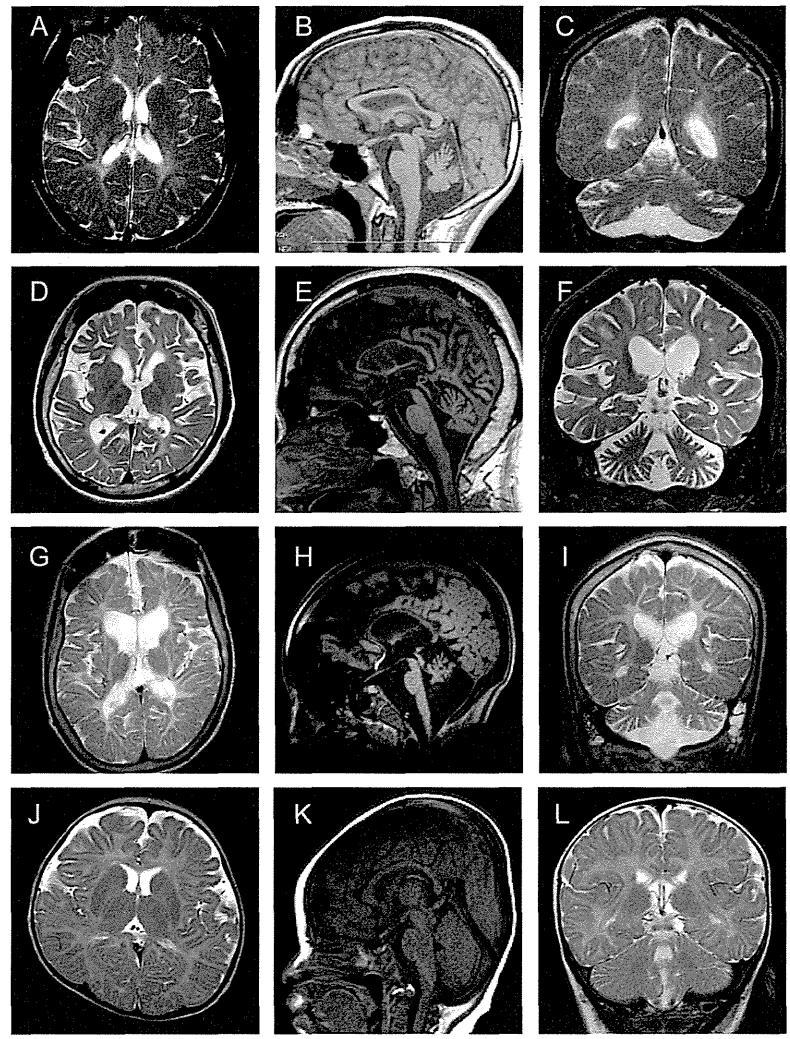
Figure 3 Brain MRI of patients with *TUBB4A* mutations



Axial T2-weighted (A, D, G, J), sagittal T1-weighted (B, E, H, K), and coronal T2-weighted (C, F, I, L) images. Patient 1 at 14 years of age (A); patient 1 at 16 years (B, C); patient 2 at 38 years (D–F); patient 3 at 13 years (G–I); and patient 8 at 7 months of age (J–L). All patients show diffuse cerebral white matter hypomyelination with normal (J), mildly reduced (A), or considerably reduced (D, G) white matter volumes. In patient 1, cerebral white matter hypomyelination is unchanged, comparing at 14 (A) and 16 (B, C) years of age. In patient 1, the putamen and the head of the caudate nucleus are normal in size (A). In patient 2, minimal putamen atrophy cannot be excluded (D). The putamen and the head of the caudate nucleus are small or hardly recognizable in patient 3 (G). In patient 8, the putamen is slightly small compared with a healthy control at the same age (J). The globus pallidus and thalamus are normal in size (A, D, G, J). Atrophy of the cerebellar vermis and hemisphere, and corpus callosum was variably observed in 4 patients, but not patient 8 (B, C, E, F, H, I, K, L).

with this phenotype have been reported worldwide.[18] The symptoms typically emerge in the third decade, following a highly penetrant, autosomal dominant mode of inheritance.[31] Brain MRI demonstrates normal structural findings. Arg2 resides within the autoregulatory MREI domain of β-tubulin 4A, which is necessary for autoregulation of the β-tubulin messenger RNA transcript. Site-directed mutagenesis shows that any Arg2 substitution leads to loss of autoregulated instability and increased mutant tubulin subunit levels.[32] Thus, mutations in the MREI domain have been assumed to cause DYT4 rather than H-ABC, because of the different impact on *TUBB4A*.[17] However, our study shows that mutations in the MREI domain can also cause the H-ABC phenotype. The phenotypic difference between the p.Arg2Gly and p.Arg2Gln mutations remains unsolved. Because DYT4 is an extremely rare

syndrome that has only been described in one large pedigree so far, patients of the family may have another modifying factor(s) to spare cerebral white matter abnormalities.

Diffuse hypomyelination syndromes are a heterogeneous group of disorders with overlapping clinical features. Currently, they are categorized based on brain MRI findings, which is very useful in clinical practice. Basal ganglia atrophy specifically distinguishes H-ABC from other hypomyelination disorders. Our study shows that *TUBB4A* mutations associate not only with the typical H-ABC cases but also with some hypomyelinating patients with retained basal ganglia, although notably all patients with *TUBB4A* mutations have extrapyramidal features in common. Our study implies that *TUBB4A* may cause hypomyelinating leukoencephalopathies with either a morphologically or a functionally impaired basal ganglia. Extrapyramidal features may be a key for clinicians to examine *TUBB4A* mutations in genetically unsolved hypomyelinating leukoencephalopathies.

AUTHOR CONTRIBUTIONS

Satoko Miyatake: genetic and clinical data analysis, data interpretation, and drafting/revising of the manuscript. Hitoshi Osaka: clinical data analysis and sample collection. Masaaki Shiina: structural data analysis. Masayuki Sasaki, Jun-ichi Takanashi, Kazuhiro Haginoya, Takahito Wada, Masafumi Morimoto, Naoki Ando, and Yoji Ikuta: clinical data analysis and sample collection. Mitsuko Nakashima, Yoshinori Tsurusaki, and Noriko Miyake: genetic data analysis. Kazuhiro Ogata: structural data analysis. Naomichi Matsumoto: study concept and design, data interpretation, and drafting/revising of the manuscript. Hirotomo Saitsu: study concept and design, genetic data analysis, data interpretation, and drafting/revising of the manuscript.

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DISCLOSURE

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1502/suppl/DC1
Materials and Methods
Supplementary Text
Fig. S1
Tables S1 to S7
Reference (*42*)

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NEUROMUSCULAR DISEASE

DOK7 gene therapy benefits mouse models of diseases characterized by defects in the neuromuscular junction

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The neuromuscular junction (NMJ) is the synapse between a motor neuron and skeletal muscle. Defects in NMJ transmission cause muscle weakness, termed myasthenia. The muscle protein Dok-7 is essential for activation of the receptor kinase MuSK, which governs NMJ formation, and *DOK7* mutations underlie familial limb-girdle myasthenia (*DOK7* myasthenia), a neuromuscular disease characterized by small NMJs. Here, we show in a mouse model of *DOK7* myasthenia that therapeutic administration of an adeno-associated virus (AAV) vector encoding the human *DOK7* gene resulted in an enlargement of NMJs and substantial increases in muscle strength and life span. When applied to model mice of another neuromuscular disorder, autosomal dominant Emery-Dreifuss muscular dystrophy, *DOK7* gene therapy likewise resulted in enlargement of NMJs as well as positive effects on motor activity and life span. These results suggest that therapies aimed at enlarging the NMJ may be useful for a range of neuromuscular disorders.

The neurotransmitter acetylcholine (ACh) is released from the presynaptic motor nerve terminal and binds to ACh receptors (AChRs) on the postsynaptic muscle membrane of the neuromuscular junction (NMJ), which forms in the central region of each myotube (*1*, *2*). To achieve efficient neuromuscular transmission, AChRs must be densely clustered on the postsynaptic membrane (*1*, *2*). Impaired AChR clustering is associated with disorders of neuromuscular transmission, including subtypes of congenital myasthenic syndromes and myasthenia gravis (*2*–*4*). The muscle-specific receptor tyrosine kinase MuSK is required for the formation and maintenance of NMJs (*1*, *2*).

