JIMD Reports
DOI 10.1007/8904_2016_538

CASE REPORT

ECHS1 Deficiency as a Cause of Severe Neonatal Lactic Acidosis

Rebecca D. Ganetzky • Kaitlyn Bloom • Rebecca Ahrens-Nicklas • Andrew Edmondson • Matthew A. Deardorff • Michael J. Bennett • Can Ficicioglu

Received: 19 November 2015 / Revised: 11 January 2016 / Accepted: 13 January 2016 / Published online: 27 February 2016

Abstract Mitochondrial short-chain enoyl-CoA hydratase deficiency (ECHS1D) is caused by mutations in *ECHS1* (OMIM 602292) and is a recently identified inborn error of valine and fatty acid metabolism. This defect leads to secondary mitochondrial dysfunction. The majority of previously reported patients had the Leigh syndrome, with a median life expectancy of approximately 2 years. We report two siblings born 3 years apart with prenatal findings including facial dysmorphia, oligohydramnios, intrauterine growth restriction, and premature delivery. They had severe lactic acidosis with onset within the first hours of life, had congenital dilated cardiomyopathy, and died at 16 h of life and 2 days of life, respectively.

Biochemical evaluation of these patients showed elevated butyryl-carnitine in the blood and elevated methylmalonyl/succinyl-CoA and decreased hydroxybutyryl-CoA in frozen liver of patient 2, confirming abnormal short-chain fatty acid metabolism. Elevated butyryl-carnitine has been reported only in a single previous case of ECHS1 deficiency, which also had neonatal onset. Pyruvate and lactate levels were both elevated with a normal pyruvate-lactate ratio. This supports the previous hypothesis that lactic acidosis in these patients results from secondary inhibition of the pyruvate dehydrogenase complex. The biomarker 2,3-dihydroxy-2-methylbutyric acid was detected in patient 2, but at lower levels than in previously reported cases.

These cases extend our understanding of the severe end of the phenotypic spectrum of ECHS1 deficiency, clarify the range of biochemical abnormalities associated with this new disorder, and highlight the need to suspect this disease in patients presenting with comparable metabolic derangements and dysmorphic features.

Communicated by: Jörn Oliver Sass

Competing interests: None declared

R.D. Ganetzky · R. Ahrens-Nicklas · A. Edmondson · M.A. Deardorff · C. Ficicioglu (✉)
Department of Pediatrics, Division of Human Genetics, The Children's Hospital of Philadelphia, Perelman School of Medicine at the University of Pennsylvania, 3501 Civic Center Blvd, Philadelphia, PA 19104, USA
e-mail: ficicioglu@email.chop.edu

K. Bloom · M.J. Bennett
Department of Pathology and Laboratory Medicine, Michael Palmieri Metabolic Disease Laboratory, The Children's Hospital of Philadelphia, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA

Introduction

Mutations in *ECHS1* causing deficiency of the short-chain enoyl-CoA hydratase have been recently reported to cause an inborn error of valine and fatty acid metabolism resulting in the Leigh syndrome (Peters et al. 2014). In particular, the formation of the highly reactive metabolites methyacrylyl-CoA and acryloyl-CoA is suspected to be the primary cause of toxicity through secondary disruption of the pyruvate dehydrogenase complex and electron transport chain (Peters et al. 2014; Haack et al. 2015).

Nineteen families (21 patients) affected with ECHS1 deficiency have been reported to date (Peters et al. 2014; Ferdinandusse et al. 2015; Sakai et al. 2015; Haack et al. 2015; Tetreault et al. 2015; Yamada et al. 2015). Previously affected patients have had predominant features of lactic acidosis, epilepsy, and death at a young age. The age of onset has varied; however, most cases have presented in early infancy. The median age of reported cases at either

death or at time of report is 36 months (mean 69 months). Only two previously reported patients (who were siblings) died in the neonatal period (Ferdinandusse et al. 2015).

Here we report two children from a single family affected by severe ECHS1D with evidence of prenatal onset, preterm delivery, severe and rapid onset of metabolic acidosis, and death within the first 2 days of life. Given the rarity and relatively new discovery of ECHS1D as a cause of disease, the full spectrum of disease is still not well understood. The purpose of this report is to extend understanding of the severe end of the spectrum of ECHS1D.

