus-Merzbacher Disease ; PMD）から痙性対麻痺 2 型（Spastic Paraplegia2 ; SPG2）

まで幅広い表現型を呈す。PMD は一般に乳児期もしくは幼児期早期に眼振、筋緊張低下、発達遅滞で発症し、症状は重度の痙性麻痺や運動失調へと進行する。生命予後も影響を受ける。いっぽうで、SPG2 は痙性対麻痺として発症するものの中枢神経系症状を伴わないこともあり、生命予後は比較的良好である。家族間での症状の違いはあるものの、通常は、家族内での症状は一定である。女性保因者にも軽度から中等度の症状が出現することがある。*PLP1* 関連疾患の臨床診断は、典型的な神経学的所見、X 連鎖の遺伝形式と、MRI で観察されるびまん性のミエリン形成不全の所見などによる。*PLP 1* 遺伝子の分子遺伝学的検査は臨床的に可能である。医療管理としては、対症療法が中心となる。神経内科、理学療法、整形外科、呼吸器内科、消化器内科の専門家からなる包括的医療チームによる対応が必要である。治療内容として、重症な嚥下困難に対する胃瘻増設や、痙攣に対する抗痙攣薬、理学療法や運動、薬物療法による痙直に対する日常管理、歯列矯正、関節拘縮に対する手術などがあげられる。側弯を認める場合、適切な車椅子の使用や理学療法が役に立つが、重症な場合は手術も考慮される。特別支援教育が必要であり、コミュニケーションツールの工夫が効果的なことがある。2 次的合併症の予防として、側弯を防ぐために適切な車椅子の使用や、理学療法が効果的なことがある。小児期には、発達や、痙直、整形外科的合併症などを確認するため、半年から一年ごとの神経学的評価、理学的評価を行う。*PLP1* 関連疾患はX連鎖の遺伝形式をとるが、新生突然変異もあり得る。PMD 男性は妊孕性を得ることは難しいが、SPG2 男性は妊孕性がある。男性発端者の場合、その娘は全て保因者となり、息子には変異は遺伝されない。女性保因者の息子は、50％の確率で変異を受け継ぎ発症するリスクがあり、娘は 50％の確率で保因者となる。病因である *PLP1* 遺伝子の変異が同定された家系内では、保因者診断が可能である。

２．遺伝学的検査の進め方

検査方法の選択と注意点

PLP1 関連疾患をもたらす遺伝子変異としては、点変異、微細な欠失や挿入、大きな重複と欠失などがある。*PLP1* 遺伝子が 3 コピーさらには 4 コピー存在して病因となることもある。

ほぼ５割（50－75％）の症例で *PLP1* 遺伝子重複を認めるので、定量 PCR 法や FISH 法などにより正常の２倍量の *PLP1* の存在を確かめる。MLPA（Multiplex ligation-dependent probe amplification）、マイクロアレイ染色体検査などでも、ゲノムコピー数の変化を検出できるようになり、特に前者は既に PMD 診断用にキットとして販売されている。*PLP1*

の欠失（null mutation）も、2%以下で認められる。*PLP1* のアレルコピー数変化（主に重複）を検出する FISH 法では、重複がタンデムで範囲も平均 500kb 程度なので、間期核 FISH が用いられる。しかし、この方法での観察は熟練を要することや、500kb 以下の重複や 3 重複は正確に判定することが困難なことを考慮に入れる必要があり、間期核 FISH 法での診断スクリーニングは推奨されない。間期核 FISH 法を診断に用いる場合には上述の *PLP1* 遺伝子の定量化解析法（MLPA、定量 PCR、マイクロアレイ染色体検査）と組み合わせる必要がある。さらに、*PLP1* 遺伝子を含む X 染色体領域の染色体転座例や挿入例も報告されているために、FISH 法でタンデム重複でないシグナルパターンが検出された場合は、転座を考慮して通常の染色体 G 分染法も行う。つまり、コピー数変化のみを検出する方法として、MLPA、マイクロアレイ染色体検査、定量 PCR 法はいずれも鋭敏ではあるが、転座は検出できない。逆に、FISH 法はコピー数の変化を検出するには限界があるが、転座・挿入を検出することは可能である。PMD 遺伝子診断の難しさは、このように変異の多様性を念頭に置き、検査を組み合わせる点が上げられる。

PLP1 変異の約 15〜20%を占める点変異は、シーケンス解析が有効である。ほとんどの点変異が結果としてミスセンス変異またはフレームシフト変異となるが、スプライシングに影響を及ぼす変異もある。

検査結果の解釈

検出された変異が実際に発症に関わる変異であるかは慎重に検討する必要がある。一般に、解釈からみた変異の種類として、１）既に文献・データベースで報告されている病原性変異、２）病原性と推測されるがこれまでのデータベース（文献）に登録（報告）がない変異、３）臨床的意義が不明な変異、４）病的意義がないと推測できるがデータベース（文献）に登録（報告）がない変異、５）既に登録（報告）されている臨床的意義のない多型、の 5 種類があげられる。具体的な検索方法として、Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php)、NHLBI Exome Sequencing Project (ESP) Exome Variant Server (http://evs.gs.washington.edu/EVS/)、日本人データベース Human Genetic Variation Browser (http://www.genome.med.kyoto-u.ac.jp/SnpDB/)などでの検索が有用である。

臨床的に *PLP1* 関連疾患と考えられる男性患者のうち、約 40%が *PLP1* 遺伝子内に変異が同定されない。解釈としては、変異が通常解析する領域以外、つまり遺伝子の上流または下流の領域やイントロンなどの領域に変異が起こっているか、*PLP1* 関連疾患に

似た別の疾患を想定する必要がある。

遺伝学的検査計画

最初に*PLP1*遺伝子重複を確認する検査を行う。既に述べたように、定量PCR、MLPA、マイクロアレイ染色体検査は、感度・特異度ともに高いが、間期核FISHは、重複部位が別の領域に挿入されている場合や、重複の体細胞モザイクを同定できるため、やはり望ましい検査法と言える。したがって、*PLP1*のアレルコピー数を調べて異常がある場合には間期核FISHを施行するべきである。重複を認めなかった場合には、*PLP1*遺伝子をシークエンスする。必要であれば、罹患男性に2本のX染色体がないか（クラインフェルター症候群）、もしくは、*PLP1*の発現に位置的効果をきたすようなX染色体の再構成がないかを評価するために、染色体検査を行うべきである。

女性における保因者診断では、まずその家系内の病因となる変異を同定されていることが前提となる。

3．遺伝カウンセリング

1）男性発端者の親

発端者の父親は、変異の保因者ではない。罹患児および1名の別の家系内罹患者を有する女性は、ヘテロ接合（保因者）である。家系解析で発端者が唯一の家系内罹患者であった場合は、その罹患男性の母親は保因者であるか、あるいは、その罹患男性は*PLP1*遺伝子の新生突然変異の可能性がある。実際には、家族歴の有無にかかわらず発端者の母親は殆どの場合、*PLP1*遺伝子変異の保因者である。新生突然変異例は、主に*PLP1*遺伝子の点変異例で報告されてきた。一方、*PLP1*重複変異では、母方祖父の生殖細胞系列で新生突然変異がしばしば見られるが、発端者が新生突然変異であった報告はない。

2）男性発端者の同胞

同胞におけるリスクは、母親が保因者であるか否かにより異なる。*PLP1*変異を有する保因者女性が、児に変異アレルを受け渡す可能性は50%である。変異アレルを受け継いだ男性は罹患する。また、変異アレルを受け継いだ同胞女性は保因者となるが、ときに軽度から中等度の症状を呈することもある。注意すべき点として、罹患男性において比較的軽症の神経学的症状（複雑型、あるいは純粋な痙性対麻痺）をもたらす*PLP1*アレルは、ヘテロ接合の女性での神経学的徴候（遅発性痙性対麻痺や知的退行など）と関連する傾向がある。ヘテロ接合女性が臨床的に罹患する可能性が最も高いのは、兄弟が欠失や早期終止コドンとなる点変異などの*PLP1*ナル（null）変異の場合で、可能性が最も低いのは兄弟が*PLP1*重複の場合である。*PLP1*重複のヘテロ接合女性は偏ったX

