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ORIGINAL ARTICLE

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3-Hydroxyisobutyrate dehydrogenase (*HIBADH*) deficiency—A novel disorder of valine metabolism

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Abstract

3-Hydroxyisobutyric acid (3HiB) is an intermediate in the degradation of the branched-chain amino acid valine. Disorders in valine degradation can lead to 3HiB accumulation and its excretion in the urine. This article describes the first two patients with a new metabolic disorder, 3-hydroxyisobutyrate dehydrogenase (HIBADH) deficiency, its phenotype and its treatment with a low-valine diet. The detected mutation in the *HIBADH* gene leads to nonsense-mediated mRNA decay of the mutant allele and to a complete loss-of-function of the enzyme. Under strict adherence to a low-valine diet a rapid decrease of 3HiB excretion in the urine was observed. Due to limited patient numbers and intrafamilial differences in phenotype with one affected and one unaffected individual, the clinical phenotype of HIBADH deficiency needs further evaluation.

KEYWORDS

3-hydroxyisobutyric aciduria, 3-hydroxyisobutyrate dehydrogenase, 3-hydroxyisobutyrate dehydrogenase deficiency, branched-chain amino acids, HIBADH, HIBADH deficiency, valine degradation

1 | INTRODUCTION

Amino acids serve as basic components of protein biosynthesis, can act as neurotransmitters, or as starting materials for hormone synthesis. Disorders of amino acid degradation may lead to accumulation of potentially toxic intermediates proximal to the metabolic block and elevated concentrations in the plasma and urine.

Valine is an essential branched-chain amino acid. It is subject to a multistep degradation. A block in valine degradation causes the accumulation of the intermediate 3-hydroxyisobutyric acid (3HiB) (see Figure 1) and leads to 3-hydroxyisobutyrate aciduria (3HiBuria). 3HiBuria has been observed in different clinical conditions[1,2] (for a more detailed overview see the introduction of Sass et al[3]).

Mutations in the *ALDH6A1* gene, coding for methylmalonate semialdehyde dehydrogenase (MMSDH),[3] lead to accumulation of 3HiB and 3HiBuria. Loupatty et al[4] have suggested that mutations in *HIBADH* gene, coding for 3-hydroxyisobutyrate dehydrogenase (HIBADH) may also