Clinical Case Report

Patient 1

Patient 1 was born to non-consanguineous Caucasian parents via an urgent Cesarean section at 34 weeks of gestation following a pregnancy complicated by intrauterine growth restriction (IUGR) and severe oligohydramnios. Complete agenesis of the corpus callosum was detected in the prenatal period.

Kussmaul respiration developed around 1 h after birth and an arterial blood gas showed severe metabolic acidosis (pH 6.85, lactate 20.8 mmol/L.) Acidosis was refractory to multiple administrations of intravenous bicarbonate.

On exam, the infant was dysmorphic with epicanthus, low-set ears, long philtrum, and flat midface. He did not have the facial features of Potter's sequence. He was severely hypotensive. He had hypospadias, large joint contractures, and absence of flexion creases. Echocardiogram showed severe dilated cardiomyopathy.

Despite intravenous glucose, bicarbonate, multiple pressor support, nitrous oxide, and high flow oscillator ventilation, the infant persisted with severe hypotonia, then developed wide-complex bradycardia, and passed away at 16 h of life.

Acylcarnitine profile was remarkable for mildly elevated butyryl-carnitine (1.42 μmol/L; normal <1.00). A simultaneous draw of lactate and pyruvate showed an essentially preserved ratio (lactate 18.11 mM, normal <1.6; pyruvate 0.84 mM, normal <0.14; ratio 22, normal 10–20). Plasma amino acids showed a striking elevation of alanine (1,425 μmol/L; normal <571). Urine organic acids were unable to be obtained as the patient had lifelong anuria.

Whole mitochondrial sequencing and sequencing of 101 nuclear genes associated with mitochondrial disease were negative. Clinical whole-exome sequencing identified two variants in *ECHS1*: p. A3D (c.8C>A; predicted possibly damaging by PolyPhen-2 (Adzhubei et al. 2010) and predicted damaging by SIFT (Kumar et al. 2009)) and p. V130D (c.389T>A; predicted probably damaging by PolyPhen-2 and predicted damaging by SIFT), which were in *trans*. These rare mutations were seen in 0/11072 and 4/120696 alleles in the Exome Aggregation Consortium, respectively (http://exac.broadinstitute.org, accessed November 2015). No mutations in other genes were reported by whole-exome sequencing.

Patient 2

Patient 2 is the younger sister of patient 1. She was born via vaginal delivery at 29 weeks of gestation following a pregnancy complicated by IUGR and oligohydramnios. She had milder lactic acidosis following delivery (6.4 mmol/L; normal <1.6); however, it worsened throughout the first day of life to a peak of 14 mmol/L. She additionally had complications of prematurity, including bilateral intraventricular hemorrhage.

Due to the severity of disease seen in her brother, and the poor neurologic outcome portended by her intraventricular hemorrhage, the family elected to withdraw care at 24 h of life.

Autopsy was performed and showed dilated cardiomyopathy, hepatosplenomegaly, a preauricular tag, incomplete separation of the right upper and middle lung lobes, and two splenules. Kidney morphology was normal.

Her biochemical testing was similar to that of her deceased sibling, patient 1. She had a very subtle elevation of butyryl-carnitine (1.05 μmol/L; normal <1.00) and elevated alanine (738 μmol/L, normal <571). Urine organic acids showed elevation of 2,3-dihydroxy-2-methylbutyric acid to 10 mmol/mol creatinine (normal is below the limit of detection, which is 1 mmol/mol creatinine.) Molecular testing for the known familial mutation was confirmed that she carried both of the *ECHS1* mutations identified in her brother.

Although not all features were shared between these patients, they were felt to have the same underlying condition because of the clinical similarity (oligohydramnios, IUGR, and dilated cardiomyopathy), the biochemical similarity (neonatal lactic acidosis and elevated butyryl-carnitine), and their shared mutations in *ECHS1*, with overlap in phenotype with previously reported patients with ECHS1D.

Additional family history is notable for uncomplicated pregnancies delivering normally sized term infants for two unaffected full siblings of patient 1 and patient 2, one delivered prior to patient 1 and one between patient 1 and patient 2.

