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Case report

# MR spectroscopy in 18q⁻ syndrome suggesting other than hypomyelination

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**Abstract**

We reported a 5-year-old boy with 18q⁻ syndrome who showed typical magnetic resonance imaging (MRI) findings of high signal intensity on T2-weighted imaging, and a slightly high but lower than normal signal on T1-weighted imaging of the white matter. MR spectroscopy (MRS) revealed increased concentrations of creatine, myoinositol and choline with a normal *N*-acetylaspartate one. The cerebral white matter lesions observed on MRI in patients with 18q⁻ syndrome have been considered to reflect hypomyelination due to a decrease in myelin basic protein so far, however, MRS suggested reactive astrocytic gliosis and accelerated myelin turnover, which are compatible with recent pathological reports of 18q⁻ syndrome.


*Keywords:* 18q⁻ syndrome, MR spectroscopy; Hypomyelination; Gliosis; *MBP* gene

## 1. Introduction

18q⁻ syndrome is a rare chromosomal disorder involving frequent abnormal intensity of the white matter on T2-weighted imaging (T2WI) and the following clinical features: developmental delay, growth retardation, hearing loss, hypotonia, craniofacial dysmorphism, foot deformities, and eye movement disorders. Myelin basic protein (MBP) accounts for 30–40% of the total myelin protein in the central nervous system [1], and is thought to play an important role in myelin compaction. As the gene for MBP is encoded on 18q23, the region most commonly deleted in 18q⁻ syndrome [2,3], it has been considered that the magnetic resonance imaging (MRI) findings probably reflect hypomyelination due to a haploinsufficiency of MBP [1]. We report a 5-year-old boy with 18q⁻ syndrome in whom quantitative MR spectroscopy (MRS) revealed increased choline (Cho), creatine (Cr), and myoinositol (mIns) concentrations. The increased Cho suggested accelerated myelin turnover rather than hypomyelination, and the increased Cr and mIns suggested astrocytic gliosis. The latter is compatible with a recent neuropathological report of 18q⁻ syndrome.

## 2. Case report

The patient, a 5-year-old Japanese boy, was delivered at 40 weeks gestation by means of vacuum extraction, weighing 3656 gm. He had no congenital cardiac disease or hearing loss. His motor and mental development were slow; he could only roll over at 6 months, sit alone at 9 months, and walk with support at 15 months and without support at 23 months. He did not utter any significant words at 2 years. G-Banding analysis revealed

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Original article

# Different patterns of cerebellar abnormality and hypomyelination between *POLR3A* and *POLR3B* mutations

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**Abstract**

*Background:* Mutations of *POLR3A* and *POLR3B* have been reported to cause several allelic hypomyelinating disorders, including hypomyelination with hypogonadotropic hypogonadism and hypodontia (4H syndrome). *Patients and methods:* To clarify the difference in MRI between the two genotypes, we reviewed MRI in three patients with *POLR3B* mutations, and three with *POLR3A* mutations. *Results:* Though small cerebellar hemispheres and vermis are common MRI findings with both types of mutations, MRI in patients with *POLR3B* mutations revealed smaller cerebellar structures, especially vermis, than those in *POLR3A* mutations. MRI also showed milder hypomyelination in patients with *POLR3B* mutations than those with *POLR3A* mutations, which might explain milder clinical manifestations. *Conclusions:* MRI findings are distinct between patients with *POLR3A* and *3B* mutations, and can provide important clues for the diagnosis, as these patients sometimes have no clinical symptoms suggesting 4H syndrome.