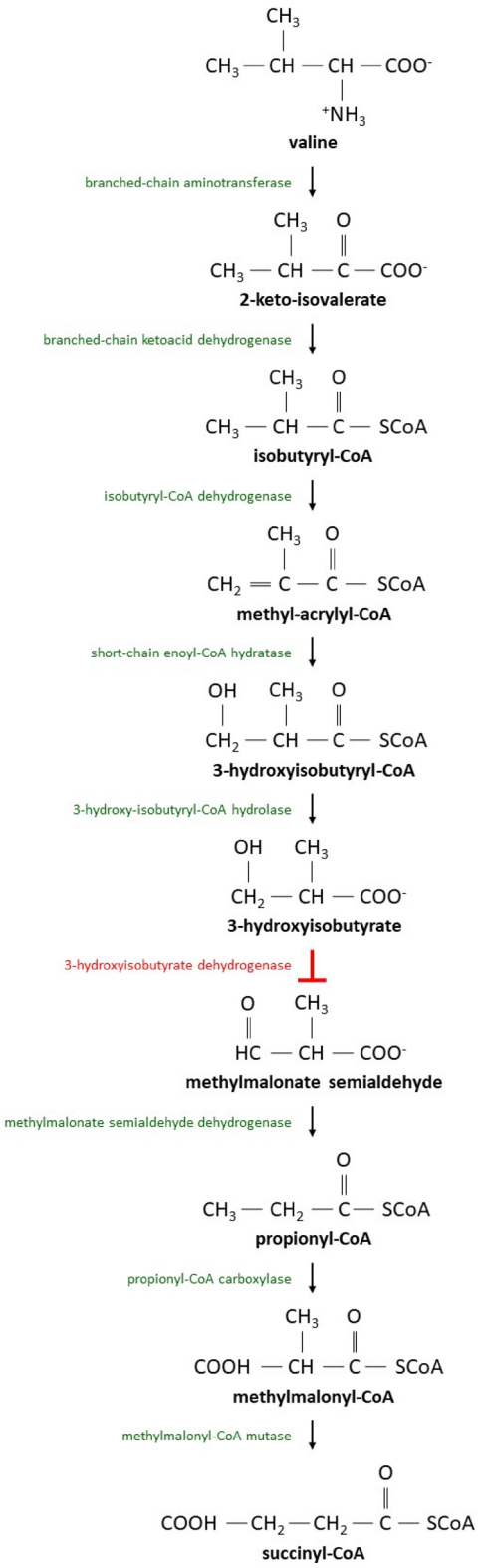


FIGURE 1 Multistep degradation of valine

cause 3HiBuria, but until now, no patient with mutations in this gene has been reported in the literature.

This article describes the first two patients with mutations in the *HIBADH* gene leading to a complete loss-of-function of its gene product. The index patient was evaluated because of psychomotor retardation. A low-valine diet decreased 3HiBuria and improved the clinical condition.

2 | MATERIALS AND METHODS

Investigations and interventions were carried out after informed consent of the patients and their parents. Data analysis was approved by the local ethics committee (Ethikkommission der Ärztekammer Westfalen-Lippe, No. 2019-199-f-S).

2.1 | Gas chromatography-mass spectrometry

Analysis of 3HiB in urine was performed by gas chromatography and mass spectrometry (GC/MS) as described previously.[5] Urine samples were normalized to the same creatinine concentration. 3HiB values were semiquantitated in relation to the internal standard phenylbutyric acid.

2.2 | DNA extraction

Genomic DNA was isolated from blood samples according to the manufacturers' recommendations using QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany). DNA quantity and quality were determined using both the Qubit Fluorometer and NanoDrop ND-8000 (Thermo Fisher Scientific, Dreieich, Germany).

2.3 | Genetic analysis

The coding and flanking intronic regions of the genes *HIBADH* and *ALDH6A1* were enriched using in solution hybridization technology and were sequenced using the Illumina HiSeq system. Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high quality reads using an internally developed method based on sequencing coverage depth. CNV calling was performed by computing the sample's normalized coverage profile and its deviation from the expected coverage. Genomic regions are called as variant if they deviate significantly from the expected coverage. Illumina bcl2fastq2 was used to demultiplex sequencing reads. Adapter removal was performed with the Skewer software. The trimmed reads were mapped to the human reference genome (hg19) using the Burrows Wheeler

Aligner. Reads mapping to more than one location with identical mapping score were discarded. Read duplicates that likely result from PCR amplification were removed. The remaining high quality sequences were used to determine sequence variants (single nucleotide changes and small insertions/deletions). Known disease-causing variants (according to HGMD) were evaluated in up to ±30 bp of flanking regions and up to 5% minor allele frequency (MAF). MAFs were taken from public databases (eg, gnomAD browser) and an in-house database. In silico variant evaluation was carried out using the prediction software MutationTaster[6] and Provean,[7] as well as PolyPhen-2.[8]

2.4 | Gene expression analysis

The contribution of the c.582dupC; p.(Asn195Glnfs*25) allele to total *HIBADH* mRNA in heterozygotes was quantified by cDNA analysis. The genomic sequence of exons 3-7 of the *HIBADH* gene in the heterozygous father of the patient was compared with the corresponding sequence of the transcript at cDNA level. RNA isolation was performed with the PAXgene Blood RNA Kit (PreAnalytiX) from whole blood and cDNA was synthesized from the isolated RNA using SuperScript III Reverse Transcriptase (Invitrogen) according to the manufacturer protocols. cDNA of the HIBADH transcript of exons 3-7 was amplified by PCR. Primers were styled with the open-source Primer3 software and are available upon request. PCR products were purified with the USB PCR Pre-Sequencing Kit (Affymetrix). Sanger sequencing was done with BigDye Terminator v3.1 (Applied Biosystems) according to the manufacturer's protocol.

