Lethal neonatal case and review of primary short-chain enoyl-CoA hydratase (SCEH) deficiency associated with secondary lymphocyte pyruvate dehydrogenase complex (PDC) deficiency

Jirair K. Bedoyan[1,2,3], Samuel P. Yang[4], Sacha Ferdinandusse[5], Rhona M. Jack[6], Alexander Miron[2], George Grahame[3], Suzanne D. DeBrosse[1,2], Charles L. Hoppel[3,7,8], Douglas S. Kerr[3,9], and Ronald J. A. Wanders[5]

[1] Center for Human Genetics, [2] Department of Genetics and Genome Sciences, [7] Department of Pharmacology, [8] Department of Medicine, [9] Department of Pediatrics, Case Western Reserve University, Cleveland, OH, USA, [3] Center for Inherited Disorders of Energy Metabolism, University Hospitals Cleveland Medical Center, Cleveland, OH, USA, [4] Clinical Genomics and Predictive Medicine, Providence Medical Group, Spokane, WA, USA, [5] Departments of Clinical Chemistry and Pediatrics, Laboratory Genetic Metabolic Diseases, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, and [6] Seattle Children's Hospital Laboratory, University of Washington, Seattle, WA, USA

ABSTRACT

Mutations in ECHS1 result in short-chain enoyl-CoA hydratase (SCEH) deficiency which mainly affects the catabolism of various amino acids, particularly valine.

We describe a case compound heterozygous for ECHS1 mutations c.836T>C (novel) and c.8C>A identified by whole exome sequencing of proband and parents. SCEH deficiency was confirmed with very low SCEH activity in fibroblasts (**Table 1**) and nearly absent immunoreactivity of SCEH (**Fig. 1**). The patient had a severe neonatal course with elevated blood and cerebrospinal fluid lactate and pyruvate concentrations, high plasma alanine and slightly low plasma cysteine (**see Timeline below)**. 2-Methyl-2,3-dihydroxybutyric acid was markedly elevated as were metabolites of the three branched-chain α-ketoacids on urine organic acids analysis (**Fig. 2A**). These urine metabolites notably decreased when lactic acidosis decreased in blood (**Fig. 2B**). Lymphocyte pyruvate dehydrogenase complex (PDC) activity was deficient, but PDC and α-ketoglutarate dehydrogenase complex activities in cultured fibroblasts were normal (**Table 1**). Mitochondrial oxidative phosphorylation analysis on intact digitonin-permeabilized fibroblasts was suggestive of slightly reduced PDC activity relative to control range in mitochondria (**Table 1**).

We reviewed 16 other cases with mutations in ECHS1 where PDC activity was also assayed in order to determine how common and generalized secondary PDC deficiency is associated with primary SCEH deficiency (**Table 2**). For reasons that remain unexplained, we find that about half of cases with primary SCEH deficiency also exhibit secondary PDC deficiency (**Table 2**). **Table 3** summarizes the currently known genetic etiologies for impaired pyruvate oxidation.

The patient died on day-of-life 39, prior to establishing his diagnosis, highlighting the importance of early and rapid neonatal diagnosis because of possible adverse effects of certain therapeutic interventions, such as administration of ketogenic diet, in this disorder. There is a need for better understanding of the pathogenic mechanisms and phenotypic variability in this relatively recently discovered disorder.

Table 1
Summary of functional assays

Enzyme/Complex/Function	Cell	Activity* Case (%mean)	Control Mean ± SD, n value	Control Ref. range
PDC-activated	Lymph	0.27 (17%)**	1.6 ± 0.5, n = 596	1.0-2.7
	FB	2.2 (90%)	2.4 ± 0.9, n= 329	1.3-4.4
KDC	FB	2.1 (100%)	2.1 ± 1.0, n = 42	0.7-4.6
SCEH	FB	<31 (BLQ)	379 ± 145	179-616
OxPhos (pyruvate, malate and ADP)	FB	22 (56%)	39 ± 6, n = 57	30-53
OxPhos (palmitoylcarnitine, malate and ADP)	FB	30 (103%)	29 ± 4, n = 49	22-39

* PDC, KDC and SCEH activities were in nmol/min/mg protein, and OxPhox activities were in pmol/sec/million cells.
**PDC/E3 = 0.4 (control mean ± SD: 2.3 ± 0.6, RR 1.4-3.6, n = 596).
Lymph, blood lymphocytes; FB, cultured fibroblasts; PDC, pyruvate dehydrogenase complex; KDC, alpha-ketoglutarate dehydrogenase complex; SCEH, short-chain enoyl-CoA hydratase; OxPhos, oxidative phosphorylation – O_2 consumption assayed in digitonin-permeabilized fibroblasts (i.e., intact cellular mitochondria); and BLQ, below limit of quantitation.