*Keywords:* Hypomyelination; MRI; Hypomyelination with hypogonadotropic hypogonadism and hypodontia (4H syndrome); Diffuse cerebral hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum (HCAHC); Cerebellum; *POLR3A*; *POLR3B*; RNA polymerase III (Pol III)

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# Reward-timing-dependent bidirectional modulation of cortical microcircuits during optical single-neuron operant conditioning

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Animals rapidly adapt to environmental change. To reveal how cortical microcircuits are rapidly reorganized when an animal recognizes novel reward contingency, we conduct two-photon calcium imaging of layer 2/3 motor cortex neurons in mice and simultaneously reinforce the activity of a single cortical neuron with water delivery. Here we show that when the target neuron is not relevant to a pre-trained forelimb movement, the mouse increases the target neuron activity and the number of rewards delivered during 15-min operant conditioning without changing forelimb movement behaviour. The reinforcement bidirectionally modulates the activity of subsets of non-target neurons, independent of distance from the target neuron. The bidirectional modulation depends on the relative timing between the reward delivery and the neuronal activity, and is recreated by pairing reward delivery and photoactivation of a subset of neurons. Reward-timing-dependent bidirectional modulation may be one of the fundamental processes in microcircuit reorganization for rapid adaptation.

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# Dystrophic *mdx* mice develop severe cardiac and respiratory dysfunction following genetic ablation of the anti-inflammatory cytokine IL-10

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**Duchenne muscular dystrophy (DMD) is a progressive muscle-wasting disease that causes respiratory and cardiac failure. Inflammation is a key pathological characteristic of dystrophic muscle lesion formation, but its role and regulation in the disease time course has not been sufficiently examined. In the present study, we used *IL-10*$^{-/-}$*/mdx* mice lacking both dystrophin and the anti-inflammatory cytokine, interleukin-10 (IL-10), to investigate whether a predisposition to inflammation affects the severity of DMD with advancing age. The IL-10 deficiency caused a profound DMD phenotype in the dystrophic heart such as muscle degeneration and extensive myofiber loss, but the limb muscle and diaphragm morphology of *IL-10*$^{-/-}$*/mdx* mice was similar to that of *mdx* mice. Extensive infiltrates of pro-inflammatory M1 macrophages in regeneration of cardiotoxin-injured muscle, altered M1/M2 macrophage phenotype and increased pro-inflammatory cytokines/chemokines production were observed in the diaphragm and heart of *IL-10*$^{-/-}$*/mdx* mice. We characterized the *IL-10*$^{-/-}$*/mdx* mice as a dystrophic model with chronic inflammation and severe cardiorespiratory dysfunction, as evidenced by decreased percent fractional shortening (%FS) and ejection fraction percent (EF%) on echocardiography, reduced lower tidal volume on whole-body plethysmography. This study suggests that a predisposition to inflammation is an important indicator of DMD disease progression. Therefore, the development of anti-inflammatory strategies may help in slowing down the cardiorespiratory dysfunction on DMD.**

## INTRODUCTION

Duchenne muscular dystrophy (DMD) is a severe X-linked muscle disease in which mutations in the gene encoding the cytoskeletal protein, dystrophin (1,2). The altered mechanical and signaling functions contribute to membrane fragility, necrosis and inflammation and result in progressive degeneration of striated muscle, manifesting as muscle weakness and, eventually, skeletal muscle atrophy (3). As the disease progresses, wheelchair and ventilatory assistance are required, and patients often succumb to cardiac dysfunction and respiratory failure (4).

Inflammation is a large component of the muscle pathology in DMD. Anti-inflammatory glucocorticoids are widely used to improve muscle strength of DMD patients (5–7), although the beneficial effects of glucocorticoids vary from patient to patient. Furthermore, long-term as well as short-term steroid treatment induces side effects. Therefore, new and improved therapeutic approaches to the treatment of DMD are needed. Moreover, it is important to determine the effect of chronic inflammation on DMD progression.

Activated immune-cell infiltrates (e.g. T lymphocytes and macrophages) are observed during early disease stages in dystrophic muscle and play critical roles in muscle wasting (8–13). Depletion or inhibition of these cells significantly improves dystrophic muscle pathology (11,14,15). The findings of these studies suggest that much of the muscle damage in dystrophin deficiency is caused by inflammatory cells as well as by direct mechanical damage, although mechanical injury and membrane defects induced by infiltration of inflammatory cells are also known to promote dystrophic pathology (16,17). *Mdx* mice are widely used as a model of DMD to evaluate various therapeutic strategies, but the pathological features in these mice are relatively mild

