COLD SPRING HARBOR
Molecular Case Studies

RAPID COMMUNICATION

Rapid whole-genome sequencing identifies a homozygous novel variant, His540Arg, in *HSD17B4* resulting in D-bifunctional protein deficiency disorder diagnosis

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Abstract Rapid whole-genome sequencing (rWGS) allows for a diagnosis to be made quickly and impact medical management, particularly in critically ill children. Variants identified by this approach are often not identified using other testing methodologies, such as carrier screening or gene sequencing panels, targeted panels, or chromosomal microarrays. However, rWGS can identify variants of uncertain significance (VUSs), which challenges clinicians in the rapid return of information to families. Here we present a case of the metabolic condition D-bifunctional protein deficiency in a neonate with epilepsy and hypotonia born to consanguineous parents. Sequencing revealed a homozygous VUS in *HSD17B4*, c.1619A > G (p.His540Arg). Preliminary results were delivered within 3 d of sample receipt. Previous parental carrier screening included the *HSD17B4* gene but was reported as negative. The molecular finding directed the clinical team to assess phenotypic overlap and investigate next steps in terms of confirmation of the findings and potential medical management of the patient. Clinical metabolic testing of fatty acids confirmed the diagnosis. Computational analysis of *HSD17B4* His540Arg showed the change to likely impact dimerization based on structural insights, with the histidine conserved and selected throughout all 223 species assessed for this amino acid. This variant clusters around several pathogenic and likely pathogenic variants in *HSD17B4*. This case demonstrates the utility of rWGS, the potential for receiving uncertain results, and the downstream implications for confirmation or rejection of a molecular diagnosis by the clinical team.

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Ontology terms: generalized neonatal hypotonia; infantile spasms; respiratory difficulties; respiratory insufficiency due to muscle weakness; very long chain fatty acid accumulation

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CASE PRESENTATION

The patient was born at 39 wk 2 d gestation by urgent cesarean section for nonreassuring fetal heart tones. Pregnancy was complicated by maternal anemia, decreased fetal movements, and parental consanguinity. Apgar scores were 3, 6, 8, and there was respiratory distress and hypotonia requiring intubation and mechanical ventilation. The patient developed

rhythmic head and hand jerking within the first 24 h of life and was loaded with phenobarbital. Magnetic resonance imaging (MRI) on day of life (DOL) 2 showed ischemic changes of the basal ganglia. Seizures continued, and she was redosed times two with phenobarbital at 3 d of life and started on maintenance. Seizures continued, and levetiracetam was added on DOL 12 with increase in dose on DOL 18. Repeat MRI on DOL 12 showed no evidence of hypoxic ischemic encephalopathy. Oxcarbazepine was added on DOL 24. Seizures finally stopped, and levetiracetam was discontinued DOL 32. The seizures returned DOL 44, and phenobarbital was loaded again. The patient was then transferred to a higher-acuity center for genetics, metabolic, and additional neurology consultation. At that time, exam was notable for generalized hypotonia, flat fontanel, no hepatosplenomegaly, and no dysmorphic features. (See Table 1.)

Selected metabolic workup was completed prior to transfer. This included normal results of serum amino acids, urine organic acids, pyruvate, carnitine profile, and ammonia. Lactate, vitamin B_6, biotin, and cerebrospinal fluid (CSF) studies including neurotransmitters were pending (and ultimately returned normal). The State of Michigan newborn screen was also normal.

Genetic testing had already been initiated prior to birth; parental expanded carrier screening was performed at ~20 wk gestation. Parents were Indian and first cousins. Father was identified as a carrier of biotinidase deficiency (*BTD* gene—p.Asp444His) and mother as a carrier for inclusion body myopathy (*GNE* gene—p.Val696Met). The patient was eventually tested and also found to carry one mutation for biotinidase deficiency. An epilepsy gene panel was also sent prior to transfer and showed multiple variants of uncertain significance that did not provide assistance regarding the ultimate diagnosis.

Family history was notable for full 4-yr-old brother in good health and a paternal aunt who died at 5 yr of age with neonatal epilepsy, hypotonia, and feeding intolerance. There was also a distant paternal family history of one male with childhood-onset epilepsy.