The cytoplasmic protein Dok-7 (downstream of tyrosine kinases 7) is an essential activator of the receptor kinase MuSK, and mice lacking Dok-7 form no NMJs (*5*–*8*). Recessive loss- or reduction-of-function mutations in the human *DOK7* gene underlie a limb-girdle type of congenital myasthenic syndrome, *DOK7* myasthenia, a disorder characterized by NMJs that are about half the normal size (*7*, *9*, *10*). In contrast to many NMJ channelopathies (*11*), *DOK7* myasthenia is not associated with abnormalities in the function and local density of AChRs or the quantal release per unit size of the endplates (the region of synaptic specialization on the myotube). These observations suggest that *DOK7* myasthenia should be classified as a synaptopathy rather than a channelopathy (*2*, *7*). Interestingly, there is accumulating evidence that NMJ structural defects may be a common feature of other neuromuscular disorders (*12*–*18*), including muscular dystrophy (MD), amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), and age-related muscle weakness or sarcopenia. Indeed, studies of patients with autosomal dominant Emery-Dreifuss muscular dystrophy (AD-EDMD) and a mouse model of this disease have produced data suggestive of inefficient neuromuscular transmission (*12*). Because the size of NMJs is an important determinant of NMJ function (*2*), these observations raise the possibility that enlargement of the synaptic area may mitigate muscle weakness associated with defective NMJ structure.

We previously generated Dok-7 transgenic (Tg) mice that overexpress Dok-7 uniformly throughout the skeletal muscle under the control of the human skeletal α-actin (HSA) promoter (*6*). Using these mice, we found that forced expression of Dok-7 in vivo enhanced the activation of muscle-specific kinase MuSK and subsequent NMJ formation at the correct, central region of muscle fibers in embryos (*6*). Consistent with this, Dok-7 Tg mice showed greatly enlarged NMJs at 12 weeks of age (fig. S1A). Because exogenous Dok-7 was expressed only in the skeletal muscle (*6*), these data indicate that forced expression of Dok-7 in muscle triggers not only intramuscular signaling but also retrograde signaling that enlarges motor axon terminals. Interestingly, although these mice have enlarged NMJs, they did not exhibit obvious defects in motor activity, as determined by wire-hang and rotarod tests (fig. S1, B and C). Together, these findings suggest that Dok-7–mediated enhancement of NMJ formation merits investigation as a possible therapeutic approach for neuromuscular disorders associated with an NMJ synaptopathy.

To facilitate Dok-7–mediated NMJ formation in the muscle, we generated AAV-D7, a recombinant adeno-associated virus (AAV) serotype 9 (AAV9) vector carrying the human *DOK7* gene tagged with enhanced green fluorescent protein (EGFP) under the control of the cytomegalovirus (CMV) promoter. This promoter shows higher activity in skeletal muscle than the HSA promoter (*19*). The AAV vector is a powerful tool for delivering therapeutic genes to skeletal muscle and other tissues (*20*, *21*). We first treated C2C12 myotubes

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with AAV-D7 and observed an increase in the number of AChR clusters (fig. S2), as expected from previous work (*5*, *7*, *9*). We next treated 8-week-old wild-type (WT) mice with 4.0×10^{11} viral genomes (vg) of AAV-D7, delivered by a single intravenous injection, and compared them with control, untreated mice. One week after the injection, NMJs were clearly enlarged in the central region of the diaphragm muscle of AAV-D7–treated mice, with exogenous expression of Dok-7 throughout the myotubes (fig. S3 and Fig. 1, A and B). MuSK activation was augmented in the muscle as judged by elevated phosphorylation of MuSK and AChR, the latter known to be dependent on MuSK activation (Fig. 1, C and D). These results demonstrate that forced expression of Dok-7 in adult mice promotes MuSK-mediated formation of NMJs, leading to their enlargement within a week of AAV-D7 treatment. WT mice treated with AAV-D7 did not show any abnormalities in motor activity, as determined by grip strength, wire-hang, and rotarod tests (fig. S4), or in histology of the skeletal muscle, heart, and liver, which are the major target tissues of this AAV9 vector in mice (fig. S5) (*22*). We confirmed exogenous expression of Dok-7 in the heart (fig. S6) and in skeletal muscle (fig. S3 and Fig. 1A) after AAV-D7 treatment.

To investigate whether forced expression of Dok-7 and subsequent enlargement of NMJs in vivo mitigates disease progression after onset of *DOK7* myasthenia, we generated Dok-7 knock-in (KI) mice homozygous for the frameshift mutation (c.1124_1127dupTGCC), which corresponds to the most prevalent mutation in patients (fig. S7) (*7*, *10*, *23*–*25*). Dok-$7^{+/KI}$ and littermate WT mice displayed no obvious abnormal phenotype. By contrast, Dok-$7^{KI/KI}$ mice (Dok-7 KI mice) exhibited characteristic features of severe muscle weakness: These mice died between postnatal day 13 (P13) and P20, exhibited about 25% of the body weight of WT mice at P12, and developed apparent disturbance in gait by P9 (fig. S8). Unlike Dok-$7^{+/KI}$ and littermate WT mice, Dok-7 KI mice were too weak for the measurement of muscle strength. Furthermore, they showed abnormally small NMJs lacking postsynaptic folding (figs. S9 and S10), a pathological feature seen in patients with *DOK7* myasthenia (*26*). The c.1124_1127dupTGCC mutation is a reduction-of-function mutation in terms of MuSK activation in C2C12 myotubes (*7*, *9*). Consistent with this, Dok-7 KI mice exhibited decreased MuSK activity in skeletal muscle, as judged by attenuated phosphorylation of AChR and MuSK (fig. S11) (see below). Thus, the Dok-7 KI mice develop defects similar to those found in patients with *DOK7* myasthenia, although the mice (hereafter referred to as "*DOK7* myasthenia mice") exhibit a more severe phenotype.

To determine whether *DOK7* gene therapy provides beneficial effects to *DOK7* myasthenia mice, we administered 2.0×10^{11} vg of AAV-D7 by intraperitoneal injection to the animals at P9 to P12. At P9, these mice required at least 10 s to right themselves after being placed on their side, confirming disease onset. A single-dose treatment with AAV-D7 led to marked recovery of the *DOK7*

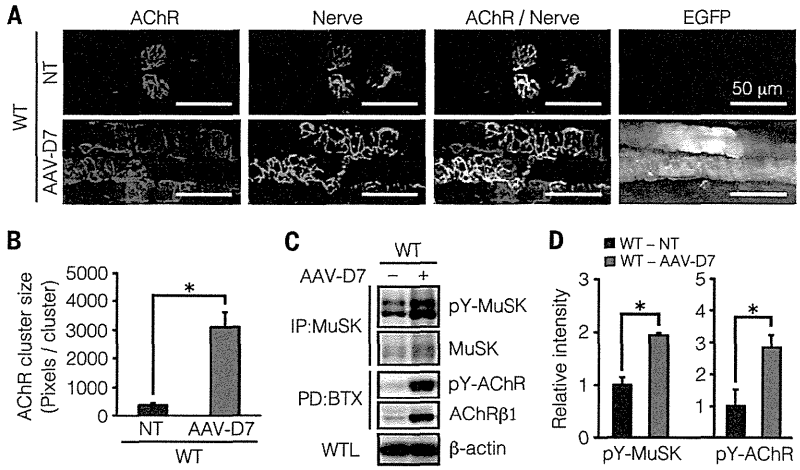

Fig. 1. AAV-D7 treatment promotes MuSK-mediated NMJ formation. WT mice were treated or untreated with 4.0×10^{11} vg of AAV-D7 at P56 and subjected to the following assays at P63. (**A**) Whole-mount staining of NMJs on the diaphragm muscle. Axons and nerve terminals (green) were stained with antibodies to neurofilament and synaptophysin, and AChRs (red) were labeled with α-bungarotoxin (BTX). Expression of Dok-7 tagged with EGFP (gray) was monitored by EGFP. NT, not treated. (**B**) Quantified data for the size of each AChR cluster in the diaphragm muscle (n = 30 microscopic fields in 6 mice; male = 3, female = 3). (**C**) Immunoblotting for tyrosine phosphorylation of MuSK or AChR and for β-actin in the hind-limb muscle. MuSK immunoprecipitates (IP) from whole-tissue lysates (WTL) of the hind-limb muscle were subjected to immunoblotting for phosphotyrosine (pY) and MuSK. AChRs pulled down with BTX-Sepharose (PD) from WTL were subjected to immunoblotting for pY or the β1 subunit of AChR. (**D**) Quantified data for tyrosine phosphorylation of MuSK and AChR in the hind-limb muscle (n = 3 mice; male = 1 to 2, female = 1 to 2). Values in (B) and (D) are means ± SD. *P < 0.05 by Student's t test.

