Exonic deletion of *OPHN1* resulting in seizures, intellectual disability, and brain malformations

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Abstract: We report the case of a 9-year-old boy with autism, intellectual disability, and complex partial seizures as well as cerebellar vermal hypoplasia, caudate nucleus hypoplasia, and ventriculomegaly. He was found to have a deletion within the oligophrenin 1 gene (*OPHN1*), affecting exons 2–5. *OPHN1* mutations result in a rare but well-characterized syndrome of neuroanatomical anomalies, epilepsy, and intellectual disability. This is a novel mutation in *OPHN1* that adds to the spectrum of pathogenic variants of the gene. Additionally, the case illustrates the significant benefit that patients and families can derive from a definitive genetic diagnosis, even in the absence of direct therapeutic interventions.

Keywords: X-linked intellectual disability, autism, cerebellar hypoplasia, chromosomal microarray, oligophrenin 1

Introduction

The number of diagnostic tests available to the clinical geneticist has exploded in recent years. Many patients who were initially evaluated in genetics, neurology, or developmental pediatric clinics with the available cytogenetic studies prior to the advent of chromosomal microarray (CMA) and next-generation sequencing panels did not receive genetic diagnoses and were diagnosed with idiopathic autism or epilepsy. Although the majority of children with epilepsy or autism remain without genetic diagnoses, those who are diagnosed may derive significant benefit from determination of the genetic etiology of their symptoms.¹ CMA is considered a first-line diagnostic test for children with developmental disabilities or congenital anomalies. CMA has a diagnostic yield of 5%–35%, depending on the indication for the test, the clinical setting, the resolution of the CMA, and the criteria used to define pathogenic variants.² As large databases of CMA data are compiled for affected patients and unaffected controls, the ability of the clinician to interpret the detected variants improves.³ In some cases, such as the 1q21.1 deletion syndrome, there is a broad array of resultant phenotypes, and prognostic information for families can be vague.⁴ In other cases, such as the one reported here, a variant may be detected that results in a narrower range of phenotypes and provides the ability for the clinician to make a more specific prognosis. In this case, a novel deletion was found within oligophrenin 1 (*OPHN1*), a gene that encodes a Rho guanosine triphosphatase (Rho GTPase) activating protein that is necessary for maintenance of neuronal synapses.⁵

Case report

The patient is a 9-year-old male who was born full-term via uncomplicated vaginal delivery to a 30-year-old woman with one prior unremarkable pregnancy

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and delivery. He was macrosomic at birth, weighing 4.65 kilograms. His initial clinical presentation was for strabismus and nystagmus at 6 months of age. Magnetic resonance imaging (MRI) of the brain obtained by his ophthalmologist showed inferior vermian hypoplasia and retrocerebellar cyst consistent with a Dandy–Walker spectrum malformation. In addition, there was mild diffuse ventriculomegaly and distinctive hypoplasia of the caudate nuclei, as shown in Figure 1. The corpus callosum was intact. He underwent surgery for strabismus at 18 months of age.

Figure 1 Sagittal and axial T1-weighted magnetic resonance images showing cerebellar hypoplasia, ventriculomegaly, and caudate atrophy.

Global developmental delays were apparent from an early age. The patient did not walk until 26 months of age, and currently cannot pedal a tricycle (a typical 3-year-old skill). At age 9 years, he is unable to feed or dress himself. Verbal communication skills are at the level of a 2-year-old. Behaviors consistent with autism are present, including echolalia, hand-flapping, self-stimulatory behaviors, poor eye contact, and lack of shared attention. He was diagnosed with autism at the age of 3 years. He did not have episodes of developmental regression. Anxious behaviors and poor sleep were particularly difficult to manage for the patient's family.

At 17 months of age the patient had his first apparent seizures, characterized as complex partial seizures with secondary generalization. He was found to have an abnormal electroencephalogram (EEG) with left temporal spike-and-wave discharges. He has been treated with multiple anticonvulsant medications with only fair control of his seizures. With diagnoses of idiopathic autism and epilepsy but no definitive underlying genetic diagnosis, he was being given large doses of a nutritional supplement containing over 500% of daily value for many vitamins as empiric therapy by his family with hope for improvement in autistic symptoms.

Karyotype and testing for fragile X syndrome were carried out in the patient's second year of life and were normal. Apart from EEG and MRI, no other diagnostic testing was performed until the age of 9 years. Evaluation in a genetics clinic revealed normal lactate, pyruvate, carnitine, and acylcarnitine profiles. Physical exam was pertinent for mildly dysmorphic facial features with downslanting palpebral fissures, epicanthal folds, and large prominent pinnae, as shown in Figure 2. Strabismus was not apparent on exam after surgical correction. There was symmetrical fifth finger clinodactyly, truncal hypotonia, and a mildly ataxic gait. Behavior was generally anxious and agitated, using occasional single words for communication. His height and head circumference were at about the 50th percentile, with weight at the 75th percentile. Family history was significant for the absence of epilepsy or intellectual disability (ID). CMA obtained at that visit revealed a deletion on chromosome X that included part of the gene *OPHN1*.