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Mini Review

# Cell therapeutic approaches using multipotent mesenchymal stromal cells for muscular dystrophy

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**Multipotent mesenchymal stromal cells (MSCs) have potential therapeutic uses owing to their ability to differentiate *in situ* into various cell types with immunosuppressive properties. Clinically, MSCs have been used to treat inflammatory diseases, such as steroid-resistant graft-versus-host disease. We previously reported a strategy to expand MSC cultures and to induce these cells to undergo myogenic differentiation, which is promising for the treatment of muscular diseases. Muscular dystrophy is an incurable genetic disease with early mortality and causes skeletal muscle weakness with chronic inflammation. Here, we focused on the beneficial properties of MSCs, namely, they can undergo mesoderm differentiation, have the ability to fuse with dystrophic muscles, and have anti-inflammatory activities. In this review, we highlight and discuss MSC-based therapeutic approaches for muscular dystrophy.**

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Key words multipotent mesenchymal stromal cells, anti-inflammation, muscular dystrophy, muscular differentiation, cell transplantation

## Introduction

Multipotent mesenchymal stromal cells (MSCs) from bone-marrow are conventionally defined as adherent non-hematopoietic cells that express several cell surface antigenic markers, such as CD44, CD73, CD90, and CD105, but not the hematopoietic markers CD34 or CD45[1)]. Although they were originally identified in bone-marrow[1)], MSCs can be extracted from numerous tissues including

# Two distinct layer-specific dynamics of cortical ensembles during learning of a motor task

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**The primary motor cortex (M1) possesses two intermediate layers upstream of the motor-output layer: layer 2/3 (L2/3) and layer 5a (L5a). Although repetitive training often improves motor performance and movement coding by M1 neuronal ensembles, it is unclear how neuronal activities in L2/3 and L5a are reorganized during motor task learning. We conducted two-photon calcium imaging in mouse M1 during 14 training sessions of a self-initiated lever-pull task. In L2/3, the accuracy of neuronal ensemble prediction of lever trajectory remained unchanged globally, with a subset of individual neurons retaining high prediction accuracy throughout the training period. However, in L5a, the ensemble prediction accuracy steadily improved, and one-third of neurons, including subcortical projection neurons, evolved to contribute substantially to ensemble prediction in the late stage of learning. The L2/3 network may represent coordination of signals from other areas throughout learning, whereas L5a may participate in the evolving network representing well-learned movements.**



M1 is the most prominent motor-output area of the cerebral cortex. In M1, L2/3 and L5a constitute intermediate layers upstream of layer 5b (L5b), the major motor-output layer. L2/3 and L5a transmit excitatory flow to L5b, but much less information is transmitted in the reverse direction, that is, from L5b to L2/3 or L5a[1,2]. Although L2/3 and L5a are reciprocally connected in M1, only L5a projects to subcortical regions such as the striatum[2]. During motor learning, the microcircuits of M1 are thought to self-organize to integrate various types of signals related to motor planning, motor primitives and sensory feedback into motor output[3–6]. In fact, when repetitive training is used to improve motor performance, M1 is functionally and structurally reorganized[7–11]. However, it is challenging to identify L5a neurons and record their activity *in vivo*, and it remains unknown how the neuronal activities of L2/3 and L5a of M1 are reorganized during learning of a motor task.

The ability to predict movement by monitoring motor cortical ensemble activity often improves with motor learning[12–15]. Furthermore, representation of movement in single L2/3 neurons in M1 changes dynamically during motor learning (over six sessions of a sensorimotor discrimination task)[16]. The single-neuron representation of movement in L5 of M1 is unreliable during the first 3 d of a two-choice movement task, but the accuracy of prediction improves during this period[14]. Functional and structural reorganization in L5 of M1 is prominent in the late stages of motor learning, after performance has plateaued[10,11]. We hypothesize that this change in the predictive accuracy of neuronal ensembles is achieved by one of two models in which the predictive accuracies of single-neuron activities variously change[12–17]. The first model is the rank-stable evolving model, which is based on the concept that single-neuron representations of movement are relatively stable[18,19]. In this model, a subset of neurons greatly contributes to the ensemble representation in both the early and late stages of learning. Neurons representing motor primitives or sensory feedback that are necessarily linked to movement continuously contribute to the ensemble representation, and their predictive accuracy rank[20] remains high throughout learning. The second model is the rank-changing evolving model, in which a subset of neurons rises through the predictive accuracy rankings[19]. In the late stage of learning, these neurons contribute to the ensemble representation more substantially than the neurons that were highly ranked in the early stages of learning.