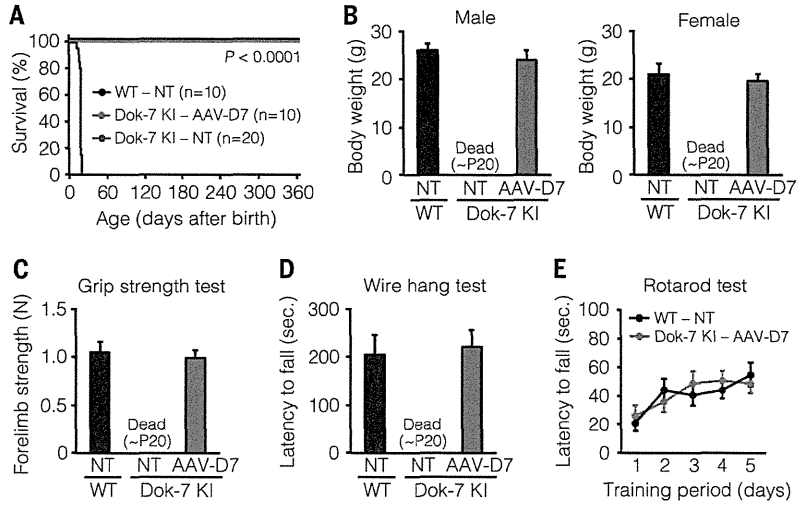

Fig. 2. Dok-7 therapy restores motor activity and survival of *DOK7* myasthenia mice. Mice were treated or untreated with 2.0×10^{11} vg of AAV-D7 at P9 to P12. (**A**) Kaplan-Meier survival curves of WT littermates and Dok-7 KI mice (*DOK7* myasthenia mice) (n = 10 to 20 mice; male = 4 to 10, female = 6 to 10). NT, not treated. (**B**) Body weight at P56 (n = 4 to 6 mice; male = 5, female = 4 to 6). (**C** to **E**) Motor activity at P56 determined by (C) grip strength, (D) wire-hang, and (E) rotarod tests (n = 10 mice; male = 4 to 5, female = 5 to 6). P value (A) was calculated by log-rank test (Dok-7 KI – NT versus Dok-7 KI – AAV-D7). Values in (B) to (E) are means ± SD.

myasthenia mice. All AAV-D7–treated mice survived for at least 1 year with no apparent abnormality, whereas all untreated and AAV-EGFP–treated control mice died by P20 (Fig. 2A and fig. S12). Indeed, body weight and motor activity—as determined by grip strength, wire-hang, and rotarod tests—of AAV-D7–treated mice approximated those of age-matched WT controls by P56 (Fig. 2, B to E). Similarly, forced expression of a *DOK7* transgene

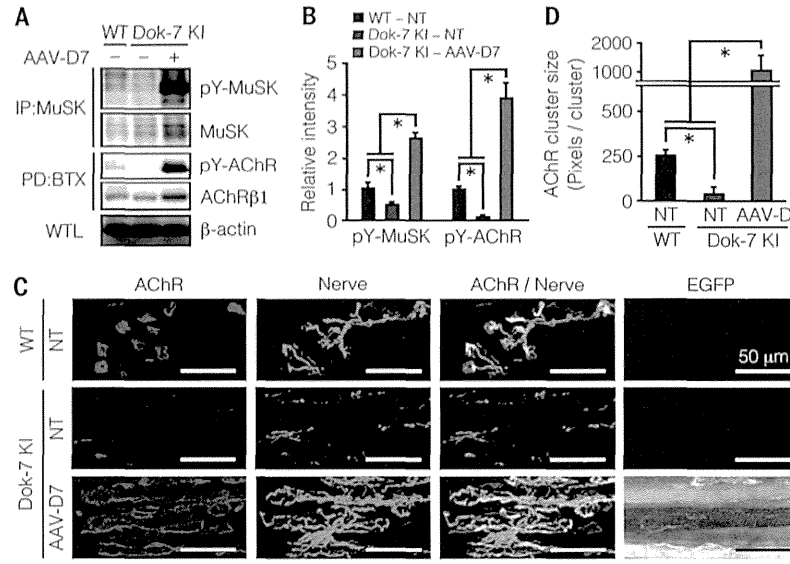

Fig. 3. Dok-7 therapy promotes MuSK-mediated NMJ formation in *DOK7* myasthenia mice. Mice were treated or untreated with 2.0×10^{11} vg of AAV-D7 at P9 and subjected to the following assays at P14. (**A**) Immunoblotting for tyrosine phosphorylation of MuSK or AChR and for β-actin in the hind-limb muscle. Experiments were performed as in Fig. 1C. (**B**) Quantified data for tyrosine phosphorylation of MuSK and AChR in the hind-limb muscle (n = 3 mice; male = 1 to 2, female = 1 to 2). (**C**) Whole-mount staining of NMJs on the diaphragm muscle. Axons and nerve terminals (green), AChRs (red), and Dok-7 tagged with EGFP (gray) were visualized as in Fig. 1A. (**D**) Quantified data for the size of each AChR cluster in the diaphragm muscle (n = 30 microscopic fields in 6 mice; male = 3, female = 3). Values in (B) and (D) are means ± SD. *P < 0.05 by analysis of variance (ANOVA) and Dunnett's test.

