Expanding the Phenotype of *TUBB2A*-Related Tubulinopathy: Three Cases of a Novel, Heterozygous *TUBB2A* Pathogenic Variant p.Gly98Arg

Lindsey Schmidt^a Karen E. Wain^b Catherine Hajek^c
Juvanee I. Estrada-Veras^d Maria J. Guillen Sacoto^e Ingrid M. Wentzensen^e
Alka Malhotra^f Amanda Clause^f Denise Perry^f Andres Moreno-De-Luca^g
Megan Bell^h

^aSanford Health, Augustana-Sanford Genetic Counseling Program, Sioux Falls, SD, USA; ^bGeisinger Autism & Developmental Medicine Institute, Lewisburg, PA, USA; ^cSanford Health, Sioux Falls, SD, USA; ^dHenry M Jackson Foundation for the Advancement of Military Medicine and Walter Reed National Military Medical Center, Bethesda, MD, USA; ^eGeneDx, Gaithersburg, MD, USA; ^fIllumina Clinical Services Laboratory, San Diego, CA, USA; ^gDepartment of Radiology, Geisinger Autism & Developmental Medicine Institute, Genomic Medicine Institute, Lewisburg, PA, USA; ^hSanford Health, Sioux Falls, SD, USA

Established Facts

- *TUBB2A*-related tubulinopathy has been associated with brain malformations including cortical dysplasia and dysmorphic corpus callosum.
- Intellectual disability, hypotonia, and developmental delay are common features of *TUBB2A*-related tubulinopathy.
- The 9 previous reported individuals with *TUBB2A* variants have been diagnosed between ages 8 months and 23 years of age.

Novel Insights

- These 3 patients are the first reported with the *TUBB2A* variant p.Gly98Arg.
- Pachygyria was present in all 3 patients, while the 2 with neuroimaging studies available for detailed review had hypoplastic right caudate nucleus and signal abnormality in the periaqueductal gray matter, which are neuroimaging findings that have not previously been reported in other individuals with *TUBB2A* variants.
- All 3 patients share evidence of developmental brain dysfunction and intellectual disability, but they differ in their presentations as patient 1 and 3 presented with hyperactivity, and patient 1 and 2 with autism spectrum disorder which are features that have not been reported in *TUBB2A*-related tubulinopathy.

Keywords

TUBB2A · Tubulinopathy · Expanding phenotype · Autism · Hypoplastic right caudate nucleus body · Novel pathogenic variant · Neuroimaging · Whole-exome sequencing · Whole-genome sequencing

Abstract

Tubulinopathies are a group of conditions caused by variants in 6 tubulin genes that present with a spectrum of brain malformations. One of these conditions is *TUBB2A*-related tubulinopathy. Currently, there are 9 reported individuals with pathogenic variants within the *TUBB2A* gene, with common manifestations including, but not limited to, global developmental delay, seizures, cortical dysplasia, and dysmorphic corpus callosum. We report 3 patients identified by exome and genome sequencing to have a novel, pathogenic, missense variant in *TUBB2A* (p.Gly98Arg). They presented similarly with intellectual disability, hypotonia, and global developmental delay and varied with respect to the type of cortical brain malformation, seizure history, diagnosis of autism spectrum disorder, and other features. This case series expands the natural history of *TUBB2A*-related tubulinopathy while describing the presentation of a novel, pathogenic, missense variant in 3 patients.

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Introduction

Historically, malformations of cortical brain development, which can be found in some patients with developmental delay and epilepsy, had been described as having malformations of cortical brain development which were classified based on abnormal neuronal and glial proliferation, neuronal migration, and abnormal post-migrational development [Barkovich, 1996; Barkovich et al., 2012]. Recently, the classification system has become more precise as genetic studies have provided molecular insight to the complexity of cortical development. One such group of conditions, tubulinopathies, are caused by variants within the tubulin genes, which encode various alpha- and beta-tubulin isoforms. There are 6 tubulin genes (*TUBA1A*, *TUBB2A*, *TUBB2B*, *TUBB3*, *TUBB*, and *TUBG1*) that contribute to phenotypic overlap including complex cortical malformations such as microlissencephaly, lissencephaly, central pachygyria and polymicrogyria like cortical dysplasia, and a simplified gyral pattern [Bahi-Buisson et al., 2014; Ejaz et al., 2017]. Tubulinopathies have been reported as autosomal dominant

disorders caused by de novo heterozygous variants [Bahi-Buisson and Cavallin, 1993].