2.5 | Biochemistry

Enzyme activity measurements in fibroblast homogenates were performed as described,[3] with the exception that the Lowry test for protein quantitation was replaced by the Bradford assay. Following protein separation by SDS-PAGE, Western blot analyses of the homogenates were done with a PVDF membrane (Bio RadL002045A) and using the following antibodies: anti-HIBADH (GeneTex GTX64844, LOT 821705878), 1:1500 in 5% skim milk in Tris-buffered saline with Tween 20 (TBST) for 2 hours at room temperature (RT); anti-Rabbit IgG (PE Health Care UKNA934V, LOT 13997044), 1:10 000 in 5% skim milk TBST 1:10 000 for 1 hour at RT; anti-alpha tubulin (Abcam ab7291), 1:5 000 in 5% skim milk TBST overnight at 4°C; anti-mouse IgG linked with HRP (PE Health Care NA931V, LOT 16953154), 1:10 000 in 5% skim milk for 75 minutes at RT. TBST- Bands were detected using the HRP-based West-Femto kit from Thermo Fisher (#34095). Molecular weight standard was Roti-Mark Western Plus (Carl Roth 2245.1, LOT 119281812).

2.6 | Therapy

The older patient was treated with a low-valine diet. This diet was refused by the parents for the asymptomatic younger sister who is also affected.

A protein-reduced diet was started with 0.8 g natural protein/kg b.w. with addition of an isoleucine, leucine and valine-free amino acid mixture (Milupa MSUD 2 PRIMA) and supplementation of isoleucine and leucine according to WHO guidelines with 23 mg isoleucine and 44 mg leucine per kg b.w. (reference: WHO: Protein and Amino Acid Requirements in Human Nutrition 2007, p. 180). In order to see whether an additional protein reduction would further decrease 3HiBuria, the supply of natural protein was stepwise decreased to a minimum of 0.2 g/kg b.w. with a corresponding increase of the valine-free amino acid mixture.

In order to avoid catabolism with endogenous valine release from protein breakdown, sufficient calories were supplied. The diet was monitored by weekly urine tests for 3HiB.

3 | RESULTS

3.1 | Patients

The 11-year-old boy is the second child of a healthy consanguineous couple of Syrian origin. He was born spontaneously in the 40th week of pregnancy. At the age of 7 months he was able to sit freely, he walked by himself at 14 months and spoke his first two-word sentences at the age of two. His further psychomotor development was slower than in his siblings.

The boy shows hypomimia with an open mouth and muscular hypotension. His movements are clumsy. The gait pattern is wavering and shuffling with flapping arms. Running is hardly possible, as the feet are only slightly lifted from the ground. He shows a dystonic position of both hands. During finger-tapping there is mirror movement of the other hand and bradydysdiadochokinesia appears on both sides. Orders to follow simple tasks have to be repeated and demonstrated several times until they can be followed. His speech is unclear and muffled.

Examination of the cognitive development status at the age of 6 years measured a SON-IQ of 63 in the speech-free SON-R (Snijders-Oomen Non-verbal Intelligence Test). This corresponded to a reference age of 4.6 years and was in the range of mental disability. A renewed examination of the cognitive development status at the age of 8 years used the CFT 1-R (Culture Fair Intelligence Test, Basic Intelligence Test Scale 1) and measured a total IQ of 85 (percentile rank = 16). An additional test at the age of 9 years confirmed the lower than average total IQ. Apart from a small cyst of the pineal gland, the cranial MRI showed no abnormalities.

Laboratory tests showed normal serum concentrations of amino acids, lactate and ketone bodies. Urinary organic acid analysis revealed a high excretion of 3HiB (Figure 2).

His 3-year-old sister also showed elevated excretion of 3HiB in the urine. She was not formally tested but reached developmental milestones within the normal time frames. Unlike her brother, she shows no phenotypic abnormalities.