After transfer to Helen DeVos Children's Hospital, proband rapid whole-genome sequencing was initiated with the patient now 6 wk old. Samples from proband, mother, and father were sent to Rady Children's Institute for Genomic Medicine, and testing was started the following day upon arrival of samples. A verbal preliminary result was communicated 2 d later as a c.1619A > G (p.His540Arg) homozygous variant of uncertain significance in *HSD17B4* was identified. This classification was made following ACMG guidelines (Richards et al. 2015, PMID: 25741868), and relying on the following pieces of evidence: the variant is absent from the gnomAD v2 population database (PM2), and multiple computation tools support a deleterious effect of the variant on the gene product (PP3; Mutation Taster: 1; PolyPhen-2—HDIV: 0.987; SIFT: 0.012). Sanger sequencing studies confirming the biparental inheritance pattern of the variant were completed and returned 3 d after the preliminary report was delivered. (See Table 2.)

Based on this potential diagnosis of D-bifunctional protein deficiency, additional biochemical testing was recommended to help confirm the diagnosis suggested by these variants. At the time of verbal results, the patient had been transferred back to her originating NICU center per family request.

Preliminary results and recommendations for confirmatory biochemical testing were relayed to that NICU immediately. At the same time, variants were sent to the Prokop laboratory for analysis using computational workflows. The analysis described in this article was completed in <24 h and provided to the clinical team to help guide determination of the clinical importance of the tentative diagnosis. This was complete both before parental testing showed biparental inheritance of the VUS and 2 wk before very long chain fatty acid (VLCFA) profile and bile acid testing returned abnormal at age 3.5 mo, confirming the diagnosis. The addition of VLCFA and bile acid studies supporting the molecular diagnosis of D-bifunctional protein deficiency allowed reclassification of this variant to Likely Pathogenic (PS4443) (Richards et al. 2015 PMID: 25741868).

Table 1. Phenotypic features

D-bifunctional protein deficiency clinical features[a]	Proband	Mother	Father
Failure to thrive	No	No	No
Macrocephaly	No	No	No
Scaphocephaly	No	No	No
Large fontanels	Yes	No	No
Delayed closure of fontanelles	Yes	No	No
Facial dysmorphism	No	No	No
Frontal bossing	No	No	No
High forehead	No	No	No
Micrognathia	No	No	No
Retrognathia	No	No	No
Long philtrum	No	No	No
Low-set ears	No	No	No
Loss of hearing	No	No	No
Upslanting palpebral fissures	No	No	No
Epicanthal folds	No	No	No
Hypertelorism	No	No	No
Visual impairment	No	No	No
Nystagmus	No	No	No
Strabismus	No	No	No
Failure to fixate on objects	No	No	No
Loss of vision	No	No	No
Abolished ERG	Unknown	No	No
Depressed nasal bridge	No	No	No
High-arched palate	No	No	No
Funnel chest	No	No	No
Long, small thorax	No	No	No
Abnormal liver function	Yes	Unknown	Unknown
Hepatomegaly	No	No	No
Histology shows normal numbers of peroxisomes	Unknown	Unknown	Unknown
Abnormal peroxisomes	Unknown	Unknown	Unknown
Absence of peroxisomes	Unknown	Unknown	Unknown
Cholestasis	Unknown	Unknown	Unknown
Steatosis	Unknown	Unknown	Unknown
Fibrosis	Unknown	Unknown	Unknown
Hemosiderosis	Unknown	Unknown	Unknown
Proliferation of bile canaliculi	Unknown	Unknown	Unknown
Poor feeding	Yes	No	No
Renal cysts	No	Unknown	Unknown
Adrenal cortex atrophy	No	Unknown	Unknown
Generalized osteopenia	No	Unknown	Unknown
Delayed bone maturation	No	Unknown	Unknown
Calcific stippling	No	Unknown	Unknown
Claw hands	No	Unknown	Unknown

(*Continued on next page.*)

Table 1. (*Continued*)