染色体不活化状態を有していることが報告されている。

また、性腺モザイクも注意する必要性がある。この場合は、母親の末梢血 DNA で病因となる変異が検出されなくても、発端者の同胞が病因となる変異を受け継ぐ可能性は一定の確率である。

３）男性発端者の子孫

典型的な PMD 男性は子孫を残せないが、SPG2 の患者では妊孕性がある。罹患男性の変異アレルは必ず娘に受け継がれ、息子は受け継ぐことはない。

４）男性罹患者の他の家族

発端者の母方おばやその子孫は、保因者あるいは罹患者になる可能性がある（性や家系内の近さ、発端者の母親が保因者かどうかにもよる）。罹患男性の症状が比較的軽症の場合には、ヘテロ接合女性は神経学的徴候（遅発性痙性対麻痺おなど）を呈することがある。

５）保因者診断

ヘテロ接合女性は一般的には神経学的に正常だが、ときに軽度から中等度の症状を呈することもある。分子遺伝学的検査で保因者診断が可能なのは、*PLP1* の病因変異が罹患家族で同定されている場合か、家系内連鎖解析で明らかになっている場合である。

４．遺伝カウンセリングに関連したその他の問題

１）表現型の多様性

児が罹患者になる可能性があるカップルに、同じ家系内の同胞や血縁者間でもさまざまな表現型（症状）があることを認識させることは重要である。男性罹患者が軽症である家系では、次世代では重症となることもある。

２）Xq22 から離れた座位への挿入重複

もともとの *PLP1* 遺伝子座である Xq22 から離れた染色体部位に新たな挿入が起こって生ずる *PLP1* 重複変異は、*PLP1* 関連疾患の稀な原因となることがあるが、遺伝カウンセリング上難しい問題をもたらす。理由は、遺伝様式として必ずしも X 連鎖でなくなるからである。

３）家族計画

遺伝的リスクを決定し、保因状況を明らかにし、出生前診断の適用を相談する時期は、妊娠前が望ましい。

４）倫理的課題

遺伝学的検査は、十分な遺伝カウンセリングを行った後に実施する。関連したガイドライン（注）を遵守することが求められる。

まとめ

Pelizaeus-Merzbacher 病の遺伝カウンセリングの難しさは、X 連鎖劣性遺伝であることと、発症メカニズムが重複（タンデム、転座挿入）や点変異、ナル変異など多岐にわたり複雑であることが上げられる。適切な遺伝カウンセリングを進めるために正確な診断は不可欠と考えられる。

注）

「遺伝学的検査に関するガイドライン」　遺伝医学関連学会（平成 15 年 8 月）

「医療における遺伝学的検査・診断に関するガイドライン」　日本医学会（2011 年 2 月）

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
髙梨潤一	小児神経疾患	Proton MRS の臨床有用性検討会	Proton MRS の臨床有用性コンセンサスガイド 2013 年度版	日本磁気共鳴医学会	東京	2013	P36-48
山本俊至	遺伝子解析とマイクロアレイ染色体検査	奥村彰久, 浜野晋一郎 編	子どものけいれん・てんかんガイド	中山書店	東京	2013	p89-100

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Morimura T, Numata Y, Nakamura S, Hirano E, Gotoh L, Goto Y, Urushitani M, Inoue K.	Attenuation of endoplasmic reticulum stress in Pelizaeus-Merzbacher disease by an anti-malaria drug, chloroquine.	***Exp Biol Med.***		doi: 10.1177/1535370213520108	2014
Numata Y, Gotoh L, Iwaki A, Kurosawa K, Takanashi J, Deguchi K, Yamamoto T, Osaka H, Inoue K.	Epidemiological, clinical, and genetic landscapes of hypomyelinating leukodystrophies.	***J Neurol.***		DOI : 10.1007/s00415-014-7263-5	2014
Ishii K, Doi T, Inoue K, Okawada M, Lane GJ, Yamataka A, Akazawa C	Correlation between multiple *ret* mutations and severity of hirschsprung's disease.	***Pediatr Surg Int***	29(2)	157-63	2013
Lassuthová P, Zaliová M, Inoue K, Haberlová J, Sixtová K, et al.	Three new PLP1 splicing mutations demonstrate pathogenic and phenotypic diversity of Pelizaeus-Merzbacher disease.	***J Child Neurol.***		doi: 10.1177/0883073813492387.	2013
Matsufuji M, Osaka H, Gotoh L, Shimbo H, Takashima S, Inoue K.	Partial *PLP1* deletion causing X-linked dominant spastic paraplegia type 2	***Pediatr Neurol.***	49(6)	477-81	2013
Numata Y, Morimura T, Nakamura S, Hirano E, Kure S, Goto YI, Inoue K	Depletion of molecular chaperones from the endoplasmic reticulum and fragmentation of the Golgi apparatus associated with	***J Biol Chem***	288(11)	7451-66	2013

	pathogenesis in Pelizaeus-Merzbacher disease.				
Takanashi J, Osaka H, Saitsu H, Sasaki M, Mori H, Shibayama H, Tanaka M, Nomura Y, Terao Y, Inoue K, Matsumoto N, Barkovich J.	Different patterns of cerebellar abnormality and hypomyelination between POLR3A and POLR3B mutations	***Brain Dev.***	36(3)	259-63	2014
Gotoh L, Inoue K, Helman G, Mora S, Maski K, Soul JS, Bloom M, Evans SH, Goto Y, Caldovic L, Hobson GM, Vanderver A.	GJC2 promoter mutations causing Pelizaeus-Merzbacher-like disease.	***Mol Genet Metab***		doi: 10.1016/j.ymgme.2013.12.001	2013
Ravenscroft G, Miyatake S, Lehtokari VL, et al.	Mutations in KLHL40 Are a Frequent Cause of Severe Autosomal-Recessive Nemaline Myopathy.	***Am J Hum Genet***	93	6-18	2013
Anselm I, Azzouz H, Bratkovic D, de Brouwer A, Hamel B et al.	Phenotype and genotype in 101 males with X-linked creatine transporter deficiency.	***J Med Genet***	50	463-472	2013
Mitani T, Aida N, Tomiyasu M, Wada T, Osaka H.	Transient ischemic attack-like episodes without stroke-like lesions in MELAS.	***Pediatr Radiol***	43	1400-1403.	2013
Kato H, Miyake F, Shimbo H, Ohya M, Sugawara H, Aida N, Anzai R, Takagi M, Okuda M, Takano K, Wada T, Iai M, Yamashita S, Osaka H.	Urine screening for patients with developmental disabilities detected a patient with creatine transporter deficiency due to a novel missense mutation in SLC6A8.	***Brain Dev***		doi: 10.1016/j.braindev.2013.08.004.	2013
Nakamura K, Kato M, Osaka H, Yamashita S, Nakagawa E, et al.	Clinical spectrum of SCN2A mutations expanding to Ohtahara syndrome.	***Neurology***	81	992-998	2013
Nakamura K, Kodera H, Akita T, Shiina M, Kato M, et al.	De Novo Mutations in GNAO1, Encoding a Galphao Subunit of Heterotrimeric G Proteins, Cause Epileptic Encephalopathy.	***Am J Hum Genet***	93	496-505	2013
Imagawa E, Osaka H, Yamashita A, Shiina M, Takahashi E, et al.	A hemizygous GYG2 mutation and Leigh syndrome: a possible link?	***Hum Genet***	133(2)	225-34	2014
Ohba C, Osaka H, Iai M, Yamashita S, Suzuki Y, et al.	Diagnostic utility of whole exome sequencing in patients showing cerebellar and/or vermis atrophy in childhood.	***Neurogenetics***	14	225-232	2013
Kodera H, Nakamura K, Osaka H, Maegaki Y, Haginoya K, et al.	De novo mutations in SLC35A2 encoding a UDP-galactose transporter cause early-onset epileptic encephalopathy.	***Hum Mutat.***	34	1708-1714	2013