3.2 | Genetic analysis

No mutations in the *ALDH6A1* gene were found. In the *HIBADH* gene, the homozygous mutation c.582dupC p.(Asn195Glnfs*25) in exon 5 was identified in both siblings. This mutation leads to a frame shift and to the formation of a premature stop-codon in exon 6. The parents are heterozygous carriers and healthy.

3.3 | Gene expression analysis

In order to investigate a possible nonsense-mediated RNA decay of the allele carrying the c.582dupC p.(Asn195Glnfs*25) mutation, the alleles of the *HIBADH* gene were sequenced at the genomic and at the cDNA level in leukocytes of the patient's heterozygous father. If the mRNA transcribed from the normal and mutant alleles would be equally stable, almost identical sequence patterns in the upper and lower panel of Figure 3 should be seen. However, different quantitative ratios in the cDNA vs genomic DNA of the bases to one another at and downstream of the mutation site were observed (Figure 3). To estimate the difference we choose a position (green arrow Figure 3) where in the genomic sequence the bases T and C (mutant allele) have almost identical signal intensities (ie, 50:50, almost perfect overlap of the signals), while in the cDNA there is a ratio C/T of about 11.5:88.5. Based on these differences it was concluded that the majority of transcript of the mutant allele (represented by the "C" at the green arrow position) is degraded by nonsense-mediated mRNA decay.

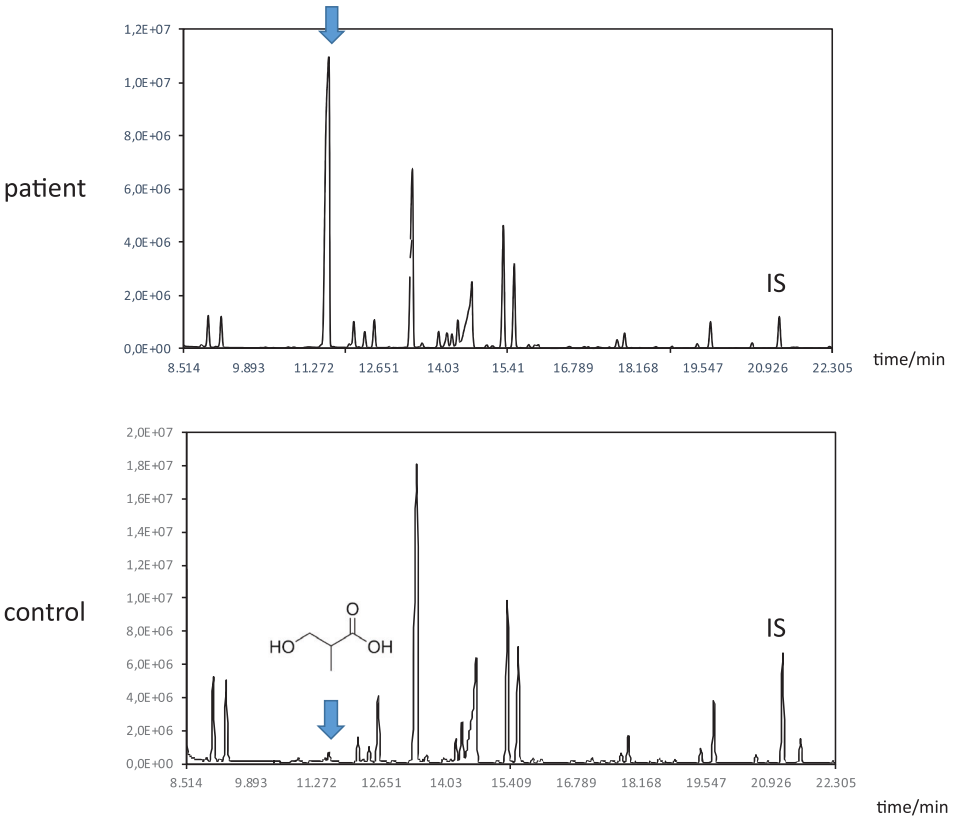


FIGURE 2 Organic acids in urine of the index patient analyzed by GC/MS. 3-Hydroxyisobutyric acid at 11.61 minutes (blue arrow). IS (internal standard): 3-Phenylbutyric acid at 21.18 minutes

