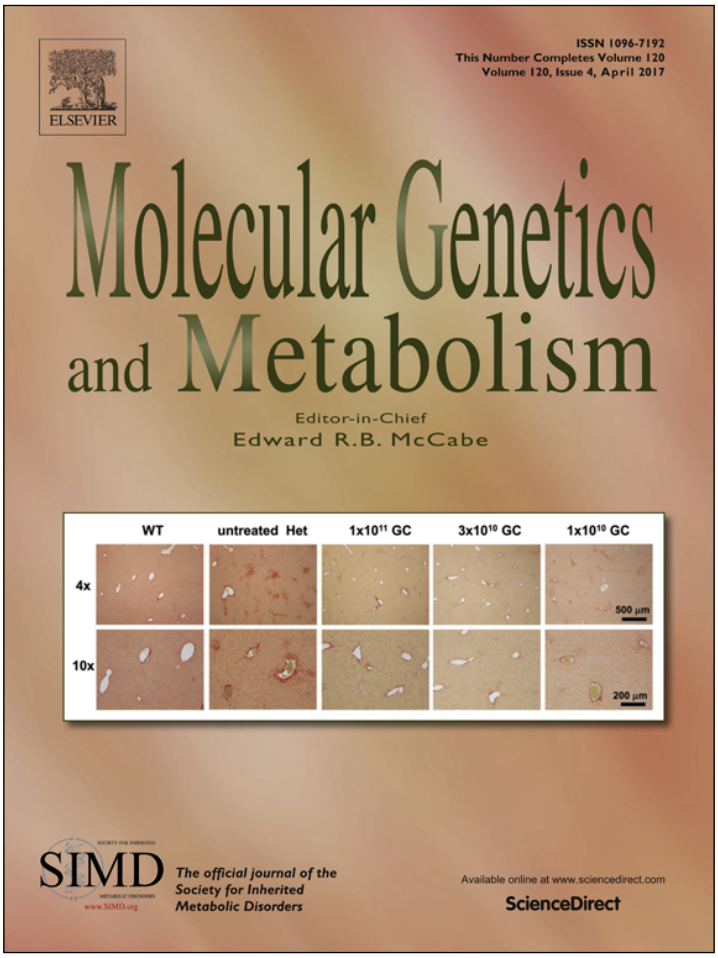

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Lethal neonatal case and review of primary short-chain enoyl-CoA hydratase (SCEH) deficiency associated with secondary lymphocyte pyruvate dehydrogenase complex (PDC) deficiency

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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 and nearly absent immunoreactivity of SCEH. The patient had a severe neonatal course with elevated blood and cerebrospinal fluid lactate and pyruvate concentrations, high plasma alanine and slightly low plasma cystine. 2-Methyl-2,3-dihydroxybutyric acid was markedly elevated as were metabolites of the three branched-chain α-ketoacids on urine organic acids analysis. These urine metabolites notably decreased when lactic acidosis decreased in blood. Lymphocyte pyruvate dehydrogenase complex (PDC) activity was deficient, but PDC and α-ketoglutarate dehydrogenase complex activities in cultured fibroblasts were normal. Oxidative phosphorylation analysis on intact digitonin-permeabilized fibroblasts was suggestive of slightly reduced PDC activity relative to control range in mitochondria. 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. For reasons that remain unexplained, we find that about half of cases with primary SCEH deficiency also exhibit secondary PDC deficiency. 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.

1. Introduction

Short-chain enoyl-CoA hydratase (SCEH, EC4.2.1.17; also known as crotonase and encoded by *ECHS1* on chromosome 10) is a 290 amino acid protein localized in the mitochondrial matrix as a 160 kDa homohexameric enzyme [1]. SCEH functions to hydrate the double bond between the second and third carbons of enoyl-CoAs in many metabolic pathways, including mitochondrial short- and medium-chain fatty acid β-oxidation and branched-chain amino acid catabolic pathways, as well as in catabolism of methionine and threonine [1–7]. Human SCEH has broad substrate specificity for acyl-CoAs, including crotonyl-CoA (from β-oxidation), acryloyl-CoA (from metabolism of various amino acids), 3-methylcrotonyl-CoA (from leucine metabolism), tiglyl-CoA (from isoleucine metabolism), and methacrylyl-CoA (from valine metabolism) [8]. Although SCEH binds tiglyl-CoA, the

Abbreviations: SCEH, short-chain enoyl-CoA hydratase; PDC, pyruvate dehydrogenase complex; KDC, α-ketoglutarate dehydrogenase complex; MDHB, 2-methyl-2,3-dihydroxybutyric acid; WES, whole exome sequencing; RR, reference range; DOL, day-of-life.

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rate of hydration is relatively low [8]. SCEH deficiency was first identified as a disorder of valine metabolism since the main accumulating metabolites are derived from the valine degradation pathway.