Ohshiro-Sasaki A, Shimbo H, Takano K, Wada T, Osaka H.	A Three-Year-Old Boy With Glucose Transporter Type 1 Deficiency Syndrome Presenting With Episodic Ataxia.	***Pediatr Neurol***	0(1)	599-100.	2014
Akiyama T, Osaka H, Shimbo H, Nakajiri T, Kobayashi K, Oka M, Endoh F,Yoshinaga H.	A Japanese Adult Case of Guanidinoacetate Methyltransferase Deficiency.	***JIMD Rep.***	12	65-9	2014
Takanashi J, Taneichi H, Misaki T, Yahata Y, Okumura A, Ishida Y, Miyawaki T, Okabe N, Sata T, Mizuguchi M.	Clinical and radiological features of encephalopathy during 2011 E. coli O111 outbreak in Japan.	***Neurology***	82(7)	564-72	2014
Takanashi J, Nitta N, Iwasaki N, Saito S, Tanaka R, Barkovich AJ, Aoki I.	Neurochemistry in Shiverer mouse depicted on MR spectroscopy.	***J Magn Reson Imaging***		doi: 10.1002/jmri.24306.	2013
Yamamoto T, Sato H, Lai PS, Nurputra DK, Harahap NIF, et al.	Intragenic mutations in SMN1may contribute more significantly to clinical severity than SMN2 copy numbers in some spinal muscular atrophy (SMA) patients.	***Brain Dev***		doi: 10.1016/j.braindev.2013.11.009.	2013
Lopez E, Thauvin-Robinet C, Reversade B, Khartoufi NE, Devisme L, et al.	C5orf42 is the major gene responsible for OFD syndrome type VI.	***Hum Genet***	133(3)	367-77	2014
Tada H, Takanashi J.	MR spectroscopy in 18q-syndrome suggesting other than hypomyelination.	***Brain Dev***	36(1)	57-60	2014
Shiihara T, Miyake T, Izumi S, Sugihara S, Watanabe M, Takanashi J, Kubota M, Kato M.	Serum and CSF biomarkers in acute pediatric neurological disorders.	***Brain Dev***		doi: 10.1016/j.braindev.2013.06.011.	2013
Miyake N, Koshimizu E, Okamoto N, Mizuno S, Ogata T, et al.	MLL2 and KDM6A mutations in patients with Kabuki syndrome.	***Am J Med Genet A***	161	2234-2243	2013
Shinohara M, Saitoh M, Nishizawa D, Ikeda K, Hirose S, Takanashi J, et al.	ADOR2A polymorphism predisposes children to encephalopathy with febrile status epilepticus.	***Neurology***	80	1571-1576	2013
Okumura A, Hayashi M, Shimojima K, Ikeno M, Uchida T, Takanashi J, et al.	Whole-exome sequencing of a unique brain malformation with periventricular heterotopia, cingulate polymicrogyria, and midbrain tectal hyperplasia.	***Neuropathology***	33	553-560.	2013
Sasaki A, Sumie M, Wada S, Kosaki R, Kurosawa K, Fukami M, Sago H, Ogata T, Kagami M.	Prenatal genetic testing for a microdeletion at chromosome 14q32.2 imprinted region leading to UPD(14)pat-like phenotype.	***Am J Med Genet A***	164A(1)	264-6	2014

Mori T, Mori K, Ito H, Goji A, Miyazaki M, Harada M, Kurosawa K, Kagami S.	Age-Related Changes in a Patient With Pelizaeus-Merzbacher Disease Determined by Repeated 1H-Magnetic Resonance Spectroscopy.	***J Child Neurol.***	29(2)	283-8.	2014
Ueda H, Sugiura T, Takeshita S, Ito K, Kakita H, Nagasaki R, Kurosawa K, Saitoh S.	Combination of Miller- Dieker syndrome and VACTERL association causes extremely severe clinical presentation.	***Eur J Pediatr***		Aug 11. [Epub aheadof print]	2013
Ishikawa A, Enomoto K, Tominaga M, Saito T, Nagai J, Furuya N, Ueno K, Ueda H, Masuno M, Kurosawa K.	Pure duplication of 19p13.3.	***Am J Med Genet A.***	161(9)	2300-4.	2013
Aoki Y, Niihori T, Banjo T, Okamoto N, Mizuno S, Kurosawa K, et al.	Gain-of-function mutations in RIT1 cause Noonan syndrome, a RAS/MAPK pathway syndrome.	***Am J Hum Genet.***	93(1)	173-80.	2013
Wada T, Ban H, Matsufuji M, Okamoto N, Enomoto K, Kurosawa K, Aida N.	Neuroradiologic features in X-linked α-thalassemia/mental retardation syndrome.	***AJNR Am J Neuroradiol.***	34(10)	2034-8.	2013
Nagase H, Ishikawa H, Kurosawa K, Furuya N, Itani Y, Yamanaka M.	Familial severe congenital diaphragmatic hernia: left herniation in one sibling and bilateral herniation in another.	***Congenit Anom.***	53(1)	54-7.	2013
Yamamoto T, Shimojima K:	Pelizaeus-Merzbacher disease as a chromosomal disorder.	***Congenit Anom***	53	3-8	2013
Shimada S, Okamoto N, Ito M, Arai Y, Momosaki K, Togawa M, Maegaki Y, Sugawara M, Shimojima K, Osawa M, Yamamoto T	MECP2 duplication syndrome in both genders.	***Brain Dev***	35	411-419	2013
Okumura A, Shimojima K, Kubota T, Abe S, Yamashita S, Imai K, Okanishi T, Enoki H, Fukasawa T, Tanabe T, Dibbens LM, Shimizu T, Yamamoto T	PRRT2 mutation in Japanese children with benign infantile epilepsy.	***Brain Dev***			In press
Shimada S, Maegaki Y, Osawa M, Yamamoto T	Mild developmental delay and obesity in two patients with mosaic 1p36 deletion syndrome.	***Am J Med Genet***			In press

Yamamoto T, Togawa,M, Shimada S, Sangu N, Shimojima K, Okamoto N	Narrowing of the responsible region for severe develop-mental delay and autistic behaviors in WAGR syndrome down to 1.6 Mb including PAX6, WT1, and PRRG4.	***Am J Med Genet***			
Ishii A, Shioda M, Okumura A, Kidokoro H, Sakauchi M, Shimada S, Shimizu T, Osawa M, Hirose S, Yamamoto T	A recurrent KCNT1 mutation in two sporadic cases with malignant migrating partial seizures in infancy.	***Gene***	531	467-71	2013
Sangu N, Shimojima K, Shimada S, Ando T, Yamamoto T	Growth patterns of patients with 1p36 deletion syndrome.	***Congenit Anom***			In press
Eto K, Sakai N, Shimada S, Shioda M, Ishigaki K, Hamada Y, Shinpo M, Azuma J, Tominaga K, Shimojima K, Ozono K, Osawa M, Yamamoto T	Microdeletions of 3p21.31 characterized by developmental delay, distinctive features, elevated serum creatine kinase levels, and white matter involvement.	***Am J Med Genet***	161A	3049-56	2013
Okamoto N, Ohmachi K, Shimada S, Shimojima K, Yamamoto T	109 kb deletion of chromosome 4p16.3 limited to WHSCR2 in a patient with mild phenotype of Wolf-Hirschhorn syndrome.	***Am J Med Genet***	161A	1465-9	2013
Yamamoto T, Shimada S, Shimojima K	Fiber-FISH analyses as a diagnostic application for orientation of the microduplications.	***World J Med Genet***	3	5-8	2013
Okumura A, Hayashi M, Tsurui H, Yamakawa Y, Abe S, Kudo T, Suzuki R, Shimizu T, Yamamoto T	Lissencephaly with marked ventricular dilation, agenesis of corpus callosum, and cerebellar hypoplasia caused by TUBA1A mutation.	***Brain Dev***	35	274-279	2013
Shichiji M, Ito Y, Shimojima K, Nakamu H, Oguni H, Osawa M, Yamamoto T	A cryptic microdeletion including MBD5 occurring within the breakpoint of a reciprocal translocation between chromosomes 2 and 5 in a patient with developmental delay and obesity.	***Am J Med genet A***	161A	850-855	2013
Kobayashi S, Inui T, Wakusawa K, Tanaka S, Nakayama T, Uematsu M, Takayanagi M, Yamamoto T, Haginoya K	A case of atypical benign partial epilepsy with action myoclonus.	***Seizure***	22	242-245	2013