D-bifunctional protein deficiency clinical features[a]	Proband	Mother	Father
Talipes equinovarus	No	No	No
Hammertoes	No	No	No
Decreased muscle mass	Yes	No	No
Hypotonia, neonatal	Yes	No	No
Seizures	Yes	No	No
Delayed psychomotor development, severe	Yes	No	No
Polymicrogyria	Yes	Unknown	Unknown
Ventricular dilatation	No	Unknown	Unknown
White matter dysmyelination/demyelination	No	Unknown	Unknown
Neocortical dysplasia	Yes	Unknown	Unknown
Hypoplastic/atrophic corpus callosum	No	Unknown	Unknown
Heterotopic neurons in white matter	No	Unknown	Unknown
Generalized cerebral hypoplasia/atrophy	No	Unknown	Unknown
Cerebellar hypoplasia/atrophy	No	Unknown	Unknown
Gliosis	No	Unknown	Unknown
Delayed peripheral nerve motor conduction velocities	Unknown	Unknown	Unknown
Adrenocortical insufficiency	No	Unknown	Unknown
Polyhydramnios	No	Unknown	Unknown
Fetal ascites	No	Unknown	Unknown
Increased plasma levels of VLCFA	Yes	Unknown	Unknown
Increased plasma levels of bile acid intermediates	Yes	Unknown	Unknown
Decreased peroxisomal fatty acid β-oxidation	Unknown	Unknown	Unknown
Decreased or absent D-bifunctional protein activity and protein	Unknown	Unknown	Unknown
Normal serum plasmalogen	Unknown	Unknown	Unknown
Onset in infancy	Yes	No	No
Early death, usually before age 2 yr	No	No	No

[a]The list of clinical features are based on the OMIM clinical synopsis (#261515; D-bifunctional protein deficiency).
(ERG) electroretinographic, (VLCFA) very long chain fatty acid.

Of note, *HSD17B4* was included on the expanded carrier screening panel that was performed prenatally on this patient's parents. No variants were reported from this gene on either parental report. Even upon request of clinicians of the laboratory to re-review this testing prior to rapid whole-genome sequencing, nothing was identified as reportable, as the variant was only classified of uncertain significance. Carrier screens are often limited to reporting only known pathogenic variants; thus, publications such as this are important for adding pathogenic variants to the literature to improve the yield of that testing. The epilepsy gene panel performed on the patient's sample prior to WGS did not include *HSD17B4*.

Chromosome microarray was also performed during hospital admission. It returned without significant deletion or duplication but showed that 6.34% of the genome contained regions of homozygosity (ROH). *HSD17B4* is located at 5q23.1, which is within one of the large ROH blocks in this patient.

Upon diagnosis, appropriate medical care was able to be structured based on the specific syndrome including cessation of additional diagnostic testing, initiation of gastrostomy tube for feeding, and genetic counseling for the family. The family was able to engage with palliative care to help ensure their wishes were being followed. The patient remains medically complex and alive at age 9 mo, and she is followed by multiple subspecialists.

Table 2. Genomic findings

Gene	Genomic location	HGVS cDNA	HGVS protein	Predicted effect	Zygosity	Parent of origin	Variant interpretation	ClinVar ID
HSD17B4	Chr 5: 118860951 (GRCh37) Chr 5: 119525256 (GRCh38)	NM_001199291.2: c.1619A > G	p.His540Arg	Substitution	Homozygous	Biparental	Likely pathogenic	VCV000974782.1

TECHNICAL ANALYSIS AND METHODS

The patient was referred to Medical Genetics at 6 wk of age. Whole blood was collected and sent to Rady Children's Institute for Genomic Medicine (RCIGM) for rapid whole-genome sequencing (Supplemental Fig. 1). The methods for sequencing, alignment, variant calling, and variant curation and reporting have been previously described (Farnaes et al. 2018; Kingsmore et al. 2019; Sanford et al. 2019). After delivery of the genomic results, variants were assessed through our previously published sequence-to-structure-to-function workflow (Prokop et al. 2017), comparing the patient variant to all gnomAD and ClinVar missense variants for *HSD17B4*, assessing each with PolyPhen-2, Provean, and SIFT. Variant impact scores were created by adding the calls of each of these tools with our conservation score and multiplied by the 21-codon sliding window conservation.