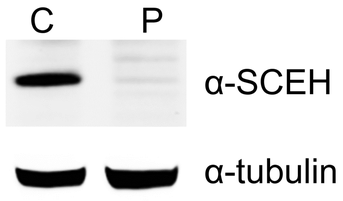

Fig. 1. Protein expression in patient fibroblasts. Immunoblot analysis using antibodies against SCEH and alpha tubulin (loading control). Patient fibroblast (P) shows significantly reduced SCEH protein expression *vs* a control sample (C).

Timeline of clinical course and biochemical testing

DOL	Clinical	Biochemical/functional
1-2	Male born to 35 yo G_1P_{0-1} by C/S b/c fetal distress 37 wk GA, normal BW and length Apgar 2^1, 5^5, 8^{10} Generalized hypotonia EEG burst suppression, no seizures MRI diffuse cortical thinning, T2 hyperintensity of WM	Blood lactate 10.6-12.8 mM (RR 0.5-1.6) Blood pyruvate 0.56 mM (5x upper limit of normal) L/P ratio: 19-23 Plasma alanine 1015 µM (RR 145-480) Plasma cystine 12 µM (RR 15-55) Normal BCAA Plasma acylcarnitines, borderline elevation of C5:1 UOA (slide), 2-methyl-2,3-dihydroxybutyrate peak not yet recognized
4	ECHO, normal	CK 868 U/L (RR 55-400)
11	NG-tube feed, started	
18		UOA (slide), 2-methyl-2,3-dihydroxybutyrate peak not yet recognized
25	Ophthalmology evaluation, normal	CSF lactate 8.3 mM (RR 0.8-2.4) CSF pyruvate >34 µM (RR 6-19) CSF alanine 144 µM (RR 25-39) CSF glycine 19 µM (RR 6-10) CSF valine 88 µM (RR 19-30) CSF isoleucine 45 µM (RR 5-12) CSF leucine 85 µM (RR 8-21) Normal neurotransmitters
26	Thiamine, started	
29	Ketogenic diet (KetoCal 3:1), started	Low lymphocyte PDC activity: 0.27 nmol/min/mg protein, 17% of control mean (control mean ± SD: 1.6 ± 0.5, RR 1.0-2.7, n = 596) Low PDC/E3 ratio: 0.4 (control mean ± SD: 2.3 ± 0.6, RR 1.4-3.6, n = 596) Blood β-hydroxybutyrate 0.3 mM (RR 0.0-0.3) Blood lactate 3.6 mM (RR 0.5-1.6)
33		Blood β-hydroxybutyrate 2.3 mM Blood lactate 1.4 mM (RR 0.5-1.6)
35	Bilateral sensorineural deafness noted No gag reflex Less responsive More apneas	
39	Died	Diagnosis not yet established, although functional PDC deficiency noted!

Intermittently received carnitine (50 mg/kg/day) and was administered folinic acid (1 mg/kg/day) for a brief period. Coenzyme Q10 supplementation was considered but not administered. Mitochondrial DNA sequencing identified a homoplasmic m.15434C>T (MT-CYB; L230F), representing a rare but a benign polymorphism.

