表1　先天性大脳白質形成不全症の分類

疾患名	OMIM	略号	HLD 分類	遺伝形式	遺伝子座	原因遺伝子
第1群；　主として中枢神経の先天性髄鞘形成不全を呈する疾患群						
（1）Pelizaeus-Merzbacher 病	#312080	PMD	HLD1	X 劣	Xq22	*PLP1*
（2）Pelizaeus-Merzbacher 様病1	#608804	PMLD1	HLD2	常劣	1q42.13	*GJC2*
（3）基底核および小脳萎縮を伴う髄鞘形成不全症	%612438	HABC	HLD6	不明	不明	不明
（4）18q 欠失症候群	#601808	18qDEL		染色体欠失	18q22-qter	*MBP*
（5）Allan-Herndon-Dudley 症候群	#300523	AHDS		X 劣	Xq13.2	*SLC16A2*
（6）Hsp60 shaperon 病	#612233	MITCHAP60	HLD4	常劣	2q33.1	*HSPD1*
（7）Salla 病	#604369	SD		常劣	6q13	*SLC17A5*
（8）小脳萎縮と脳梁低形成を伴うび漫性大脳白質形成不全症	未	HCAHC		不明	不明	不明
第2群；　中枢神経および末梢神経の先天性髄鞘形成不全を呈する疾患群						
(1) 先天性白内障を伴う髄鞘形成不全症	#610532	HCC	HLD5	常劣	7p15.3	*FAM126A*
(2) 失調，歯牙低形成を伴う髄鞘形成不全症	%612440	ADDH	HLD7	不明	不明	不明
(3) 脱髄型末梢神経炎，中枢性髄鞘形成不全症，Waardenburg 症候群，Hirschsprung 病	#609136	PCWH		常優	22q13	*SOX10*

HLD;Hypomyelinating Leukodystrophy の略．OMIM では先天性大脳白質形成不全症に含まれる疾患の一部を HLD として分類しているので，本分類でもこれに倣った．X 劣;X 連鎖劣性，常劣;常染色体劣性，常優;常染色体優性．

II 治療指針

先天性大脳白質形成不全症では、現在のところ根本的な治療がないため、各症状に対応した治療を行う。

（1） 精神運動発達遅滞

知的障害に運動障害を伴うことから、より罹患率の高い脳性麻痺児と同様の療育を受けることが実際的である。保健所あるいは診断を行った病院より、療育センターや病院のリハビリテーション科を紹介する。聴性脳幹反応が異常であるために、聴覚異常と判定される場合があるが、通常聴力に異常は認めない。言語理解まで獲得された場合、表出性言語能力よりも受容性言語理解がまさっている。脳性麻痺との違いは、発達退行が脳性麻痺よりもはっきりと現れる点である。

（2） てんかん

先天性大脳白質形成不全症の患者のうち、どれほどの割合にてんかんを認めるかについては、統計的な報告はないが、10-20％程度と推定される。治療は一般的な小児のてんかんの治療法に基づく。発作のタイプにより、部分発作にはカルバマゼピン（5-15 mg/kg, 2X)を第一選択とし、第二次選択薬としてはラモトリジン、トピラマート(4-10 mg/kg, 2X)、ゾニサミド(4-10 mg/kg, 2X)、バロプロ酸(15-40 mg/kg, 3X)、クロバザム(0.2-1 mg/kg, 2X)等のベンゾジアゼピン系抗痙攣薬を用いる。全般発作には第一選択薬バロプロ酸、フェノバルビタール（2-5 mg/kg, 1-2X)を用い、第二次選択薬としてはラモトリジン、トピラマート、ゾニサミド、クロバザム等のベンゾジアゼピン系抗痙攣薬を用いる。

（3） ジストニア

全身性のジストニアに関してはエペリゾン（ミオナール）(1-4mg/kg, 3X), ジアゼパム(0.1-0.3mg/kg, 1-3X)、バクロフェン（リオレサール）(0.1-0.3-0.6mg/kg, 1-3X)、ダントロレンナトリウム（ダントリウム）(0.5mg/kg-3mg/kg, 2-3X)、ジサニジン（テルネリン）(0.05-0.1-0.15mg/kg, 1-3X)などフェノバルビタール(2-5 mg/kg, 1-2X)を用いる。局所性のジストニアでは、ボツリヌス毒素(1-3U/Kg)を用いる（最大3ヶ月毎)。

