COLD SPRING HARBOR
Molecular Case Studies

RAPID COMMUNICATION

Biallelic *SEPSECS* variants in two siblings with pontocerebellar hypoplasia type 2D underscore the relevance of splice-disrupting synonymous variants in disease

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Abstract Noncoding and synonymous coding variants that exert their effects via alternative splicing are increasingly recognized as an important category of disease-causing variants. In this report, we describe two siblings who presented with hypotonia, profound developmental delays, and seizures. Brain magnetic resonance imaging (MRI) in the proband at 5 yr showed diffuse cerebral and cerebellar white matter volume loss. Both siblings later developed ventilator-dependent respiratory insufficiency and scoliosis and are currently nonverbal and nonambulatory. Extensive molecular testing including oligo array and clinical exome sequencing was nondiagnostic. Research genome sequencing under an institutional review board (IRB)-approved study protocol revealed that both affected children were compound-heterozygous for variants in the *SEPSECS* gene. One variant was an initiator codon change (c.1A > T) that disrupted protein translation, consistent with the observation that most disease-causing variants are loss-of-function changes. The other variant was a coding change (c.846G > A) that was predicted to be synonymous but had been demonstrated to disrupt mRNA splicing in a minigene assay. The *SEPSECS* gene encodes O-phosphoseryl-tRNA(Sec) selenium transferase, an enzyme that participates in the biosynthesis and transport of selenoproteins in the body. Variations in *SEPSECS* cause autosomal recessive pontocerebellar hypoplasia type 2D (PCHT 2D; OMIM #613811), a neurodegenerative condition characterized by progressive cerebrocerebellar atrophy, microcephaly, and epileptic encephalopathy. The identification of biallelic pathogenic variants in this family—one of which was a synonymous change not identified by prior clinical testing—not only ended the diagnostic odyssey for this family but also highlights the contribution of occult pathogenic variants that may not be recognized by standard genetic testing methodologies.

[Supplemental material is available for this article.]

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Ontology terms: delayed gross motor development; generalized neonatal hypotonia; intellectual disability, profound; pontocerebellar atrophy; respiratory insufficiency

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CASE PRESENTATION

The 16-yr-old female proband and 9-yr-old male sibling described in this report were both delivered by spontaneous vaginal delivery with an unremarkable prenatal history. Hypotonia was noted shortly after birth. Their neonatal periods were marked by feeding issues and significant developmental delays by 6 mo of age. The family history was considered noncontributory (Fig. 1A). Their subsequent clinical course was nearly identical with dramatic reduction in head circumference and seizures associated with fever, which later progressed into clusters of focal seizures. The proband's initial brain magnetic resonance imaging (MRI) indicated vasogenic edema of the frontal lobes, probably secondary to venous sinus thrombosis. A follow-up MRI indicated improvement. The initial diagnosis for the female proband was a static encephalopathy. However, the sibling was born with a similar phenotype with severe hypotonia, epilepsy, and developmental delay.

At 16 yr of age and 9 yr of age, the proband and male sibling, respectively, are described as having static encephalopathy, hypotonia, epilepsy, and intellectual disability (Table 1). Per clinician notes, both of their facies are hypotonic with tented mouth, but otherwise exhibit no specific dysmorphic features. They are both nonverbal and nonambulatory, with progressive chronic respiratory insufficiency, eventually requiring a tracheostomy and are currently ventilator-dependent. They also have intractable epilepsy. See Supplemental Information for detailed clinical information.

Mitochondrial studies on the proband's muscle biopsy revealed a reduction in mitochondrial complex I and II activity and an increased number a of type 1 fibers in the muscle. Both