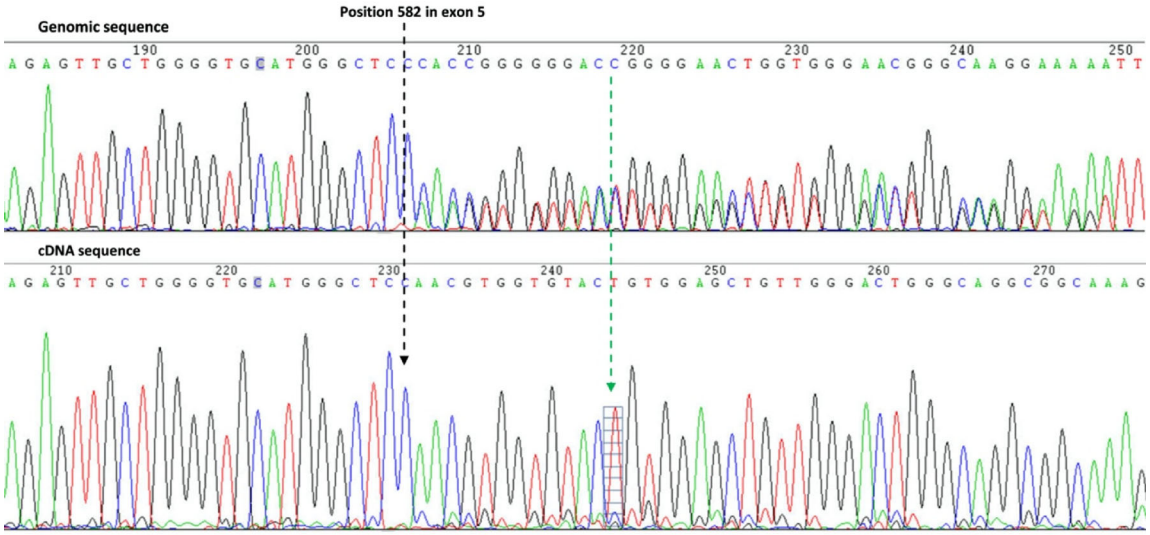
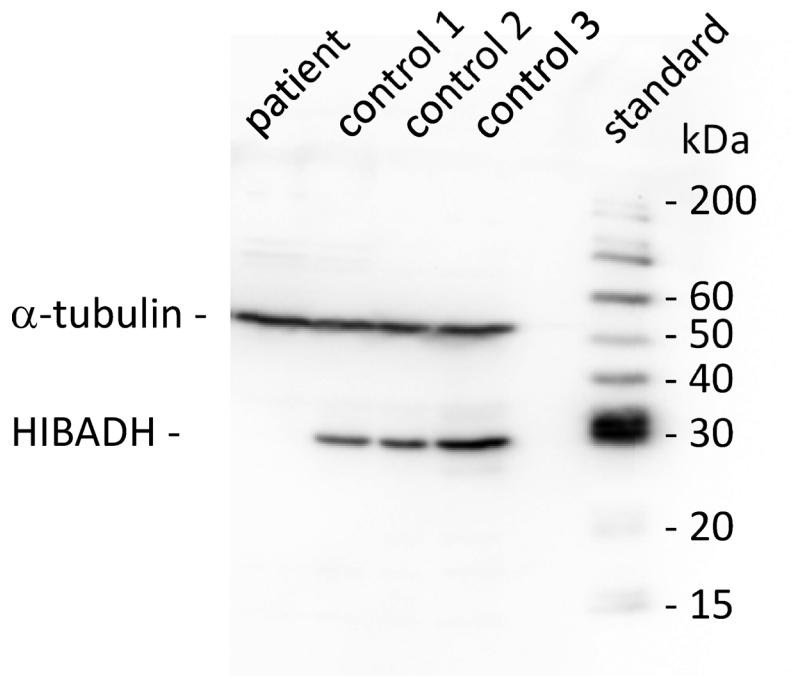