The deletion was determined to be a de novo variant and his family was counseled about their low recurrence risk and the patient's prognosis. Alternative therapies for autism (vitamin supplements) were stopped by the family at that time.

Figure 2 Photographs of the patient showing epicanthal folds, downslanting palpebral fissures, and large pinnae.

Methods

CMA was performed on deoxyribonucleic acid (DNA) extracted from leukocytes in peripheral blood. Testing was performed in a licensed clinical laboratory using standard techniques. CMA was performed using the CytoChip oligonucleotide 180K platform (BlueGnome, Cambridge, UK). Patient and pooled same-sex reference DNA (Promega Corporation, Fitchburg, WI, USA) were labeled with Cy3-dUTP and Cy5-dUTP, respectively, and hybridized to the array platform, as specified by the manufacturer's protocol. The array was scanned using the G2505C microarray scanner (Agilent Technologies, Santa Clara, CA, USA). Data analysis was performed using BlueFuse Multi v2.6 with the ADM-2 algorithm set at a threshold of 5.0 and a minimum of four continuous probes (BlueGnome). Findings were mapped to Genome Reference Consortium Human Build 37 (GRCh37).

The deletion was confirmed using a bacterial artificial chromosome clone corresponding to the sequence RP3-360E18 for fluorescent in situ hybridization (FISH). The same FISH probe was used to evaluate the patient's mother for the deletion. The FISH probe spanned linear positions 67,342,256–67,474,160 and largely overlapped with the detected deletion from CMA (see Results section).

Results

CMA revealed a deletion within chromosome Xq12 spanning linear positions 67,362,279–67,552,882, as shown in Figure 3. This is an intragenic deletion affecting exons 2–5 of the gene *OPHN1*. Given that the patient is male and hence hemizygous for *OPHN1*, an exonic deletion would be expected to be pathogenic. His mother did not carry the deletion in leukocyte DNA. Figure 4 demonstrates confirmation of the presence of the deletion in the proband via FISH and the absence of the deletion in the mother of the proband.

Discussion

Loss of function of *OPHN1* was discovered to cause X-linked ID in 1998.⁶ Though initially thought to result in ID without other features, additional phenotypic data found that *OPHN1* mutations cause cerebellar hypoplasia.⁷ The clinical phenotype has been further expanded to include epilepsy, hypotonia, and strabismus.⁸ The facial dysmorphisms associated with *OPHN1* mutations and deletions are variable and have been reported to include deep-set eyes, infraorbital creases, short philtrum, broad nasal root, prominent chin, and large pinnae.⁹ MRI findings in patients with *OPHN1* mutations include inferior cerebellar vermis hypoplasia, malformations of the

Figure 3 Chromosomal microarray representation showing copy number loss for probes corresponding to linear positions 67,362,279–67,552,882 in Genome Reference Consortium Human Build 37.

Note: The red mark indicates that the deletion is within band Xq12.

cerebellar hemispheres, enlargement of the lateral ventricles, caudate atrophy, and mild cortical thinning.¹⁰ The radiological phenotype of caudate atrophy with cerebellar vermian hypoplasia is relatively distinctive and should promote strong consideration of *OPHN1* mutations, particularly in the setting of a male with epilepsy and ID.

Figure 4 Fluorescent in situ hybridization analysis showing absence of the red probe on the X chromosome in the proband and presence of the red probe on both X chromosomes of the proband's mother. Note that the red probe hybridizes with the Y chromosome centromere in the proband and does not correspond to the sequences within *OPHN1*.

Since its initial description, about 50 cases of *OPHN1* mutations have been published.¹¹ In an attempt to determine the prevalence of *OPHN1* mutations in males with ID, mutation and deletion analysis was conducted for 196 males with at least one other affected male relative, as well as 17 males with ID and cerebellar abnormalities. In each group, two individuals were diagnosed with *OPHN1* mutations for a prevalence of 1 percent (two of 196) in X-linked ID generally and 12% (two of 17) in males with ID and posterior fossa abnormalities.¹² Next-generation sequencing has made possible the diagnostic use of sequencing via large panels of ID-associated genes. As *OPHN1* is included in many clinically available panels, diagnoses of *OPHN1* mutations are likely to increase in coming years. As clinical use of CMA continues to expand for patients with autism and epilepsy, *OPHN1* deletions and duplications will increasingly be detected as well.