Mouse forelimb M1, also known as the caudal forelimb area[21,22], is a subdivision of M1 in which low-intensity intracortical microstimulation elicits forelimb movements rather than other body movements[21,22]. Its activity is necessary for the performance of a forelimb movement (lever pull) task and many neurons in the rodent forelimb M1 exhibit forelimb movement-associated activity[21,23]. We conducted two-photon calcium imaging in L2/3 and L5a of the forelimb M1 while mice practiced a self-initiated lever-pull task (requiring forelimb use) for 14 consecutive days. L2/3 and L5a were identified according to the cortical depth of the imaging plane. We found that ensemble and single-neuron activities that predicted the lever trajectory were dynamically reorganized in two distinct layer-specific manners during long-term training.

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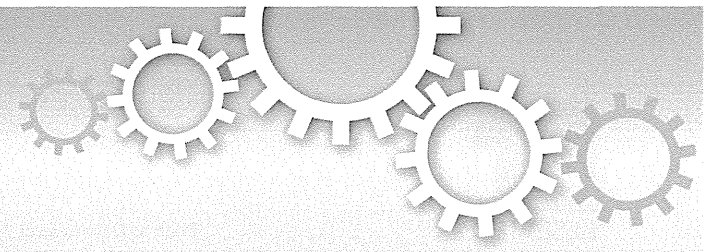

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# Fukutin is prerequisite to ameliorate muscular dystrophic phenotype by myofiber-selective LARGE expression

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**α-Dystroglycanopathy (α-DGP) is a group of muscular dystrophy characterized by abnormal glycosylation of α-dystroglycan (α-DG), including Fukuyama congenital muscular dystrophy (FCMD), muscle-eye-brain disease, Walker-Warburg syndrome, and congenital muscular dystrophy type 1D (MDC1D), etc. LARGE, the causative gene for MDC1D, encodes a glycosyltransferase to form [-3Xyl-α1,3GlcAβ1-] polymer in the terminal end of the post-phosphoryl moiety, which is essential for α-DG function. It has been proposed that LARGE possesses the great potential to rescue glycosylation defects in α-DGPs regardless of causative genes. However, the in vivo therapeutic benefit of using LARGE activity is controversial. To explore the conditions needed for successful LARGE gene therapy, here we used *Large*-deficient and *fukutin*-deficient mouse models for MDC1D and FCMD, respectively. Myofibre-selective LARGE expression via systemic adeno-associated viral gene transfer ameliorated dystrophic pathology of *Large*-deficient mice even when intervention occurred after disease manifestation. However, the same strategy failed to ameliorate the dystrophic phenotype of *fukutin*-conditional knockout mice. Furthermore, forced expression of *Large* in *fukutin*-deficient embryonic stem cells also failed to recover α-DG glycosylation, however coexpression with *fukutin* strongly enhanced α-DG glycosylation. Together, our data demonstrated that fukutin is required for LARGE-dependent rescue of α-DG glycosylation, and thus suggesting new directions for LARGE-utilizing therapy targeted to myofibres.**