Broad genomic testing methods have allowed for the discovery of variants in tubulin genes. *TUBB2A*-related tubulinopathy (p.Asn247Lys) was first described in a patient with a phenotype of infantile spasms, global developmental delay, and brain malformations including a simplified gyral pattern with abnormalities of the corpus callosum, basal ganglia, thalami, brainstem, and cerebellum [Cushion et al., 2014]. Since then, there have been 8 other reported individuals with *TUBB2A*-related tubulinopathy attributed to 6 different pathogenic variants (Table 1) [Lee et al., 2014; Ejaz et al., 2017; Rodan et al., 2017; Sferra et al., 2018; Cai et al., 2020]. Here, we present 3 individuals with *TUBB2A*-related tubulinopathy, due to a novel, pathogenic missense *TUBB2A* variant (NM_001069.2:c.292G>A; p.Gly98Arg). These individuals provide variant-specific phenotype insight and expand understanding of the natural history and phenotypic variability of *TUBB2A*-related tubulinopathy in older individuals.

Case Presentations

Patient 1

Patient 1 is a 17-year-old male with a history of seizures, intellectual disability, developmental delay, and autism spectrum disorder. Born at 40 weeks gestation after an uncomplicated pregnancy and birth, the patient had a birth weight of 7 lbs, 11 oz. At 4 months of age, he was diagnosed with visual cortical impairment. Brain magnetic resonance imaging (MRI) at age 2 years showed ill-defined signal abnormality involving the bilateral peritrial white matter and what appeared to be more prominent areas of slow myelination. At age 3 years, he was diagnosed with a mild neuronal migration disorder responsible for his microcephaly, cortical visual impairment, feeding difficulties, and global developmental delay with receptive and expressive speech and language deficits. At age 8 years, he presented with grand mal seizures, confirmed by electroencephalograph (EEG). A brain MRI at age 12 years showed thinning of the posterior body of the corpus callosum, signal abnormality in the bilateral periventricular white matter most pronounced at the peritrial regions, mild associated white matter volume loss, globular configuration and asymmetric rotation of the basal ganglia and thalami, shallow sulcation, broad gyri, and thickened cortex consistent with pachygyria at the bilateral frontal and temporal lobes, hypoplasia of the right