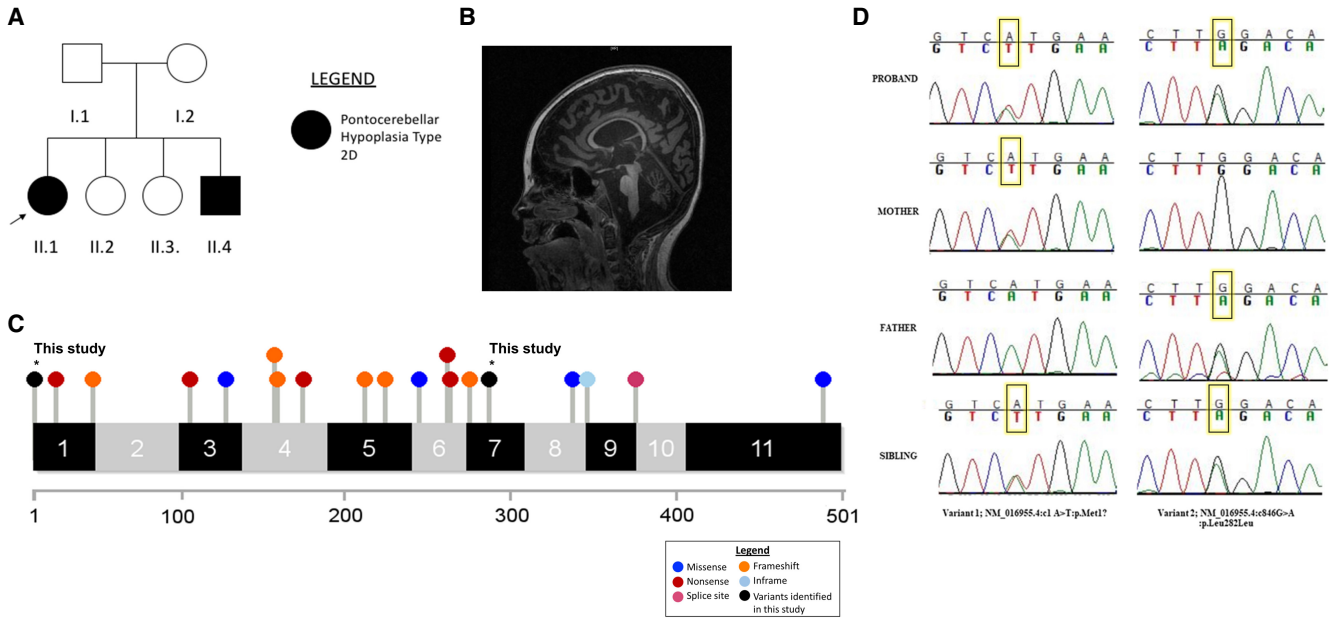

Figure 1. Whole-genome sequencing of the family quad reveals biallelic variants in the *SEPSECS* gene in the proband and her sibling. (*A*) Pedigree of the affected siblings. (*B*) Diffuse cerebral and cerebellar cortical and white matter volume loss observed in male sibling's magnetic resonance imaging (MRI). (*C*) Lollipop plots that map all known pathogenic/likely pathogenic *SEPSECS* variants found in the ClinVar database as of January 1, 2021 as well as novel variants found in this study (black dots) on the exons encoding the SEPSECS protein. (*D*) Confirmatory Sanger sequencing traces (reverse primer) demonstrating the biallelic *SEPSECS* variants in both siblings, whereas the unaffected parents were both carriers for one of the *SEPSECS* variant alleles. Highlighted bases indicate the single-nucleotide variation (SNV) in respective family members.

Table 1. Patient clinical characteristics using HPO terms

HPO ID	Phenotype	Proband (16-yr-old female)	Sibling (9-yr-old male)
HP:0001290	Generalized hypotonia	+	+
HP:0002194	Delayed gross motor development	+	+
HP:0001263	Global developmental delay	+	+
HP:0002187	Intellectual disability; profound	+	+
HP:0012736	Profound global developmental delay	+	+
HP:0001250	Seizures	+	+
HP:0002650	Scoliosis	+	+
HP:0000505	Visual impairment	+	+
HP:0002579	Gastrointestinal dysmotility	+	+
HP:0002093	Respiratory insufficiency	+	+
HP:0011922	Abnormal activity of mitochondrial respiratory chain	+	Unk
HP:0008347	Decreased activity of mitochondrial complex IV	+	Unk
HP:0000252	Microcephaly	+	+
HP:0008936	Muscular hypotonia of the trunk	+	+
HP:008872	Feeding difficulties in infancy	+	+

(HPO) Human Phenotype Ontology, (+) presence, (–) absence, (Unk) unknown.

siblings had extensive molecular testing including karyotype, oligonucleotide chromosomal microarray, fragile X, and several single gene tests (see Supplemental Information), all of which were nondiagnostic. Following discussion of the case with the steering committee, the proband, sibling, mother, and father were enrolled in an institutional review board (IRB)-approved research study (IRB11-00215) for genome sequencing.