The *OPHN1* clinical phenotype results from loss of protein function due to deletion of the entire gene,¹³ intragenic deletions,^{14,15} intragenic insertions (without frameshift),¹⁶ chromosomal translocations with breakpoints within the gene, and nonsense and frameshift mutations.¹⁰ One patient with severe ID has been reported with a duplication of the entire *OPHN1* gene. His phenotype differed from males with null alleles due to his absence of cerebellar hypoplasia.¹⁷ The androgen receptor gene *AR* is immediately proximal (centromeric) to *OPHN1*, and there are reports of a contiguous gene deletion syndrome resulting in 46, XY disorder of sex development due to complete androgen insensitivity as well as the manifestations of *OPHN1* deletion.^{18,19} A separate contiguous gene deletion syndrome includes *OPHN1* as well as the distal gene *EFNB1*. Deletion of *EFNB1* results in craniofrontonasal syndrome with hypertelorism, nasal clefting, and skeletal asymmetry, with the distinctive feature that the phenotype is expressed largely in females with mutations and not males.¹³ As would be expected in an X-linked condition, there is wide variability in the phenotype of female carriers of *OPHN1* mutations, ranging from asymptomatic to isolated strabismus to moderate ID with similar brain malformations to affected males.^{8,20} More severely affected females have been found to have skewed X chromosome inactivation with increased expression of the mutated allele.²¹

OPHN1 is a large gene, consisting of 25 exons and spanning about 390 kilobases of genomic DNA.²² The gene is expressed in developing fetal brain tissue as well as in mature structures. In particular, its protein product is found on both the axonal and dendritic sides of synapses and is found in all major types of neurons in the brain.^{23,24} It acts as a Rho GTPase activating protein, regulating G protein signaling in the neuron as it relates to the cytoskeleton.^{5,25} The result of disrupted function of the cytoskeleton is shortened or immature dendritic spines, likely resulting in impaired synaptic plasticity. There are several major functional domains within the protein product of *OPHN1*: a GTPase activating protein (GAP) domain, a Homer-binding domain, an actin-binding domain at the C-terminus, and, at the N-terminus of the protein, a Bin/amphiphysin/Rvs (BAR) domain.^{23,24} The GAP domain interacts with Rho-GTPases in the neuron to inhibit RhoA and other GTPases. GTPase inhibition preserves dendrite spine length, and the Homer-binding domain mediates interaction with the glutamate receptors of the synapse.²³ The BAR domain facilitates interaction of the protein with the cell membrane and may provide autoinhibition on the GAP activity of the protein.²⁶ Additional smaller proline-rich domains near

the C-terminus of the protein interact with regulators of endocytosis of synaptic vesicles.²⁷ Analysis of messenger ribonucleic acid transcripts was not performed for our patient, so it is unknown whether the mutated allele is transcribed. If it were translated, the resultant protein would lack a functional BAR domain, given the deletion of exons 2–5. Pirozzi et al¹⁶ showed that a 16 amino acid in-frame insertion in the BAR domain resulted in phenotypes equivalent to patients with frameshift and nonsense mutations. Thus, it is highly likely that the deletion of exons 2–5 of *OPHN1* results in a pathogenic allele.

An *Ophn-1* knockout mouse showed hyperactivity, impaired social and procedural learning, and inappropriately decreased aggression. Brain malformations included ventriculomegaly but not cerebellar hypoplasia in the mouse model.²⁸ An in vitro model using interfering ribonucleic acid knockdown in rat hippocampal neurons showed that excitatory glutamatergic synapses could be neither formed nor maintained normally in the absence of *Ophn-1* expression.²⁹ *Ophn-1* knockdown also prevents long-term depression in the hippocampus by impairing α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor endocytosis, representing a distinct mechanism of dysfunction of synaptic plasticity.³⁰ Using a similar assay, it was shown that neurons not expressing *Ophn-1* are unable to perform normal synaptic vesicle recycling, further refining the understanding of the molecular pathogenesis of *Ophn-1* dysfunction.²⁷ Using this model, it was shown that synaptic function in *Ophn-1*-knockdown neurons could be rescued with application of Rho-kinase inhibitors. Thus, separate mechanisms of impaired long-term potentiation and long-term depression have been elucidated in models of *OPHN1* dysfunction. Given that these processes are a fundamental mechanism of learning at a cellular and molecular level, it is not surprising that patients with *OPHN1* mutations have severe ID.³¹

The determination of a genetic diagnosis for the patient described in this report significantly altered his care. Prior to the diagnostic finding on CMA, with diagnoses of idiopathic autism and epilepsy, his parents treated him with large doses of vitamins well in excess of recommendations. Subsequent to the diagnosis, with an improved understanding of the underlying cause of his autism and ID, his parents chose to forgo alternative therapies. As in this case, a thorough diagnostic evaluation of individuals considered to have idiopathic epilepsy, autism, or ID may provide significant benefit to families and patients via improved understanding of prognosis, therapy, and recurrence risk.

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Disclosure

The authors report no conflicts of interest in this work.

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