（4） 股関節の痙性脱臼

大腿骨が内転・内旋・屈位になりやすいためにおこる。外転位保持夜間装具が必要となる場合がある。高度例では整形外科的な腸腰筋延長・切離術をおこなう。

（5） 呼吸障害・摂食障害

喉頭咽頭機能不全のために、誤嚥性肺炎を起こしやすい。経口摂取が難しい症例では、経胃管あるいは胃瘻からの栄養補給が行われる。筋緊張亢進のために、胃食道逆流を伴う症例では、噴門形成術を併用する。

（6） 遺伝カウンセリング

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 III

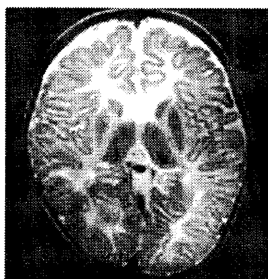
第２回市民公開セミナーのお知らせ

先天性大脳白質形成不全症の克服へ向けて

～患者さんを取巻く医療と研究の進歩～

第２回市民公開セミナー

先天性大脳白質形成不全症は、ペリツェウスーメルツバッハ病など、非常に稀ながら重度の障害を伴う小児難治性神経疾患です。現在、医療現場ではその診断や症状、予後などについてどこまでわかっているのか。病気の原因はどこまで解明されているのか。そして、治療を目指してどのような研究が行われていているのか。本セミナーでは、患者さんご家族など一般の方々を対象に、専門の医師や研究者がわかりやすく解説をします。



日時；平成 22 年 7 月 1 7 日土曜日　1 3 時～1 5 時

所；神奈川県立こども医療センター　講堂（新棟２階）

【第一部　教育講演（いずれも仮題）】

1．診断のための検査と医療的ケア

神奈川県立こども医療センター神経内科　小坂　仁

2．こどもの健康と遺伝

神奈川県立こども医療センター遺伝科　黒澤健司

3．先天性大脳白質形成不全症の全国調査について

国立精神・神経医療研究センター神経研究所　井上　健

【第二部　懇親会】

お茶とお菓子を用意します。

気軽な相談や、ご家族同士のご歓談に！

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「先天性大脳白質形成不全症の診断と治療に向けた研究」班　代表研究者　井上　健

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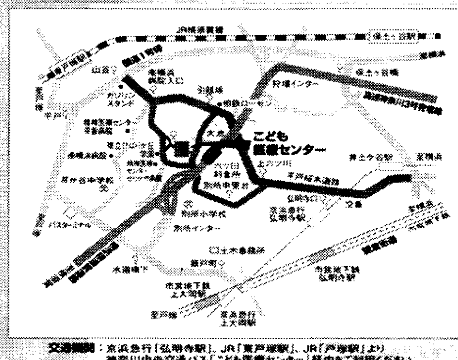
アクセスの詳細は神奈川県立こども医療センターホームページをご参照ください。



研究成果の刊行に関する一覧表

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研究成果の刊行物・別刷（抜粋）

Disrupted *SOX10* Regulation of *GJC2* Transcription Causes Pelizaeus-Merzbacher-Like Disease

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Mutations in the gap junction protein gamma-2 gene, *GJC2*, cause a central hypomyelinating disorder; Pelizaeus-Merzbacher-like disease (PMLD; MIM311601). Using a homozygosity mapping and positional candidate gene approach, we identified a homozygous mutation (c.-167A>G) within the *GJC2* promoter at a potent SOX10 binding site in a patient with mild PMLD. Functionally, this mutation completely abolished the SOX10 binding and attenuated *GJC2* promoter activity. These findings suggest not only that the *SOX10*-to-*GJC2* transcriptional dysregulation is a cause of PMLD, but also that *GJC2* may be in part responsible for the central hypomyelination caused by *SOX10* mutations.

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Congenital hypomyelinating disorders are a heterogeneous group of central nerve system (CNS) leukoencephalopathies, most of which are inherited disorders of myelin formation. The prototype condition is Pelizaeus-Merzbacher disease (PMD; MIM312080), an X-linked disorder caused by mutations in the proteolipid protein 1 gene (*PLP1*).[1] Patients with PMD have nystagmus, impaired motor development, ataxia, choreoathetotic movements, dysarthria, and progressive spasticity. However, ~20 to 50 % of patients clinically diagnosed with PMD have no detectable abnormalities in the *PLP1* gene, and some have a distinct disease, Pelizaeus-Merzbacher-like disease (PMLD; MIM311601).

