

Novel *PLP1* Mutations Identified With Next-Generation Sequencing Expand the Spectrum of *PLP1*-Associated Leukodystrophy Clinical Phenotypes

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Abstract

Next-generation sequencing was performed for 2 families with an undiagnosed neurologic disease. Analysis revealed X-linked mutations in the *proteolipid protein 1 (PLP1)* gene, which is associated with X-linked Pelizaeus-Merzbacher disease and Spastic Paraplegia type 2. In family A, the novel *PLP1* missense mutation c.617T>A (p.M206K) was hemizygous in the 2 affected male children and heterozygous in the mother. In family B, the novel *de novo PLP1* frameshift mutation c.359_369del (p.G120fs) was hemizygous in the affected male child. Although *PLP1* mutations have been reported to cause an increasingly wide range of phenotypes inclusive of the dystonia, spastic paraparesis, motor neuronopathy, and leukodystrophy observed in our patients, atypical features included the cerebrospinal fluid deficiency of neurotransmitter and pterin metabolites and the delayed appearance of myelin abnormalities on neuroimaging studies. Next-generation sequencing studies provided a diagnosis for these families with complex leukodystrophy disease phenotypes, which expanded the spectrum of *PLP1*-associated leukodystrophy clinical phenotypes.

Keywords

Pelizaeus-Merzbacher disease, proteolipid protein 1, spastic paraplegia type 2, *PLP1*, leukodystrophy, PMD, SPG2, dystonia, brain, developmental delay, genetics, mutation, next-generation sequencing, spasticity

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Next-generation sequencing has identified causal mutations in families with inherited disease of unknown etiology and, in some cases, has facilitated intervention with disease-modifying therapies.¹⁻⁴ Genome and exome sequencing is especially helpful when the phenotype is genetically heterogeneous and complex, as in the hereditary spastic paraparesis and spinocerebellar ataxia syndromes.⁵⁻⁷ Next-generation sequencing has also proved instrumental in associating novel phenotypes with known genes. For instance, we used exome sequencing to identify *de novo* pathogenic mutations in *ATPIA3* in alternating hemiplegia of childhood; dominantly inherited mutations in this gene had been previously associated with rapid-onset dystonia parkinsonism.⁴

PLP1 is a transmembrane myelin proteolipid protein and is the predominant protein in central nervous system myelin. Pathogenic *proteolipid protein 1 (PLP1)* mutations, including

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missense mutations, duplications, and deletions, are known to cause X-linked Pelizaeus-Merzbacher disease and Spastic Paraplegia type 2.⁸ *PLP1* mutations are increasingly associated with a broad spectrum of disease severity and age of onset.^{9–11} The congenital and classic forms of Pelizaeus-Merzbacher disease are associated with congenital or early-onset nystagmus and progressive spasticity in association with a leukodystrophy consisting of a severe hypomyelination. Spastic Paraplegia type 2 falls at the milder end of this spectrum, in which central nervous system myelin abnormalities are more subtle or absent, becoming evident only later in the clinical course. In the severe congenital form of Pelizaeus-Merzbacher disease, eye movement abnormalities are present from birth, bulbar and respiratory insufficiency present early, and progressive spasticity and cognitive impairment are universal. Death can occur from infancy to the third decade, and these children typically do not achieve independent sitting or verbal communication. Seizures may or may not be present. In a pediatric population cohort of leukodystrophies, 7.4% had Pelizaeus-Merzbacher disease, the second most frequently observed cause of leukodystrophy.¹² In Spastic Paraplegia type 2 phenotypes associated with *PLP1* mutations, progressive spastic paraparesis is the predominant phenotype, and affected males are reported to have normal or near-normal cognition. A null phenotype related to missense mutations has also been described; patients reported tend to have a milder Pelizaeus-Merzbacher disease–like phenotype or pure or complicated spastic paraparesis phenotype and may manifest an associated demyelinating peripheral neuropathy. In *PLP1*-associated syndromes, approximately 60% to 70% are associated with gene duplication events of variable size and 10% to 20% with intragenic mutations or smaller deletions/duplications.⁹

The phenotypes in the affected children in the 2 families described in this report were complex, with unique phenotypic features in each case. To discover the genetic cause for these families which could lead to diagnosis, genomic analyses were performed via genome sequencing on a research basis in family A, and in family B, clinical exome sequencing was performed. By this approach, novel *PLP1* mutations were identified in both families. The clinical phenotypes detailed in this report highlight the unique phenotypic features in these patients (eg, delayed appearance and recognition of central nervous system myelin abnormalities) that led to the diagnostic odyssey these families experienced that spanned more than a decade.