VLCFA and bile acid testing was performed at Mayo Laboratories. Peroxisomal fatty acid analysis in this patient revealed marked elevations in C26:0 (13.31 nmol/mL, reference value ≤1.30 nmol/mL), C24:0/C22:0 (1.98 nmol/mL, reference value ≤1.39 nmol/mL), C26:0/C22:0 (0.379 nmol/mL, reference value ≤0.023 nmol/mL), pristanic acid (2.14 nmol/mL, reference value ≤0.60 nmol/mL), and pristanic acid to phytanic acid ratio (2.74 nmol/mL, reference value ≤0.35 nmol/mL). Bile acid analysis demonstrated elevations in both di- and trihydroxycholestanoic acids (DHCA and THCA) (total bile acids 7.84 nmol/mL, reference value <19.00 nmol/mL; DHCA 2.67 nmol/mL, reference value ≤0.10 nmol/mL; THCA 2.74 nmol/mL, reference value ≤1.30 nmol/mL), consistent with the molecular diagnosis.

VARIANT INTERPRETATION

Sequencing revealed a homozygous c.1610A > G (p.His540Arg) variant in *HSD17B4*. *HSD17B4* is located on the long arm of Chromosome 5 and encodes the enzyme 17-β-hydroxysteroid dehydrogenase IV, also referred to as multifunctional protein-2, or D-bifunctional protein. D-bifunctional protein deficiency impairs the catabolism of very long chain fatty acids, DHCA and THCA, and pristanic acid resulting in accumulation of these metabolites. HSD17B4 is essential in peroxisomal fatty acid β-oxidation, and mutations in *HSD17B4* are associated in multiple autosomal recessive disorders, including D-bifunctional protein deficiency and Perrault syndrome. Most common features are neonatal hypotonia for D-bifunctional protein deficiency and ovarian dysgenesis in females, sensorineural deafness, and other neurological abnormalities in Perrault syndrome (Ferdinandusse et al. 2006; Pierce et al. 2010).

HSD17B4 forms a dimer structure with a high level of conservation on the internal hydrophobic collapse and the dimer contacts (Fig. 1A). Histidine 540 (515 in HSD17B4 isoform 2) falls within the conserved core near the dimer contact (Fig. 1B). Using 233 open reading