Yamamoto T, Matsuo M, Shimada S, Sangu N, Shimojima K, Aso S, Saito K:	De novo triplication of 11q12.3 in a patient with developmental delay and distinctive facial features.	***Mol Cytogenet***	6	15	2013
Shimojima K, Shimada S, Sugawara M, Yoshikawa N, Niijima S, Urao M, Yamamoto T	Challenges in genetic counseling because of intra-familial phenotypic variation of oral-facial-digital syndrome type 1.	***Congenit Anom***	53	155-159	2013
Usui D, Shimada S, Shimojima K, Sugawara M, Kawasaki H, Shigematu H, Takahashi Y, Inoue Y, Imai K, Yamamoto T	Interstitial duplication of 2q32.1-q33.3 in a patient with epilepsy, developmental delay, and autistic behavior.	***Am J Med Genet A***	161A	1078-1084	2013
Sangu N, Shimosato T, Inoda H, Shimada S, Shimojima K, Ando T, Yamamoto T:	A novel nucleotide mutation leading to a recurrent amino acid alteration in SH3BP2 in a patient with cherubism.	***Congenit Anom***	53	166-169	2013
Okumura A, Hayashi M, Shimojima K, Ikeno M, Uchida T, Takanashi J, Okamoto N, Hisata K, Shoji H, Saito A, Furukawa T, Kishida T, Shimizu T, Yamamoto T:	Whole-exome sequence for a unique brain malformation with periventricular heterotopia, cingulate polymicrogyria, and midbrain tectal hyperplasia.	***Neuropathology***	33	553-560	2013
Okamoto N, Ohmachi K, Shimada S, Shimojima K, Yamamoto T:	101 kb deletion of chromosome 4p16.3 limited to WHSCR2 in a patient with mild phenotype of Wolf-Hirschhorn syndrome.	***Am J Med Genet A***	161A	1465-1469	2013
Shimada S, Okamoto N, Nomura S, Fukui M, Shimakawa S, Sangu N, Shimojima K, Osawa M, Yamamoto T	Microdeletions of 5.5 Mb (4q13.2-q13.3) and 4.1 Mb (7p15.3-p21.1) associated with a saethre-chotzen-like phenotype, severe intellectual disability, and autism.	***Am J Med Genet A***	161A	2078-2083	2013
Shimojima K, Tanaka R, Shimada S, Sangu N, Nakayama J, Iwasaki N, Yamamoto T:	A novel homozygous mutation of GJC2 derived from maternal uniparental disomy in a female patient with Pelizaeus–Merzbacher-like disease.	***J Neurol Sci***	330	123-126	2013
Abe Y, Kobayashi S, Wakusawa K, Tanaka S, Inui T, Yamamoto T, Kunishima S, Haginoya K:	Bilateral periventricular nodular heterotopia with megalencephaly: a case report.	***J Child Neurol***			in press
Shimojima K, Shimada S, Tamasaki A, Akaboshi S, Komoike Y, Saito A, Furukawa T, Yamamoto T	Novel compound heterozygous mutations of POLR3A revealed by whole-exome sequencing in a patient with hypomyelination.	***Brain Dev***			in press

研究成果の刊行物・別刷（抜粋）

Molecular Genetics and Metabolism xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

GJC2 promoter mutations causing Pelizaeus–Merzbacher-like disease

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ARTICLE INFO

Article history:
Received 16 October 2013
Received in revised form 3 December 2013
Accepted 3 December 2013
Available online xxxx

Keywords:
Leukodystrophy
Glia
Myelin
GJC2
Pelizaeus–Merzbacher

ABSTRACT

Objective: Pelizaeus–Merzbacher-like disease is a rare hypomyelinating leukodystrophy caused by autosomal recessive mutations in *GJC2*, encoding a gap junction protein essential for production of a mature myelin sheath. A previously identified *GJC2* mutation (c.-167G > A) in the promoter region is hypothesized to disrupt a putative SOX10 binding site; however, the lack of additional mutations in this region and contradictory functional data have limited the interpretation of this variant.
Methods: We describe two independent Pelizaeus–Merzbacher-like disease families with a novel promoter region mutation and updated in vitro functional assays.
Results: A novel *GJC2* mutation (c.-170G > A) in the promoter region was identified in Pelizaeus–Merzbacher-like disease patients. In vitro functional assays using human *GJC2* promoter constructs demonstrated that this mutation and the previously described c.-167G > A mutation similarly diminished the transcriptional activity driven by SOX10 and the binding affinity for SOX10.
Interpretation: These findings support the role of *GJC2* promoter mutations in Pelizaeus–Merzbacher-like disease. *GJC2* promoter region mutation screening should be included in the evaluation of patients with unexplained hypomyelinating leukodystrophies.

1. Introduction

Hypomyelinating leukodystrophies are a rare cause of disease of the central nervous system (CNS) characterized by abnormal myelin formation [1]. The prototype condition for hypomyelinating leukodystrophies is Pelizaeus–Merzbacher disease (PMD) (OMIM 312080), an X-linked condition [2] that is due to a mutation in the proteolipid protein 1 gene (*PLP1*) (OMIM 300401). Pelizaeus–Merzbacher-like disease (PMLD) (OMIM 608804) is a clinically similar disease without detectable abnormalities within the *PLP1* gene. PMLD is instead an autosomal recessive hypomyelinating leukodystrophy that was shown to be caused by mutations in the gap junction protein gamma-2 gene (*GJC2*) (OMIM 608803) that encodes the connexin 47 protein (Cx47), a connexin family member and gap junction protein important in astrocytes and oligodendrocytes [3,4]. Mutation of *GJC2* does not allow Cx47 to reach the membrane, resulting in loss of function [5]. Additionally, the *GJC2* promoter region contains SOX10 transcriptional factor binding sites, which allow for SOX10 to play a role in myelin formation [5].

More than twenty different coding mutations have so far been identified in the *GJC2* coding region [2,4,6–11]. An additional mutation, c.-167A > G, was identified in the putative promoter region in individuals with the phenotype of PMLD [3,5,12,13]. This promoter mutation was first identified in the homozygous state, has now been reported in 15 individuals from 5 families [3,5,12,13], and has additionally been found in two patients [12] in the heterozygous state with another previously published mutation [7] within the coding sequence of *GJC2*. There is evidence suggesting that some c.-167A > G cases arose from a single founder [3,13] and this mutation is thought to account for nearly a third of *GJC2*-PMLD phenotypes [13]. *GJC2* mutations account overall for only 10% of unsolved cases of hypomyelination, suggesting that mutations in *GJC2* and its promoter region at the SOX10 binding site are a rare cause of this phenotype [6]. Mutation c.-167A > G was demonstrated to result in decreased SOX10 dependent transcription of the luciferase reporter gene in constructs containing mouse *Gjc2* promoter region [5]. However,

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1096-7192/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.ymgme.2013.12.001

previous studies using the human *GJC2* regulatory region did not show that the c.-167A > G mutation disrupts the SOX10-dependent transcription [12].

Here we present two PMLD-affected individuals with a novel homozygous mutation (c.-170A > G) in the SOX10 binding site within the *GJC2* regulatory region. We also demonstrate that both the c.-167A > G and c.-170A > G mutations reduce transcription of human *GJC2* using a new luciferase reporter assay. Together, these studies further our understanding of the underlying causes of PMLD and the role of SOX10 in regulation of *GJC2* in CNS myelin formation.