Mutations in the gap junction protein gamma-2 gene (*GJC2*, also known as *Cx47* or *GJA12*) have been reported as a cause of PMLD.[2–8] Twenty-four different mutations (8 frameshift, 10 missence, 5 nonsense, and 1 missense/insertion alterations) have been reported to date, and most if not all result in a loss of channel function.[7,9]

By combining homozygosity mapping and a candidate gene approach, we found a homozygous mutation that disrupts a SOX10 transcriptional activation site in the *GJC2* promoter region in a family showing a mild PMLD phenotype. SOX10 is an high mobility group (HMG) family transcription factor that plays a critical role in peripheral nervous system (PNS) and CNS myelination. In addition, a subset of *SOX10* mutations cause peripheral and central hypomyelination, Waardenburg syndrome, and Hirschsprung disease (PCWH; MIM609136).[10] This study reports the first case of PMLD caused by a mutation in the *GJC2* promoter and suggests that *SOX10* transcriptional regulation of *GJC2* plays a critical role in CNS myelination.

Patients and Methods

Detailed clinical information of a Japanese female patient with PMLD, who is now 25-years-old, was previously reported.[11] In brief, her healthy parents were second cousins. She had congenital pendular nystagmus as a neonate, but otherwise developed normally and was educated at a normal school. At the age of 10 years, she developed a spastic gait that worsened and made her wheelchair bound by the age of 12 years. Her disease progressed to mild athetosis of the upper limbs and ataxia by age 13 years and dysarthria by age 15 years. She cannot speak and understands only easy commands now. Brain magnetic resonance imaging at age 15 and 20 years showed diffuse hyperintensity of white matter on T2-weighted images with interval progression of brain atrophy (Fig 1). Electrophysiological examinations showed extensive nerve conduction slowing in the CNS, although this was less severe than usually seen in male patients with PMD.[11] Peripheral nerve conduction velocities were nor-

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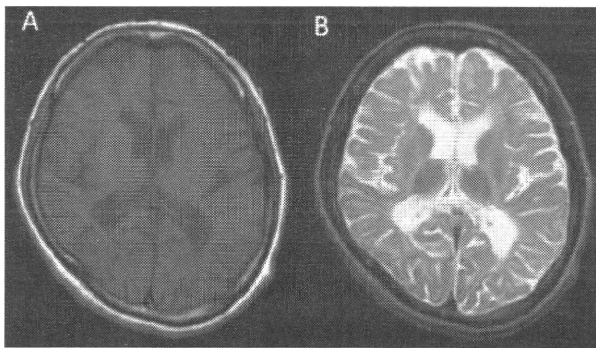
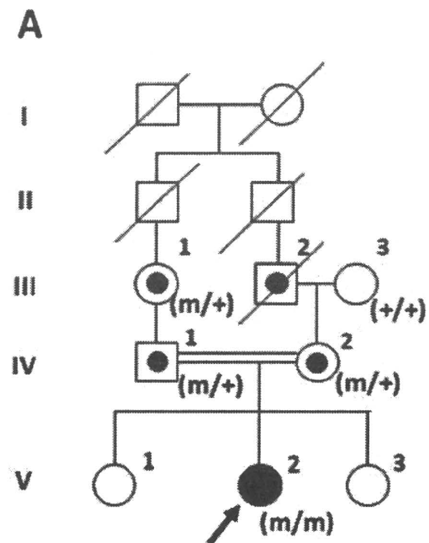


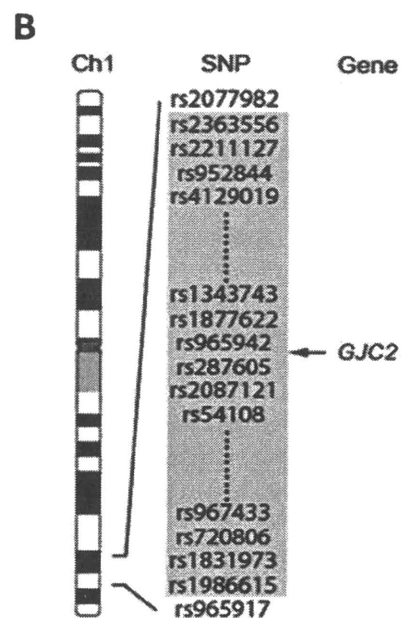
FIGURE 1: Magnetic resonance imaging of the cerebrum. (A) T1-weighted image of the proband at 20 years shows cerebral atrophy with ventricular dilatation and widening of a subarachnoidal space. Disappearance of contrast between cortex and white matter, which suggested incomplete myelination throughout the cerebrum, was evident. (B) T2-weighted image reveals diffuse hyperintensity in the white matter, suggesting the arrest of myelination. Note that the inner capsule, which is usually myelinated in the neonate, was not myelinated in this patient.

mal. Molecular examinations excluded *PLP1* exonic mutations, large duplications, and deletions.