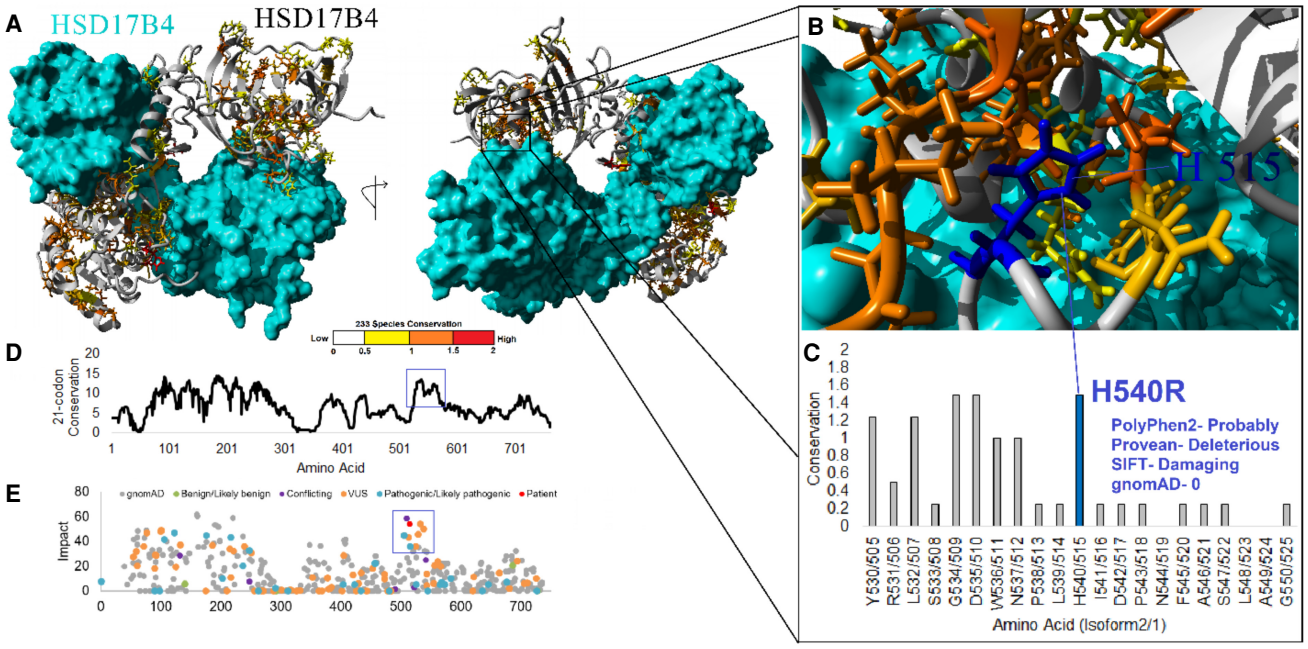

Figure 1. Structure and evolution of HSD17B4 H540R. (*A*) Dimer model of HSD17B4 with one monomer shown as a surface plot and the other as cartoon with colors based on the conservation of 233 species. (*B*) Zoom-in of H540 (blue) found in a core of conserved amino acids near the dimer contact point. (*C*) Conservation of amino acids near 540 (blue) with assessment from multiple tools shown to the side. (*D*) Conservation throughout 233 species put on a 21-codon sliding window with the region near the variant boxed in blue. Conservation score is calculated as published in Prokop et al. (2017), using binned scoring of selection rate based on dN-dS *z*-score in addition to amino acid conservation. (*E*) Assessment of known variants in gnomAD (gray) and ClinVar (benign, green; conflicting, magenta; variant of uncertain significance [VUS], orange; pathogenic, cyan) and the patient variant (red). Impact score is calculated as the sum of damaging calls in PolyPhen-2, Provean, and SIFT with our calculated conservation score (*C*) multiplied by the 21-codon conservation score (*D*).

frames of *HSD17B4*, all species have a conserved histidine at this site, with several species having codon wobble such that the selection rate at this site is 1.7 standard deviations above the mean for all sites indicating high selection on the histidine. Selection rate is calculated using a dN-dS metric placed on a *z*-score, calculating the rate of nonsynonymous variants minus the rate of synonymous variants at each amino acid and then how many standard deviations that value is above or below the mean of all sites within the gene. The His540Arg is predicted probably damaging in PolyPhen-2, deleterious in Provean, and damaging in SIFT with no occurrences in gnomAD (Fig. 1C). The region around amino acid 540 is one of the highest conserved segments in the entire gene (Fig. 1D), with a clustering of high impact variants from ClinVar for likely pathogenic and pathogenic variation (Fig. 1E).

SUMMARY

Rapid whole-genome sequencing is an extremely useful tool for diagnosis of critically ill newborns (Clark et al. 2019). This includes improved clinical utility of standard genetic testing as well as impact on the total cost of care (Farnaes et al. 2018; Kingsmore et al. 2019). However, with the expanded use of genetic and genomic testing, the incidence of finding variants of uncertain significance will only grow. Standards have been established to characterize

variants found on testing (Richards et al. 2015), but uncertain results can be especially difficult to interpret when they may suggest a potentially explanatory diagnosis for a critically ill patient. Genes associated with biochemical phenotypes that can be readily assessed with clinically available assays could be considered appropriate for lower threshold reporting as that additional testing can fairly easily provide resolution for the variants of uncertain significance. Acting on clinical judgement in an intensive care setting is standard practice for interventions, treatments, medications, and more. However, a process for integrating genomics into that workflow is still evolving (Bennett et al. 2019). Developing tools to provide near real-time analysis of variants of uncertain significance using computational methods is a crucial step in the practice of precision medicine in genomics. This case example serves as a model for successful implementation which improved patient care through collaboration and innovation.

ADDITIONAL INFORMATION

Database Deposition and Access

Sequence data and variant have been deposited in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) under accession number VCV000974782.1.

Ethics Statement

Informed consent for research and publication was obtained from the patient's parents.

Author Contributions

S.R. and C.B. designed the study and developed the protocol. K.J. and S.C. performed rWGS testing and analysis. J.W.P. performed variant interpretation. S.D.A. and C.B. investigated the patient. L.S. and C.B. created the first draft of the manuscript. All authors reviewed all drafts of the manuscript including the final draft.

Competing Interest Statement

The authors have declared no competing interest.

Referees

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