Patients with a defect in SCEH can present with encephalopathy, generalized hypotonia, respiratory insufficiency, sensorineural deafness, epilepsy, optic atrophy, cardiomyopathy, and/or developmental delay. Blood and cerebrospinal fluid (CSF) lactate and pyruvate are usually elevated and brain MRI may show white matter changes or a Leigh syndrome-like pattern affecting brainstem and basal ganglia resembling other inherited disorders of energy metabolism [5,9–13]. A number of metabolites when present in the urine (and presumably blood) are important diagnostic markers for this disorder, including S-(2-carboxypropyl)-L-cysteine and S-(2-carboxypropyl)cysteamine (which are derived from methacrylyl-CoA), S-(2-carboxyethyl)-L-cysteine and S-(2-carboxyethylcysteamine (which are derived from acryloyl-CoA), and 2-methyl-2,3-dihydroxybutyric acid (MDHB) [5,8,14]. Although MDHB is thought to be derived from acryloyl-CoA [5,14] and/or possibly methacrylyl-CoA (also known as 2-methylprop-2-enoyl-CoA), the exact origin of this metabolite is currently unknown. The inclusion of some of the above biomarkers in newborn screening (NBS) panels for early neonatal diagnosis of SCEH deficiency has been suggested [8,14]. To date, almost half of cases diagnosed with this autosomal recessive disorder perish within the neonatal or infantile period, but survival into adulthood is reported. To date, at least 20 missense exonic, one nonsense, and a few splice site and frame shift mutations in *ECHS1* have been reported associated with this relatively novel disorder [5,8–13,15–17].

Because almost all individuals affected with this disorder present with lactic acidosis, initial workup often involves investigation(s) into disorders of pyruvate metabolism and/or other mitochondrial oxidative enzyme defects. Therefore, secondary functional pyruvate dehydrogenase complex (PDC) deficiency has been reported in several cases with primary SCEH deficiency. In some cases, secondary PDC deficiency appeared to be due to reduction of the E2 protein component of PDC [11]. In this report, we describe a patient with severe neonatal primary SCEH deficiency with two pathogenic *ECHS1* mutations (one novel) identified by whole exome sequencing (WES) with subsequent biochemical and functional confirmation of SCEH deficiency and low PDC activity in lymphocytes but not fibroblasts. We review reported cases with mutations in *ECHS1* where PDC activity was also assayed to determine how common secondary PDC deficiency is associated with primary SCEH deficiency.

2. Case report

The Caucasian proband was a male born to a 35 year-old G_1P_{0-1} mother by C-section due to fetal distress at 37 weeks gestational age with birth weight, height and head circumference 2,550 g (26–50%ile), 48 cm (51–75%ile) and 30 cm (4–10%ile), respectively. Apgar scores were 2, 5 and 8 at 1, 5 and 10 min, respectively. At birth, he was noted to have mild jaundice, decreased overall tone and activity, especially truncal tone with significant head lag. Labs at day-of-life (DOL) #1–2 showed metabolic acidosis with a very high anion gap and trace ketones but normal liver function on comprehensive metabolic panel testing, high blood lactate 10.6–12.8 mM (RR 0.5–1.6), high blood pyruvate 0.56 mM (5× upper limit of normal), with lactate to pyruvate ratio ranging 19–23, and normal plasma ammonia for a neonate (55 μM). Plasma amino acids (PAAs) showed very high alanine 1,015 μM (RR 145–480) with no other abnormalities except for slightly low cystine 12 μM (RR 15–55). Other metabolic findings included intermittent borderline elevation of C5:1 on plasma acylcarnitines (PACs). No elevations of C5-OH or C4-OH were noted on PACs. Urine organic acids (UOAs) on DOL #2 showed massive amounts of lactate, pyruvate and ketones. Branched-chain α-ketoacids (BCKAs) and 3-methylglutaconic acid were also present (see also Results 4.3).

His electroencephalogram repeatedly showed burst suppression but no electrographic seizures. His brain MRI scans demonstrated diffuse cortical thinning and T2 hyperintensity of the white matter with sparing of the brainstem, cerebellum and basal ganglia. Over time, diffusion restriction abnormalities were noted, especially over the occipital regions and along the corpus callosum. These findings did not suggest Leigh syndrome, but increased lactate peaks were consistently noted by MRS in the frontal gray and white matters, as well as elevated CSF lactate (8.3 mM; RR 0.8–2.4) and CSF pyruvate (>34 μM; RR 6–19) on DOL #25. CSF neurotransmitter metabolites (5-hydroxyindolacetic acid, homovanillic acid and 3-*O*-methyldopa) and 5-methyltetrahydrofolate were within their respective reference ranges. CSF amino acids were significant for high alanine 143.8 μM (RR 24.7–39.0), with slightly high glycine 18.5 μM (RR 5.8–9.5), and high branched-chain amino acids (BCAAs) with valine 87.6 μM (RR 19.3–29.7), isoleucine 45.1 μM (RR 4.7–11.7) and leucine 85.1 μM (RR 8.4–20.9). There was no evidence of cardiac dysfunction although serum creatine phosphokinase (CPK) was slightly high at 868 U/L (RR 55–400) on DOL #4. On DOL #25, an ophthalmologic evaluation was normal. On DOL #35, bilateral sensorineural deafness was noted by both otoacoustic emissions and auditory brainstem response methods. Mitochondrial DNA sequencing (Transgenomic Laboratory, New Haven, CT) identified a homoplasmic m.15434C>T (MT-CYB; L230F), representing a rare but benign polymorphism.