Materials and Methods

Samples

Patients and their family members were consented under the University of Utah institutional review board protocol #25651. For family A, DNA extraction from peripheral blood samples was performed by the genomic core facility at the University of Utah Clinical Center for Translational Science and submitted to ARUP Laboratories for genomic analysis. Family A analysis included the 2 affected male children (II-2 and II-3), an unaffected female half-sibling (II-1), and the unaffected parents (I-2 and I-3; pedigree in Figure 1A). For family B, peripheral blood samples were submitted to the ARUP Laboratories

Genomics Section for clinical exome sequencing. Family B consists of 1 affected male child (II-1) and the unaffected parents (I-1 and I-2; pedigree in Figure 1B).

Next-Generation Sequencing

Five members of family A (I-2, I-3, II-1, II-2, and II-3; Figure 1A) underwent genome sequencing on the HiSeq 2000 instrument (Illumina Inc, San Diego, California) with 100 base length pair end reads. DNA from the parents and affected child in family B (Figure 1B) were exome sequenced using methods presented by Wooderchak-Donahue et al¹³ with the following exceptions: the Agilent SureSelectXT Human All Exon V4 kit (Agilent Technologies, Inc, Santa Clara, California) was used on the automated library generation Bravo instrument option A (Agilent), and the sequencing was done on the HiSeq 2500 instrument (Illumina).

Data Analysis

Illumina Fastq files were aligned to the human reference genome (GRCh37) and variants from the reference were called using BWA, Samtools, and GATK software. After the first alignment, a second refined alignment was done, which removed polymerase chain reaction duplicate reads, identified read bias, and realigned around deletions and insertions.

For family A, analyzed via genome sequencing, the candidate gene identification for the expected X-linked or recessive disease inheritance was approached using 2 methods. Golden Helix software (SNP and Variation Suite; Golden Helix Inc, Bozeman, Montana) used heuristic filtering (such as requiring $>3\times$ read depth) and family intersects or subtractions to identify suspect genes. VAAST is a probabilistic algorithm^{14,15} that performs family inheritance analysis and comparison of variants to a background variant file of 250 Caucasian data sets. After applying VarBin¹⁶ to identify false-positive variants, requiring Mendelian segregation with disease, limiting to exons ± 10 bases, and eliminating genes with functions that are unlikely to be phenotypically relevant, these methods yielded 16 candidate genes. These 16 genes were further analyzed with respect to minor allele population frequency, predicted pathogenicity using SIFT¹⁷ scores, variant's potential to affect gene function, literature searches, disease database information, gene function, and potential phenotype relevance.

Analyses of exome data for family B excluded variants with a variant quality score <10 and with $>1\%$ population frequency from further analysis. Family members' variants were intersected or subtracted for analysis of different inheritance patterns possible for family B. Then high-priority variants were identified based on predicted variant pathogenicity and phenotypic correlation with the proband. Variants were further analyzed for those that were predicted to be pathogenically most relevant using SIFT scores and variant's potential to affect gene function, as well as the gene function being phenotypically relevant to symptoms of the proband.