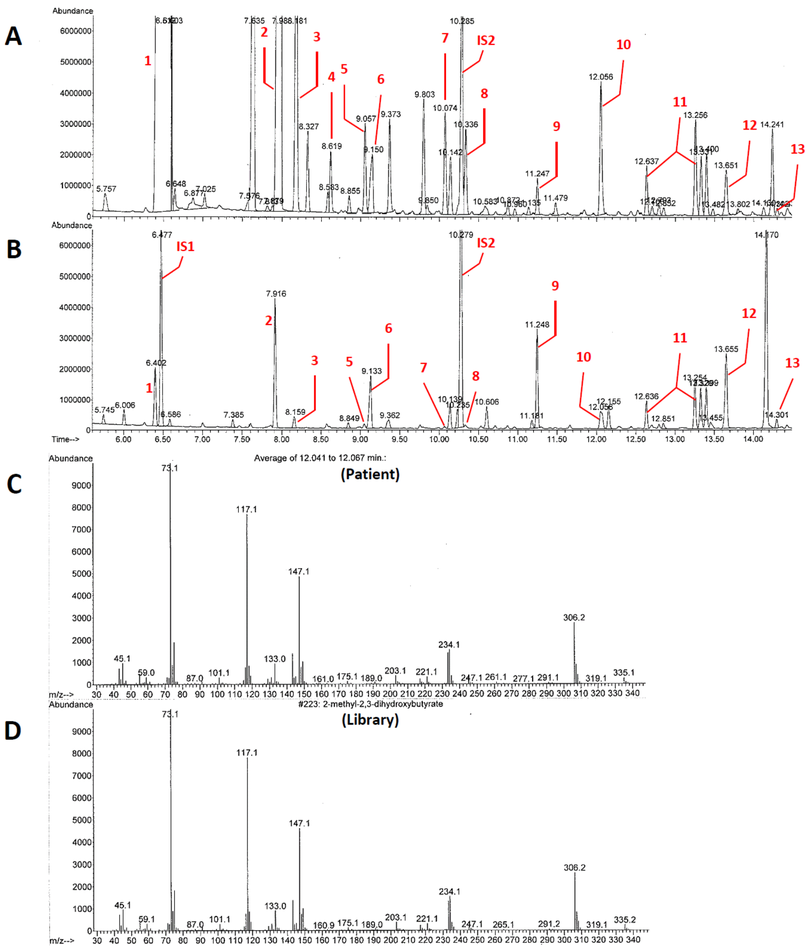

Fig. 2. Urine organic acid profiles of patient. Total ion chromatograms (A and B from DOL #3 and #19, respectively) and mass spectra (C and D). A, blood lactate 10.6-12.8 mM and UOA lactate 15800 mg/g creatinine (RR <125); B, UOA lactate 137 mg/g creatinine; C, Average of 12.1 minutes, identifying the peak as 2-methyl-2,3-dihydroxybutyric acid; and D, m/z spectrum of 2-methyl-2,3-dihydroxybutyric acid. Noted in red: 1, lactic acid; 2, pyruvic acid; 3, 3-hydroxybutyric acid; 4, acetoacetic acid; 5, 2-ketoisovaleric acid; 6, urea; 7, 2-ketomethylvaleric acid; 8, 2-ketoisocaproic acid; 9, fumaric acid; 10, 2-methyl-2,3-dihydroxybutyric acid; 11, 3-methylglutaconic acid (peaks 1 and 2); 12, adipic acid; and 13, tiglylglycine. Internal standards 1 and 2 (IS1 and IS2) are caproic acid, and cyclohexylacetic acid, respectively.

CONCLUSIONS

The *ECHS1* c.836T>C and c.8C>A mutations in a compound heterozygous state are pathogenic, leading to very low SCEH activity and immunoreactivity of SECH as well as a lethal neonatal phenotype. Lymphocyte PDC activity was low in this patient but PDC and KDC activities in cultured fibroblasts were normal. Oxidative phosphorylation analysis on intact digitonin-permeabilized fibroblasts showed moderate impairment that could be due to reduced pyruvate oxidation in intact mitochondria. Urine 2-methyl-2,3-dihydroxybutyric acid was markedly elevated but markedly decreased when lactic acidosis diminished.

Soon after initiation of ketogenic diet the patient's clinical course deteriorated and he died, prior to his diagnosis with SCEH deficiency. Others have also reported lack of success with use of ketogenic diet for this disorder (1). Administration of a ketogenic diet may not be completely effective to control lactic acidosis and/or may be harmful in cases where PDC deficiency is secondary to 1) impairment of formation of acetyl-CoA in defects of fatty acid β-oxidation (e.g., SCEH deficiency), 2) decreased oxidation of acetyl-CoA due to primary oxidation defects distal to PDC (e.g., the tricarboxylic acid cycle including succinyl-CoA synthetase deficiency), or 3) combined defects of PDC and α-ketoglutarate dehydrogenase complex (e.g., E3, thiamine pyrophosphate, or lipoate deficiencies).

Early and rapid neonatal diagnosis of this disorder through inclusion in NBS panels or by targeted gene-panel or WES testing of ill neonates with lactic acidosis is crucial because of the possible adverse impact of certain therapeutic interventions in outcome. For mechanisms that remain unexplained, about half of previously reported cases with primary SCEH deficiency also exhibit secondary PDC deficiency.