TECHNICAL ANALYSIS AND METHODS

Genome Sequencing and Analysis

Following consent and enrollment, genomic DNA from the peripheral blood of the proband, her sibling, and their parents was extracted. Paired-end genome sequencing libraries were constructed using NEBNext Ultra II FS DNA Library Prep Kit (New England BioLabs) according to manufacturer's protocols. Whole-genome sequencing was performed using an Illumina NovaSeq6000 instrument according to manufacturer's protocols. Reads were mapped to the GRCh38 reference sequence and secondary data analysis was performed using our in-house Churchill pipeline (Kelly et al. 2015), which implements the GATK "best practices" workflow for alignment, variant discovery, and genotyping. We generated ~136 Gbp per sample on average. Mapping the sequence reads to the GRCh38 reference sequence yielded ~41× haploid coverage per sample (range, 33×–53×; see Supplemental Table 1 for detailed metrics). Variants were joint-called in all family members using GATK 4.0.5.1, and the resulting VCF file was annotated with gene, transcript, function class, damaging scores, and population allele frequencies using VarHouse, an in-house annotation tool built around the SNPeff annotation tool (Cingolani et al. 2012).

Variant annotation and prioritization were performed as previously described (Koboldt et al. 2018). Multisample variant calling with GATK HaplotypeCaller yielded 6,753,946

small sequence variants (single-nucleotide variants [SNVs] and indels) in the family quad. Of these, 5095 were in or near coding/untranslated region (UTR) and splice site regions in the proband's sample and 2414 were shared by the proband and sibling. After removing common variants (minor allele frequency [MAF] > 0.001 in the gnomAD and ExAC database), all splice site, frameshift, and nonsense variants, as well as missense variants predicted to be damaging by SIFT (score < 0.05), PolyPhen (score > 0.453), GERP (score > 2.0), or CADD (Phred score > 15), were selected for further analysis. Given the family history, rare variants that segregated with disease under a recessive inheritance model (i.e., homozygous or compound-heterozygous) were prioritized and closely scrutinized. Out of four candidate genes (all in compound-heterozygous state in the siblings) that remained following our filtering, two were, at the time, not associated with human disease according to the OMIM database. The other two included *SEPSECS* (associated with autosomal recessive pontocerebellar hypoplasia type 2D) and *DNAH17* (associated with autosomal recessive spermatogenic failure). As the latter was a poor phenotypic match for our proband and sibling's presentation, that left *SEPSECS* (Fig. 1D) as the only candidate gene consistent with the expected inheritance model that matched the clinical presentation.

Variant Interpretation

The maternally inherited variant (also identified in a prior clinical whole-exome sequencing [WES] on proband; see Supplemental Information) is a novel SNV in the initiator codon (NM_016955.4:c.1A > T, evidence PVS1) that is absent from public databases (0 in gnomAD despite good coverage; evidence PM2). Although this exact nucleotide change has not been previously reported, Zhu et al. (2015) identified another *SEPSECS* variant that affected the same codon (p.M1?) via a different nucleotide change (ENST00000382103.2; NM_016955.4: c.1A > G; evidence PS1). This change is predicted to result in protein translation start loss and is associated with a diagnosis of pontocerebellar hypoplasia type 2D in the proband (Zhu et al. 2015). The paternally inherited variant in *SEPSECS* (NM_016955.4: c.846G > A) is a rare SNV according to the gnomAD database (maximum population frequency 0.00019; evidence PM2). Although predicted to be a synonymous change, this variant was shown experimentally to disrupt mRNA splicing (evidence PS3) using the affected proband's blood RNA sequencing (RNA-seq) data in a recent study by the Undiagnosed Diseases Network (Lee et al. 2020) and reported as likely pathogenic to the ClinVar database (evidence PP5; ClinVar number SCV000746576.2). Briefly, Lee et al. demonstrated that the synonymous variant (located on exon 7 of *SEPSECS* gene product), which was 42 bp downstream from a splice acceptor site, caused exon 7 skipping in nearly all of the *SEPSECS* transcripts in proband and mother. Although there was an indication of exon 7 skipping in unrelated control samples, this occurred in a significantly fewer transcripts, suggesting that this exon is prone to skipping, and the synonymous change greatly increased the chances of this event (Lee et al. 2020). Although the mechanisms underlying this altered splicing event are unclear, one possible explanation for this skipping could be that this synonymous change may be altering an exonic splice enhancer or silencer within the exon. Ramser et al. (2005) have previously reported a synonymous change in the *ATP6AP2* gene, leading to exon skipping via the alteration of a putative exonic splice enhancer. Further, such exon skipping events are well-established disease-causing mechanisms, as seen in spinal muscular atrophy and other conditions (Lorson et al. 1999; Anna and Monika 2018). Although this protein change is not novel, ours is the first study that reports the occurrence of this variant in combination with a different *SEPSECS* variant in *trans*. Both variants were compound heterozygous in the two affected children, consistent with recessive inheritance (evidence PP1). Taken together, under American College of Medical Genetics and Genomics (ACMG) guidelines, the maternally inherited