Clinical Report

Family A

The proband, II-2, was born at 37 weeks' gestation to unrelated parents of European Caucasian ancestry. He was delivered via vacuum-assisted vaginal birth following a prolonged labor lasting 3 days, with Apgar scores of 5 and 6, and a birth weight of 7 lbs 10 oz. He had hypotonia and a weak suck from birth and

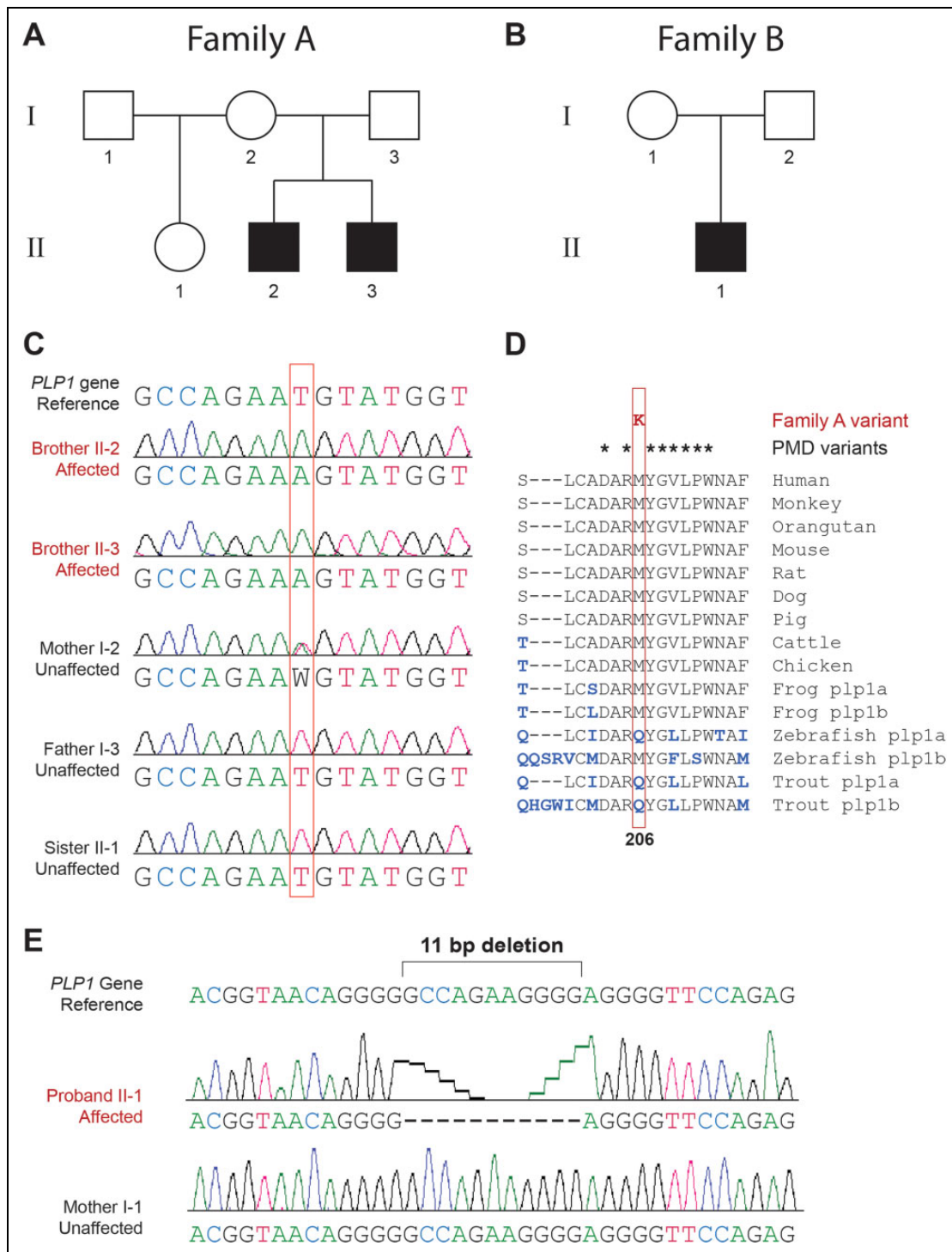


Figure 1. A-B, Family pedigrees. The circles represent female and the squares represent male. The affected male family members are represented by filled squares. A, Family A pedigree. B, Family B pedigree. C-D, Family A mutation data. C, Sanger sequencing traces for the *PLP1* c.617T>A mutation (location boxed in red) are shown for five family A members. D, Multiple sequence alignment. *PLP1* protein sequences for several species shown. Amino acid differences from the human *PLP1* sequence are in bold blue text. Amino acid 206, where the family A mutation “K” (p.M206K) is located, is boxed in red. The other locations of nearby known Pelizaeus-Merzbacher disease (PMD) causative mutations are starred. E, Sanger sequencing traces are shown for the family B proband II-1 and mother I-1 at the *PLP1* mutation position (11 base pair *de novo* mutation in bracket).