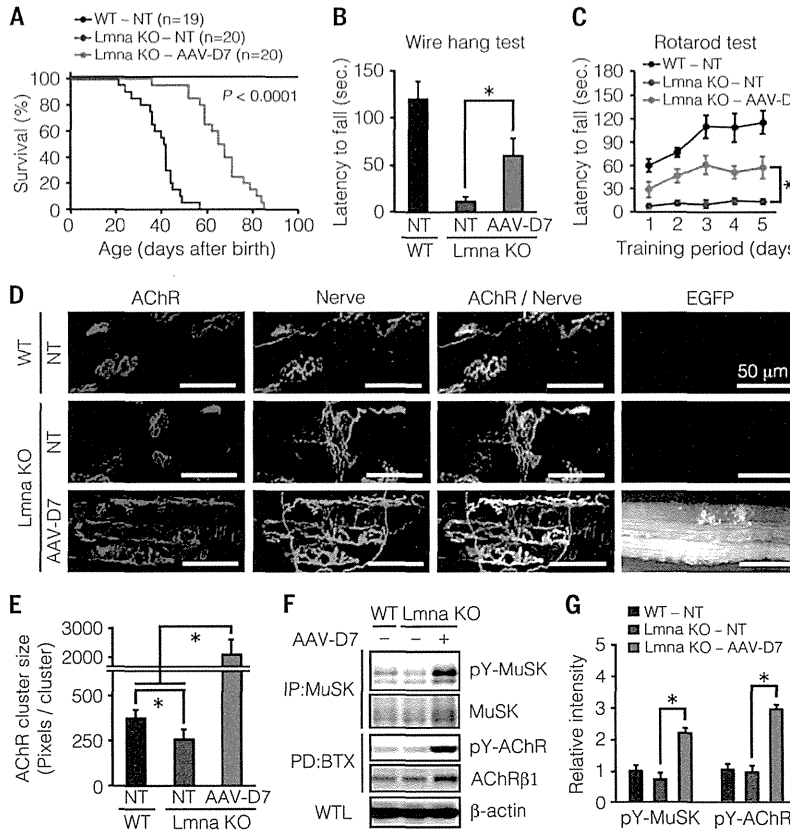

Fig. 4. The effect of Dok-7 therapy in a mouse model of AD-EDMD. Mice were treated or untreated with 4.0×10^{11} vg of AAV-D7 at P16 and subjected to the following assays. (**A**) Kaplan-Meier survival curves of WT littermates and Lmna KO mice (AD-EDMD mice) (n = 19 to 20 mice; male = 9 to 12, female = 8 to 11). NT, not treated. (**B** and **C**) Motor activity at P35, determined by (B) wire-hang and (C) rotarod tests (n = 8 to 10 mice; male = 4 to 6, female = 4 to 5). (**D**) Whole-mount staining of NMJs on the diaphragm muscle at P42. Axons and nerve terminals (green), AChRs (red), and Dok-7 tagged with EGFP (gray) were visualized as in Fig. 1A. (**E**) Quantified data for the size of each AChR cluster in the diaphragm muscle at P42 (n = 30 microscopic fields in 6 mice; male = 3 to 4, female = 2 to 3). (**F**) Immunoblotting for tyrosine phosphorylation of MuSK or AChR and for β-actin in the hind-limb muscle at P42. Experiments were performed as in Fig. 1C. (**G**) Quantified data for tyrosine phosphorylation of MuSK and AChR in the hind-limb muscle at P42 (n = 3 mice; male = 1 to 2, female = 1 to 2). P value (A) was calculated by log-rank test (Lmna KO – NT versus Lmna KO – AAV-D7). Values in (B), (C), (E), and (G) are means ± SD. *P < 0.05 by analysis of variance (ANOVA) and Dunnett's test.

specifically in skeletal muscle restored survival and motor activity of *DOK7* myasthenia mice as determined by wire-hang and rotarod tests, indicating that muscle-specific expression of Dok-7 is sufficient to rescue these mice (fig. S13, A to C).

We next investigated whether AAV-D7 treatment promotes activation of MuSK and subsequent enlargement of NMJs in *DOK7* myasthenia mice. We found that MuSK and AChR phosphorylation was strongly elevated in *DOK7* myasthenia mice just 5 days after treatment with AAV-D7 (Fig. 3, A and B). Consistent with this, NMJs were greatly enlarged in *DOK7* myasthenia mice within 5 days of treatment (Fig. 3, C and D, and fig. S14) and remained enlarged at 8 weeks of age (fig. S15). Together, these data demonstrate that treatment with AAV-D7 (hereafter referred to as "Dok-7 therapy") facilitates MuSK-mediated NMJ formation, resulting in stable enlargement of NMJs, restoration of motor activity, and enhanced survival of *DOK7* myasthenia mice.

Because AAV-D7 enlarges NMJs not only in *DOK7* myasthenia mice but also in WT mice, we hypothesized that Dok-7 therapy might be applicable to other types of neuromuscular disorders that are associated with abnormalities of NMJ structure but not caused by *DOK7* mutations. As noted above, AD-EDMD is one such candidate. AD-EDMD is caused by mutations in the *LMNA* gene, which encodes lamin A/C, an important determinant of interphase nuclear architecture (*27*). Patients with AD-EDMD develop cardiac defects and skeletal muscle weakness (*28*). Although pacemaker and implantable cardioverter defibrillator (ICD) insertion helps address the cardiac defects (*29*), there is no effective treatment for skeletal muscle weakness. Histology and gene expression profiles of muscle biopsies from patients with AD-EDMD are suggestive of alterations in NMJ structure (*12*, *30*, *31*).

We studied a mouse model of AD-EDMD (hereafter referred to as "AD-EDMD mice") that is genetically deficient in lamin A/C and that has structurally abnormal and functionally inefficient NMJs (*12*, *30*). At P16, we administered 4.0×10^{11} vg of AAV-D7 into the AD-EDMD mice by a single intraperitoneal injection. Disease onset was confirmed in each AD-EDMD mouse at P16, when the animals showed hind-limb paralysis and required at least 10 s to right themselves after being placed on their sides. We found that Dok-7 therapy prolonged survival of AD-EDMD mice (Fig. 4A). Mice receiving Dok-7 therapy, but not those receiving AAV-EGFP treatment, lived an average of 29 days longer than untreated mice ($P < 0.0001$, log-rank test) (Fig. 4A and fig. S16). In addition, Dok-7 therapy increased latency to fall of AD-EDMD mice, as determined by wire-hang and rotarod tests by 49 s and 44 s, respectively, over untreated mice ($P < 0.05$, Dunnett's test) (Fig. 4, B and C), indicating enhanced motor activity. Forced expression of Dok-7 specifically in skeletal muscle via a transgene also enhanced AD-EDMD mouse survival and motor activity, as determined by wire-hang and rotarod tests, indicating that muscle-specific expression of Dok-7 is sufficient to benefit these mice (fig. S13, D to F). Dok-7 therapy enhanced MuSK activation and enlarged NMJs in the skeletal muscle within 26 days of treatment (Fig. 4, D to G), and the NMJs in control, untreated AD-EDMD mice were significantly smaller than those in WT mice (Fig. 4, D and E). Electrocardiographic and histological analyses showed that Dok-7 therapy did not benefit heart function in AD-EDMD mice (fig. S17). It is possible that the beneficial effects of Dok-7 therapy on muscle weakness are partially masked in these mice by heart failure, which, as noted above, would be treatable in patients with AD-EDMD by pacemaker and ICD insertion (*29*). These findings suggest that Dok-7 therapy might be beneficial for patients with AD-EDMD.

The mechanisms through which Dok-7 therapy alleviates muscle weakness in mouse models of *DOK7* myasthenia and AD-EDMD remain to be determined. We speculate that, in addition to an effect on the postsynaptic region of muscle, the mechanism likely involves retrograde signaling from the muscle to the nerve. This idea is consistent with the observation that enlargement of the nerve terminals at NMJs is seen in the Dok-7 Tg mice that overexpress Dok-7 only in the skeletal muscle, as well as in AAV-D7-treated mice.

Recent studies of mouse models of ALS and SMA revealed that peripheral motor nerve degeneration first manifests as reduction of the nerve terminal area with subsequent denervation at NMJs, and then proceeds proximally, a pattern known as "dying-back" pathology (*32*, *33*). Consistent with this, autopsies of patients with ALS or SMA suggest that motor neuron pathology begins at the distal axon and proceeds proximally (*32*, *34*). Because AAV-D7 has the potential to enlarge the nerve terminals at NMJs, it is tempting to speculate that Dok-7 therapy may counteract the "dying-back" pathology at NMJs and be beneficial in these multifactorial disorders of mostly unknown etiology. Interestingly, in a recent study of the SOD1 (superoxide dismutase 1) G93A Tg mouse model of ALS, it was reported that a modest, muscle-specific increase in MuSK expression via a transgene delayed denervation at NMJs and improved motor activity, but not survival, of the mice (*35*). However, a previous study had shown that higher-level expression of MuSK in the muscle induces scattered NMJ formation throughout myotubes, leading to severe muscle weakness and ultimately to death (*36*). Dok-7 gene therapy may be a safer approach because it greatly facilitates correct, centrally localized NMJ enlargement without lethal effects for more than 1 year in *DOK7* myasthenia mice (Figs. 2A and 3C and figs. S14 and S15).

AAV-mediated gene transfer to skeletal muscle can result in long-term expression of the therapeutic gene. For instance, in a patient with hemophilia B, AAV-mediated expression of factor IX was detected even 10 years after a single intramuscular injection (*37*). In addition, although AAV capsid, rather than transgene, is the antigen that is targeted by the host immune responses in humans, this process can be controlled by a short course of treatment with immunosuppressant without loss of transgene expression (*38*), raising prospects for long-term use of these vectors in therapies.