Informed consent was obtained from the patient and family members in accordance with human study protocols approved by the institutional review board of Kanagawa Children's Medical Center. Genomic DNA was extracted from peripheral lymphocytes. A genome-wide single nucleotide polymorphism (SNP) genotyping was undertaken for III-1, III-3, IV-1, IV-2, V-1, and V-2 (Fig 2A) using the GeneChip Human Mapping 10K Array *X*ba 142 2.0 (Affymetrix, Santa Clara, CA) containing 10,204 SNPs according to the manufacture's protocols (Supplementary Materials and Methods). Polymerase chain reaction and DNA sequencing are described in the Supplementary Materials and Methods (Supplementary Table 1).

Mammalian cell expression plasmids for the wild-type and E189X mutant human *SOX10* cDNA were reported previously.[12] Luciferase reporter plasmids containing mouse *Gjc2* promoters (kindly provided from Dr M. Wegner) were utilized





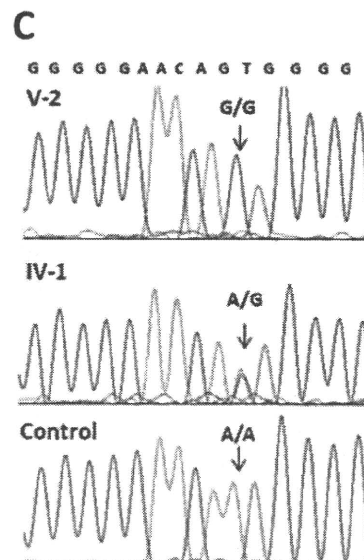
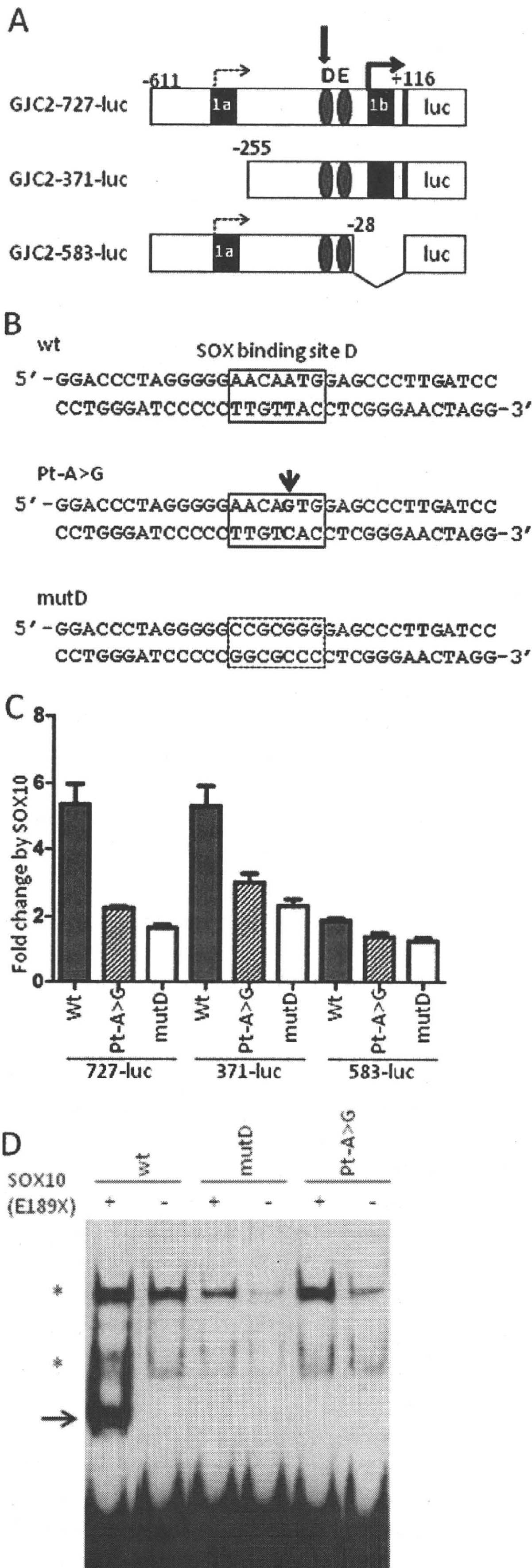