α-Dystroglycanopathy (α-DGP) is a genetically and clinically heterogeneous group of muscular dystrophy[1,2] for which more than 15 causative genes have been identified[3–21]: *POMT1, POMT2, POMGnT1, fukutin, FKRP, LARGE, ISPD, GTDC2* (*POMGnT2*), *DAG1, TMEM5, B3GALNT2, SGK196* (*POMK*), *B3GNT1* (*B4GAT1*), *GMPPB, DOLK, DPM1, DPM2 and DPM3*. Regardless of the causative gene, α-DGP is characterized by abnormal glycosylation of α-DG, indicating that the disease is associated with defects in the glycosylation pathway for α-DG. α-DG is a cell surface receptor for matrix and synaptic proteins such as laminins, agrin, perlecan, neurexin, and pikachurin[22,23]. A unique *O*-mannosyl glycosylation is required for the ligand-binding activity of α-DG, and abnormal glycosylation leads to reduced ligand-binding activity[24,25]. α-DG also interacts with a transmembrane β-DG, which in turn binds to intracellular dystrophin[23]. Thus, proper glycosylation of α-DG is necessary for the connection between the basement membrane and cytoskeleton. Disruption of this linkage is thought to cause myofibre membrane weakness, leading to disease-predisposing muscle cell necrosis[26]. Although myofibres can regenerate after necrosis, it has been shown that muscle regeneration activity is impaired in α-DGP[27]. Thus, α-DG glycosylation is important for maintenance of skeletal muscle viability and defects in this process underlie the pathogenesis of α-DGP.

α-DGP includes Fukuyama congenital muscular dystrophy (FCMD), muscle-eye-brain disease (MEB), Walker-Warburg syndrome (WWS), and several types of congenital muscular dystrophies (MDCs) and limb-girdle muscular dystrophies (LGMDs)[1,2]. The clinical spectrum of α-DGP is wide; the most severe cases exhibit congenital muscular dystrophy with structural abnormalities in the brain and eyes, whereas the mildest form presents as adult-onset LGMD with no central nervous system involvement[28–30]. In addition, there is no clear genotype-phenotype correlation. Thus, it has been proposed that α-DGPs can be classified into three broad phenotypic groups, MDDG (muscular dystrophy dystroglycanopathy) type A, B and C[29]: MDC with brain/eye abnormalities (A), MDC with milder brain structural abnormalities (B), and LGMD (C). FCMD is the first

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Original article

# A de novo *TUBB4A* mutation in a patient with hypomyelination mimicking Pelizaeus–Merzbacher disease

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**Abstract**

*Objective:* Hypomyelinating leukoencephalopathy is a heterogeneous disorder caused by mutations in several-different genes. Clinical entity of hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) is one of them.

*Method:* A male patient showed pendular nystagmus, infantile hypotonia, an abnormal pattern of brain auditory evoked potential, and hypomyelination on brain magnetic resonance imaging, which suggested Pelizaeus–Merzbacher disease (PMD) as the candidate diagnosis; however, no abnormality was found in the proteolipid protein 1 gene (*PLP1*) that is responsible for PMD. Whole exome sequencing was performed to identify pathogenic mutations in this patient.

*Results:* A de novo mutation was identified in the tubulin 4a gene (*TUBB4A*), which has been recently reported to be associated with H-ABC. Although the patient did not show any neurological features suggesting H-ABC, such as extrapyramidal or cerebellar signs, radiological findings demonstrated the finding of cerebellar atrophy at the age of 36 months.

*Conclusion:* This study suggested us the difficulty of clinical diagnosis for H-ABC early in the life of the patient, which makes predication of prognosis and genetic counseling difficult.


*Keywords:* Hypomyelinating leukoencephalopathy; *TUBB4A*; Hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC); Pelizaeus–Merzbacher disease (PMD); Genetic counseling

## 1. Introduction

There are heterogeneous genetic backgrounds in patients with hypomyelinating leukoencephalopathy (HLD), which are classified into eight groups by Online Mendelian Inheritance in Man (OMIM; http://www.omim.org/). Pelizaeus–Merzbacher disease (PMD; MIM# 312080 [HLD1]) is the most well-known disorder

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Clinical Observations

# Clinical Course and Images of Four Familial Cases of Allan-Herndon-Dudley Syndrome With a Novel Monocarboxylate Transporter 8 Gene Mutation

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ABSTRACT

**BACKGROUND:** Allan-Herndon-Dudley syndrome, an X-linked condition characterized by severe intellectual disability, dysarthria, athetoid movements, muscle hypoplasia, and spastic paraplegia, is associated with defects in the monocarboxylate transporter 8 gene (*MCT8*). The long-term prognosis of Allan-Herndon-Dudley syndrome remains uncertain. **PATIENTS:** We describe the clinical features and course of four adults in a family with Allan-Herndon-Dudley syndrome with athetoid type cerebral palsy. **RESULTS:** We identified an *MCT8* gene mutation in this family. Two of the four affected family members died at 32 and 24 years of age. **CONCLUSIONS:** Individuals with Allan-Herndon-Dudley syndrome are at increased risk for recurrent infection, such as aspiration pneumonia. These individuals require careful management with consideration for this increased risk of recurrent infection.