Our findings demonstrate that elevated Dok-7 expression, or any equivalent method that stably and safely enlarges the NMJ, has potential as a therapy for a variety of neuromuscular disorders that feature defects in NMJ structure, including those of unknown etiology. Such NMJ-targeted therapies could be administered alone or in combination with other therapies.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1505/suppl/DC1
Materials and Methods
Figs. S1 to S17
Reference (*39*–*43*)

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DATA REPORT

SLC16A2 mutations in two Japanese patients with Allan–Herndon–Dudley syndrome

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Allan–Herndon–Dudley syndrome (AHDS) is a neurodevelopmental disorder that manifests as intellectual disability and motor developmental delay. Thyroid hormone transporter dysfunction due to *SLC16A2* mutation is the underlying cause of this disorder. We identified a novel (P537del) and a recurrent (A150V) *SLC16A2* mutation in Japanese AHDS patients from two different families. A150V co-segregated with S33P. Both patients showed similar clinical features including severe neurological features and delayed myelination. Thyroid function showed a common finding of elevated T3 levels. No clear genotype–phenotype correlation was observed in patients with *SLC16A2* alterations.

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Allan–Herndon–Dudley syndrome (AHDS; MIM #300523) is recognized as a neurodevelopmental disorder that manifests in intellectual disability and motor developmental delay and is associated with infantile hypotonia. In 2004, the solute carrier family 16 member 2 gene (*SLC16A2*) was identified as the gene responsible for this disorder.[1] *SLC16A2* encodes a T3- and T4-transporter that is expressed in neuronal cells (monocarboxylate transporter 8; MCT8). MCT8 plays a decisive role in the transport of T3 into neurons.[2] Therefore, the abnormal thyroid function observed in AHDS patients is the consequence of transporter dysfunction in neuronal cells.

In 2009, *SLC16A2* mutations were identified in a number of patients initially presenting with mimicking phenotypes of Pelizaeus–Merzbacher disease (PMD) during the infantile period, in association with delayed myelination.[3,4] Similar to PMD, AHDS is inherited as an X-linked recessive trait because *SLC16A2* is located on Xq13.2. Many *SLC16A2* mutations have been reported.[5] In the present study, we identified *SLC16A2* mutations in two different patients who initially presented with phenotypes mimicking those of PMD.

Patient 1 is a 7-month-old Japanese boy, who was born at 41 weeks of gestation with a birth weight of 3,040 g (mean). He was the only child of non-consanguineous parents. The family had no history of neuromuscular disorders. The pregnancy was not remarkable, and no asphyxic events occurred during birth. Although he showed social smiling, he did not show age-appropriate head control at the 5-month health checkup and was referred to our hospital. He showed hypertonus of his extremities and often displayed an asymmetrical tonic neck reflex posture with opisthotonus. He could neither turn over nor sit. Laboratory examinations revealed abnormal thyroid function with a normal TSH level of 3.647 μg/ml (normal range: 0.46–3.73), a high free T3 level of 7.73 pg/ml (normal range: 2.51–4.12) and a low free T4 level of 0.52 ng/dl (normal range: 0.88–1.5). Brain magnetic resonance imaging (MRI) of the patient at the age of 7 months showed markedly delayed myelination and mildly reduced brain volume (Figure 1a, b).

Patient 2 is a 21-month-old Japanese boy who was born as the first child of healthy and non-consanguineous parents at 42 weeks of gestation with a birth weight of 3,686 g (90–97th percentile). At the age of 8 months, he was referred to our hospital because of a motor developmental delay. At that time, he had no head control and could not turn over. Physical examinations showed no abnormalities. The neurological examination showed conflicting findings with mild axial hypotonia and mild spasticity in his extremities, as indicated by accelerated deep tendon reflexes. The auditory brainstem response clearly showed all five response peaks, but a mild deafness pattern was indicated by the fact that the response could be recorded over 50 dB. Almost all laboratory examinations showed no abnormalities, but thyroid functions showed abnormal thyroid function with a high TSH level of 5.93 μg/ml (normal range: 0.49–4.94), a high free T3 level of 6.37 pg/ml (normal range: 1.71–3.71) and a normal free T4 level of 0.75 ng/dl (normal range: 0.7–1.48). A brain MRI examined at the age of 8 months showed no marked abnormality, but subsequent analysis at 17 months showed a delayed myelination pattern and mild dilatation of the extra-cerebrum space (Figure 1c, d). Chromosomal examination showed a normal male karyotype of 46,XY. At present, the patient shows severe developmental delay with no head control and no turning over. He is declared bedridden.

We suspected that *SLC16A2* mutations would be related in these two patients because of the combined presentation of delayed myelination and thyroid dysfunction with an elevated T3 pattern. After obtaining written informed consent, blood samples were obtained from both families for the purpose of molecular diagnosis. This study was approved by the ethical committee in Tokyo Women's Medical University. Genomic DNA extracted using a QIAamp DNA extraction kit (Qiagen, Hilden, Germany) was used for PCR-based direct Sanger sequencing of *SLC16A2*.

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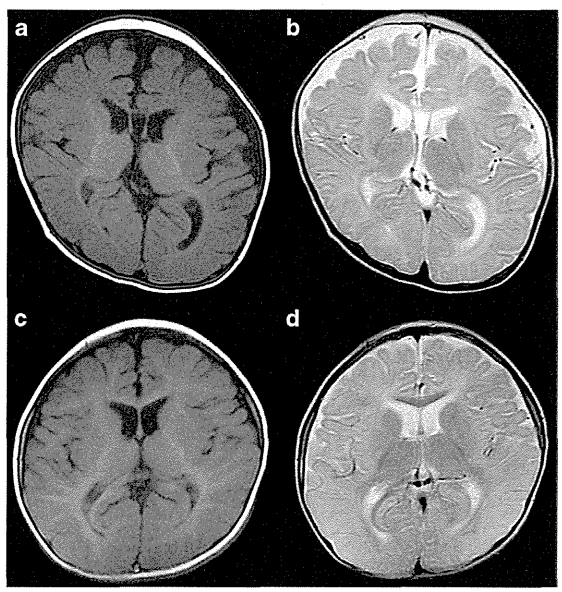

Figure 1. Brain magnetic resonance imaging of two patients.T1- and T2-weighted axial images of patient 1 examined at 7 months of age (**a** and **b**, respectively). Dilatation of the extra-cerebrum space and lateral ventricles is indicative of reduced brain volume. The genu of the corpus callosum is thin. Myelination is noted only in the posterior limb of the internal capsule and in the optic radiations, as shown in both of the T1- and T2-weighted images. T1- and T2-weighted axial images of patient 2 examined at 17 months of age (**c** and **d**, respectively). The extra-cerebrum space is remarkable. The T1-weighted image shows a normal myelination pattern with high intensity in the white matter (**c**); however, the T2-weighted image shows a delayed myelination pattern with low intensity only in the genu of the corpus callosum, anterior and posterior limbs of the internal capsule, and optic radiations (**d**).

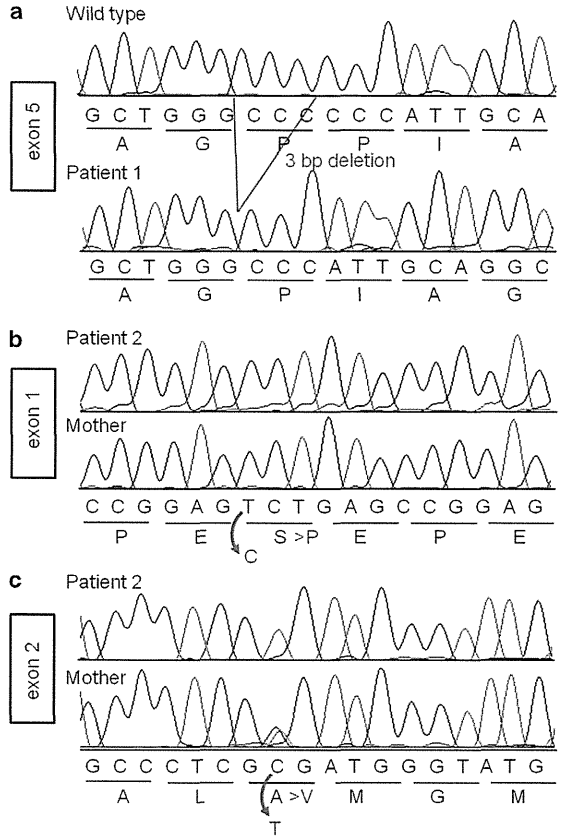

Figure 2. Electrophoregrams of the mutations identified in this study. (**a**) c.1390_1392delCCC is shown in *SLC16A2* exon 5 in patient 1. (**b**) Patient 2 and his mother are hemizygous and homozygous for c.97T>C in *SLC16A2* exon 1 (S33P), respectively. (**c**) Patient 2 and his mother are hemizygous and heterozygous for c.449C>T in *SLC16A2* exon 2 (A150V).