Family history was non-contributory. Maternal breast milk feeding via NG tube was started on DOL #11, then switched to a ketogenic diet (Nutricia KetoCal 3:1 with PDM/MBM-22 kcal/oz) on DOL #29. Thiamine was started at 100 mg daily on DOL #26, the dosage doubled on DOL #29 and then maintained throughout the remaining hospital course. He 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 done after checking the blood CoQ10 level which came back normal.

Although the patient was noted to be hypotonic from birth, he was responsive and moved all extremities spontaneously throughout the majority of his hospital course. Around DOL #35, the neurological status started to rapidly deteriorate becoming less responsive and losing the gag reflex. He developed recurrent apneas at which time comfort measures only were instituted and he eventually succumbed to his disorder on DOL #39.

3. Materials and methods

Informed consent was obtained from the parents/guardians for investigative studies by inclusion in the University Hospitals Cleveland Medical Center IRB-approved *Disorders of Pyruvate Metabolism* study, for additional functional and/or molecular analyses including trio whole exome sequencing (WES) of proband and parents.

3.1. Whole exome sequencing (WES) analysis

Next-generation sequencing (NGS) of 23 genes associated with pyruvate metabolism was negative for the following genes: *BOLA3*, *DLAT*, *DLD*, *LIAS*, *LIPT1*, *LIPT2*, *NFU1*, *PDHA1*, *PDHB*, *PDHX*, *PDK1*, *PDK2*, *PDK3*, *PDK4*, *PDP1* (*PPM2C*), *PDP2* (*PPM2C2*), *PC*, *PCK1*, *PCK2*, *SLC19A2*, *SLC19A3*, *SLC25A19*, and *TPK1*. This led to performance of trio WES analysis using DNA from parents and proband. The WES pipeline used was described before [18], except for using updated Omicia Opal version 4.23.2 and Omicia VAAST Trio Report algorithm in this case.

3.2. Functional and biochemical assays

Assay of PDC, both activated-dephosphorylated and inactivated-phosphorylated, α-ketoglutarate dehydrogenase complex (KDC) and dihydrolipoamide dehydrogenase (E3) activities in disrupted blood lymphocytes and cultured skin fibroblasts were as previously described [19,20]. Assay of SCEH activity and analysis of SCEH protein content by immunoblotting were as previously described [5,11]. Quantitative

oxidative phosphorylation in harvested cultured skin fibroblasts permeabilized with digitonin was measured as described previously [21]. UOAs and PACs analyses were performed by standard clinical gas chromatography–mass spectrometry and tandem mass spectrometry methods.

4. Results

4.1. Identification of two ECHS1 variants by WES

The depth of coverage for the trio WES analysis identifying the *ECHS1* gene variants were as follows: 96.2% average target bases covered at ≥10x (range was 96.0–96.2% for the trio samples) and 144 average depth of coverage (range was 138–152 for the trio samples). Omicia Opal identified 14,349 variants, VAAST Trio Report ranked 268 variants as recessive and 8 as X-linked (with *ECHS1* by VAAST ranked #3 out of 137), and Phevor ranked 137 genes with *ECHS1* as #2, after the input of the following phenotype characteristics: lactic acidosis and decreased activity of PDC. Phevor and phenotype/gene association scores were 3.37 and 0.482, respectively.

The candidate *ECHS1* variants identified by VAAST were c.836T>C (p.F279S) and c.8C>A (p.A3D) with Omicia scores of 0.93 and 0.42, respectively. The proband was compound heterozygous for these mutations while his mother and father were carriers of the p.F279S and p.A3D substitutions, respectively. The read depth for the c.836T>C and c.8C>A variants were 156 and 30, respectively, distributed essentially equally (78:78 and 14:16, respectively) between both DNA strands.