Table 2. Genomic findings and variant interpretation

Gene	Variant	HGVS	Proband	Mother	Father	Sibling	Interpretation
SEPSECS	Chr 4: 25160369 T/A	NM_016955.4:c.1A > T	Het	Het	Ref	Het	P (PVS1, PS1, PM2, PP1)
SEPSECS	Chr 4: 25145092 C/T	NM_016955.4:c.846G > A	Het	Ref	Het	Het	LP (PS3, PM2, PP1, PP5)

Genomic coordinates reflect build GRCh38.

variant was classified "pathogenic," whereas the paternally inherited variant was classified "likely pathogenic" (Table 2).

These genomic findings were discussed with the referring clinician, who concluded that the clinical presentation of the siblings was a good match to the phenotype of pontocerebellar hypoplasia type 2D associated with *SEPSECS* gene variants. The results were shared with the family, who declined segregation testing for the unaffected siblings.

SUMMARY

Pontocerebellar hypoplasia (PCH; OMIM #607596) is a group of heterogenous and often lethal autosomal recessive neurodegenerative disorders that affect the growth and function of the pons (a major part of the brainstem) and cerebellum. Since its initial classification into type 1 and 2 PCH based on accompanying clinical phenotypes; (Barth 1993), there are currently nine different subtypes with varying clinical presentations and genetic causes. Specifically, pontocerebellar hypoplasia type 2 (PCH2) involves a lack of voluntary motor development. Individuals with PCH2 present with impaired vision, dysphagia, severe impairment of cognitive and gross motor function, and seizures of varying severity (Namavar et al. 2011). There are currently variants in six unique genes known to cause PCH2, resulting in a subclassification of A through F (van Dijk et al. 2018). The first report of PCH2D (OMIM #613811) was found in a cohort of Sephardic Jews who presented with intellectual disability, progressive microcephaly, spasticity, and progressive cerebellar–cerebral atrophy (Ben-Zeev et al. 2003); the disorder was later linked to the *SEPSECS* gene (Agamy et al. 2010). Pontocerebellar hypoplasia type 2D is characterized by progressive microcephaly, cerebellar atrophy, early-onset epileptic encephalopathy, and optic nerve atrophy (van Dijk et al. 2018) complicated by heterogeneity in symptoms and severity. Our patients' presentation was consistent with the classical phenotypic spectrum comprising progressive microcephaly, cerebellar atrophy (Fig. 1B; Supplemental Figures 1 and 2), vision loss, epilepsy, and dysphagia.

The identification of biallelic pathogenic *SEPSECS* variants (one of which was a synonymous change) in the siblings led to a critical diagnostic breakthrough for the family and demonstrates the contribution of occult splicing variants to monogenic disorders.

ADDITIONAL INFORMATION

Data Deposition and Access

The *SEPSECS* variants and our interpretation have been submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession numbers SCV001960820.1 (NM_016955.4:c.1A > T) and SCV001960821.1 (NM_016955.4:c.846G > A).

Ethics Statement

Written informed consent was obtained for all participants in this study under a research protocol approved by the Institutional Review Board at Nationwide Children's Hospital (IRB11-00215 Study: Using Genome Sequencing to Identify Causes of Rare Birth Defects and Rare Disorders).

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Competing Interest Statement

The authors have declared no competing interest.

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Author Contributions

All authors contributed to scientific discussion, variant interpretation, and manuscript review.

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