

# Homozygous Mutation in *PRUNE1* in an Oji-Cree Male with a Complex Neurological Phenotype

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The *PRUNE1* gene encodes a member of the phosphoesterases (DHH) protein superfamily that is highly expressed in the human fetal brain and involved in the regulation of cell migration. Homozygous or compound heterozygous *PRUNE1* mutations were recently identified in five individuals with brain malformations from four families. We present a case of a 2-year-old male with a complex neurological phenotype and abnormalities on brain MRI. Re-annotation of clinical whole-exome sequencing data revealed a homozygous likely pathogenic variant in *PRUNE1* (c.521-2A>G). These results further delineate a new *PRUNE1*-related syndrome, and highlight the importance of periodic data re-annotation in individuals who remain without a diagnosis after undergoing genome-wide testing. © 2017 Wiley Periodicals, Inc.

**Key words:** *PRUNE1*; *PRUNE*; *HTCD37*; *DRES17*; founder effect; Cree; Ojibwe; brain structure; infantile spasms; whole-exome sequencing

## INTRODUCTION

Whole-exome sequencing (WES) is a powerful diagnostic tool in clinical genetics practice. Our ability to accurately interpret the genome-wide data is improving at a rapid pace. We report on a child with a complex neurological phenotype and no diagnosis after undergoing WES. Re-annotation of the data approximately 1 year later revealed a homozygous likely pathogenic variant in *PRUNE1*, a gene implicated in the interval period in causing a similar phenotype to that of our patient.

## CLINICAL REPORT

### Clinical History

The proband was the product of a natural conception by non-consanguineous parents of Cree (maternal) and Ojibwe-Cree (paternal) descent. His mother was a healthy 40-year-old gravida 7 para 4 woman, and the pregnancy itself was unremarkable, with

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no teratogenic exposures, no known in utero infections, and normal routine prenatal ultrasounds. There was no gestational diabetes mellitus. After spontaneous onset of labor at 41-week gestation, he was delivered via Cesarean section because of failure to progress and the presence of meconium. Birth parameters were weight 5,180 g (+4 standard deviations), length 52 cm (75–90th centile), and head circumference 38 cm (+3 standard deviations). He was well with normal Apgar scores in the immediate postpartum period, but then briefly required positive pressure ventilation because of poor respiratory effort. He subsequently developed central hypoventilation requiring intubation in the first hours of life. His first newborn examinations were notable for bilateral talipes equinovarus and generalized hypotonia.

Additional issues identified in his first year of life include severe global developmental delay without regression, cortical blindness, and infantile spasms with characteristic hypsarrhythmia and an

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active focus in the right posterior temporal region on EEG. Somatosensory evoked potentials showed absent median nerve response bilaterally, while auditory and visual evoked potentials were within normal limits. Echocardiogram showed a structurally normal heart and great vessels. Ophthalmologic exam including dilated funduscopy was unremarkable. There were no renal or other abnormalities on abdominal ultrasound.