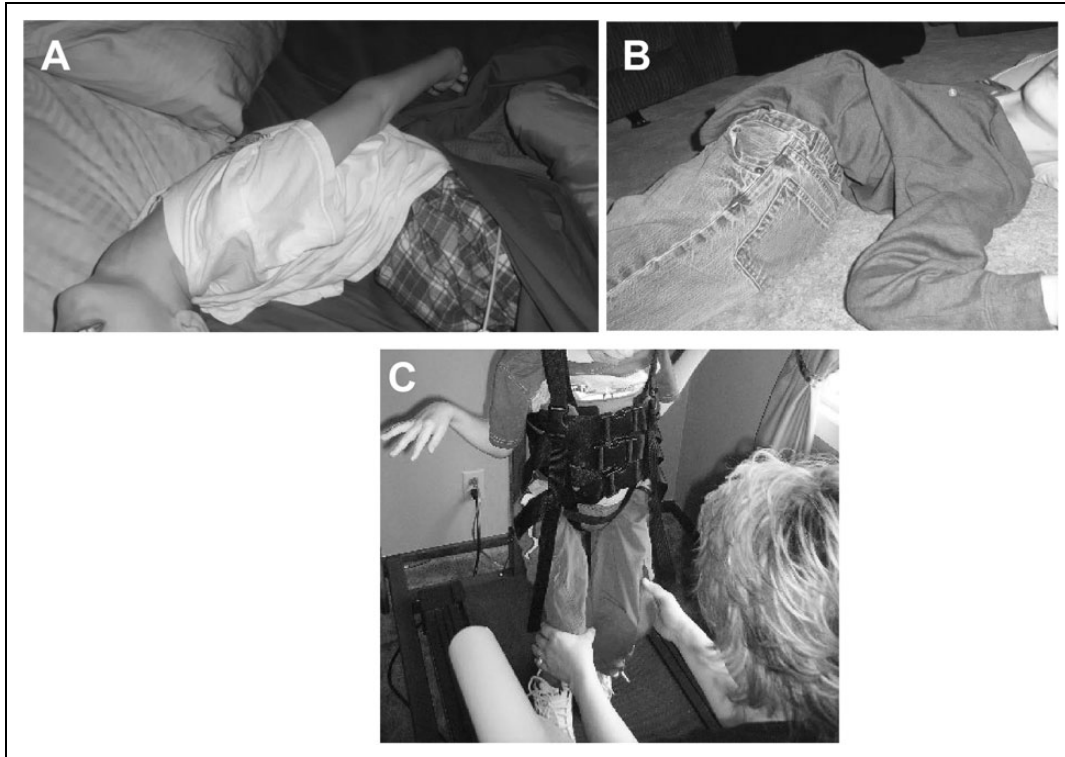


Figure 2. A, Family A proband II-2 in opisthotonic crisis. B, Family A proband II-2 showing persistent postural deformity associated with progressive kyphoscoliosis and lumbar lordosis. C, Family A affected brother II-3 showing axial hypotonia, significant dystonia, and lower limb spasticity.