Material and Methods

Tissue Extraction for Acyl-CoA Analysis

Human liver tissues from patient 2 and control samples were collected and immediately flash frozen in liquid nitrogen. Acyl-CoA profiling on tissue samples was performed and analyzed for acyl-CoA species as previously described (Palladino et al. 2012). The patient and control samples were done in triplicate. Acyl-CoA profiling identifies long, medium, short, and 3-hydroxyacyl-CoA species in tissues using flow-injection tandem mass spectrometry. The complete acyl-CoA profiles are identified using neutral loss of m/z 507 (Palladino et al. 2012). The student's *t*-test was used for statistical analysis.

Results

Acyl-CoA Analysis

The free acyl-CoA profile demonstrated a pattern consistent with fasting in the liver. There was elevated methylmalonyl/succinyl-CoA (C4-DC) (patient (measure in triplicate), 1208 ± 736 nmol/g; control (measure in triplicate), 776 ± 136 nmol/g; $p = 0.2884$), although these results did not reach statistical significance. 3-Hydroxybutyryl-CoA (C4-OH) was significantly decreased (patient (measure in triplicate), 1456 ± 512 nmol/g; control (measure in triplicate), 5306.3 ± 958.6 nmol/g; $p = 0.0016$) using student's *t*-test. Although this result was significant, it was not replicated in the previously published patient (Ferdinandusse et al. 2015).

Discussion

Here we report two siblings with prenatal onset of ECHS1D manifesting as severe oligohydramnios resulting in fetal akinesia sequence, intrauterine growth retardation, and dysmorphic facial features reminiscent of fetal alcohol syndrome, as well as multiple minor anomalies including hypospadias and splenule formation. Severe and ultimately fatal biochemical deterioration occurred within the first day of life in both cases.

These siblings are the first reported cases of prenatal onset of disease in ECHS1D; compared to previously reported cases, their presentation and death occurred rapidly after birth (Table 1.) Despite previously reported intrafamilial variability, both of these patients had similar and severe manifestations. In their severity, these two cases extend the range of the previously reported phenotype associated with ECHS1D. The underlying genotype-phenotype correlation remains unclear. Both mutations in this case are missense mutations and do not alter import into the mitochondria, so the cause of their severity is not readily apparent (Claros and Vincens 1996). More cases will help clarify mutations associated with severe disease.

Striking components of the phenotype included dysmorphic features and multiple minor congenital anomalies. This underscores the importance of considering inborn errors of mitochondrial metabolism in children with structural anomalies. ECHS1D should be considered in children with dysmorphia or congenital anomalies and lactic acidosis.

Acyl-CoA profiling is a very sensitive assay used for identifying subtle changes in specific biomarkers linked to disease. The analysis of the CoA data for patient 2 indicated accumulation of the abnormal short-chain CoA species C4-DC and decreased C4-OH. One previously reported patient with ECHS1D on whom CoA analysis has been performed had similar results. The meaning of these findings is limited because of small number of patients, but over time collection of these data from additional patients will allow for identification of consistent variations that will contribute to our understanding of the pathobiochemistry of this disease.

Diagnosis in patient 1 was made by whole-exome sequencing; however, the biochemical diagnosis was strongly suspected in the second sibling due to elevations in butyryl-carnitine on the acylcarnitine profile and 2,3-dihydroxy-2-methylbutyric acid on urine organic acids. Elevated lactate and pyruvate with a preserved ratio in this case support the previously hypothesized mechanism of the disease: the inhibition of the pyruvate dehydrogenase enzyme (Peters et al. 2014). Elevated butyryl-carnitine was noted in the previously reported family with neonatal onset (Ferdinandusse et al. 2015), suggesting that this marker is more sensitive in the severe cohort. Taken together, these biochemical features suggest that this condition is diagnosable on standard biochemical testing,