In patient 1, a 3-bp deletion (c.1390_1392delCCC), leading to an in-frame amino-acid deletion (P464del), was identified in exon 5 (Figure 2a). This deletion has never been previously reported. Because his mother declined to be genotyped, we do not know whether this mutation is *de novo* or familial.

In patient 2, we identified two single-nucleotide variants (SNVs) that lead to amino-acid changes: c.97T>C in exon 1 (S33P) and c.449C>T in exon 2 (A150V) (Figure 2b,c). The SNVs were listed in dbSNP build 138 as rs6647476 and rs104894936, respectively. The damaging effect scores of the SNVs were calculated using PolyPhen-2 and SIFT; however, no difference was observed between the SNVs. The PolyPhen-2 scores were 0.898 (S33P) and 0.673 (A150V), indicating that they were benign. SIFT scores were 0.47 (S33P) and 0.25 (A150V), indicating that the SNVs were TOLERATED. The minor allele frequency (MAF) of S33P was 36.632%, indicating that it is a common SNP with $\geqslant$1% MAF. In comparison, the MAF of A150V is low, at $<$1%, indicating that it is a flagged SNP. Based on these data, S33P was considered benign, whereas A150V was considered pathogenic. The mother of patient 2 was homozygous and heterozygous for S33P and A150V, respectively, showing that these variants co-segregated in the maternally derived allele.

In the present study, we diagnosed two patients with AHDS who showed severe developmental delay, especially with respect to motor development. Neither patient acquired head control. The results of the thyroid function examination showed elevated T3 levels in both patients despite variable TSH and T4 levels. This is the typical pattern for AHDS. Brain MRI showed severely delayed myelination in both patients. Additionally, the total brain volume was mildly reduced in both patients.

Molecular analysis identified a novel single-amino-acid deletion arising from a 3-bp nucleotide deletion in patient 1 (P464del).

A missense mutation, P464L, affecting this residue had been previously reported.[6] A150V identified in patient 2 has been recurrently reported,[7–9] and functional analysis of this mutation confirmed that it results in a complete loss of specific T3 uptake[8] and damaged T3 metabolism.[10] To our knowledge, co-segregation of S33P and A150V was reported for the first time in the present study. Although we had no evidence of whether A150V was inherited in this family or occurred *de novo*, the relatively frequent occurrence of A150V in AHDS patients suggests that this region containing a "CGCG" repeat sequence may be a mutation hot spot.

In conclusion, we identified an in-frame/single-amino-acid deletion as well as a recurrent missense mutation in AHDS patients. Because both patients showed similar clinical manifestations, no clear genotype–phenotype correlation was observed for *SLC16A2* alterations.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.507, http://dx.doi.org/10.6084/m9.figshare.hgv.509 and http://dx.doi.org/10.6084/m9.figshare.hgv.511.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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ORIGINAL COMMUNICATION

Epidemiological, clinical, and genetic landscapes of hypomyelinating leukodystrophies

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Abstract To determine the epidemiological, clinical, and genetic characteristics of congenital hypomyelinating leukodystrophies, including Pelizaeus–Merzbacher disease (PMD), we conducted a nationwide epidemiological survey in Japan. A two-step survey targeting all medical institutions specializing in pediatric neurology and childhood disability (919 institutes) in Japan was performed. Detailed information was collected for 101 patients (86 males and 15 females) with congenital hypomyelinating leukodystrophies. The prevalence of congenital hypomyelinating disorders was 0.78 per 100,000 people (0–19 years old), and the incidence was 1.40 per 100,000 live births. Molecular testing was performed in 75 % of patients, and *PLP1* gene abnormalities were observed in 62 %. The incidence of PMD with *PLP1* mutations was estimated to be 1.45 per 100,000 male live births and that for congenital hypomyelinating disorders with unknown cause to be 0.41 per 100,000 live births. Patients with *PLP1* mutations showed a higher proportion of nystagmus and hypotonia, both of which tend to disappear over time. Our results constitute the first nationwide survey of congenital hypomyelinating disorders, and provide the epidemiological, clinical, and genetic landscapes of these disorders.

Keywords Hypomyelinating leukodystrophy · Epidemiology · Pelizaeus–Merzbacher disease

Introduction

Congenital hypomyelinating leukodystrophies are a heterogeneous group of inherited diseases characterized by a substantial deficit in myelin deposition in the brain. These disorders are distinguished from classic demyelinating leukodystrophies characterized by myelin degeneration. Therefore, clinical manifestations, pathogenesis, and potential treatment approaches can be distinct for these two groups of disorders. Recently, at least 11 congenital

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GJC2 promoter mutations causing Pelizaeus–Merzbacher-like disease

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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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ORIGINAL INVESTIGATION

A hemizygous *GYG2* mutation and Leigh syndrome: a possible link?

Eri Imagawa · Hitoshi Osaka · Akio Yamashita · Masaaki Shiina · Eihiko Takahashi · Hideo Sugie · Mitsuko Nakashima · Yoshinori Tsurusaki · Hirotomo Saitsu · Kazuhiro Ogata · Naomichi Matsumoto · Noriko Miyake

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Abstract Leigh syndrome (LS) is an early-onset progressive neurodegenerative disorder characterized by unique, bilateral neuropathological findings in brainstem, basal ganglia, cerebellum and spinal cord. LS is genetically heterogeneous, with the majority of the causative genes affecting mitochondrial malfunction, and many cases still remain unsolved. Here, we report male sibs affected with LS showing ketonemia, but no marked elevation of lactate and pyruvate. To identify their genetic cause, we performed whole exome sequencing. Candidate variants were narrowed down based on autosomal recessive and X-linked recessive models. Only one hemizygous missense mutation (c.665G>C, p.W222S) in glycogenin-2 (*GYG2*) (isoform a: NM_001079855) in both affected sibs and a heterozygous change in their mother were identified, being consistent with the X-linked recessive trait. *GYG2* encodes glycogenin-2 (GYG2) protein, which plays an important role in the initiation of glycogen synthesis. Based on the structural modeling, the mutation can destabilize the structure and result in protein malfunctioning. Furthermore, in vitro experiments showed mutant GYG2 was unable to undergo the self-glucosylation, which is observed in wild-type GYG2. This is the first report of *GYG2* mutation in human, implying a possible link between GYG2 abnormality and LS.

Introduction

Glycogen is a large branched polysaccharide containing linear chains of glucose residues. Glycogen deposits in skeletal muscle and liver serve as shorter-term energy storage in mammals, while fat provides long-term storage. Glycogen biosynthesis begins with self-glucosylation of glycogenins by covalent binding of UDP-glucose to tyrosine residues of the glycogenins and the subsequent extension of approximately ten glucose residues (Pitcher et al. 1988; Smythe et al. 1988). Glycogen particles are formed by the continued addition of UDP-glucose to the growing

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A rapid screening with direct sequencing from blood samples for the diagnosis of Leigh syndrome

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ABSTRACT

Large numbers of genes are responsible for Leigh syndrome (LS), making genetic confirmation of LS difficult. We screened our patients with LS using a limited set of 21 primers encompassing the frequently reported gene for the respiratory chain complexes I (ND1–ND6, and ND4L), IV(SURF1), and V(ATP6) and the pyruvate dehydrogenase E1α-subunit. Of 18 LS patients, we identified mutations in 11 patients, including 7 in mDNA (two with ATP6), 4 in nuclear (three with SURF1). Overall, we identified mutations in 61% of LS patients (11/18 individuals) in this cohort. Sanger sequencing with our limited set of primers allowed us a rapid genetic confirmation of more than half of the LS patients and it appears to be efficient as a primary genetic screening in this cohort.