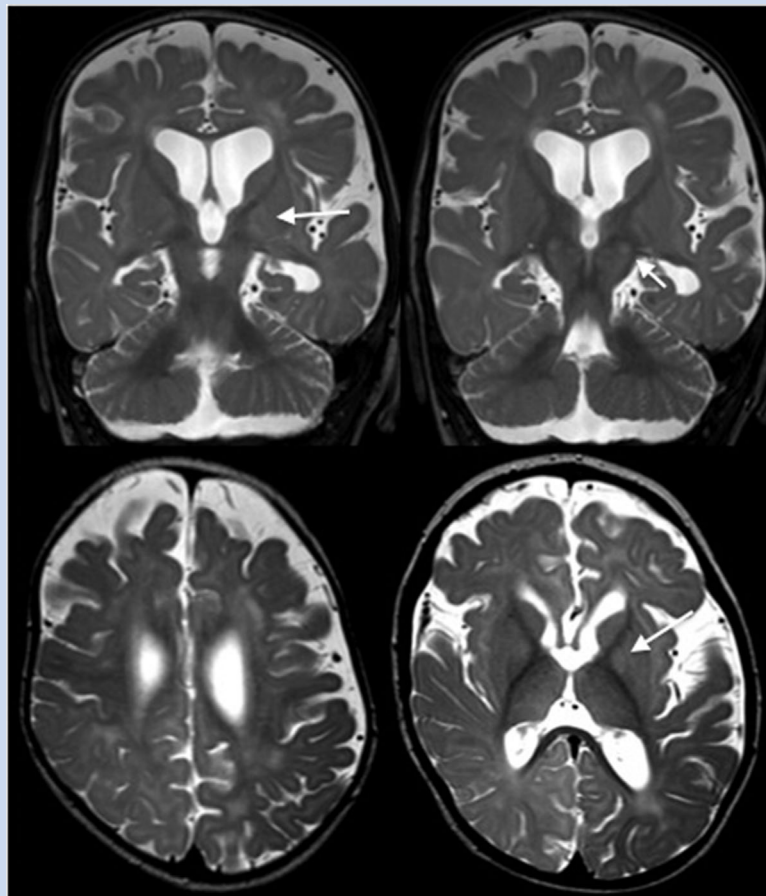
Past infancy, central hypoventilation, and sleep apnea have remained a core issue. He is treated with vigabatrin for his seizure disorder. Sialorrhea and bulbar palsy with absent swallow led to gastrostomy tube insertion. He has progressive infantile levoscoliosis of the thoracolumbar spine, with an otherwise normal MRI spine and electromyogram/nerve conduction studies showing neurogenic muscle changes. At chronological age 18 months, his skeletal maturation was at the lower end of normal to slightly delayed, in the setting of persistent growth acceleration.

He has had three MRI brain scans, at ages 8 days, 7 weeks, and 10 months, each showing non-specific abnormalities. The most recent MRI study was notable for cortical atrophy and a small cerebellum (Fig. 1). There was bilateral cerebral white matter loss

with thinning of the corpus callosum, and patchy T2 hyperintensity in the cerebral white matter with a frontal and parietal predominance (Fig. 1). Bilateral symmetric areas of diffusion restriction and T2 hyperintensity involving the globi pallidi and the subthalamic nuclei (Fig. 1) were attributed to vigabatrin use [Pearl et al., 2009]. Time-of-flight MR angiography and single voxel MR spectroscopy in the left basal ganglia region were normal.

He has three older full siblings and one older maternal half-sister who are all alive and well. The three full siblings (one male, two female) were born large for gestational age, and the father has macrocephaly (63 cm; +4 standard deviations [Bushby et al., 1992]). Otherwise the family history was non-contributory.

On examination at age 2 years 2 months, his weight was 16.5 kg (95th centile), height 90 cm (75th centile), and head circumference 49.5 cm (50th centile). His appearance was mildly dysmorphic, with a tall forehead, bitemporal narrowing, low set ears, flat nasal bridge, and a narrow high arched palate (Fig. 2). Development was severely delayed, with partial head support while sitting, inconsistent hand grasp, and a few vocalizations without any words.



**FIG. 1.** Axial and coronal T2 weighted MRI brain images at 10 months of age showing mild generalized volume loss with ventriculomegaly and slight lag in myelination. In this study, there was also diffuse thinning of the corpus callosum and the appearance of a small cerebellar vermis. Subtle T2 hyperintense signal changes (arrows) in the globus pallidus are presumably related to vigabatrin [Pearl et al., 2009].



**FIG. 2.** Photograph of the patient at age 2 years 5 months. His appearance is notable for mild dysmorphic features including a tall forehead, bitemporal narrowing, a flat nasal bridge, and low-set ears. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

### Genetic and Metabolic Studies

Initial genetic investigations included an oligonucleotide chromosomal microarray, and targeted testing for Beckwith–Wiedemann syndrome, Prader–Willi syndrome, spinal muscular atrophy with respiratory distress type 1 (SMARD1), myotonic dystrophy type 1, and congenital central hypoventilation syndrome. Work-up for genetic metabolic disorders included assays of plasma and CSF amino acids, urine organic acids, serum acylcarnitines and free and total carnitine, and transferrin glycosylation status. All results were within normal limits.