Table 1 Clinical features of previously reported patients with ECHS1 deficiency

Reference	Current Pt 1	Current Pt 2	1 Peters et al. (2014)	2	3 Sakai et al. (2015)	4 Ferdinandusse et al. (2015)	5	6	7	8 Haack et al. (2015)	9	10	11	12	13
Onset	Prenatal	Prenatal	Birth	Birth	2 months	1 day	1 day	Early infancy	1 year	Birth	Birth	Birth	Birth	5 days	Birth
Dysmorphia	+	+/−	−	−	−	−	−	−	−	−	−	−	−	−	−
IUGR	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Oligohydramnios	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Premature delivery	(Emergent c-section)	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Hypoplastic corpus callosum	++	−	+	−	−	−	−	+	−	−	−	−	+	+	−
Structural anomalies	Hypospadias	Splenules	−	VSD	−	−	Periventricular cysts	−	−	−	−	−	−	−	−
Neonatal lactic acidosis	+++	++	+	−	−	+	+	−	−	+++	+	+	−	++	−
Cardiomyopathy	DCM	DCM	−	HCM	−	Poor contractility	−	−	−	HCM	HCM	N/A	N/A	HCM	DCM
Acylcarnitine profile	Elevated C4	Elevated C4	Normal	Normal	Normal	Mild elevated C3, C4, C5	Elevated C3, C4	N/A	Elevated C4OH	Normal	Normal	Normal	N/A	Normal	Normal
Lactate	High	High	High	High	High	High	High	High	Intermittently high	High	High	High	N/A	High	High
Pyruvate	High	High	High	High	Nl	High	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Lactate/pyruvate ratio	Nl	Nl	Nl	Nl	High	Nl									
Urinary 2-methyl, 2,3-dihydroxybutyrate	N/A	Small peak	1,560–2,500 µmol/mmol creatine	782–972 µmol/mmol creatine	−	N/A	Large peak	+, not quantified	+, not quanitied	Normal	229-fold ULN	N/A	N/A	39-fold ULN	Nl
Death	16 h	2 days	4 months	8 months	Alive at 4 years	24 h	2 days	Alive at 7 years	Alive at 1 year	4 months	11 months	2.3 years	7.5 years	Alive at 2 years	Alive at 3 years
Genetic mutation	c.8C>A/c.389T>A	c.8C>A/c.389T>A	c.414+3G>C/c.473T>C	c.414+3G>C/c.473T>C	c.2T>G/c.5C>T	c.817G>A/c.817G>A	c.817G>A/c.817G>A	c.433C>T/c.476A>G	c.673T>C c.674G>C	c.176A>G/c.476A>G	c.197T>C/c.449A>G	c.476A>G/c.476A>G	c.161G>A/c.817A>G	c.673T>C/c.673T>C	c.98T>C/c.176A>G
Protein effect	A3D/V130D	A3D/V130D	A158D/splicing	A158D/splicing	M1R/A2V	K273E/K273E	K273E/K273E	L145F/Q159R	C225R/C225S	N59S/Q159R	I66T/D150G	Q159R/Q159R	R54H/K273E	C225/C225R	F33S/N59S

Reference	14 Haack et al. (2015)	15	16	17	18 Yamada et al. (2015)	19	20 Tetreault et al. (2015)	21	22	23
Onset	2 years	1 year	18 months	11 months	10 months	7 months	2.5 months	2.9 years	10 months	6 months
Dysmorphia	−	+	−	−	−	−	−	−	−	−
IUGR	−	−	−	−	−	−	−	−	−	−
Oligohydramnios	−	−	−	−	−	−	−	−	−	−
Premature delivery	−	−	−	−	−	−	−	−	−	−
Hypoplastic corpus callosum	−	−	−	−	−	−	−	−	−	−
Structural anomalies	−	Gastroschisis	−	−	−	−	−	−	−	−
Neonatal lactic acidosis	−	−	−	−	−	−	−	−	−	−
Cardiomyopathy	N/A	N/A	−	−	N/A	N/A	N/A	N/A	N/A	N/A
Acylcarnitine profile	N/A	N/A	N/A	N/A	N/A	N/A	Normal	Normal	Normal	Normal
Lactate	N/A	High	High	High	Normal	High in urine	High	Intermittently high	Intermittently high	Normal
Pyruvate	N/A	N/A	N/A	N/A	Normal	N/A	N/A	N/A	N/A	N/A
Lactate/pyruvate ratio					Normal	N/A	N/A	N/A	N/A	N/A
Urinary 2-methyl, 2,3-dihydroxybutyrate	Sixfold ULN	N/A	N/A	Normal	Upper limit of normal	Slightly elevated	Normal	Normal	Normal	Normal
Death	Alive at 5 years	Alive at 8 years	Alive at 16 years	Alive at 31 years	Alive at 7 years	5 years	10 months	Alive at 18 years	Alive at 13 years	Alive at 12 years
Genetic mutation	c.268G>A/c.583G>A	c.161G>A/c.394G>A	c.161G>A/c.431dup	c.229G>C/c.476A>G	c.176A>G/c.413C>T	c.176A>G/c.413C>T	c.538A>G/c.583G>A	c.538A>G/c.713C>T	c.538A>G/c.713C>T	c.473C>T/c.414+3G>C
Protein effect	G90R/G195S	R54H/A132T	R54H/L154Afs*6	E77Q/Q159R	N59S/A138V	N59S/A138V	T180A/G195S	T180A/A238V	T180A/A238V	p.A581I/splicing

which has the possibility for providing families with a more rapid diagnosis than molecular testing.

