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# Successful diagnosis of HIBCH deficiency from exome sequencing and positive retrospective analysis of newborn screening cards in two siblings presenting with Leigh's disease

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ABSTRACT

*Purpose:* 3-Hydroxyisobutryl-CoA hydrolase (HIBCH) deficiency is a rare disorder of valine metabolism. We present a family with the oldest reported subjects with HIBCH deficiency and provide support that HIBCH deficiency should be included in the differential for elevated hydroxy-C4-carnitine in newborn screening (NBS).
*Methods:* Whole exome sequencing (WES) was performed on one affected sibling. HIBCH enzymatic activity was measured in patient fibroblasts. Acylcarnitines were measured by electrospray ionization tandem mass spectrometry (ESI–MS/MS). Disease incidence was estimated using a cohort of 61,434 individuals.
*Results:* Two siblings presented with infantile-onset, progressive neurodegenerative disease. WES identified a novel homozygous variant in *HIBCH* c.196C>T; p.Arg66Trp. HIBCH enzymatic activity was significantly reduced in patients' fibroblasts. Acylcarnitine analysis showed elevated hydroxy-C4-carnitine in blood spots of both affected siblings, including in their NBS cards, while plasma acylcarnitines were normal. Estimates show HIBCH deficiency incidence as high as 1 in ~130,000 individuals.
*Conclusion:* We describe a novel family with HIBCH deficiency at the biochemical, enzymatic and molecular level. Disease incidence estimates indicate HIBCH deficiency may be under-diagnosed. This together with the elevated hydroxy-C4-carnitine found in the retrospective analysis of our patient's NBS cards suggests that this disorder could be screened for by NBS programs and should be added to the differential diagnosis for elevated hydroxy-C4-carnitine which is already measured in most NBS programs using MS/MS.


## 1. Introduction

HIBCH deficiency (OMIM #250620) is a rare inborn error of metabolism caused by a defect in the HIBCH enzyme resulting in deficiency in the conversion of 3-hydroxy-isobutryl-CoA to 3-hydroxy-isobutyric acid, a critical step in valine catabolism [1]. Only 9 patients from 7 unrelated families, 3 of which are consanguineous, have been reported [1–7]. This autosomal recessive condition is characterized by developmental delay of motor milestones in early infancy and neurological regression within the first year of life. MRI abnormalities are striking for bilateral involvement of the basal ganglia with varying degrees of white matter atrophy [1,2,4]. Strikingly, in all HIBCH patients reported to date, the clinical presentation and MRI findings led to a general diagnosis of Leigh syndrome (OMIM 256000). Leigh's is a relatively common neurometabolic condition associated with several different gene disorders which feature infantile onset progressive encephalopathy and characteristic brain MRI findings including bilateral basal ganglia and white matter changes. Leigh syndrome is caused mainly, but not exclusively, by defects in oxidative phosphorylation and may be accompanied with elevated lactic acid [8]. Due to this, mitochondrial diseases are naturally considered in the top differential for children presenting with Leigh's disease while HIBCH, which is considered to be exceedingly rare, is not typically investigated.

A definitive diagnosis of HIBCH deficiency can be achieved through measurement of HIBCH enzymatic activity in patient tissues. Affected individuals have significantly decreased enzymatic activity as compared to healthy controls [1,4]. However, this assay is not readily performed in clinical laboratories. A significant clinical diagnostic finding for HIBCH

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deficiency is elevation of 3-hydroxy-isobutyryl-carnitine, which occurs secondary to the accumulation of 3-hydroxy-isobutyryl-CoA and has been reported in almost all subjects with HIBCH deficiency in whom this metabolite was measured [1,4,6]. In addition, urinary excretion of 2-methyl-2,3-dihydroxybutyric acid can be seen in small quantities by urine organic acid analysis and methacrylyl-CoA metabolites, *S*-(2-carboxypropyl)cysteine and *S*-(2-carboxypropyl)cysteamine, can be detected through urine MS/MS analysis [7,9]. However, these metabolites, which are not routinely measured in most laboratories, are also present in individuals with a deficiency of short-chain enoyl-CoA hydratase (SCEH), a mitochondrial enzyme immediately upstream of HIBCH in the valine catabolic pathway encoded by *ECHS1* [10]. Further obfuscation of the diagnosis of HIBCH deficiency occurs when diagnostic measurement of acylcarnitine levels does not include hydroxy-C4-carnitine in the report, and/or when hydroxy-C4-carnitine falls within normal limits [6,7]. All of these factors may lead to the under-diagnosis of HIBCH deficiency.

Here, we report a novel family with two siblings affected with HIBCH deficiency confirmed by enzymatic, biochemical and molecular studies. We present detailed biochemical profiling of these cases including retrospective evaluation of newborn screening cards showing elevated hydroxy-C4-carnitine. We also estimate incidence for this disorder and find compelling results that imply HIBCH deficiency is under-diagnosed and more common than currently held. Our report highlights the clinical and biochemical presentations of this disease and provides evidence that HIBCH deficiency should be included in the differential diagnosis for elevated hydroxy-C4-carnitine in NBS programs using MS/MS.