had failed to achieve head control by 3 months of age. Shortly thereafter, he began manifesting dystonic posturing of limbs, involuntary head turning, and eye movement abnormalities. High-resolution karyotype and Fluorescence In Situ Hybridization analysis for Prader-Willi syndrome were negative. Brain magnetic resonance imaging (MRI) at 4 months of age was reported as normal. He received a tentative diagnosis of cerebral palsy but had additional diagnostic testing including normal plasma very long chain fatty acids, lysosomal enzymes, cerebrospinal fluid and blood lactate, carnitine and acylcarnitine studies, and urine organic acids. By 12 months, he was noted to track poorly and was thought to have optic nerve pallor; electroretinogram testing was normal but visual-evoked responses were impaired. By 18 months of age, he was severely developmentally delayed from a gross motor standpoint and was unable to sit or roll. By that point, his younger brother had been born and appeared similarly affected, and further diagnostic workup included muscle and skin biopsies to exclude defects in energy metabolism proved unrevealing. A follow-up brain MRI at 23 months demonstrated an interval increase in the size of the lateral and third ventricles and diffuse reversal of the normal gray and white matter signal intensity. Over the ensuing years, he made little developmental progress with regard to gross or fine motor skills but could spell his name and knew his colors. At that time, the parents saw a television program featuring a child with an inherited defect in neurotransmitter biosynthesis who responded dramatically

to treatment with L-dopa/carbidopa therapy. Thus, the family pursued further diagnostic workup including cerebrospinal fluid neurotransmitter metabolite, pterin, and folate studies. Levels of homovanillic acid (a dopamine metabolite), 5-hydroxyindoleacetic acid (a serotonin metabolite), and tetrahydrobiopterin levels in the cerebrospinal fluid were low. He was begun on treatment with L-dopa/carbidopa, and parents reported increased alertness, a reduction in abnormal limb and trunk posturing, improved use of his hands, reduced arching, and improved visual tracking. He was referred to our institution for further evaluation at 5 years of age. Although he appeared bright and attentive, he was unable to speak due to bulbar involvement; he had severe axial hypotonia, fluctuating limb hypertonia, and dystonic posturing and brisk deep tendon reflexes. However, he had no nystagmus. A cytokine-stimulated fibroblast assay demonstrated impaired tetrahydrobiopterin biosynthesis thought to be consistent with GTP cyclohydrolase 1 deficiency. However, Sanger sequencing and Multiplex Ligation-dependent Probe Amplification analysis of the *GTP cyclohydrolase 1* gene did not reveal mutations. Despite an initial response to L-dopa/carbidopa therapy, over the ensuing years, his symptoms progressed. By age 9, he began manifesting painful opisthotonic crises (Figure 2A). He also developed a persistent postural deformity associated with progressive kyphoscoliosis and lumbar lordosis (Figure 2B). At 11 years of age, he died in his sleep following an episode of pneumonia and was found with his neck hyperextended.

His brother, II-3, was born at 39 weeks following an unremarkable pregnancy and vaginal delivery, with a birth weight of 8 lbs 1 oz and Apgar scores of 7¹ and 10.⁵ He first manifested abnormal eye movements at 2 weeks of age and had poor suck, head control, and gross motor delays comparable to his brother. Brain MRI at 7 months of age noted possible hypoplasia of the anterior lobe of the pituitary and optic chiasm, but was otherwise deemed unremarkable. At almost 4 years of age, he had severe motor developmental delay, axial hypotonia, poor head control, significant dystonia, and lower limb spasticity (Figure 2C). A lumbar puncture revealed low cerebrospinal fluid homovanillic acid, 5-hydroxyindoleacetic acid, and pterin abnormalities. He demonstrated a modest improvement in generalized dystonia with treatment with L-dopa/carbidopa. At the age of 10 years, we pursued further diagnostic evaluation, as the clinical features and evident progression of symptoms were not consistent with GTP cyclohydrolase 1 deficiency. Cytokine-stimulated fibroblast culture failed to confirm a defect in tetrahydrobiopterin biosynthesis, in contrast to his brother's result. A brain MRI revealed diffuse signal intensity throughout the central nervous system myelin and generalized cortical atrophy consistent with a leukodystrophy. He remains unable to speak, sit unsupported, or roll and has developed progressive spasticity over time.

Family history was notable for a maternal aunt with intellectual disability and a possible mild gait disorder; she had been born a twin, and the maternal grandmother reported that she had been told her daughter had "arrested hydrocephalus." No records or neuroimaging studies dating back to infancy or childhood were available for review. However, the family arranged for her to undergo a lumbar puncture for cerebrospinal fluid analysis. Her studies, in contrast to her nephews, did not reveal evident abnormalities in cerebrospinal fluid dopamine, serotonin, or pterin metabolites. The boy's mother denied any symptoms, was of normal intelligence, and her neurologic examination was unremarkable.