FIGURE 3 Comparison of the sequences of the *HIBADH* gene at genomic level with the cDNA level in a heterozygote carrier of the c.582dupC; p.(Asn195Glnfs*25) mutation. At the position of the green arrow the ratio of cytosine to thymine in the genomic sequence is 50:50. At the corresponding position in the cDNA of the HIBADH transcript, the ratio of cytosine to thymine is 11.5:88.5

TABLE 1 HIBADH enzyme activities: Mean ± SD

	Patient	Control 1	Control 2	Control 3
Enzyme activity µmol/(min*g)	Not detectable (n = 5)	6.17 ± 2.87 (n = 5)	1.42 ± 0.90 (n = 4)	3.50 ± 0.93 (n = 5)

FIGURE 4 Western blot. 20 µg total protein (determined with the Bradford method) were loaded onto the SDS-PAGE gel. HIBADH (33 kDa) runs at the position of the 30 kDa marker, the alpha tubulin loading control at 50 kDa. Molecular weight standard at the right side of the figure



3.4 | Enzyme activity

HIBADH enzymatic activity was determined in fibroblasts of patient 1 and of healthy controls (Table 1). Residual activity in the patient was below the detection level.

In Western blots, no HIBADH protein was detectable in the patient cells (Figure 4).

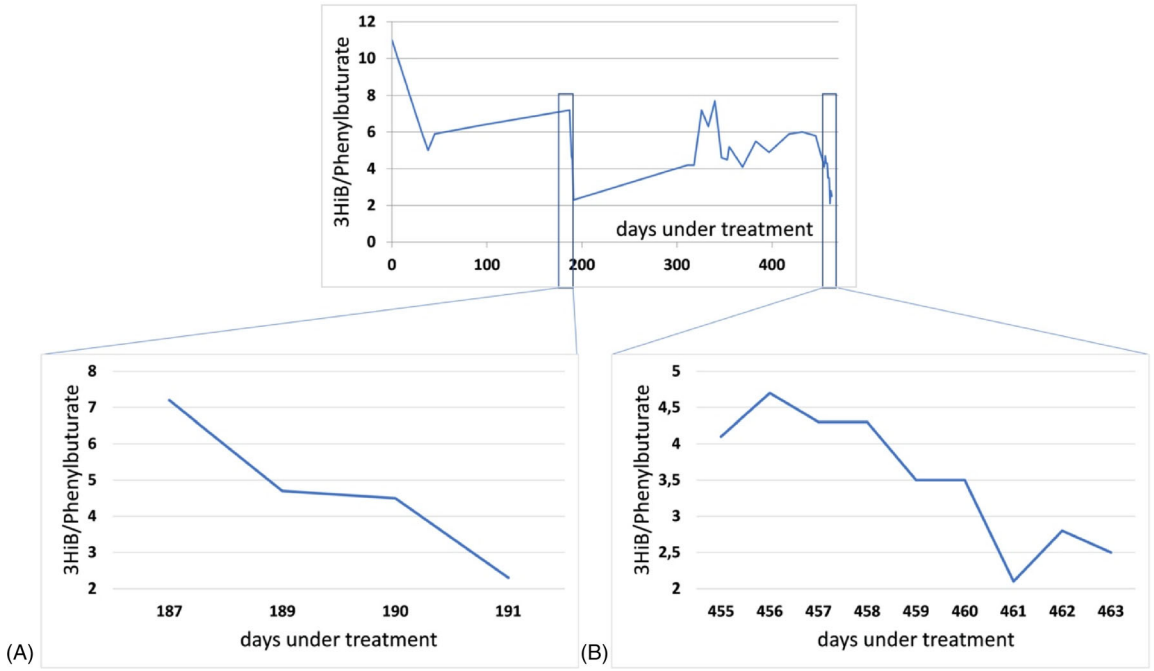


FIGURE 5 Measurement of urinary 3HiB excretion with low valine diet. The 3HiB values in urine were determined by GC/MS and presented in relation to a phenylbutyric acid standard. A, First and B, second inpatient stay