2. Materials and methods

2.1. Clinical material collection and evaluation

Individual 1 and his unaffected brother were identified prospectively, as part of evaluation of individuals with unsolved leukodystrophies in the IRB-approved Myelin Disorders Bioregistry Project at Children's National Medical Center. Individual 2 and her unaffected family members were enrolled in an IRB-approved research protocol at Nemours Alfred I. duPont Hospital for Children. Affected individuals were examined by author AV (1) and authors JSS and KM (2). Informed consent was obtained. PMLD molecular diagnostic testing was performed in the Molecular Diagnostics Laboratory at the Nemours Alfred I. duPont Hospital for Children.

2.2. Clinical scoring of PMLD affected by promoter region mutations

Existing literature was reviewed for cases of *GJC2* promoter region mutations, and where clinical data was sufficient, cases were scored according to the motor function score previously used in the PMD and PMLD patient groups [1,14].

2.3. Bioinformatic analysis of GJC2 promoter region

The UCSC human genome browser was queried to identify *GJC2* on chromosome 1, intron–exon boundaries, sequence conservation and predicted transcription factor binding sites. Transcription start site (TSS) database [15–17] was queried with the *GJC2* UniGene identifier Hs.100072 to identify *GJC2* TSS. Additional TSS were identified by aligning human *GJC2* ESTs with the human genome (Supplement 1) [18].

2.4. In vitro functional analyses of mutations in the GJC2 promoter

We generated a luciferase reporter plasmid pGL4-hGJC2_SS-WT, which contains a 384 bp DNA fragment from human *GJC2* promoter. Mutations c.-167A > G and c.-170A > G were introduced at the putative SOX binding site to evaluate their effect on the *GJC2* promoter activity. Human SOX10 expression plasmid pCDNA-hSOX10, and luciferase reporter plasmids were co-transfected into human glioblastoma cells, U138, or human embryonic kidney cells, HEK239 (see Supplementary Materials and Methods). SOX10 binding ability of *GJC2* promoter regions was determined using electro-mobility shift assay (EMSA), as previously described [5] (Supplement 1).

3. Results

3.1. Clinical data

Case histories are detailed in supplemental data (Supplement 2) and in Table 1. Both affected individuals presented in the newborn period with congenital nystagmus, and had significant motor delays in early childhood. Neither individual gained independent walking, but both walked supported for some time. Receptive language appeared relatively spared, as with cognition, but expressive language was severely limited by dysarthria. Both individuals had a combination of pyramidal and extrapyramidal motor abnormalities and abnormal oculomotor function on physical examination. In both cases, neuroimaging using MRI (Figs. 1A and B) demonstrates increased T2 and isointense T1 white matter signal consistent with hypomyelination, along with the brainstem signal abnormalities classically seen in PMLD [19].

Table 1
Clinical features of affected individuals.

Finding	Individual 1	Individual 2
Gender	Male	Female
Ethnicity	Polish descent	Portuguese descent
Age at presentation	Newborn	Newborn
Current age	39 years	9 years
Presenting sign	Congenital nystagmus	Congenital nystagmus
Initial motor development	Crawling at 19 months and cruising by 24 months. He never established independent walking	At age 6 years she was unable to sit or stand without assistance even though she could commando crawl
Onset of motor deterioration	Gradual deterioration in early childhood related to spasticity	No episodes of deterioration
Loss of supported walking ability	Loss of supported walking around 4–5 years	At age 9 years still could ambulate with a walker with truncal support
Spasticity	+	+
Rigidity	+	+
Dystonia	+	+
Ataxia	−	+
Tremor	−	+
Choreoathetosis	−	−
Dysarthria	+	+
Scoliosis	+	+
Oculomotor abnormalities	Rotatory nystagmoid eye movements	Impaired smooth pursuit, continuous, pendular nystagmus and intermittent exotropia
Vision	Severe myopia since early childhood	Severe myopia and astigmatism since early childhood
Hearing	Normal	Normal
Cognitive decline	+	NA
Language development	At 9 years of age, he was reported to use 3 to 4 word sentences, but that ability was diminished over time by dysarthria. Currently only able to say yes or no.	At 9 years she had 6 sign language words and was able to say 2 words with dysarthric speech.
Epilepsy	+ mild and easily controlled	none

Diagnostic testing of affected individuals 1 and 2, including sequence analysis of *GJC2*, identified a homozygous variant (c.-170A > G) in the regulatory region of *GJC2*. Parental testing of the region for both individuals was not available, though an unaffected brother of individual 1 was heterozygous for the variant.

Clinical data from patients with *GJC2* promoter region mutations in the literature suggest that promoter region mutations may result in a milder phenotype than that commonly reported for PMLD patients in general [14]. Patients with c.-167A > G mutations achieved walking with support (three cases) or autonomous walking (13 cases) [3,5,12,13]. though in most cases independent ambulation was ultimately lost. In one case data was insufficient to determine best motor function. Our two patients, who achieved walking with support, but never walked independently, are similar to these previously reported cases.

3.2. Bioinformatic analysis of the GJC2 regulatory region

Bioinformatic tools were used to determine the location of the c.-170A > G variant in the human *GJC2* gene. The human *GJC2* gene

Please cite this article as: L. Gotoh, et al., GJC2 promoter mutations causing Pelizaeus–Merzbacher-like disease, Mol. Genet. Metab. (2013), http://dx.doi.org/10.1016/j.ymgme.2013.12.001

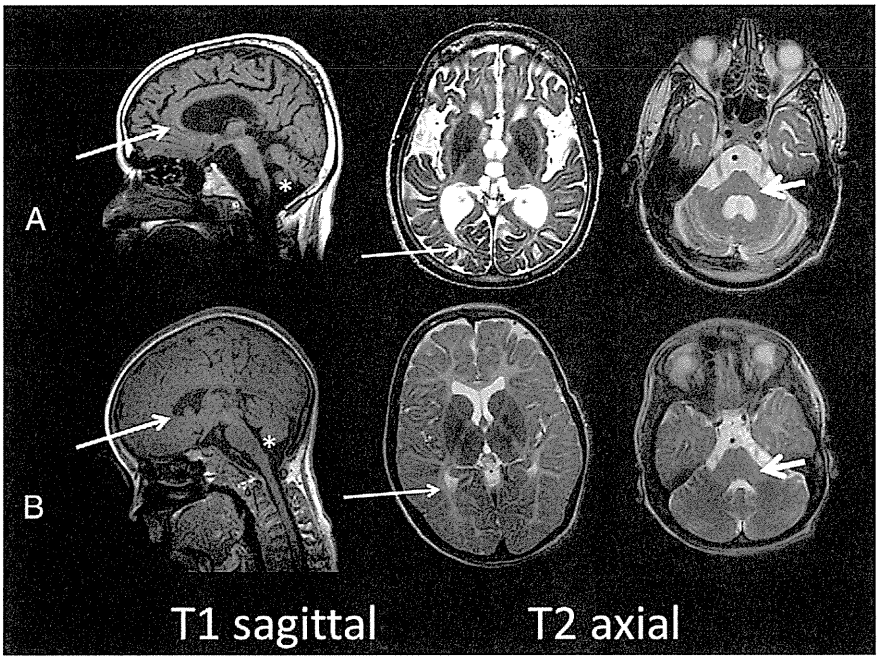

Fig. 1. Imaging results. A. Neuroimaging of individual 1 at age 32 revealed severe loss of the cerebral periventricular white matter with associated prominence of the supratentorial sulci (thin arrow T2 Axial) with a diffusely thin corpus callosum (thin arrow T1 sagittal). There was, to a lesser degree, volume loss of the cerebellum with associated mild prominence of the sulci (asterisk). There was T2 hyperintensity of the residual cerebral white matter diffusely suggesting a persistent deficit of myelin. B. A brain MRI performed on individual 2 at 2 years of age in Brazil reportedly showed abnormal signal in the white matter, thalamus and basal ganglia. Brain MRI scans at age 8 years showed T2 prolongation in the white matter throughout the entire brain (thin arrow T2 Axial) and brainstem (T2 axial thick arrow), with a diffusely thin corpus callosum (thin arrow T1 sagittal). Spectroscopy demonstrated an increased N-acetyl aspartate (NAA) to choline/creatine peak (not shown) in affected white matter. The cerebral and cerebellar sulci were mildly prominent, with no significant change over the two-year interval (asterisk).

spans approximately 19 kb and consists of two exons. Exon 1 is non-coding while exon 2 contains part of the 5′-UTR, the entire connexin 47 coding sequence and the 3′-UTR. The c.-170A > G variant resides within non-coding exon 1 (Fig. 2). Human *GJC2* exon 1 may contain two Sox10 transcription factor binding sites [5,20,21], as predicted in the mouse *Gjc2* gene, as well as various positions of predicted transcription start sites (TSS) (Figs. 2B–D). Both the c.-170A > G variant, identified in this study, and the previously identified c.-167A > G mutation [5] reside in the region of the human gene that corresponds to one of the two mouse Sox10 transcription factor binding sites (Figs. 2C–D and Supplemental Fig. 1), which are highly conserved in mammals. Positions of the c.-170A > G and c.-167A > G variants within predicted Sox10 binding site and relative to predicted TSS (Figs. 2B–D) suggest that these two variants reside either in the promoter or in the 5′-UTR of the human *GJC2* gene. The c.-170A > G variant was not found in either the latest release of dbSNP (version Build 139, Oct 25 2013) or in sequences of 1094 genomes sequenced in the 1000 Genomes Project.