To further elucidate the genetic basis of these neurological symptoms in the 2 affected male siblings, whole-genome sequencing was performed for 5 family members (Figure 1A). Based on the family A pedigree, X-linked and autosomal recessive inheritance patterns were considered most likely, and data were analyzed as described in Materials and Methods. Based on this analysis, a missense mutation in the *PLP1* gene appeared to be the best candidate for the causal gene within the family, particularly since mutations, gene duplications, and deletions in *PLP1* are associated with several of the features noted in our patients, including dystonia, spasticity, abnormal eye movements, and leukodystrophy.

The identified *PLP1* missense mutation c.617T>A (NM_000533, p.M206K) is found at genomic position chrX:103,042,890 (NC_000023.10, human reference genome GRCh37). This *PLP1* mutation is found within exon 4; hence, the mutation is present in both PLP1 and the alternatively spliced PLP1 isoform, DM20. The presence and the segregation of this mutation with disease in the family were confirmed by Sanger sequencing (Figure 1C). This mutation was not

found in several human mutation databases: NCBI dbSNP database (build 151),¹⁸ 1000 Genomes,¹⁹ Exome Sequencing Project,²⁰ gnomAD,²¹ ClinVar,²² or Human Gene Mutation Database Professional 2018.1²³; so it appears to be novel. Of note, an adjacent, extremely rare variant c.618G>A (chrX:103,042,891, NM_000533, p.M206I) was present in the gnomAD database at 1 allele in 178,486 alleles tested. This c.618G>A variant was found in a hemizygous sample, but associated clinical information was not available. Several mutations of known pathogenicity for Pelizaeus-Merzbacher disease have been reported in the literature and in Human Gene Mutation Database flanking either side of the novel p.M206K mutation (Figure 1D).^{10,11,24-26} Although the PLP1 protein is 100% conserved between mice and humans,²⁷ for some more divergent species, the amino acid at position 206 is glutamine (eg, Q in zebrafish PLP1a; Figure 1D). Even so, lysine (K) was not seen in these multiple sequence alignments at amino acid residue 206.

Family B

The proband, II-1, was born at term to unrelated parents of European Caucasian heritage following an unremarkable gestation and vaginal delivery. Parents became concerned when he failed to achieve independent sitting by 8 months of age. When first examined at 11 ½ months of age, he was diffusely hypotonic, unwilling to bear weight on his lower extremities, unable to sit independently, or crawl. He had preserved deep tendon reflexes but hypotonic lower extremities. An electromyogram and nerve conduction study revealed evidence of chronic and ongoing denervation in the lower extremities, suggesting a possible motor neuron disorder. A muscle biopsy showed "subtle myopathic features". By 2 ½ years of age, he had speech delay and had developed significant lower extremity spasticity. A brain MRI at 24 months of age was reported as normal. Additional metabolic screening was performed including cerebrospinal fluid quantitative amino acids and neurotransmitter and pterin metabolites; methyltetrahydrofolate, glucose, protein, and cell count; serum creatinine kinase, leukocyte lysosomal enzymes, thin layer chromatography for mucopolysaccharides, and urine organic acids; all of which were unremarkable. A tentative diagnosis of hereditary spastic paraparesis was made, but no further genetic testing pursued at that time. Subsequently, spasticity and weakness continued to progress and he developed ataxia and was manifesting more obvious significant cognitive impairment and slow speech. He had undergone more than 16 tendon releases and/or transfers. At age 5 years, he could speak in sentences but was dysarthric. He could scribble and knew a few letters of the alphabet; he could eat with a fork or spoon. His leg weakness and spasticity had progressed, and he had axial hypotonia, oculomotor apraxia, increased deep tendon reflexes, but no frank ataxia. He was able to walk on his toes very slowly and with much effort using a walker. Additional workup included a normal spine MRI. Brain MRI revealed diffuse signal abnormalities of myelin throughout the central nervous system, especially the parieto-occipital areas. An inpatient trial of intrathecal

baclofen resulted in a loss of his ability to ambulate. An electromyography revealed a progressive distally predominant motor axonal neuropathy. He received a clinical diagnosis of hereditary spastic paraparesis with motor neuronopathy, but genetic testing was deferred due to the lack of insurance coverage. Follow-up at age 7 years indicated some gains in understandable speech, but he was having increasing difficulties using his walker. His trunk was weaker and he had developed an upper extremity tremor. At 10 years of age, sequencing of *SPG11* was negative for mutations. At that point, exome sequencing was recommended.