Table 1. Review of reported *TUBB2A*-related tubulinopathy patients

Most recent reported patients to date with <i>TUBB2A</i> variants	Patient age at diagnosis	Imaging abnormalities	Clinical characteristics	Variant nomenclature	Ref.
1	17 years	Thinning of the posterior body of the corpus callosum, signal abnormality in the bilateral periventricular white matter most pronounced at the peritriangular regions, mild associated white matter volume loss, globular configuration and asymmetric rotation of the basal ganglia and thalami, pachygyria at the bilateral frontal and temporal lobes, hypoplasia of the right caudate nucleus body, and signal abnormality in the periaqueductal gray matter	Seizures, global developmental delay (receptive and expressive speech delays), severe intellectual disability, attention deficit hyperactivity disorder, autism spectrum disorder, microcephaly, cortical visual impairment, progressive weakness, coordination and balance deficits, muscle twitching, feeding difficulties, recurrent respiratory infections	NM_001069.2:c.292G>A p.Gly98Arg	Current study
2	14 years	Cortical thickening of the frontal region and features suggestive of pachygyria	Global developmental delay, intellectual disability and central nervous system abnormalities, hypotonia, autism spectrum disorder, microcephaly	NM_001069.2:c.292G>A p.Gly98Arg	Current study
3	13 years	Pachygyria most pronounced at the bilateral frontal and temporal lobes, signal abnormality in the bilateral peritriangular white matter, mild volume loss of the posterior body of the corpus callosum, hypoplasia of the right caudate nucleus body, slightly globular configuration and asymmetric rotation of the basal ganglia and thalami, and subtle signal abnormality in the periaqueductal gray matter	Seizure disorder, global developmental delay, mild intellectual disability, hypotonia, hyperactivity, sleep disturbance, central hypothyroidism	NM_001069.2:c.292G>A p.Gly98Arg	Current study
4	6 years	Nonspecific white matter myelination delay, slightly enlarged anterior horn of lateral ventricle	Seizures, moderate global developmental delay, OFC between 25th and 50th percentile	NM_001069.2:c.728C>T p.Pro243Leu	Cai et al., 2020
5	3 years	Bilateral pachygyria and corpus callosum dysplasia	Seizures, severe global developmental delay, no language, OFC between 3rd and 25th percentile	NM_001069.2:c.743C>T p.Ala248Val	Cai et al., 2020
6	23 years	Cerebellar atrophy and hypointense stripes in the pons suggestive of saccinopathy	Prominent progressive spastic ataxia, sensory motor axonal neuropathy, bilateral optic macular dystrophy, mild intellectual disability	NM_001069.2:c.1249G>A p.Asp417Asn	Sferra et al., 2018
7	2 years 3 months	Bilateral posterior sylvian, polymicrogyria with asymmetric ventricles and abnormal frontal gyration, microcephaly with motor neuronal cytomegaly at the brainstem and spinal cord, small brainstem and cerebellar vermis, thin corpus callosum and moderate ventriculomegaly	Seizures (episodic limb shaking and eye twitching), axial hypotonia and appendicular hypertonia, severe global intellectual disability, and evolving contractures	NM_001069.2:c.785G>A p.Arg262His	Ejaz et al., 2017
8	4 years	Shallow sulcation, broad gyri, thickened cortex (focal pachygyria), mildly decreased cerebral white matter volume (left greater than right), thinning of body and splenium of corpus callosum	Profound language delay, cortical visual impairment, asymmetric lower extremity rigidity and pyramidal signs (right greater than left), and an exaggerated startle response	NM: not provided, c. not provided p.Ala248Val	Rodan et al., 2017
9	1 year	Cortical dysplasia, complex, perisylvian polymicrogyria with other brain malformations	Seizures, developmental delay, infantile spasms, microcephaly, plagiocephaly	NM: not provided, c. not provided p.Ile345Phe	Lee et al., 2014
10	11 years	Cortical dysplasia, complex, with other brain malformations	Seizures, developmental delay, short stature, ureteropelvic junction obstruction, constipation, hydronephrosis, clinodactyly, growth hormone deficiency, Russell Silver syndrome	NM: not provided, c. not provided p.Gln291Pro	Lee et al., 2014
11	3 years	Dysmorphic corpus callosum	Seizures (subtle vertical eye movements), nonverbal, nonambulatory with generalized hypotonia, OFC on 25th percentile	NC_000006.11:c.743C>T p.Ala248Val	Cushion et al., 2014
12	8 months	Diffuse simplified gyral pattern, reduced volume of white matter, globular basal ganglia and thalami, moderately enlarged 3rd and lateral ventricles, thin and dysmorphic corpus callosum, mild brain stem hypoplasia with flat pons, mild cerebellar vermis hypoplasia, and mildly enlarged posterior fossa	Seizures, global developmental delay, OFC between 10 and 25th percentile	NC_000006.11:c.741C>G p.Asn247Lys	Cushion et al., 2014

Fig. 1. Neuroimaging findings in patients 1 (a–e) and 3 (f–j). Sagittal T1-weighted (a, f), axial T2-weighted (a–d, g–i), and coronal T2-weighted (e, j) images show shallow sulcation, broad gyri, and thickened cortex consistent with pachygyria at the bilateral frontal and temporal lobes (c–e, g–j), thinning of the posterior body of the corpus callosum (a, f), hypoplasia of the right caudate nucleus (b, e, g, j), globular configuration and asymmetric rotation of the basal ganglia and thalami (c, e, h, j), and signal abnormality in the bilateral periventricular white matter (b, c, g, h) and periaqueductal gray matter (d, i).

caudate nucleus body, and signal abnormality in the periaqueductal gray matter (Fig. 1). Previous negative clinical testing included: metabolic evaluations, chromosomal microarray, karyotyping, and an epilepsy gene panel.

At age 17 years, his head circumference was 56 cm (76th percentile) and height and weight were at 0.64 percentile and 0.60 percentile, respectively ($Z = -2.51$). Dysmorphic facial features were not appreciated, but he was noted to have mild 2–3 toe syndactyly. His main health concerns included decreased respiratory function and recurrent respiratory infections. His recurrent pneumonias and chronic cough required the use of a high frequency oscillation vest daily. He relied on a gastrostomy tube for nutrition and struggled with fatigue, coordination, and balance deficits. He had diagnoses of attention deficit hyperactive disorder and autism spectrum disorder, and exhibited defiant and self-injurious behaviors.