3.3. Luciferase assays

We utilized a 384 bp minimal *GJC2* promoter construct to determine the effect of mutations in the putative SOX10 binding site using U138 cells, which do not express endogenous SOX10. We observed increased activation of the wild-type *GJC2* promoter activity in U138 cells when SOX10 is co-expressed. When c.-167A > G and c.-170A > G mutations were introduced, this activation was significantly diminished to a level similar to the mutD construct in which one of the two SOX10 binding sites was completely disrupted (Fig. 3A). We also observed similar results in HEK293 cells (Supplemental Fig. 2). These findings suggested that both c.-167A > G and c.-170A > G mutations are similarly deleterious. It should be noted that the use of non-glial HEK293 cells resulted in much smaller fold changes than in U138 cells, and the relative activity of c.-167A > G mutant was not significantly different from WT when it was compared to basal activity of WT promoter construct (Supplemental Fig. 1). We also tested some longer promoter constructs which extended to the upstream region, including one that Combes et al. reported[12] (Supplemental Fig. 3). However, we did not observe efficient up-regulation of transcriptional activities of those promoters in the presence of SOX10, as reported.[12]

3.4. EMSA

The effect of mutations on the SOX10 binding ability was determined by EMSA. A 33 bp probe containing wild type human SOX10 binding site showed strong SOX10 binding (Figs. 3B, C), as observed for the corresponding region of the mouse *Gjc2* promoter [5]. Addition of SOX10 antibody revealed a super shifted band, indicating specificity of the binding. Both c.-167A > G and c.-170A > G mutations completely abolished SOX10 binding to the mutated SOX10 binding sites, similar to the absence of SOX10 binding to its mutated consensus binding site (mutD).

4. Discussion

In this report, we identified a new c.-170 A > G mutation in the *GJC2* promoter region in two independent families with PMLD. The mutation is located in the putative SOX10 binding site, which lies within a highly conserved segment and is predicted to play an important role in *GJC2* transcription. Previously, another promoter mutation, c.-167A > G, was identified in multiple families. Although the c.-167A > G mutation was shown to be deleterious using well-studied mouse promoter constructs, another functional study using human promoter constructs revealed that this alteration had no effect on transcription activity, leading to contradictory interpretation on the effect of the c.-167A > G sequence change. There had been only one promoter mutation, c.-167A > G, reported to cause PMLD, leading to ambiguous interpretation of this alteration. The identification of the c.-170A > G mutation, yet another alteration that putatively disrupt the same SOX10 binding site, in two patients from two families with PMLD, is supportive of the pathogenic role of these sequence alterations in PMLD.

Please cite this article as: L. Gotoh, et al., GJC2 promoter mutations causing Pelizaeus–Merzbacher-like disease, Mol. Genet. Metab. (2013), http://dx.doi.org/10.1016/j.ymgme.2013.12.001

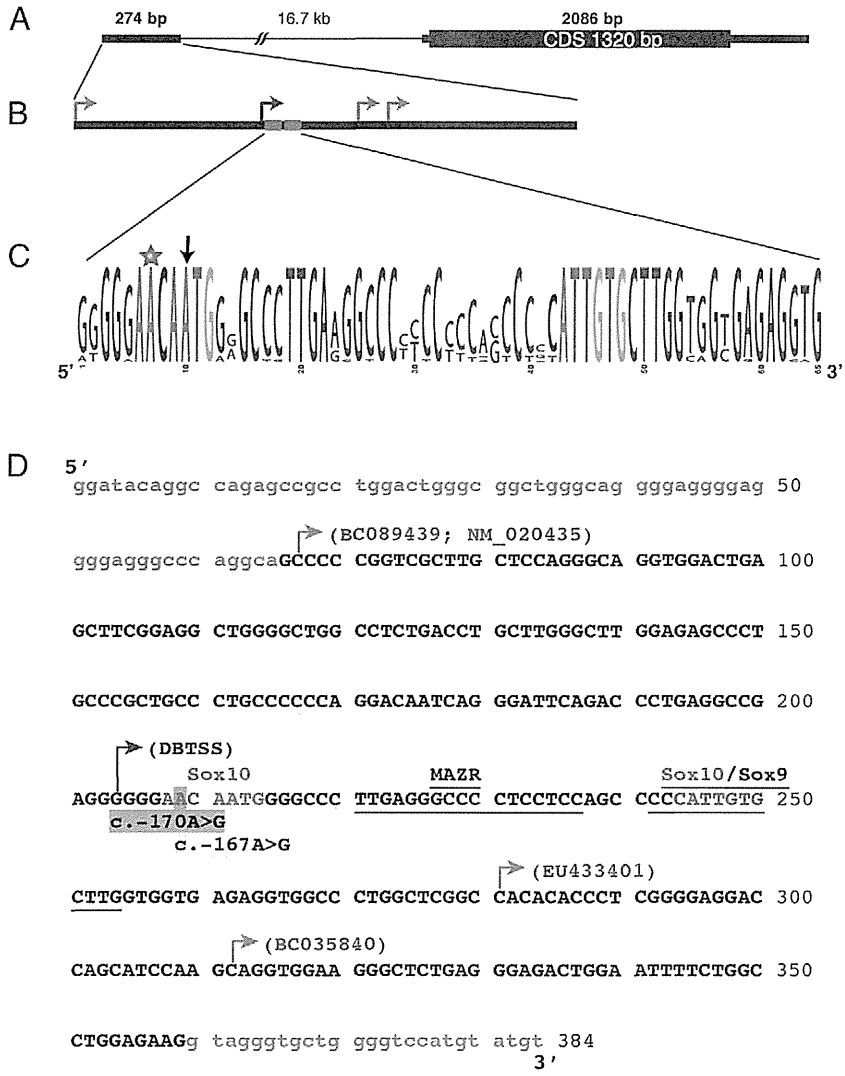

Fig. 2. Map of the human GJC2 gene, positions of the c.-170A > G and c.167A > G variants, and transcription factor binding sites. A. Two exons (solid boxes) and an intron (line) of the human GJC2 gene. Sizes of exons, an intron and coding sequence (CDS) are shown. B. Map of the non-coding exon 1. Red boxes indicate positions of two predicted Sox10 binding sites in the mouse *Gjc2* gene. Angled arrows indicate predicted Transcription start sites (TSS); Purple arrows are TSS predicted in the database of TSS (DBTSS); Green arrows are TSS predicted based on the alignment of human GJC2 ESTs. Locations of TSS were determined based on sequencing of whole length mRNA in the TSS database and on the alignment of human *GJC2* ESTs with genomic sequence in mice. C. LOGO alignment of sequences from 16 mammals (see S1) that include two Sox10 binding sites and flanking sequences. Sox10 binding sites are shown in colored letters. Variant c.-170A > G is indicated with the star. Variant c.-167A > G is indicated with down-arrow. The size of letters in the alignment corresponds to the degree of conservation at that position in the alignment. All sequences from 16 mammals have an A at positions that correspond to the c.-170A > G and c.-167A > G variants in the human *GJC2* gene. D. Sequence of exon 1 of the human GJC2 gene (upper case) and its flanking sequences (lower case and gray typeface). Predicted TSS are shown as angled arrows. Accession numbers of corresponding human GJC2 ESTs are in parenthesis. Two predicted Sox10 binding sites are shown in red. Predicted Sox9 and MAZR transcription factor binding sites are underlined (from the transcription factor binding site track of the UCSC genome browser). Variants c.-170A > G and c.-167A > G are highlighted in green and yellow, respectively. In addition to the two predicted Sox10 binding sites, *GJC2* also has predicted Sox9 and MAZR transcription factor binding sites (Fig. 2D).