Clinical exome sequencing was performed for all 3 family B members (Figure 1B). Based on the absence of family history of a similar condition, X-linked and autosomal recessive inheritance as well as *de novo* mutation were considered for family B. Two blinded, independent analyses both identified an 11 base pair deletion in the *PLP1* gene as the top candidate. This conclusion was primarily based on the clear pathogenicity of this variant (expected to cause a frameshift in the resulting messenger RNA [mRNA]), the apparent *de novo* nature of the variant in the proband, and phenotypic match to the proband's symptoms.

The *PLP1* frameshift variant c.359_369delGCCA-GAAGGGG (NM_000533, p.G120fs) is caused by an 11 base pair deletion from chrX:103,041,561 to chrX:103,041,571 (NC_000023.10). This *PLP1* variant is found within exon 3B, which is retained in the full length PLP1 transcript, but is spliced out of the shorter DM20 isoform. Next-generation sequencing analyses demonstrated this variant arose *de novo* in the proband, as it was not observed in either parent. Sanger sequencing of this locus confirmed the next-generation sequencing data (Figure 1E). As this variant was not found in NCBI dbSNP database, 1000 Genomes, Exome Sequencing Project, gnomAD, ClinVar, or Human Gene Mutation Database, it appears to be novel as well as *de novo*. This frameshift deletion is near the location of a previously published pathogenic frameshift deletion c.354_355delAG (presented by Osaka *et al.* as c.352_353delAG).²⁸ Both frameshift deletions resulted in the same alternate reading frame after amino acid residue 124 until a stop codon is reached.

Discussion

The unique clinical features in each of the families reported here contributed to the diagnostic odyssey they experienced, and interestingly, both families were found to have mutations in the well-described *PLP1* gene. The diagnostic odyssey was compounded by features in each proband that delayed the proper diagnosis by several years, because the phenotypes have not been reported previously in patients with *PLP1* mutations. In family A, the normal brain myelination led clinicians to discount Pelizaeus-Merzbacher disease and subsequently the abnormal cerebrospinal fluid neurotransmitter and bipterin studies supporting a putatively reasonable alternative diagnosis of GTP cyclohydrolase 1 deficiency. In family B, the diagnosis of motor neuronopathy in infancy, which manifested well prior

to the spastic paraparesis, was the primary factor delaying diagnosis. This was confounded further by the lack of insurance coverage early on for diagnostic confirmation of the suspected clinical diagnosis of a variant of hereditary spastic paraparesis with motor neuronopathy. These cases markedly illustrate how increased access to exome and genome sequencing continues to transform clinical diagnosis and care for children with complex neurologic phenotypes.

The PLP1 protein is the predominant myelin protein present in the central nervous system and it plays a role in the stabilization and maintenance of myelin sheaths, as well as in oligodendrocyte development and axonal survival. Mutations in *PLP1* cause the X-linked disorders: Spastic Paraplegia type 2, PLP1 null syndrome, and Pelizaeus-Merzbacher disease.^{8–11,26} These disorders present with varying severity but include symptoms of spasticity, hypotonia, and motor delay. Other symptoms may include nystagmus, dystonic posturing, ataxia, intellectual disability, absent or dysarthric speech, and impaired ambulation. Peripheral nervous system myelin may be affected, causing symptoms suggestive of spinal muscular atrophy.

