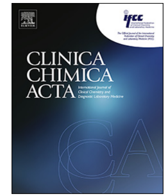
Clinica Chimica Acta 487 (2018) 133–138

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca



Clinical, biochemical and genetic analysis of Chinese patients with isobutyryl-CoA dehydrogenase deficiency

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ARTICLE INFO

Keywords:
Isobutyryl-CoA dehydrogenase deficiency
ACAD8
Newborn screening
3D crystal structure
Valine catabolism

ABSTRACT

Isobutyryl-CoA dehydrogenase deficiency (IBDHD) is a rare autosomal recessive metabolic disorder related to valine catabolism and results from variants in *ACAD8*. Here, we present the clinical, biochemical, and genotypes of seven patients with IBDHD in China for the first time. Five patients remained asymptomatic during follow-up, whereas one juvenile had speech delay and one newborn exhibited clinical symptoms. All patients showed remarkably increased concentrations of C4-aclycarnitine with elevated C4/C2 and C4/C3 ratios. In urine organic acid tests, only one patient presented with an increased concentration of isobutyrylglycine excretion. Genetic testing was performed to detect the causative variants. Five previously unreported variants, c.235C > G, c.286G > A, c.444G > T c.1092 + 1G > A, and c.1176G > T, and one known variant, c.1000C > T, in *ACAD8* were identified. These previously unreported variants in *ACAD8* were predicted to be disease-causing and the c.1092 + 1G > A variant was confirmed to cause skipping of exon 9 by reverse transcription PCR. The most common variant was c.286G > A, which showed an allelic frequency of 50% (7/14), and thus may be a prevalent variant among Chinese patients. Our results broaden the mutational spectrum of *ACAD8* and improve the understanding of the clinical phenotype of IBDHD.

1. Introduction

Isobutyryl-CoA dehydrogenase (IBD) is a mitochondrial enzyme that catalyzes the third step of degradation of the branched chain amino acid valine [1,2]. This protein is encoded by *ACAD8* (MIM 604773), which is located on chromosome 11q25 [3]. IBD deficiency (IBDHD, MIM #611283) is a very rare autosomal recessive metabolic disorder of valine metabolism. Most patients with IBDHD were identified through newborn screening (NBS) programs which rely on the detection of elevated C4 acylcarnitine concentrations by tandem MS/MS. However, elevations in C4 acylcarnitine are not specific to IBDHD and are also observed in short-chain acyl-CoA dehydrogenase deficiency and ethylmalonic encephalopathy [4–7]. Thus, the diagnosis of IBDHD depends on isobutyryl-CoA dehydrogenase activity determination or genetic testing. Symptoms of IBDHD generally appear until late in infancy or in childhood, and the symptoms can include poor feeding, developmental delay, dilated cardiomyopathy, seizures, and anemia [1,8]. Recently, Nygaard et al. [9] reported a patient with IBDHD presenting with significant clinical symptoms in adulthood, indicating that asymptomatic children with IBDHD are at risk of developing clinical manifestations in adulthood. Moreover, in another study, Sabbagha et al. [10] found that alternative splicing in *ACAD8* caused a mitochondrial defect and progressive hepatic steatosis in mice, suggesting a relationship between IBDHD and fatty liver. Thus, the clinical importance of IBDHD is unclear. Systematic assessment of this disease is particularly urgent and patients with IBDHD should be monitored carefully. However, few patients with IBDHD have been reported worldwide and no Chinese cases of IBDHD have been reported previously in English literature. In this study, we present clinical and biochemical information as well as the genotypes of seven patients with IBDHD in China for the first time.

Abbreviations: IBDHD, Isobutyryl-CoA dehydrogenase deficiency; NBS, Newborn screening; MS/MS, Tandem mass spectrometry; NGS, Next-generation sequencing; PCR, Polymerase chain reaction; SNPs, Single-nucleotide polymorphisms; 3D, Three-dimensional; ACMG, American College of Medical Genetics Association of Clinical Genetics; RT-PCR, Reverse transcription PCR

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https://doi.org/10.1016/j.cca.2018.09.033
Received 24 June 2018; Received in revised form 24 August 2018; Accepted 21 September 2018
Available online 22 September 2018
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Table 1
Data on individuals with IBDHD in this study.