3.5 | Therapy

The low-valine diet was started at home (Figure 5). Insufficient adherence to dietary recommendations resulted in 2 periods of controlled dietary adherence in an inpatient setting (Figure 5A,B) leading to a rapid decrease in 3HiB excretion in the urine. 3HiB excretion did not reach normal levels, which was close to zero in healthy controls.

In the course of therapy, the parents reported an improvement in language skills, as well as in the ability to concentrate and in school performance. The parents consider changing the school type from a special school to a regular school.

4 | DISCUSSION

HIBADH deficiency due to mutations in HIBADH has been suspected as a possible cause of 3HiBuria.[2,4] Wanders et al[9] stated that they identified the first patient with HIBADH deficiency, but did not provide further details about genetic analysis or clinical phenotype. Mutations in the *ALDH6A1* gene and secondary causes which influence the presence or function of enzymes involved in valine degradation—MMSDH and HIBADH—have been published.

3HiBuria caused by homozygous missense mutations in the *ALDH6A1* gene was described by Chambliss et al and Sass et al.[3,10] Secondary inhibition of MMSDH due to an increase of certain metabolites was described in glyoxylate reductase/hydroxypyruvate reductase and AGXT2 enzyme deficiency.[11]

HIBADH can be inhibited because of defects in the respiratory chain and thus cause 3HiBuria. If the respiratory chain is defective, a disturbed oxidation of NADH leads to an increased NADH/NAD^+-ratio.[4] The increased NADH/NAD^+-ratio may cause an inhibition of HIBADH activity (discussed in Sass et al[3]). This should be considered particularly in case of increased lactate values in urine and plasma.

Heterogeneous symptoms have been described in 3HiBuria: Facial dysmorphisms such as a small triangular face, a long philtrum, small low-set ears,[1] clinodactyly of the toes,[12] delayed psychomotor development,[2] delayed myelination and narrow corpus callosum as well as intracranial calcification,[13] eccentric hypertrophy of the right ventricle and persistent pulmonary hypertension,[4] seizures,[1] fatal episodes of ketoacidosis.[2] The range of symptoms in 3HiBuria is wide and can extend from an inconspicuous development[14] to a severe developmental disorder and also shows intrafamilial variability.[13] Obviously, individuals with 3HiBuria represent a heterogeneous group with different underlying mechanisms.

The patient described here presented with psychomotor retardation, low average total IQ values and a high amount of 3HiB in urine. The already known causes of 3HiBuria could be excluded by an unobtrusive analysis of the *ALDH6A1* gene and normal lactate concentrations in blood and urine. Expression analysis of *HIBADH* showed degradation of the transcript of the mutated allele by nonsense-mediated mRNA decay and a

complete loss of enzyme function. Altogether, our data confirm that HIBADH deficiency is another cause for a 3HiBuria.

The 3-year-old sister of the index patient, who has the same mutations and also has increased urinary excretion of 3HiB, has reached all developmental milestones in normal time frames. The intrafamilial variability—with one phenotypically affected brother and one phenotypically unaffected sister—raises the possibility that HIBADH deficiency is a biochemical alteration that may not always cause symptoms. It cannot be excluded that the phenotype of the index patient could be due to a second, so far undiscovered disorder.

More patients with HIBADH deficiency have to be found in order to fully evaluate the spectrum of clinical symptoms and the value of valine restriction.

In conclusion, we report on the first patients with 3HiBuria caused by a homozygous loss-of-function mutation in the *HIBADH* gene. Therapy with a low-valine diet leads to a decrease of 3HiBuria. Our results expand the previously known causes of 3HiBuria by HIBADH deficiency.

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