Patient 2

Patient 2 is a 14-year-old female with history of congenital microcephaly, hypotonia, autism spectrum disorder, global developmental delay, intellectual disability, and central nervous system abnormalities. She was born

at presumed 36 weeks gestation via vaginal delivery, though gestational age was estimated at between 28 and 32 weeks after postnatal assessment. The pregnancy was complicated by inaccurate dates and alcohol exposure during the first trimester, decreased fetal movement, and preterm labor. Birth weight was 5 lbs, 11 oz, and the patient presented with microcephaly, hypotonia, and respiratory distress requiring resuscitation, ventilatory support, and a 2-week neonatal intensive care unit stay. At age 1 year, she was diagnosed with global developmental delay, including prominent fine motor and speech delay. Brain MRI at age 3 years showed cortical thickening of the frontal region and features suggestive of pachygyria, and repeat brain MRI at age 6 years showed signal abnormality suggestive of periventricular leukomalacia (findings extracted from both MRI reports; images were not available for independent review). Her head circumference at 16 months was reported at 43 cm, (<3rd percentile). Previous negative testing included: Prader-Willi/Angelman methylation testing, *MECP2* and *UBE3A* gene sequencing, 22q11.2 deletion syndrome via FISH analysis, fragile X syndrome, congenital disorders of glycosylation through carbohydrate deficient transferrin, and creatine

disorders through creatine and guanidinoacetate measurements. Chromosomal microarray yielded a paternally inherited duplication (338 kb) of 12q24.33 of uncertain clinical significance.

At age 14 years, she continued to have hypotonia and microcephaly. Notable facial features included bilateral epicanthal folds, long prominent ears, and a broad nasal bridge. Her main health concerns seemed to be related to her underlying neurodevelopmental issues. She required support with ambulation, was nonverbal, and had major deficits with social skills, including lack of danger awareness. She struggled with frequent constipation and eczema. She had diagnoses of autism spectrum disorder and intellectual disability.

Patient 3

Patient 3 is a 13-year-old female with a history of hypotonia, seizure disorder, mild intellectual disability, sleep disturbance, central hypothyroidism, and hyperactivity. The pregnancy was largely uncomplicated, with the exception of maternal hypertension. The patient was born at 38 weeks gestation by vacuum-assisted vaginal delivery. Birth weight was 7 lbs, 3 oz. Mild jaundice was noted but did not require treatment, and she was discharged after 2 days. Early developmental milestones were globally delayed. She was noted to be clumsy but not ataxic. At age 2 years, she used about 20 single words, combined words at age 3 years, and used phrases at age 6 years. The patient was diagnosed with partial complex and generalized tonic seizure disorder at age 4 years. An initial EEG noted a single burst of left hemispheric spike-wave discharges during sleep; subsequent EEGs have been normal. Her seizures were not fully controlled with Keppra or Trileptal, and Lamictal was found to be a superior treatment. Brain MRI at age 4 years showed shallow sulcation, broad gyri, and cortical thickening suggestive of pachygyria most pronounced at the bilateral frontal and temporal lobes, signal abnormality in the bilateral periauricular white matter, mild volume loss of the posterior body of the corpus callosum, hypoplasia of the right caudate nucleus body, slightly globular configuration and asymmetric rotation of the basal ganglia and thalami, and subtle signal abnormality in the periaqueductal gray matter (Fig. 1). Previous negative clinical testing included: Prader-Willi/Angelman methylation testing, fragile X syndrome testing, and chromosomal microarray. Family history was negative for neurodevelopmental disorders.

At age 13 years, she required assistance to dress and undress, brush teeth, and use the toilet. She spoke in short

sentences with syntactic and articulation errors. Physical growth was normal; at age 13 years, her height was in the 31st percentile and weight was at the 78th percentile. On physical examination, no dysmorphic features or minor anomalies were observed. Her hypotonia and coordination had improved with age. Behavior problems, including hyperactivity and outbursts, were noted, particularly at school, and triggered by frustration or demands. Self-injurious behaviors included skin-picking and self-biting. She was noted to be very social, have a bright affect, and was able to engage with good eye contact.