***Keywords:*** Allan-Herndon-Dudley syndrome, monocarboxylate transporter 8 (MCT8) gene, brain MRI, cerebral palsy

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## Introduction

Allan-Herndon-Dudley syndrome is an X-linked syndrome characterized by severe intellectual disability, dysarthria, athetoid movements, muscle hypoplasia, and spastic paraplegia.[1] It is caused by mutations in the monocarboxylate transporter 8 gene (*MCT8*).[2,3] *MCT8* encodes an active thyroid hormone transporter, which induces a greater than 10-fold increase in the uptake of triiodothyronine ($T_3$) into cells and to a lesser extent thyroxine ($T_4$), and is expressed differentially in human tissues.[4] Mutations in *MCT8* are associated with endocrine findings, including elevated bioactive $T_3$ and decreased $T_4$ levels in the presence of a normal thyrotropin (thyroid-stimulating hormone [TSH]) secretion. *MCT8* is present in many organs, such as liver, kidneys, pituitary, and thyroid gland, and is also expressed in brain.[5] Affected male patients show hypotonia and muscle weakness at birth.

Few reports document the natural history of Allan-Herndon-Dudley syndrome due to *MCT8* mutations, although a few reports describe the cause of death. Here we report a family with four adults diagnosed with athetoid type cerebral palsy. A mutation in *MCT8* was identified in one of the affected family members, two of whom died at 32 and 24 years of age.

## Patient Descriptions

*Patient 1*

This 26-year-old man (III-3; Fig 1) is the third child in his family and was born healthy to nonconsanguineous parents after a term pregnancy without asphyxia. A male infant in the second generation died in early infancy.

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Original article

# Novel compound heterozygous mutations of *POLR3A* revealed by whole-exome sequencing in a patient with hypomyelination

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**Abstract**

*Objective:* Congenital white matter disorders are a heterogeneous group of hypomyelination disorders affecting the white matter of the brain. Recently, mutations in the genes encoding the subunits of RNA polymerase III (Pol III), *POLR3A* and *POLR3B*, have been identified as new genetic causes for hypomyelinating disorders.

*Method:* Whole-exome sequencing was applied to identify responsible gene mutations in a 29-year-old female patient showing hypomyelination of unknown cause. To investigate the pathological mechanism underlying the hypomyelination in this patient, the expression level of 7SL RNA, a transcriptional target of Pol III, was analyzed in cultured skin fibroblasts derived from the patient with *POLR3A* mutations.

*Results:* Novel compound heterozygous mutations of *POLR3A* were identified in the patient, who started to show cerebellar signs at 3 years, lost ambulation at 7 years, and became bedridden at 18 years. Brain magnetic resonance imaging showed severe volume loss in the brainstem, the cerebellum, and the white matter associated with hypomyelination. In addition to hypodontia and hypogonadism, she showed many pituitary hormone-related deficiencies. The expression level of 7SL RNA in cultured skin fibroblasts derived from this patient showed no significant abnormality.

*Conclusion:* The many pituitary hormone-related deficiencies identified in this patient may be an essential finding for the Pol III-related leukodystrophies spectrum. Further investigation is needed for a better understanding of the disease mechanism.


*Keywords:* Hypomyelination; Leukodystrophy; Hypomyelination with hypodontia and hypogonadotropic hypogonadism (4H) syndrome; *POLR3A*; Whole-exome sequencing; RNA polymerase III (Pol III)

## 1. Introduction

Congenital white matter disorders are a heterogeneous group of dysmyelination or hypomyelination disorders of the brain white matter and are visible by brain magnetic resonance imaging (MRI) [1,2]. Pelizaeus-Merzbacher disease (PMD; MIM#312080) is a major

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