The *PLP1* locus also encodes a splice isoform called DM20, which lacks exon 3B in the mature transcript.²⁹ Both PLP1 and DM20 proteins are major components of myelin. PLP1 is the predominant postnatal expressed isoform, but DM20 isoform plays a key role in the formation of myelin in the prenatal period. PLP1 is 276 amino acids long, and the DM20 isoform lacks 35 amino acids encoded by *PLP1* exon 3B. Nonsense mutations within exon 3B affect only the full length PLP1 isoform and have been reported in milder forms of Pelizaeus-Merzbacher disease.^{10,28} However, missense mutations that affect both isoforms of the protein (PLP1 and DM20) tend to result in more severe phenotypes. In either case, the course of the disease can be heterogeneous.

The family A missense mutation, p.M206K, is found in both protein isoforms (DM20 and PLP1). PLP1 is highly conserved among mammalian species, with 100% identity at the amino acid level between humans, rats, and mice.²⁷ Changes in the more conserved amino acids across species lead to more severe disease.¹⁰ The PLP1 amino acid 206 is conserved across many species including the zebra-fish PLP 1b protein, which has only 51% overall identity to the human PLP1 protein. The family A mutation is found in the C-D extracellular loop of the protein, where many Pelizaeus-Merzbacher disease-causative mutations are located.¹⁰ Also many known Pelizaeus-Merzbacher disease mutations are adjacent to PLP1 amino acid 206, at amino acids 203, 205, and 207.^{10,11,24–26}

Family B has a *de novo* frameshift mutation (c.359_369del; p. Gly120fs). This mutation deletes 11 nucleotides from the coding region of the *PLP1* gene, resulting in a shift in the reading frame of the mRNA starting at codon 120. The c.359_369del mutation is located in exon 3B and is therefore present only in the PLP1 isoform and not in the DM20 isoform, which lacks exon 3B. Considering the available clinical evidence and a pathogenic deletion causing a *PLP1* frameshift at a

similar location,²⁸ this identified mutation in the *PLP1* gene is predicted to be causative for the patient's clinical findings.

In the 2 cases presented in this report, genome or exome sequencing enabled a specific clinical diagnosis where traditional diagnostic and clinical practices had not. Although phenotypic heterogeneity is a well-known entity for certain genetic diseases, the application of exome and genome sequencing is expanding our understanding of phenotypic heterogeneity in a broader spectrum of disorders. The lack of obvious abnormalities in central nervous system myelin on the initial neuroimaging studies contributed to the delay in diagnosis; in the affected child in family B, the brain MRI was reported as normal, but the digital images were not reviewed. Family A had 2 affected male siblings who presented with infantile onset dystonia in association with abnormalities in cerebrospinal fluid neurotransmitter and bipterin metabolites, with delayed onset of progressive central nervous system demyelination, spasticity, and premature death in 1 of the 2 boys. Family B had an affected boy who presented in infancy with hypotonia and delayed motor development in association with a motor neuronopathy, only later to manifest progressive spastic paraparesis, ataxia, cognitive impairment, and leukodystrophy. Although all 3 boys ultimately manifested abnormalities of central nervous system myelin on neuroimaging studies, the delayed appearance of abnormalities until after 2 years of age contributed to diagnostic confusion. Both families in this report had endured more than a decade without answers prior to the identification of the novel *PLP1* mutations discovered by next-generation sequencing technologies. Thus, genomic studies proved extremely useful in providing a diagnosis for these families.

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Author Contributions

RM contributed to design, contributed to acquisition, analysis, and interpretation, drafted the manuscript, and gave final approval; JD contributed to analysis, critically revised the manuscript, and gave final approval; BK contributed to conception, contributed to analysis, critically revised the manuscript, and gave final approval; TN contributed to acquisition, critically revised the manuscript, and gave final approval; JB contributed to acquisition and interpretation, critically revised the manuscript, and gave final approval; KV contributed to conception, contributed to interpretation, critically revised the manuscript, and gave final approval; P B-T contributed to conception, contributed to interpretation, critically revised the manuscript, and gave final approval; RL contributed to acquisition and interpretation, critically revised the manuscript, and gave final approval; KJS contributed to conception and design, contributed to acquisition, analysis, and interpretation, drafted the manuscript, critically revised the manuscript, and gave final approval. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

Patients and their family members were consented under the University of Utah institutional review board protocol #25651.

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