Methods and Variant Interpretation

Trio clinical exome (for patients 2 and 3) and genome (for patient 1) sequencing was performed at an average of 18-fold, 10-fold, and 3-fold coverage, respectively, in CLIA-certified and CAP-accredited laboratories. In all 3 cases, testing identified the heterozygous, missense c.292G>A, p.Gly98Arg, variant in *TUBB2A*. The p.Gly98Arg variant was confirmed by Sanger sequencing.

The *TUBB2A* c.292G>A (p.Gly98Arg) variant was classified as pathogenic based on published sequence variant interpretation guidelines and pathogenic criteria as defined by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP), noted with each category [Richards et al., 2015]. The variant is absent from population databases, including the Genome Aggregation Database (PM2), and is conserved across multiple species [Kent et al., 2002; Lek et al., 2016]. It results in a nonconservative amino acid substitution, which is likely to impact secondary protein structure as these residues differ in polarity, charge, size and/or other properties. In silico analyses, including protein predictors and evolutionary conservation, support a deleterious effect (Provean, -4.56; CADD, 29.4; MutTaster, damaging; REVEL: 0.916) (PP3). Missense variants in *TUBB2A* occur at a lower frequency than expected among large population data sets (MisZ, 5.26), suggesting this gene is intolerant to missense variation and may have potential importance in biological function [Lek et al., 2016]. Missense variants in this gene are often considered pathogenic (PP2) [Stenson et al., 2014], and this particular variant has been reported as such in ClinVar (VCV000421739.5). Further supporting the pathogenic classification, this de novo variant is present in 3 unrelated probands with specific MRI abnormalities and similar clinical presentation reported here (PS2, PP4). The p.Gly98Arg variant in a

highly homologous gene, *TUBB2B* (98% amino acid sequence homology), has been reported in a heterozygous de novo state in at least 2 individuals with a tubulinopathy phenotype, increasing the evidence for a pathogenic classification [Cushion et al., 2013; Bahi-Buisson et al., 2014].

Discussion and Conclusion

Tubulin gene products are involved in the cellular process of neuronal generation, migration, and differentiation in cortical development [Bahi-Buisson et al., 2014]. The tubulin molecule is a polymer composed of alpha-tubulin and beta-tubulin subunits that assemble the microtubule structure of the cytoskeleton [Mitchison and Kirschner, 1984]. Variants in 6 tubulin genes, including both alpha- and beta-tubulin subunits, are known to cause tubulinopathies. The phenotypic variability of brain malformations, ranging from complete agyria to a mild simplified cortical gyral pattern, caused by tubulin gene variants points to the essential role of these molecules and their complex interactions [Cushion et al., 2014]. This impact on structural brain development, and subsequent underlying brain dysfunction, can ultimately manifest as a variety of brain disorders such as intellectual disability, autism spectrum disorder, seizures, and other brain disorders [Moreno-De-Luca et al., 2013]. *TUBB2A* is one of the most recent tubulinopathy-related genes discovered, and although it has less early fetal expression than other beta-tubulin isoforms, it constitutes 30% of all beta-tubulin in the brain [Leandro-García et al., 2010; Breuss et al., 2012]. The unique expression pattern of each tubulin isoform also likely contributes to the differences in cortical phenotypes amongst tubulinopathies [Cushion et al., 2014].

Variability within TUBB2A-Related Tubulinopathy

The phenotypic variability seen in the broader group of tubulinopathies is also observed in individuals with *TUBB2A* variants. The main characteristic features of *TUBB2A*-related tubulinopathy are consistent with the other known tubulinopathies including brain malformations, seizures, developmental delay, and intellectual disability. Across the 12 reported patients with *TUBB2A* variants, the specific brain abnormalities, however, have more variability (Table 1). Brain malformations described to date include diffuse simplified gyral pattern, dysmorphic corpus callosum, cortical dysplasia, asymmetric caudate nucleus, and cortical thickening suggestive of pachy-

gyria. These varying brain malformations may represent genotype-phenotype correlation, though the small sample size limits this conclusion.