Acknowledgments The authors thank the family for their participation in this work. The authors also thank the National Phenylketonuria Alliance, which provided salary support for RDG through the Koch Memorial Fellowship.

Synopsis

We present two siblings with the most severe form of short-chain enoyl-CoA hydratase deficiency, including oligohydramnios, preterm delivery, dysmorphic features, agenesis of the corpus callosum, neonatal cardiomyopathy, and primary lactic acidosis with biochemical features including elevated C4, elevated pyruvate and lactate, and mild elevation of 2,3-dihydroxy-2-methylbutyric acid.

Compliance with Ethics Guidelines

Conflict of Interest

Rebecca D. Ganetzky, Kaitlyn Bloom, Rebecca Ahrens-Nicklas, Andrew Edmondson, Matthew A. Deardorff, Michael J. Bennett, and Can Ficicioglu declare that they have no conflicts of interest.

Informed Consent/Animal Rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

Details of the Contributions of Individual Authors

RDG performed clinical and biochemical evaluation for patient 1, analyzed the molecular data, and conceived and wrote the manuscript and collated data for Table 1. KB performed CoA analysis and wrote the method and discussion sections related to CoA data. RAN, AE, and MAD performed clinical evaluation and designed biochemical testing strategy for patient 2. MJB designed CoA analysis and edited the manuscript. CF conceived and edited the manuscript, provided oversight, and serves as the guarantor for the article.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Details of Funding

RDG received salary support from the National Phenylketonuria Alliance through the Koch Memorial Fellowship. MB and KB were supported in their work by the Evelyn Willing Bromley Endowed Chair in Clinical Laboratories and Pathology.

References

Adzhubei IA, Schmidt S, Peshkin L et al (2010) A method and server for predicting damaging missense mutations. Nat Methods 7:248–249. doi:10.1038/nmeth0410-248

Claros MG, Vincens P (1996) Computational method to predict mitochondrially imported proteins and their targeting sequences. Eur J Biochem 241:779–786

Ferdinandusse S, Friederich MW, Burlina A et al (2015) Clinical and biochemical characterization of four patients with mutations in ECHS1. Orphanet J Rare Dis 10:79. doi:10.1186/s13023-015-0290-1

Haack TB, Jackson CB, Murayama K et al (2015) Deficiency of ECHS1 causes mitochondrial encephalopathy with cardiac involvement. Ann Clin Transl Neurol 2:492–509. doi:10.1002/acn3.189

Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4:1073–1081. doi:10.1038/nprot.2009.86

Palladino A, Chen J, Kallish S et al (2012) Measurement of tissue acyl-CoAs using flow-injection tandem mass spectrometry: acyl-CoA profiles in short-chain fatty acid oxidation defects. Mol Genet Metab 107:679–683. doi:10.1016/j.ymgme.2012.10.007

Peters H, Buck N, Wanders R et al (2014) ECHS1 mutations in Leigh disease: a new inborn error of metabolism affecting valine metabolism. Brain 137:2903–2908. doi:10.1093/brain/awu216

Sakai C, Yamaguchi S, Sasaki M et al (2015) ECHS1 mutations cause combined respiratory chain deficiency resulting in Leigh syndrome. Hum Mutat 36:232–239. doi:10.1002/humu.22730

Tetreault M, Fahiminiya S, Antonicka H et al (2015) Whole-exome sequencing identifies novel ECHS1 mutations in Leigh syndrome. Hum Genet 134:981–991. doi:10.1007/s00439-015-1577-y

Yamada K, Aiba K, Kitaura Y et al (2015) Clinical, biochemical and metabolic characterisation of a mild form of human short-chain enoyl-CoA hydratase deficiency: significance of increased N-acetyl-S-(2-carboxypropyl)cysteine excretion. J Med Genet 52:691–698. doi:10.1136/jmedgenet-2015-103231