FIGURE 2: Family pedigree, largest region of interest on chromosome 1, and the *CJC2* mutation. (A) Pedigree of the Pelizaeus-Merzbacher-like disease family with the proband *(filled circle with arrow)*. DNA from III-1, III-3, IV-1, IV-2, V-1, and V-2 were used for single nucleotide polymorphism (SNP) genotyping. Carriers are indicated as circles with black dots. m = mutant allele; + = wild-type allele. (B) The largest region of interest by homozygosity SNP mapping at 1q41-q42.2. The homozygous interval is shown as a shaded square with SNP identifiers. The location of *GJC2* is shown with an arrow. The region between rs2077982 and rs965917 was 18.2 Mb in size (University of California, Santa Cruz genome browser coordinate, chromosome 1: 215150317-233384165, February 2009 version). (C) Sequencing chromatograms from the patient (V-2, top), a carrier (IV-1, middle), and a normal control (bottom). The c.-167A>G mutation in the promoter region of *CJC2* is shown with arrows.



for site-directed mutagenesis (see Supplementary Materials and Methods). We measured *GJC2* transcriptional activity by luciferase reporter assays using human glioblastoma U138 cells (see Supplementary Materials and Methods). SOX10 binding affinity was determined by electrophoretic mobility shift assay (EMSA) using synthetic oligonucleotide probes and nuclear extracts from HeLa cells transfected with pCMV-SOX10-E189X, as previously described.[13]

Results

The largest region with homozygosity identified by SNP genotyping on chromosome 1q42.13 was our primary focus for candidate gene scanning (see Fig 2B, Supplementary Table 2, Supplementary Fig 1). Among 115 refseq genes mapped within this region, 34 gene products were identified from mouse whole brain proteomics studies (Supplementary Table 3).[14,15] After we sequenced all coding regions and intron-exon boundaries of these 34 genes to exclude any disease-causing mutations, we extended our analysis to promoter regions. We found a homozygous mutation, c.-167A>G, in the proximal promoter re-

FIGURE 3: Functional consequence of the c.-167A>G point mutation in the *GJC2* promoter. (A) Schematic diagram of the luciferase reporter constructs of mouse *Gjc2* promoter region utilized in this study. Exon 1b contains the major transcription start site *(thick arrow)*, whereas exon 1a contains the minor site *(dotted arrow)*. GJC2-727-luc contains a full proximal promoter, whereas GJC2-371-luc lacks exon 1a and the upstream portion and GJC2-583-luc lacks exon 1b. Two SOX10 binding site, D and E, are shown as shaded ovals with a thick arrow pointing to site D, where the mutation was identified. (B) Sequences of the probes used for electrophoretic mobility shift assays (EMSAs). Top: *wt* probe containing the wild-type site D *(square)*. Middle: Pt-A>G probe carrying c.-167A>G mutation *(arrow)*. Bottom: mutD probe in which site D was changed to abolish SOX10 binding. (C) Transcriptional activities of different *GJC2* promoter constructs carrying either wt, Pt-A>G, or mutD at site D shown as fold changes obtained by presence or absence of SOX10 determined by luciferase reporter assay. Note that the *wt* constructs for 727-luc and 371-luc, harboring the major start site in exon 1b, were activated by SOX10 >5-fold. In contrast, a much smaller effect was observed when either Pt-A>G or mutD was introduced. The 583-luc constructs, which only harbor a minor transcription start site, remain inactivated by SOX10 regardless of changes in site D. Each bar represents average ± standard deviation. Each experiment was performed 3×, each in triplicate. Results from a representative experiment were shown. (D) DNA binding affinity of each probe (shown in B) was determined by EMSA using nuclear extracts from HeLa cells transfected with plasmid expressing truncated SOX10 protein (E189X) or empty plasmid (as a negative control). The *wt* probe showed a strong binding to E189X SOX10 protein, which retains enhanced DNA binding ability *(arrow)*. In contrast, we observed no binding of the mutant probes, either mutD or Pt-A>G. Asterisks show nonspecific binding. Free probes were observed at the bottom of the picture.

gion of *GJC2* that segregated with PMLD in the family members (see Fig 2A and C) and was absent in 122 normal Japanese chromosomes. Analysis of this region in 10 additional female PMLD patients without mutations in the open reading frame of *GJC2* detected no abnormalities.