Table 3
Currently known and potential etiologies of impaired pyruvate oxidation

Enzyme/complex/function/pathway	Gene: Known	Gene: Potential
Pyruvate dehydrogenase complex:	*PDHA1, PDHB, DLAT, DLD, PDHX*	
Pyruvate dehydrogenase phosphatase:	*PDP1, PDP2, PDP3 (PDPR)*[a]	
Pyruvate carrier (mitochondrial):	*MPC1*	
Thiamine pyrophosphokinase:	*TPK1*	
Thiamine/thiamine pyrophosphate transporters:	*SLC25A19*	*SLC19A2, SLC19A3*
Lipoamide synthesis/transfer/degradation:	*LIAS, LIPT2, LIPT1, SIRT4*	
Fe-S Cluster proteins:	*BOLA3, NFU1, GLRX5, IBA57*	*ISCA2, ISCU*
Fatty acid β-oxidation:	*ECHS1*	
Branched-chain amino acid (valine) metabolism:	*ECHS1, HIBCH*	
Tricarboxylic acid (TCA) cycle:	*SUCLA2*	*SUCLA1*[b], *SUCLG2*[b]
Phosphoenolpyruvate carboxykinase:	*PCK2*[c]	

[a] Christodoulou et al. PgmNr 376 abstract presented at the 2015 ASHG meeting, Baltimore, MD
[b] Presumed, based on PDC deficiency secondary to primary succinyl-CoA synthetase (*SUCLA2*) deficiency (6).
[c] Bedoyan et al., unpublished data

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Table 2
Reports of patients with *ECHS1* mutations where PDC activity in various tissues was evaluated.

Patient genotype	Elevated lactate	Elevated alanine	Urine MDHB	SCEH Tissue	SCEH Activity*	SCEH Mean ± SD	SCEH RR	PDC Tissue	PDC Activity* (%mean)	PDC Mean ± SD	PDC RR	Reference
c.817A>G/c.817A>G[1]	Yes	Yes	Large	FB	<9 (BLD)	379 ± 145	179-616	FB	**0.83** (50%)**	1.66 ± 067	0.87-3.03	1
								FB	**1.11** (46%)**	2.42 ± 0.88	1.26-4.42	
								Liver	**0.33** (15%)**	2.17 ± 0.77	1.23-3.89	
								SM	**0.10** (3%)**	3.17 ± 1.49	1.20-6.52	
c.817A>G/c.817A>G[1]	Yes	Yes	Large	FB	<9 (BLD)	379 ± 145	179-616	FB	0.88 (53%)	1.66 ± 067	0.87-3.03	
								Liver	**0.88** (41)**	2.17 ± 0.77	1.23-3.89	
								SM	**0.29** (9%)**	3.17 ± 1.49	1.20-6.52	
c.433C>T/c.476A>G	Yes	Yes	Large		ND			SM	5.8		5.6-9.4	
c.673T>C/c.674G>C	Yes	NR	Large		ND			SM	129		110-130	
c.197T>C/c.449A>G	Yes	NR	229 fold		ND			FB	Normal			2
c.673T>C/ c.673T>C	Yes	Yes	39 fold		ND			SM	Normal			
c.268G>A/c.583G>A	ND	No	6 fold		ND			FB	Normal			
c.161G>A/c.431dup	Yes	No	ND		ND			SM	Normal			
c.538A>G/c.583G>A	No	Yes	ND		ND			FB	**Mildly reduced****			3
c.538A>G/c.713C>T[2]	Mildly increased	No	NR		ND			FB	Normal			
c.538A>G/c.713C>T[2]	Mildly increased	ND	NR		ND				ND			
c.538A>G/c.476A>G	No	No	NR		ND			FB	Normal			
c.473C>T/c.414+3G>C[3]	Yes	NR	62-100 fold	FB	<9 (BLD)	379 ± 145	179-616	FB	**0.15****		0.23-0.53	4
c.473C>T/c.414+3G>C[3]	NR	NR	31-39 fold	FB	<9 (BLD)	379 ± 145	179-616	FB	**0.04****		0.23-0.53	
c.88+5G>A/ c.88+5G>A[4]	Yes	Yes	NR		ND			FB	**7.6** mU/CS**		9.7-36	5
c.88+5G>A/ c.88+5G>A[4]	Yes	Yes	NR		ND			FB	Normal			
c.8C>A/c.836T>C	Yes	Yes	Large	FB	<31 (BLQ)	379 ± 145	179-616	Lymph	**0.27** (17%)**	1.63 ± 0.53	0.98-2.72	This report
								FB	2.17 (90%)	2.42 ± 0.88	1.26-4.42	

Corresponding numbered superscripts indicate siblings; * indicates activity reported as nmol/min/mg protein; low/reduced PDC activity are noted in bold and **.
BLD, below limit of detection; BLQ, below limit of quantitation; CS, citrate synthase; FB, fibroblasts; Lymph, lymphocytes; MDHB, 2-methyl-2,3-dihydroxybutyric acid; ND, not determined; NR, not reported; PDC, pyruvate dehydrogenase complex; RR, reference range; SCEH, short-chain enoyl-CoA hydratase; SD, standard deviation; and SM, skeletal muscle.