Subsequent WES and initial annotation through a commercial laboratory (GeneDx; <http://www.genedx.com/>) highlighted one likely pathogenic variant in *DNM1L* and two variants of uncertain significance in *TRAPPC9*. Copy number of the *DNM1L* gene was normal. Follow-up testing of parental samples revealed that the *DNM1L* mutation was paternally inherited, and that both of the *TRAPPC9* mutations were maternally inherited. Mean depth of WES coverage was 125×, with 98.8% of the defined target region with read depth at least 10×.

Re-analysis of the WES data in 2016 identified a novel homozygous splice site variant in the *PRUNE1* gene that destroys a canonical splice acceptor site in intron 4 (c.521-2A>G; IVS4-2A>G; NM\_021222.1). This finding was confirmed using standard DNA sequencing methods. Both parents were heterozygous for the *PRUNE1* variant. This change is predicted to cause abnormal gene splicing, and was not observed in the >60,000 unrelated individuals in the Exome Aggregation Consortium (ExAC) database (Cambridge, MA; <http://exac.broadinstitute.org>, accessed May 2016). Molecular confirmation of aberrant splicing was not deemed necessary for clinical reporting. A de novo pathogenic variant in *PEX12* was also noted upon WES data re-analysis; no second variant in *PEX12* was identified. There were no other homozygous variants of potential clinical significance.

### DISCUSSION

The *PRUNE1* gene encodes prune exopolyphosphatase, a member of the phosphoesterases (DHH) protein superfamily, that is, highly expressed in the human fetal brain and involved in the regulation of cell migration [Kobayashi et al., 2006; Karaca et al., 2015]. Homozygous and compound heterozygous missense variants were recently identified in five individuals with complex neurological phenotypes from four unrelated families [Karaca et al., 2015]. Common features were microcephaly, fronto-temporal cortical atrophy, thin or hypoplastic corpus callosum, and cerebellar atrophy. In addition, three had seizures, three had severe developmental delay, and two had developmental regression. Two families from Turkey and one family from Saudi Arabia were consanguineous; the proband with compound heterozygous mutations was from a non-consanguineous American family. All reported variants were located within the catalytic DHH domain of the *PRUNE1* protein, as is the c.521-2A>G change in this case. Our patient did not have absolute microcephaly, possibly because of other competing inherited factors. Otherwise, his history and imaging results are either consistent with or expand on the general features reported by Karaca et al. [2015]. The identification of additional cases will help to refine the phenotypic spectrum.

The Cree and the Ojibwe are two of the largest groups of First Nations in North America. Historical intermarriage was common, and led to the Oji-Cree people. There are known founder effects leading to autosomal recessive conditions in this population, for example, Cree leukoencephalopathy (OMIM #603896) [Fogli et al., 2002] and Cree encephalitis (OMIM #225750) [Black et al., 1988; Crow et al., 2003]. As with the c.316G>A variant in *PRUNE1* in the Turkish population [Karaca et al., 2015], the c.521-2A>G variant may be a founder mutation specific to the Cree and Ojibwe tribes. Carrier prevalence remains to be determined.

Use of WES in research and clinical settings facilitates the discovery of new genes integral to normal human neurodevelopment [Lee et al., 2014; Soden et al., 2014; Yang et al., 2014; Karaca et al., 2015; Sawyer et al., 2016]. WES or whole-genome sequencing as a single universal genetic test may ultimately become a cost-effective first-tier testing option in patients with complex non-specific neurological phenotypes [Soden et al., 2014; Stavropoulos et al., 2016]. Interpretation of WES findings is not static, however. This case highlights the importance of periodic data re-annotation in individuals who remain without a diagnosis after undergoing genome-wide testing.

Expanding the clinical application of WES promises to end the diagnostic odyssey for many families [Rosell et al., 2016; Sawyer et al., 2016]. For our patient, a diagnosis would not have been possible without hypothesis-free testing. His parents were pleased to have an explanation for their son's condition. The parents have written a letter in which they reflect on their experience with WES, which we provide as Supplemental Material (Box S1). As expected [Rosell et al., 2016], the diagnosis also generated new questions about his prognosis and the implications for their extended family. In this way, our case is emblematic of the issues faced in this era of genomic medicine by the growing cohort with individually rare or novel genetic diagnoses.

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