1. Introduction

Leigh syndrome (LS) (OMIM 256000) is an early onset, devastating neurodegenerative disease of the central nervous system (CNS) characterized by symmetrical necrotic lesions in the brainstem, basal

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CASE REPORT

A Japanese Adult Case of Guanidinoacetate Methyltransferase Deficiency

Tomoyuki Akiyama • Hitoshi Osaka • Hiroko Shimbo • Tomoshi Nakajiri • Katsuhiro Kobayashi • Makio Oka • Fumika Endoh • Harumi Yoshinaga

Received: 12 April 2013 / Revised: 12 May 2013 / Accepted: 26 May 2013

Abstract Guanidinoacetate methyltransferase (GAMT) deficiency is a rare disorder of creatine synthesis resulting in cerebral creatine depletion. We present a 38-year-old patient, the first Japanese case of GAMT deficiency. Developmental delay started after a few months of age with a marked delay in language, which resulted in severe intellectual deficit. She showed hyperactivity and trichotillomania from childhood. Epileptic seizures appeared at 18 months and she had multiple types of seizures including epileptic spasms, brief tonic seizures, atypical absences, complex partial seizures with secondary generalization, and "drop" seizures. They have been refractory to multiple antiepileptic drugs. Although there have been no involuntary movements, magnetic resonance imaging revealed T2 hyperintense lesions in bilateral globus pallidi. Motor regression started around 30 years of age and the patient is now able to walk for only short periods. Very low serum creatinine levels measured by enzymatic method raised a suspicion of GAMT deficiency, which was confirmed by proton magnetic resonance spectroscopy and urinary guanidinoacetate assay. *GAMT* gene analysis revealed that the patient is a compound heterozygote of c.578A>G, p.Gln193Arg and splice site mutation, c.391G>C, p.Gly131Arg, neither of which have been reported in the literature. We also identified two aberrant splice products from the patient's cDNA analysis. The patient was recently started on supplementation of high-dose creatine and ornithine, the effects of which are currently under evaluation. Although rare, patients with developmental delay, epilepsy, behavioral problems, and movement disorders should be vigorously screened for GAMT deficiency, as it is a treatable disorder.

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Introduction

Guanidinoacetate methyltransferase (GAMT; OMIM 601240) deficiency is a rare autosomal recessive disorder of creatine synthesis resulting in cerebral creatine depletion (Stöckler et al. 1994, 1996b). Guanidinoacetate (GAA) accumulates in body fluids. Symptoms of GAMT deficiency usually emerge after a few months of life, such as intellectual disability, speech delay, autistic behaviors, epileptic seizures, and involuntary movements (Mercimek-Mahmutoglu et al. 2006). Making a diagnosis of GAMT deficiency is challenging; nonetheless, early diagnosis is crucial because this disorder is treatable (Stöckler et al. 1996a). Only approximately 80 cases have been reported to date, mostly from Europe and the Middle East. Here we report on the first Japanese patient with GAMT deficiency with two novel gene mutations.

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Short Report

Genotype–phenotype correlation of contiguous gene deletions of *SLC6A8*, *BCAP31* and *ABCD1*

van de Kamp J.M., Errami A., Howidi M., Anselm I., Winter S., Phalin-Roque J., Osaka H., van Dooren S.J.M., Mancini G.M., Steinberg S.J., Salomons G.S. Genotype–phenotype correlation of contiguous gene deletions of *SLC6A8*, *BCAP31* and *ABCD1*. Clin Genet 2015: 87: 141–147. © John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, 2014

The *BCAP31* gene is located between *SLC6A8*, associated with X-linked creatine transporter deficiency, and *ABCD1*, associated with X-linked adrenoleukodystrophy. Recently, loss-of-function mutations in *BCAP31* were reported in association with severe developmental delay, deafness and dystonia. We characterized the break points in eight patients with deletions of *SLC6A8*, *BCAP31* and/or *ABCD1* and studied the genotype–phenotype correlations. The phenotype in patients with contiguous gene deletions involving *BCAP31* overlaps with the phenotype of isolated *BCAP31* deficiency. Only deletions involving both *BCAP31* and *ABCD1* were associated with hepatic cholestasis and death before 1 year, which might be explained by a synergistic effect. Remarkably, a patient with an isolated deletion at the 3′-end of *SLC6A8* had a similar severe phenotype as seen in *BCAP31* deficiency but without deafness. This might be caused by the disturbance of a regulatory element between *SLC6A8* and *BCAP31*.

Conflict of interest

The authors have no conflict of interest.

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Key words: clinical genetics – creatine transporter deficiency – deletion – intellectual disability – liver disease – metabolic disorders – neurology – X-linked adrenoleukodystrophy

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BRIEF COMMUNICATION

PIGO mutations in intractable epilepsy and severe developmental delay with mild elevation of alkaline phosphatase levels

***Kazuyuki Nakamura, †Hitoshi Osaka, ‡Yoshiko Murakami, †Rie Anzai, *Kiyomi Nishiyama, *Hirofumi Kodera, *Mitsuko Nakashima, *Yoshinori Tsurusaki, *Noriko Miyake, ‡Taroh Kinoshita, *Naomichi Matsumoto, and *Hirotomo Saitsu**

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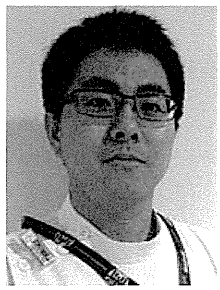

Kazuyuki Nakamura is a pediatric neurologist, and researches for epilepsy and brain malformation.

Summary

Aberrations in the glycosylphosphatidylinositol (GPI)–anchor biosynthesis pathway constitute a subclass of congenital disorders of glycosylation, and mutations in seven genes involved in this pathway have been identified. Among them, mutations in *PIGV* and *PIGO*, which are involved in the late stages of GPI-anchor synthesis, and *PGAP2*, which is involved in fatty-acid GPI-anchor remodeling, are all causative for hyperphosphatasia with mental retardation syndrome (HPMRS). Using whole exome sequencing, we identified novel compound heterozygous *PIGO* mutations (c.389C>A [p.Thr130Asn] and c.1288C>T [p.Gln430*]) in two siblings, one of them having epileptic encephalopathy. GPI-anchored proteins (CD16 and CD24) on blood granulocytes were slightly decreased compared with a control and his mother. Our patients lacked the characteristic features of HPMRS, such as facial dysmorphology (showing only a tented mouth) and hypoplasia of distal phalanges, and had only a mild elevation of serum alkaline phosphatase (ALP). Our findings therefore expand the clinical spectrum of GPI-anchor deficiencies involving *PIGO* mutations to include epileptic encephalopathy with mild elevation of ALP.

KEY WORDS: Congenital disorders of glycosylation, Epileptic encephalopathy, Glycosylphosphatidylinositol anchors, *PIGO*.

More than 100 mammalian cell-surface proteins are anchored to the plasma membrane by the addition of glycosylphosphatidylinositol (GPI) to their C-termini. More than 20 genes are involved in the GPI-anchor biosynthesis pathway[1,2] of which 7 are mutated in GPI-anchor deficiencies, a subclass of congenital glycosylation disorders, in association with neurologic impairments.[3–7] Among them, mutations in *PIGV*, *PIGO* (both are involved in the last step of GPI-anchor synthesis), and *PGAP2* (involved in fatty-acid GPI-anchor remodeling) have been identified in patients with hyperphosphatasia with mental retardation syndrome (HPMRS), also known as Mabry syndrome.[3–8]

PIGO encodes GPI ethanolamine phosphate transferase 3, which is also known as phosphatidylinositol-glycan biosynthesis class O. To date, only three HPMRS families with compound heterozygous mutations in *PIGO* have been reported. In this study, we performed whole exome sequencing of a Japanese family containing two affected siblings, one of them having epileptic encephalopathy, and identified novel *PIGO* mutations that expand the clinical spectrum of *PIGO* abnormalities to include epileptic encephalopathy.