Interestingly, this mutation is located within a critical SOX10 binding site (designated as site D) in the syntenic mouse *Gjc2* proximal promoter and diminishes the consensus of the SOX binding sequence (AACA<u>A</u>TG to AACA<u>G</u>TG, Fig 3A and B). Based on this, we predicted that this mutation disrupts *GJC2* promoter activity and measured transcription in vitro using a luciferase reporter system. Because the region harboring the mutation is highly conserved across mammals,[16] we introduced this mutation into well-studied mouse *Gjc2* promoter constructs (see Fig 3A and B). The c.-167A>G point mutation in the SOX10 binding site dramatically decreased transcription to levels similar to a completely disrupted SOX10 binding site D (see Fig 3C). These findings suggest that the c.-167A>G point mutation found in our patients results in a diminished *GJC2* transcription.

Based on these results, we hypothesized that this mutation altered SOX10 binding affinity to site D and tested this by EMSA. Because full-length SOX10 has a low binding affinity that is difficult to distinguish from background noise, we used a C-terminus truncation version of SOX10, E189X, which retains the HMG binding domain and has enhanced binding affinity.[16] Introduction of the c.-167A>G mutation into site D resulted in a complete loss of E189X SOX10 binding (see Fig 3D). Therefore, combined with the preceding observations, we find that the c.-167A>G mutation abolishes SOX10 binding to the *GJC2* promoter, resulting in a dramatic attenuation of the *GJC2* transcription.

Discussion

GJC2 encodes Cx47, a member of the connexin family. Connexins are components of gap junctions, intercellular channels that allow ions and small molecules to pass across neighboring plasma membranes. Gap junctions have diverse functions, including the propagation of electrical signals and metabolic cooperation. Two hemichannels, each built up of 6 connexin protein subunits on opposing cell membranes, form the channel. Astrocytes and oligodendrocytes are coupled by gap junctions constructed predominantly of GJC2 (Cx47) and Cx43.[17] Because Cx47 proteins carrying PMLD-causing mutations either fail to reach the membrane or have reduced transport activity, loss of function is likely the mechanism underlying the CNS hypomyelination in PMLD.[7,9] Herein we report the first *GJC2* promoter mutation,[18] c.-167A>G, in a patient with PMLD, and this is associated with allelic transcription failure.

Our female patient had nystagmus, spasticity, and choreoathetosis, clinical symptoms common to PMD and PMDL. However, she attained normal motor and intellectual developmental milestones. Because only $\frac{1}{3}$ (11 of 33) of PMLD patients with *GJC2* mutations have walked unsupported,[2–7] her clinical manifestation was mild and overlaps with that of spastic paraplegia phenotype. Of note, she lost her motor and cognitive abilities within a few years, accompanied by progressive brain atrophy (see Fig 1). Such acute regression has rarely been observed in PMD and is more characteristic of PMLD secondary to *GJC2* mutations.[6]

A recent study showed that SOX10 directly regulates *GJC2* by binding to its proximal promoter.[16] Site D, the SOX10 binding site in which our mutation was identified, plays a predominant role in *GJC2* promoter activity,[16] and the c.-167A>G mutation we identified reduces its affinity for SOX10 and abolishes *GJC2* transcription. These findings suggest that *SOX10* regulation of *GJC2* via site D is essential for proper *GJC2* expression and that its failure causes PMLD. Presumably, the relatively milder clinical phenotype observed in our patient results from reduced but not completely abolished transcriptional activity, allowing translation of a small amount of normal Cx47 protein.

This constitutes the second disorder associated with dysregulation of a SOX10 target gene. Previously, mutations within the *SOX10* binding site of the *GJB1* promoter have been shown to cause demyelinating peripheral neuropathy.