## 2. Methods

### 2.1. Human subjects

Patients 1 and 2 are similarly affected siblings with parents of Lebanese origin. The family self-reports consanguinity as parents being first cousins. Informed consent was obtained for all subjects under approved Institutional Review Boards #130990 at CHOC Children's. Genomic DNA was extracted from peripheral-blood lymphocytes or cultured fibroblasts according to standard protocols.

### 2.2. Molecular analysis

Exome sequencing was performed at the Human Genome Sequencing Center (HGSC) at Baylor College of Medicine through the Baylor–Hopkins Center for Mendelian Genomics initiative. Using 1 μg of DNA an Illumina paired-end pre-capture library was constructed according to the manufacturer's protocol. Four pre-captured libraries were pooled and then hybridized in solution to the HGSC CORE design (52 Mb, NimbleGen) according to the manufacturer's protocol *NimbleGen SeqCap EZ Exome Library SR User's Guide* (*Version 2.2*) with minor revisions. The sequencing run was performed with a sequencing yield of 9.4 Gb, the sample achieved 91% of the targeted exome bases covered to a depth of 20× or greater. Sequence data were aligned, single nucleotide variants (SNVs) and small insertions and deletions (InDels) were called by GATK [11,12]. Quality control filtering of variants was based on coverage, strand bias, mapping quality, and base quality custom Perl scripts were used to annotate variants as previously described [13]. Multiple metrics for prediction of potential functional consequences of variants were applied: CADD [14], SIFT [15] and PolyPhen2 [16], Genomic Evolutionary Rate Profiling (GERP) [17,18], and PhyloP [19]. Filtering of variants included five criteria: 1. Allele frequency must be less than 2% in any reference population. 2. Scaled CADD of ≥ 15 or two or more of PhyloP must be greater than 2 OR GERP_RS greater than 5 or maximum damaging scores for SIFT or PolyPhen2_HDIV. 3. Variants should be homozygous or compound heterozygous. 4. Variants must not be present in a segmental duplication. 5. Variant must not be present in a SNP cluster defined as the presence of 5 or more SNVs within 1 Kb of each other. The Exome Aggregation Consortium (ExAC) Cohort was used as reference population data for variant filtering of exome data (Exome Aggregation Consortium (ExAC) Cohort, Cambridge, MA, http://exac.broadinstitute.org, accessed December, 2014).

The *HIBCH* variant identified through exome sequencing in Patient 2 was orthogonally validated and recessive segregation through the subject's pedigree was confirmed using PCR-based Sanger sequencing.

All genetic alleles studied were submitted to ClinVar http://www.ncbi.nlm.nih.gov/clinvar/ and were annotated in reference to HIBCH NM_014362.3 for cDNA and NP_055177.2 for protein.

### 2.3. Enzyme analysis

HIBCH enzymatic activity was measured in primary fibroblasts at the Laboratory Genetic Metabolic Diseases at the Academic Medical Center according to published methods [1].

### 2.4. Acylcarnitine analysis

Acylcarnitines were measured at CHOC Children's Metabolic Laboratory from dried blood spots (Schleicher & Schuell 903, Keene, NH, U.S.A.), and retrospectively from the patient's newborn screening cards (stored 12–13 yrs). Ten birth-matched control samples (5 for each patient) obtained at the same time and stored in the same manner were also analyzed. The laboratory was blind regarding the sample identity (patient or control). Acylcarnitine butyl-esters were analyzed by ESI–MS/MS (Waters Alliance 2795 Quattro Micro). Precursor ion scans of the peak at m/z 85 were monitored in the range m/z 250–500.

### 2.5. Disease incidence estimate

Disease incidence was estimated based on the Hardy–Weinberg Equation, $1 = p^2 + 2pq + q^2$, using carrier allele frequencies. Any variant meeting the above criteria for being considered 'damaging' were considered pathogenic and the frequency of these 'damaging' alleles were determined in the ExAC cohort.

## 3. Results

### 3.1. Clinical report

#### 3.1.1. Patient 1

The family history was positive for six first trimester miscarriages, thought to be related to a uterine septum which was later surgically corrected but left scar tissue. A female term baby was born after the surgical procedure, but died of hypoplastic left heart at six days of age (Fig. 1A). Patient 1 (IV-2), a girl, the product of *in vitro fertilization* (IVF), was the second child born at 38 weeks gestational age by cesarean section after an uneventful pregnancy. Early developmental milestones were normal. She was able to sit unsupported but was never able to bear weight and never developed language. From 4–5 months of age, our patient began to lose previously acquired milestones. Initial neurological examination at 11 months of age noted normal head circumference, no dysmorphic features, dystonia, spastic quadriplegia and absent reflexes in the lower extremities. Visual inattention prompted an ophthalmological examination which revealed optic atrophy. A gastrostomy tube was placed at 1.6 years of age due to poor feeding and failure to thrive. At 2 years of age brain MRI demonstrated high signal lesions in the globus pallidus and head of the caudate nucleus with patchy high signal in the periventricular white matter and a focal lesion in the left cerebral peduncle (Fig. 2A, a–c). Repeat MRI at 6.3 years of age showed improved high signal abnormality in the basal ganglia, however new associated atrophy was seen (Fig. 2A, d–f). The most recent brain MRI at 12 years of age showed residual cystic changes in the globus pallidus, basal ganglia atrophy, and persistent generalized brain atrophy and