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Case Report

A Japanese girl with an early-infantile onset vanishing white matter disease resembling Cree leukoencephalopathy

Kyoko Takano [a,b,*], Yu Tsuyusaki [a], Mutsumi Sato [a], Mariko Takagi [a], Rie Anzai [a], Mitsuko Okuda [a], Mizue Iai [a], Sumimasa Yamashita [a], Tetsuhiko Okabe [c], Noriko Aida [c], Yoshinori Tsurusaki [d], Hirotomo Saitsu [d], Naomichi Matsumoto [d], Hitoshi Osaka [a,e]

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Abstract

Vanishing white matter disease (VWM)/childhood ataxia with central hypomyelination (CACH) is an autosomal recessive leukoencephalopathy caused by mutations in one of five genes, *EIF2B1–5*, encoding the 5 subunits of eukaryotic translation initiation factor 2B (eIF2B). The classical phenotype is characterized by early childhood onset and chronic progressive neurological deterioration with cerebellar ataxia, spasticity, optic atrophy and epilepsy. However, the onset of disease varies from antenatal period to adulthood. Cree leukoencephalopathy (CLE) is a severe variant of VWM and caused by a homozygous mutation (R195H) in the *EIF2B5* gene.

The patient reported in this study developed lethargy, vomiting and seizure 3 days after an oral poliovirus vaccination at the age of 4 months. She presented with rapid neurological deterioration within a month of onset. Brain MRI showed abnormal white matter intensity. Whole-exome sequencing identified two heterozygous mutations in the *EIF2B5* gene: a known mutation, c.584G>A (R195H, which is homozygous in CLE), and a novel mutation, c.1223T>C (I408T, which resides in the "I-patch"). Mutations in the "I-patch" encoded region of eIF2Bε may be related to an early-infantile onset phenotype. This patient exhibits an early-infantile onset and progressive disease course resembling CLE, suggesting a severe functional disruption of eIF2Bε caused by R195H as well as by I408T mutations.

Keywords: Vanishing white matter disease (VWM); Cree leukoencephalopathy; Eukaryotic translation initiation factor 2B; eIF2Bε; *EIF2B5*

1. Introduction

Vanishing white matter disease (VWM, OMIM# 603896)/childhood ataxia with central hypomyelination (CACH) is an autosomal recessive brain disorder showing white matter rarefaction and cystic degeneration. Neurological signs are dominated by progressive cerebellar ataxia, spasticity, optic atrophy and epilepsy. Febrile infections and minor head trauma may provoke rapid neurological deterioration following normal development [1]. Typical onset of VWM is at age 2–6 years, but varies from prenatal to adulthood. Patients with

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0387-7604/

破壊性獲得性二次性障害

大脳萎縮症

Cerebral atrophy

小坂 仁

Key words：アポトーシス，ネクローシス，白質，灰白質

1. 概念・定義

大脳が形成された後の何らかの破壊，萎縮による細胞死による大脳萎縮．

2. 疫　　学

我が国における大規模な調査はなく不明である．

3. 病　　因[1-3]

1）出生前の異常

a. 母体環境によるもの

a）母体疾患によるもの

妊娠中毒症，糖尿病，甲状腺疾患，高血圧，膠原病などは，胎盤機能不全などを通じて大脳萎縮を生じさせる可能性がある．

b）薬剤による影響

向精神薬，抗痙攣薬，アルコール，麻薬，麻酔薬など

b. 胎内感染

トキソプラズマ，風疹ウイルス，サイトメガロウイルス，単純ヘルペスウイルス感染など

c. 染色体異常，代謝性疾患などの遺伝子異常による疾患

2）周産期異常

仮死，血管障害，分娩外傷など

3）出生後の異常

(1) 代謝異常などの遺伝子異常による疾患

(2) 感染，脳症，脳血管障害，外傷など後天性疾患

4. 病　　態[1]

中枢神経系の構成細胞であるニューロン，オリゴデンドロサイト，アストロサイトなどに，不可逆的な細胞傷害が起き，細胞死が起こることによる萎縮性変化が基本病態である．未熟な細胞に生じる障害は成人期と異なる反応を示す．細胞死の分子機構を図1に示す．

細胞死には，従来顕微鏡学的に細胞膜が消失し，細胞内小器官が浮腫し，空胞化しタンパク分解酵素やDNA分解酵素が活性化しクロマチンが無秩序に分解されDNAがスメア様に分解を受けるネクローシスおよび，ATPを用い，分解酵素のカスパーゼによって担われ，DNAがラダー状に分解を受ける細胞死であるアポトーシスが知られている．新生児低酸素性虚血性障害の研究などより，アポトーシスとネクローシスは厳密に区別されるものではなく，一連の反応であることがわかっている．

5. 診断と鑑別診断(表1)[1-5]

遺伝性疾患には非常に多くの鑑別診断が存在するため，MRIにより画像的に病変の主体が灰白質(ニューロン)か白質(オリゴデンドロサイトなど)で分類し，灰白質も，皮質，深部灰白質に分け，白質も皮質下白質，深部白質かで分け分類する．また病変が，髄鞘化不全によるものはこれらから鑑別し，臨床・検査所見から原因診断に至る．

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Original Research

Neurochemistry in Shiverer Mouse Depicted on MR Spectroscopy

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Purpose: To evaluate the neurochemical changes associated with hypomyelination, especially to clarify whether increased total *N*-acetylaspartate (tNAA) with decreased choline (Cho) observed in the thalamus of *msd* mice with the *plp1* mutation is a common finding for hypomyelinating disorders.

Materials and Methods: We performed magnetic resonance imaging (MRI) and proton MR spectroscopy (^{1}H-MRS) of the thalamus and cortex of postnatal 12-week shiverer mice devoid of myelin basic protein (mbp), heterozygous and wild-type mice with a 7.0T magnet. Luxol Fast Blue staining and immunohistochemical analysis with anti-Mbp, Gfap, Olig2, and NeuN antibodies were also performed.

Results: In the thalamus, decreased Cho and normal tNAA were observed in shiverer mice. In the cortex, tNAA, Cho, and glutamate were decreased in shiverer mice. Histological and immunohistochemical analysis of shiverer mice brains revealed hypomyelination in the thalamus, white matter, and cortex; astrogliosis and an increased number of total oligodendrocytes in the white matter; and a decreased number of neurons in the cortex.

Conclusion: The reduction of Cho on ^{1}H-MRS might be a common marker for hypomyelinating disorders. A normal tNAA level in the thalamus of shiverer mice might be explained by the presence of mature oligodendrocytes, which enable neuron-to-oligodendrocyte NAA transport or NAA catabolism.

Key Words: magnetic resonance spectroscopy; *N*-acetylaspartate; choline; hypomyelination; myelin basic protein; shiverer mouse

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THE TERM HYPOMYELINATION describes a permanent, substantial deficit of myelin deposition in the brain. The protein composition of myelin in the central nervous system (CNS) is simpler than that of other membranes; the two major components are proteolipid protein (PLP) and myelin basic protein (MBP), which account for 50% and 30% of the total myelin protein, respectively. MBP, the second major structural protein of the myelin sheath of the mammalian CNS, is associated with the major dense line (1). Shiverer (*shi/shi*) is an autosomal recessive mouse mutation of the *mbp* gene, which deletes a 20-kb region including exons 3–7, resulting in the absence of mbp (1–3). Oligodendrocytes of shiverer mice fail to assemble compacted myelin (1,2), which causes an almost total lack of myelin (hypomyelination) in the CNS.

Despite progress in understanding the molecular basis and neuroimaging characteristics of Pelizaeus-Merzbacher disease (PMD) (4,5), a representative hypomyelination disease due to derangement of the *PLP1* gene, the neurochemical changes associated with hypomyelination remains unknown. We performed proton magnetic resonance spectroscopy (^{1}H-MRS) with a 7.0T magnet on the brains of *myelin synthesis-deficient* (*msd*) mice, a model of connatal PMD, one of the most severely affected murine mutants as to the *plp1* gene. ^{1}H-MRS of *msd* mice showed increased total *N*-acetylaspartate (tNAA; NAA, 2.01 ppm, and *N*-acetylaspartylglutamate [NAAG] 2.04 ppm, which are difficult to distinguish on ^{1}H-MRS) and decreased choline (Cho) (6), as observed in

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