Patient no.	Sex	Age at last f/u	MS/MS analysis			Urine isobutyrylglycine (mmol/mol creatinine)[e]	Genotype[f]		Clinical manifestation
			C4 (μmol/L)[b]	C4/C2[c]	C4/C3[d]		Allele 1	Allele 2	
1	F	1 y, 7 m	1.47/1.31(4 d/13 d)	0.14/0.21(4 d/13 d)	1.03/1.96(4 d/13 d)	0	**c.235C > G (p.R79G)**	c.1000C > T (p.R334C)	Normal
2	M	1 y, 7 m	1.94/1.69(4 d/11 d)	0.12/0.19(4 d/11 d)	0.82/2.38(4 d/11 d)	0	**c.286G > A (p.G96S)**	**c.286G > A (p.G96S)**	Normal
3	F	1 y, 5 m	1.29/1.96(7 d/21 d)	0.16/0.17(7 d/21 d)	1.63/2.09(7 d/21 d)	0	**c.286G > A (p.G96S)**	**c.286G > A (p.G96S)**	Normal
4	M	1 y, 2 m	0.98/0.77(4 d/21 d)	0.03/0.07(4 d/21 d)	0.53/0.86(4 d/21 d)	0	**c.286G > A (p.G96S)**	**c.444G > T (p.P148P)**	Normal
5[a]	F	10 m	0.83/1.38(4 d/20 d)	0.06/0.19(4 d/20 d)	0.62/1.79(4 d/20 d)	2.58	**c.286G > A (p.G96S)**	**c.1092 + 1G > A**	Normal
6[a]	M	8 y, 11 m	1.07(8 y, 8 m)	0.13(8 y, 8 m)	1.20(8 y, 8 m)	ND	**c.286G > A (p.G96S)**	**c.1092 + 1G > A**	Speech delay, learning disability
7	M	6 m	1.01/0.98(10 d/19 d)	0.14/0.38(10 d/19 d)	0.79/1.61(10 d/19 d)	0	**c.444G > T (p.P148P)**	**c.1176G > T (p.R392S)**	Haematemesis, hypotonia, poor feeding, recurrent vomiting

d: day, w: week, m: month, y: year; f/u: follow up; MS/MS: tandem mass spectrometry; ND: not determined.
[a] Siblings.
[b] Reference range: 0.08–0.45 μmol/L.
[c] Reference range: 0–0.03.
[d] Reference range: 0.04–0.39.
[e] Reference range: 0–0.4 mmol/mol creatinine.
[f] The previously unreported variants are in boldface type.

2. Materials and methods

2.1. Patients and auxiliary analysis

A total of 309,344 newborns (178,620 males and 130,724 females) were screened by MS/MS at Quanzhou Maternity and Children's Hospital between January 2014 and March 2018. Five patients (patients no. 1–5) were asymptomatic and screened for further diagnostic investigation because they showed elevated C4-aclycarnitine in NBS. Patient no. 6 is the older brother of patient no. 5. He is an 8-year-old boy who had not undergone expanded NBS at birth but presented with clinical symptoms by this time and was diagnosed later because his younger sibling has IBDHD. Patient no. 7 is the only newborn who showed some clinical manifestations in this study. Additionally, a total of 100 healthy newborns whose expanded NBS results were within the reference range from our center were randomly selected as controls. The concentrations of C4-aclycarnitine, C4/acetylcarnitine (C2), and C4/propionylcarnitine (C3) on dried blood spot filter paper cards were determined by MS/MS (ACQUITY TQD, Waters, Milford, MA, USA). Their urine samples were collected for urine organic acid analysis by GC–MS (7890B/5977A, Agilent Technologies, Santa Clara, CA, USA). Genetic analysis was conducted as a confirmatory test. Informed consent in accordance with the Declaration of Helsinki was obtained from each of the participants' parents. This study was approved by the Ethical Committee of Quanzhou Maternity and Children's Hospital and all experimental procedures were performed in accordance with relevant guidelines and regulations.

2.2. DNA isolation and next-generation sequencing (NGS)

Dried blood spots or peripheral whole blood of patients and their parents, as well as those of 100 control subjects, were collected and genomic DNA was extracted using Qiagen Blood DNA mini kits following the manufacturer's instructions (Qiagen, Hilden, Germany). DNA samples of the probands were collected for NGS. The target sequences of a metabolic disorder panel including abnormal C4-aclycarnitine related genes (*ACAD8*, *ACADS*, *ETHE1*) were enriched by multiplex polymerase chain reaction (PCR). The library concentration and amplicon size were determined using an Agilent High Sensitivity DNA Kit (Agilent Technologies). The libraries were then sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) in paired-end mode, generating 150-base pair (bp) paired-end reads and the data were analyzed by MiSeq Reporter. The paired-end reads were quality trimmed using the Trimmomatic program (http://www.usadellab.org/cms/index.php?page¼trimmomatic) and aligned with the human genome reference sequence (UCSC Genome build hg19). Single-nucleotide polymorphisms (SNPs) and insertions or deletions were identified using the SAMtools program (http://www.htslib.org/).

2.3. Bioinformatics analysis

We checked the identified variants in frequently used databases such as the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/), ExAC consortium (http://exac.broadinstitue.org/), and 1000 Genome Project database (http://www.1000genomes.org/). Missense variants were further assessed for possible pathogenicity using several bioinformatic programs including SIFT, PolyPhen-2, PROVEAN, and MutationTaster to predict the effect of three new amino acid substitutions (p.Arg79Gly, p.Gly96Ser, and p.Arg392Ser). Multiple amino acid sequences of different species were extracted from the National Center for Biotechnology Information and aligned to evaluate the evolutionary conservation of the variants using ClustalX (http://www.clustal.org/clustal2). Furthermore, homology modeling was used to build three-dimensional (3D) models of ACAD8 using Swiss Model Workspace with

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