The clinical picture in these two individuals is similar to that previously described in PMLD caused by promoter region mutations. Published PMLD patients with promoter region mutations appear to have a milder course than PMLD overall [14] with the greatest majority achieving independent ambulation, and all achieving at least supported walking, though this is typically later lost. There are currently too few patients, however, to know whether there is a correlation between promoter region mutations and a milder disease course and this requires further study.

In order to clarify the role of *GJC2* promoter mutations, we performed functional assays of the two mutations, the c.-170A > G identified in this study and the c.-167A > G, using a minimal promoter construct to study the transcription activity of the human promoter. We demonstrated that both mutations similarly reduced the transcription activity in the human promoter in U138 glioblastoma. The reduced, but not completely abolished transcription activity may allow low level of Cx47 expression, presumably resulting in a relatively mild phenotype in these two patients [5]. In our in vitro transcriptional assays, we utilized a 384 bp minimal promoter fragment, which was markedly activated in the presence of SOX10. The use of the pGL4 reporter system, which eliminated non-specific binding of transcription factors to the vector backbone, may have improved detection of specific transcriptional activities. Longer constructs containing the upstream region, including one that Combes et al. reported [12], did not show efficient up-regulation of transcriptional activities in the presence of SOX10 (supplemental Fig. 2). The reason for this difference is unknown, but it is possible that these upstream regions may contain repressor binding elements that diminish the transcriptional activity in the cell lines utilized in our studies. Further studies will be necessary to clarify this point. In conclusion, c.-170A > G and -167A > G mutations disrupt the SOX10 binding to the human *GJC2* promoter, which facilitates normal transcriptional activation of *GJC2*, presumably resulting in reduced

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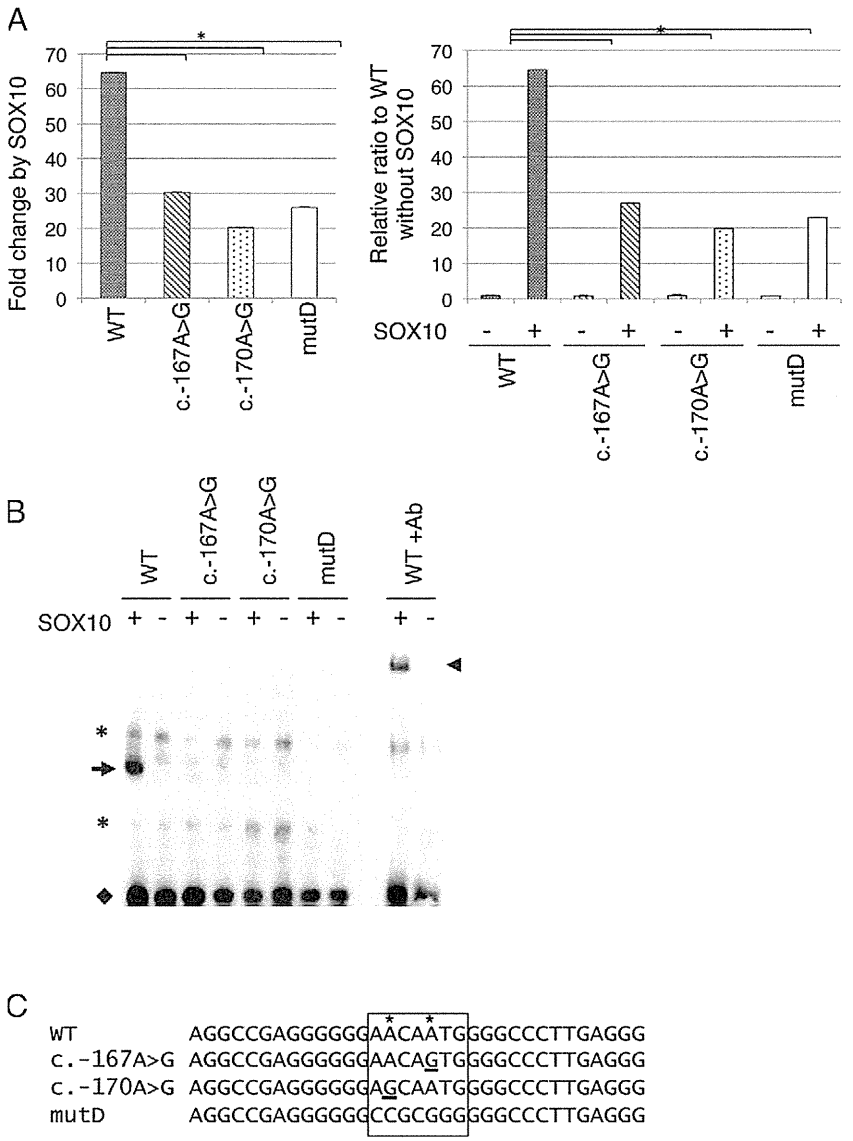

Fig. 3. *In vitro* functional assays of GJC2 mutations. A. Transcription activities determined by luciferase reporter assays in U138 cells. Left panel shows fold changes of transcriptional activity of each promoter construct between co-transfection of pCDNA-hSOX10 and an empty plasmid, pCDNA3.1. Right panel shows luciferase activities as the relative ratio above the mean activity from transfections with WT promoter and an empty plasmid, which was arbitrarily set as 1. A renilla luciferase reporter plasmid pRL (Promega) was used as an internal control to normalize transfection efficiency. The asterisk indicates the statistical significance of $p < 0.001$ by ANOVA. The experiment was repeated three times with 3 independent samples per experiment and representative results from one experiment are shown. B. EMSA showing SOX10 binding to each probe tested, as listed in C, in the presence (+) and absence (−) of SOX10. Arrow indicates the specific binding of SOX10 to the wild-type (WT) probe. Arrowhead indicates supershift by addition of anti-SOX10 antibody. Rhombus indicates free probes. Asterisks indicate nonspecific bands. The experiment was repeated three times and a representative result is shown. C. Oligo probes used in B. A square indicate putative SOX10 binding site. Asterisks show the position of mutations and nucleotide alterations are underlined.

expression of Cx47 that is important for the maintenance of myelinating oligodendrocytes.

This study has provided two important findings that demonstrate that the *GJC2* promoter mutations can cause PMLD. First, we identified a second alteration in the SOX10 binding site, c.-170A > G, in PMLD patients. Second, functional assays of these two mutations using a minimal promoter construct demonstrated that both mutations similarly reduced the transcription activity in the human promoter (Fig. 3). Together with high conservation in mammalian species (Fig. 2), these findings suggest that both PMLD-causing mutations in the SOX10 binding site are likely deleterious and probably result in a down-regulation of the *GJC2* promoter activity. These two findings together provide genetic confirmation of pathogenicity of mutations in the SOX10 binding site in the *GJC2* promoter region as causative of PMLD. *GJC2* promoter region mutation screening should therefore be included in the evaluation of patients with unexplained hypomyelinating leukodystrophies.

Authorship and contributions

Leo Gotoh, Ken Inoue, Grace Hobson and Adeline Vanderver took primary responsibility for writing and revising the paper. Leo Gotoh, Yu-ichi Goto and Ken Inoue performed experiments on promoter region function. Guy Helman, Sara Mora, Kiran Maski, Janet S. Soul, Miriam Bloom, Sarah Evans and Adeline Vanderver examined, counseled and provided patient specific information, Ljubica Caldovic and Grace M. Hobson provided critical review of sequencing results and in silico analyses.