Together the peripheral neuropathy and PMLD provide a partial understanding of the clinical manifestations of PCWH patients. Because these patients have SOX10 mutations,[10] we predict that the expression of both *GJC2* and *GJB1* is impaired. Impaired expression of both of these genes would, at least in part, respectively account for the de-/hypomyelination of the CNS and PNS observed in PCWH. Based on this, we predict that impaired expression of other target genes of SOX10 is responsible for the Hirschprung disease and other Waardenburg features.

In conclusion, we identified the first case of PMLD caused by a mutation in the *GJC2* promoter. Because this mutation disrupts SOX10 regulation of *GJC2* transcription, we hypothesize that SOX10 regulation of transcription plays a major role in nervous system myelination.

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Potential Conflicts of Interest

Nothing to report.

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Interruption of SOX10 Function in Myelinopathies

Tremendous progress in our understanding of myelin development, function, and maintenance has come from insights provided by patients manifesting myelinopathies. Mutations affecting peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ), and gap junction protein beta-1 (GJB1; also known as Cx32, encoding connexin-32) result in Charcot-Marie-Tooth (CMT) and related peripheral neuropathies such as Dejerine-Sottas neuropathy and congenital hypomyelinating neuropathy (CHN).[1] Mutations that affect proteins of the central nervous system myelin, such as proteolipid protein 1 (PLP1), cause Pelizaeus-Merzbacher disease (PMD) and spastic paraplegia.[2] Interestingly, for the most common forms of de- or dysmyelinating CMT and PMD, there are no mutant proteins at all. Instead, in about 70% of patients with either CMT1 or PMD, myelinopathies are caused by altered dosage due to gene copy number variation, resulting from duplication of either *PMP22* or *PLP1*, respectively. These findings emphasize the importance of nervous system gene regulation and expression for proper myelin function. In this issue of *Annals of Neurology*, Osaka and colleagues beautifully demonstrate the importance of myelin gene regulation by identifying point mutations in a *SOX10* transcription factor binding site upstream of the *GJC2* gene in a patient with a central hypomyelinating disorder similar to, but clinically less severe than, PMD.[3] The *GJC2* gene encodes a gap junction protein; coding region mutations have been associated with a PMD-like disorder (PMLD; Mendelian Inheritance in Man [MIM] #311601).

Key to our understanding of genes and genetic pathways critical to myelin development and function was the identification of patients with mutations in the transcription factors regulating myelin gene expression. One of the first identified by virtue of mutations that conveyed a neurological phenotype was early growth response 2 (EGR2),[4] in which, perhaps as might have been anticipated because this transcription factor is important for myelin development, mutations can cause a severe CHN. However, a surprise was to find patients with adult onset CMT1 who had mutations in *EGR2*. Such a finding suggests that this transcription factor is not only important for myelin development but also essential to maintain proper myelin function.[5]

Often a single subject, that is, a case report, can initiate a cascade of insights into neuropathologic mechanisms, as was the case in a patient with CHN, PMD, Waardenburg syndrome, and Hirschsprung disease[6] found to have *SOX10* mutations. This condition has now been extensively characterized and is referred to as PCWH (peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease; MIM #609132).[7] The complex neurological disease is caused by apparent gain-of-function mutations that act in neural crest cells during development (Fig 1A). Interestingly, some mutations of *SOX10* may have dominant-negative activity restricted to single neural crest-derived cell lineages.[8] Many of the genes downstream of *SOX10* that potentially mediate all the phenotypic consequences of PCWH remain to be elucidated.

This Brief Communication by Osaka and colleagues elucidates a potential mechanism for the central nervous system findings of hypomyelination in PCWH.[3] They studied a female patient with PMLD. PMLD has been associated with both point mutations and deletion[9] of the gap junction protein gamma-2 gene, *GJC2* (also known as *GJA12*). By homozygosity mapping and a positional candidate gene approach, they identified a homozygous mutation (c.-167A>G) within the *GJC2* promoter that functionally abolishes *SOX10* binding and attenuates *GJC2* promoter activity. Their findings not only reveal that *SOX10*-to-*GJC2* transcriptional dysregulation is a cause of PMLD, but further suggest that *GJC2* may be in part responsible for the central hypomyelination caused by *SOX10* mutations, particularly in patients with PCWH.

Regarding peripheral nerve myelin gene expression, previous work has shown that SOX10 directly regulates *MPZ*.[10] Furthermore, SOX10 in conjunction with EGR2 strongly activates *GJB1/Cx32* expression by directly binding to its promoter, a transactivation activity abolished in

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