Cai et al. [2020] suggest a potential *TUBB2A* “hot spot” mutation domain within the “T7” loop between the 7th and 8th alpha-helices of *TUBB2A* where 3 (p.Pro243Leu, p.Asn247Lys, p.Ala248Val) of the 8 reported *TUBB2A* variants, identified in 5 (patients 4, 5, 8, 11, 12) of the 12 reported patients, are located (Table 1). The *TUBB2A* p.Gly98Arg variant falls within the Tubulin/FtsZ, GTPase domain, potentially an additional hotspot location due to the essential nature of the GTPase domain for microtubule function [Cai et al., 2020].

Variability within Individuals with the TUBB2A p.Gly98Arg Variant

This report of 3 patients establishes the novel pathogenic missense *TUBB2A* variant (p.Gly98Arg) as a cause of *TUBB2A*-related tubulinopathy. It also provides insight into the natural history of this disorder into the teenage years, as these are among the oldest patients with *TUBB2A*-related tubulinopathy reported to date, in addition to a previously reported 23-year-old [Sferra et al., 2018]. Despite the same variant, their presentations vary slightly. All 3 patients share the same cortical malformation consisting of pachygyria. The MRI images from patients 1 and 3 were available for detailed evaluation by a board-certified neuroradiologist and had strikingly similar findings, which included mild thinning of the posterior body of the corpus callosum, signal abnormality in the bilateral periventricular white matter, slightly globular configuration and asymmetric rotation of the basal ganglia and thalami, pachygyria at the bilateral frontal and temporal lobes, prominent hypoplasia of the right caudate nucleus body, and signal abnormality in the periaqueductal gray matter (Fig. 1). The neuroimaging findings of hypoplasia of the right caudate nucleus body and signal abnormality in the periaqueductal gray matter have not been reported in patients with a *TUBB2A*-related tubulinopathy, and are therefore novel findings. These 3 patients presented with novel phenotypes not reported in other individuals with *TUBB2A* variants. Patients 1 and 2 had autism spectrum disorder, patients 1 and 2 had hyperactivity, while patient 1 had recurrent respiratory infections and muscle twitching, and patient 3 had sleep disturbances. The respiratory infections are thought to be secondary to decreased neurological function and hypotonia. Though these patients had varying clinical features, none of them had progressive symptoms, showed loss of skills, or neurologic decline.

Cushion et al. [2014], Rodan et al. [2017], and Cai et al. [2020] previously reported 3 separate patients with the recurrent p.A248V variant in *TUBB2A* with different phenotype presentations (Table 1). Similarly, our 3 patients harbor the p.Gly98Arg variant in *TUBB2A* and have varying phenotypes. Possible explanations for this variable expressivity include modifier genes, environmental factors in utero, and stochastic variation caused by random cellular or molecular events that affect phenotype formation, but more research is needed to understand the mechanism of phenotypic variability in tubulinopathies [Bahi-Buisson et al., 2014; Rodan et al., 2017; Tikhodeyev and Shcherbakova, 2019].

This case series expands the reported phenotype associated with *TUBB2A*-related tubulinopathy and provides insight into the natural history of the disorder in late childhood. Features including hypoplastic right caudate nucleus body, signal abnormality in the periaqueductal gray matter, autism spectrum disorder, and hyperactivity are newly reported and should be included in the spectrum of this condition. This report also highlights the importance of understanding emerging phenotypes as we continue to identify individuals with *TUBB2A* variants.

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Statement of Ethics

All patients' parents or guardians involved in this case have given their written informed consent to publish their case, including publication of images. The paper is exempt from ethical committee approval because this study was determined not to be human research by the Sanford Health Institutional Review Board.

Conflict of Interest Statement

I.W.M. and M.J.G.S. are employees of GeneDx, Inc. A.M., D.P., and A.C. are employees of Illumina, Inc. Other authors have no conflicts of interest to declare.

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Author Contributions

All authors made substantial contributions to the manuscript, in providing insight, drafting, and reviewing. L. Schmidt drafted the case report. M. Bell and K. Wain provided guidance and revisions of the manuscript. K. Wain and J. Estrada-Veras provided additional patient information. M. J. Guillen Sacoto, I. Wentzenesen, A. Malhotra, D. Perry, and A. Clause interpreted results and provided methods. C. Hajek provided insight and revisions of the manuscript. A. Moreno-De-Luca re-analyzed the MRI for patients 1 and 3.

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