Conflict of interest

Sara Mora and Dr. Grace Hobson report that they provide some services for A. I. duPont Molecular Diagnostics Laboratory. They could not determine whether a third party paid for diagnostic testing of the patients in the work under consideration. In any case, the patients

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were recruited into the study after the diagnostic testing was done (conception and planning of the study was done after the diagnostic testing), but the paper mentions the diagnostic test results. Dr. Hobson also reports grants from the NIH. Otherwise, the authors report no conflict of interest.

Acknowledgments

This work was funded in part by the Pelizaeus Merzbacher Disease Foundation (to AV and Gr Ho), grants from the Health and Labour Sciences Research Grants, Research on Rare and Intractable Diseases (H24-Nanchitou-Ippan-072, to K.I.), and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (KAKENHI: 21390103 and 23659531, to K.I.). Gr Ho is supported by NIH R01NS058978. Gu He is funded by the Delman family fund. Individual material was collected as part of the Myelin Disorders Bioregistry Project. Gr Ho would also like to thank Danielle Stubbolo for technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgme.2013.12.001.

References

[1] F. Cailloux, F. Gauthier-Barichard, C. Mimault, V. Isabelle, V. Courtois, G. Giraud, B. Dastugue, O. Boespflug-Tangu, 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. 8 (2000) 837–845.
[2] N.I. Wolf, E.A. Sistermans, M. Cundall, G.M. Hobson, A.P. Davis-Williams, R. Palmer, P. Stubbs, S. Davies, M. Endziniene, Y. Wu, W.K. Chong, S. Malcolm, R. Surtees, J.Y. Garbern, K.J. Woodward, Three or more copies of the proteolipid protein gene PLP1 cause severe Pelizaeus-Merzbacher disease, Brain 128 (2005) 743–751.
[3] N. Kamoun-Jellouli, I.H. Salem, E. Ellouz, N. Louhichi, A. Tlili, F. Kammoun, C. Triki, F. Fakhfakh, Molecular confirmation of founder mutation c.-167A > G in Tunisian patients with PMLD disease, Gene 513 (2012) 233–238.
[4] B. Uhlenberg, M. Schuelke, F. Rüschendorf, N. Ruf, A.M. Kaindl, M. Henneke, H. Thiele, G. Stoltenburg-Didinger, F. Aksu, H. Topaloğlu, P. Nürnberg, C. Hübner, B. Weschke, J. Gärtner, Mutations in the gene encoding gap junction protein α12 (Connexin 46.6) cause Pelizaeus-Merzbacher like disease, Am. J. Hum. Genet. 75 (2004) 251–260.
[5] H. Osaka, H. Hamanoue, R. Yamamoto, A. Nezu, M. Sasaki, H. Saitsu, K. Kurosawa, H. Shimbo, N. Matsumoto, K. Inoue, Disrupted SOX10 regulation of GJC2 transcription causes Pelizaeus-Merzbacher-like disease, Ann. Neurol. 68 (2010) 250–254.
[6] S.A. Al-Yahyaee, M. Al-Kindi, P.D. Jonghe, A. Al-Asmi, A. Al-Futaisi, E.D. Vriendt, T. Deconinck, P. Chand, Pelizaeus-Merzbacher like disease in a family with variable phenotype and a novel splicing gjc2 mutation, J. Child 28 (2012) 1–7.
[7] M. Henneke, P. Combes, S. Diekmann, E. Bertini, K. Brockmann, A.P. Burlina, J. Kaiser, A. Ohlenbusch, B. Plecko, D. Rodriguez, O. Boespflug-Tanguy, J. Gartner, GJA12 mutations are a rare cause of Pelizaeus-Merzbacher-like disease, Neurol. 70 (2008) 748–754.
[8] B. Bilir, Z. Yapici, C. Yalcinkaya, I. Baris, C.M.B. Carvalho, M. Bartnik, B. Ozes, M. Eraksoy, J.R. Lupski, E. Battaloglu, High frequency of GJA12/GJC2 mutations in Turkish patients with Pelizaeus–Merzbacher disease, Clin. Genet. 83 (2013) 66–72.
[9] L. Salviati, E. Trevisson, M. Baldoin, I. Toldo, S. Sartori, M. Calderone, R. Tenconi, A. Laverda, A novel deletion in the GJA12 gene causes Pelizaeus-Merzbacher-like disease, Neurogenetics 8 (2007) 57–60.
[10] J. Wang, H. Wang, Y. Wang, T. Chen, X. Wu, Y. Jiang, Two novel gap junction protein alpha 12 gene mutations in two Chinese patients with Pelizaeus-Merzbacher-like disease, Brain Dev. 32 (2010) 236–243.
[11] M. Bugiani, S. Al Shahwan, E. Lamantea, A. Bizzi, E. Bakhsh, I. Moroni, M.R. Balestrini, G. Uziel, M. Zeviani, GJA12 mutations in children with recessive hypomyelinating leukoencephalopathy, Neurol. 67 (2006) 273–279.
[12] P. Combes, N. Kammoun, A. Monnier, C. Gonthier-Guéret, G. Giraud, E. Bertini, T. Chahnez, F. Fakhfakh, O. Boespflug-Tanguy, C. Vaurs-Barrière, Relevance of GJC2 promoter mutation in Pelizaeus-Merzbacher-like disease, Ann. Neurol. 71 (2012) 146–148.
[13] E. Meyer, M.A. Kurian, N.V. Morgan, A. McNeill, S. Pasha, L. Tee, R. Younis, A. Norman, M.S. van der Knaap, E. Wassmer, R.C. Trembath, L. Brueton, E.R. Maher, Promoter mutation is a common variant in GJC2-associated Pelizaeus-Merzbacher-like disease, Mol. Genet. Metab. 104 (2011) 637–643.
[14] R. Biancheri, C. Rosano, L. Denegri, E. Lamantea, F. Pinto, F. Lanza, M. Severino, M. Filocamo, Expanded spectrum of Pelizaeus-Merzbacher-like disease: literature revision and description of a novel GJC2 mutation in an unusually severe form, Eur. J. Hum. Genet. 21 (2013) 34–39.
[15] R. Yamashita, N.P. Sathira, A. Kanai, K. Tanimoto, T. Arauchi, Y. Tanaka, S. Hashimoto, S. Sugano, K. Nakai, Y. Suzuki, Genome-wide characterization of transcriptional start sites in humans by integrative transcriptome analysis, Genome Res. 21 (2011) 775–789.
[16] R. Yamashita, H. Wakaguri, S. Sugano, Y. Suzuki, K. Nakai, DBTSS provides a tissue specific dynamic view of Transcription Start Sites, Nucleic Acids Res. 38 (2010) D98–D104.
[17] K. Tsuchihara, Y. Suzuki, H. Wakaguri, T. Irie, K. Tanimoto, S. Hashimoto, K. Matsushima, J. Mizushima-Sugano, R. Yamashita, K. Nakai, D. Bentley, H. Esumi, S. Sugano, Massive transcriptional start site analysis of human genes in hypoxia cells, Nucleic Acids Res. 37 (2009) 2249–2263.
[18] G.E. Crooks, G. Hon, J.M. Chandonia, S.E. Brenner, WebLogo: a sequence logo generator, Genome Res. 14 (2004) 1188–1190.
[19] M.E. Steenweg, A. Vanderver, S. Blaser, A. Bizzi, T.J. de Koning, G.M. Mancini, W.N. van Wieringen, F. Barkhof, N.I. Wolf, M.S. van der Knaap, Magnetic resonance imaging pattern recognition in hypomyelinating disorders, Brain 133 (2010) 2971–2982.
[20] B. Schlierf, T. Werner, G. Glaser, M. Wegner, Expression of connexin47 in oligodendrocytes is regulated by the Sox10 transcription factor, J. Mol. Biol. 361 (2006) 11–21.
[21] C.L. Anderson, M.A. Zundel, R. Werner, Variable promoter usage and alternative splicing in five mouse connexin genes, Genomics 85 (2005) 238–244.