ELSEVIER

Brain & Development xxx (2013) xxx–xxx

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

www.elsevier.com/locate/braindev

Original article

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

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Received 27 December 2012; received in revised form 16 April 2013; accepted 23 April 2013

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 disease

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0387-7604/$ - see front matter
http://dx.doi.org/10.1016/j.braindev.2013.04.011

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
J. Magn. Reson. Imaging 2013;00:000–000.

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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Contract grant sponsor: Ministry of Health, Labor and Welfare of Japan; Contract grant numbers: Grant-in-aid for the Research on Measures for Intractable Diseases (H24-Nanchi-Ippan-072), Research Grant for Nervous and Mental Disorders (24-7); Contract grant sponsor: Grant-in-Aid for Scientific Research (C), JSPS; Contract grant numbers: 17591104, 24591790; Contract grant sponsor: Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program).
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Received February 5, 2013; Accepted June 18, 2013.
DOI 10.1002/jmri.24306
View this article online at wileyonlinelibrary.com.

Journal of Child Neurology
00(0) 1-8

DOI: 10.1177/0883073813492387
jcn.sagepub.com
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Three New *PLP1* Splicing Mutations Demonstrate Pathogenic and Phenotypic Diversity of Pelizaeus-Merzbacher Disease

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Abstract

Pelizaeus-Merzbacher disease is a severe X-linked disorder of central myelination caused by mutations affecting the proteolipid protein gene. We describe 3 new *PLP1* splicing mutations, their effect on splicing and associated phenotypes. Mutation c.453_453+6del7insA affects the exon 3B donor splice site and disrupts the PLP1-transcript without affecting the DM20, was found in a patient with severe Pelizaeus-Merzbacher disease and in his female cousin with early-onset spastic paraparesis. Mutation c.191+1G>A causes exon 2 skipping with a frame shift, is expected to result in a functionally null allele, and was found in a patient with mild Pelizaeus-Merzbacher disease and in his aunt with late-onset spastic paraparesis. Mutation c.696+1G>A utilizes a cryptic splice site in exon 5, causes partial exon 5 skipping and in-frame deletion, and was found in an isolated patient with a severe classical Pelizaeus-Merzbacher. *PLP1* splice-site mutations express a variety of disease phenotypes mediated by different molecular pathogenic mechanisms.

Keywords

Pelizaeus-Merzbacher disease, *PLP1*, splice-site mutations

Received revised April 16, 2013. Accepted for publication May 9, 2013.

Mutations in the proteolipid protein 1 gene (*PLP1*) in humans cause a spectrum of X-linked dysmyelinating disorders of central nervous system, ranging from the most severe connatal form of Pelizaeus-Merzbacher disease through classical Pelizaeus-Merzbacher disease to the mildest form of spastic paraplegia type 2.[1-2]

The *PLP1* gene is highly conserved among vertebrates and lies at Xq22.2 in humans. It has 2 major alternatively spliced transcripts, PLP1 and DM20.[3] In central nervous system myelin, proteolipid protein (PLP1) and its smaller isoform (DM20) constitute the most abundant protein compartment.[4] Expression of PLP1 and DM20 is developmentally regulated in the central nervous system and peripheral nervous system.[5] In the peripheral nervous system, DM20 is expressed in early stages of development and later predominates in the adult peripheral myelin, whereas in the central nervous system, DM20 is expressed prenatally, but after birth and during the peak of myelination, expression of PLP1 is predominant.[6-7]

The *PLP1* gene is affected by various types of mutations. Most frequent are duplications, which account for about 60-70% of Pelizaeus-Merzbacher families, followed by point mutations (missense, nonsense and splicing) and other small

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Pediatric Neurology 49 (2013) 477–481

Contents lists available at ScienceDirect

Pediatric Neurology

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

Clinical Observations

Partial *PLP1* Deletion Causing X-Linked Dominant Spastic Paraplegia Type 2

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ABSTRACT

BACKGROUND: Proteolipid protein 1 gene (*PLP1*) mutations result in a continuum of neurological findings characterized by X-linked hypomyelinating leukodystrophies of the central nervous system, from mild spastic paraplegia type 2 to severe Pelizaeus–Merzbacher disease. **PATIENTS:** We report spastic paraplegia type 2 in three individuals in one family. A 29-year-old man developed progressive spastic quadriplegia from early childhood with dysarthria, ataxia, dysphagia, and intellectual delay, but he displayed no nystagmus. His mother developed adult-onset mild spastic diplegia with dementia developing in later life, whereas his sister exhibited spastic diplegia from childhood, complicated by motor developmental delay and dysphagia. All three individuals had initially mild but progressive neurological phenotypes, no nystagmus, normal brainstem auditory-evoked potentials, and demyelinating peripheral neuropathy, but with varying clinical severity. **RESULTS:** A 33-kb deletion encompassing exon 2 to 7 of *PLP1* was identified in all three patients. Cloning of the junction fragment of the genomic recombination revealed a short palindromic sequence at the distal breakpoint, potentially facilitating a double-strand deoxyribonucleic acid break, followed by nonhomologous end joining. X-inactivation study and sequencing of the undeleted *PLP1* alleles failed to explain the differences in severity between the two female patients. **CONCLUSIONS:** *PLP1* partial deletion is a rare cause of spastic paraplegia type 2 and exhibits X-linked dominant inheritance with variable expressivity.

Keywords: proteolipid protein 1, spastic paraplegia type 2, myelin, hypomyelinating leukodystrophy, deletion, Pelizaeus-Merzbacher disease, palindrome, non-homologous end joining

Pediatr Neurol 2013; 49: 477-481

Introduction

The proteolipid protein 1 gene (*PLP1*) encodes major myelin membrane proteins (PLP1 and DM20) in the central nervous system (CNS). Phenotypically, *PLP1* mutations result in a continuum of neurological findings characterized by X-linked hypomyelinating leukodystrophies of the CNS, from Pelizaeus–Merzbacher disease (PMD) with severe CNS involvement to spastic paraplegia type 2 (SPG2), showing later onset with milder phenotype, but progressive weakness and spasticity of the lower limbs.[1] Distinct types of mutations, including point mutations and genomic duplications and deletions, result in PMD and SPG2 through different molecular mechanisms.[2]

Large genomic deletions or early truncating mutations result in null *PLP1* alleles. Patients with such null mutations show a unique clinical phenotype. Their neurological symptoms are milder than that commonly observed in other types of alterations, such as missense mutations and genomic duplications; thus, they are often diagnosed with mild PMD or a complicated form of SPG2.[3,4] Contrary to the mild disease in male patients, female carriers are more frequently symptomatic in these families, often presenting with adolescent- or adult-onset mild spastic diplegia and slowly progressive leukodystrophy, with dementia developing in later life. In addition, *PLP1* null syndrome is

Article History:
Received 17 April 2013; Accepted in final form 13 July 2013
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0887-8994/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pediatrneurol.2013.07.012

ELSEVIER

Brain & Development 36 (2014) 259–263

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

www.elsevier.com/locate/braindev

Original article

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

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Received 21 December 2012; received in revised form 16 March 2013; accepted 27 March 2013

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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0387-7604/$ - see front matter © 2013 The Japanese Society of Child Neurology. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.braindev.2013.03.006