The Omicia score represents a composite score using MutationTaster, Polyphen-2, SIFT, and PhyloP-vertebrate *in silico* prediction algorithms [22]. A score of ≥0.85 ≅ 1% false-positive prediction rate [22]. Furthermore, a variant with an Omicia score of >0.85 is considered likely pathogenic, while one with a score between 0.50 and 0.85 is considered potentially pathogenic [22]. The novel p.F279S substitution was predicted to be pathogenic by all 4 *in silico* algorithms, with prediction/score value as follows: mutation taster, D; Polyphen-2, 1; SIFT, 0; and PhyloP, 4.19. In contrast, only SIFT predicted p.A3D substitution to be pathogenic while PolyPhen and PhyloP scored 0.622 (score range 0 to 1) and 2.87 (score range −11.764 to +6.424), respectively, and MutationTaster predicted this variant to be a polymorphism. Both variants were not found in the 1,000 Genomes, Exome Variant Server (EVS), Exome Aggregation Consortium (ExAC) and other common allele frequency databases implying that both alleles are quite rare.

4.2. Enzyme assays and functional confirmation of SCEH deficiency

4.2.1. Assay of PDC and other mitochondrial oxidative enzymes

Prior to identification of the two *ECHS1* mutations by WES and because of lactic acidosis and elevated plasma alanine, workup for defects in pyruvate metabolism and other mitochondrial oxidative enzymes were pursued. Assay of PDC in blood lymphocytes showed low activity with low PDC/E3 ratio consistent with PDC deficiency (Table 1). Follow-up assays of PDC and KDC in cultured skin fibroblasts from the patient showed normal activities (Table 1). These results prompted molecular testing of 23 genes related to pyruvate metabolism by NGS which were all normal and eventual enrollment of the patient in a study protocol for research WES. The identification of the two candidate *ECHS1* variants by WES prompted reexamination of UOAs for pathognomonic metabolites (Results 4.3 below) and testing of SCEH activity.

Analysis of integrated oxidative phosphorylation (OxPhos) in digitonin-permeabilized patient fibroblasts (i.e., with intact cellular mitochondria) showed a slight reduction in oxygen consumption in the presence of pyruvate, malate and ADP (Table 1). Changes in oxygen consumption in the presence of pyruvate, malate and ADP reflect composite activity of the mitochondrial pyruvate transporter, production of acetyl-CoA by PDC, coupled production of NADH, and oxidation of the NADH by Complex I. Slight reduction of oxygen consumption was also noted when glutamate was added to the solution (i.e., to the pyruvate, malate and ADP mix with permeabilized fibroblasts) (23 pmol/sec/million cells; control mean ± SD: 40 ± 6, RR 30–56, $n = 57$). Addition of glutamate in the presence of malate stimulates malate dehydrogenase activity, and coupled production of NADH, aspartate, and α-ketoglutarate. As an alternate source of intra-mitochondrial acetyl-CoA, we also examined oxygen consumption in the presence of palmitoylcarnitine, malate and ADP in the digitonin-permeabilized patient fibroblasts and this was normal (Table 1). Uncoupled Complex I respiration was normal (37 pmol/sec/million cells; control mean ± SD: 55 ± 13, RR 30–83, $n = 57$). There were no functional abnormalities of the other three (II, III or IV) enzyme complexes of mitochondrial oxidative phosphorylation (data not shown). The carnitine transport system, including carnitine acylcarnitine translocase and carnitine palmitoyltransferase II, and the enzymes for long-chain fatty acid β-oxidation were also normal (data not shown).

4.2.2. SCEH assay and protein expression

SCEH activity in cultured fibroblasts of our patient was below the limit of quantitation of the enzyme assay (<31 nmol/min/mg protein, RR 179–616), confirming that this patient has primary deficiency of SCEH (Table 1). Furthermore, follow-up protein expression analysis in patient fibroblasts by immunoblotting using antibodies against SCEH revealed a markedly reduced SCEH protein level also supporting the functional SCEH defect (Fig. 1).

4.3. Biochemical support for primary SCEH deficiency

Because many patients with SCEH deficiency show significantly large amounts of 2-methyl-2,3-dihydroxybutyric acid in urine, we re-

Table 1
Summary of functional assays.

Enzyme/complex/function	Cell	Activity[a]		
		Case (%mean)	Control	
			Mean ± SD, *n* value	Ref. range
PDC-activated	Lymph	0.27 (17%)[b]	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

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.

[a] PDC, KDC and SCEH activities were in nmol/min/mg protein, and OxPhox activities were in pmol/sec/million cells.

[b] PDC/E3 = 0.4 (control mean ± SD: 2.3 ± 0.6, RR 1.4–3.6, $n = 596$). (For results of other functional assays, the reader is referred to Results 4.2.1.)

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).

examined our patient's UOAs for the presence of 2-methyl-2,3-dihydroxybutyric acid. A urine sample collected on DOL #3 when blood lactate concentrations ranged between 10.6 and 12.8 mM (UOA approximate lactate 15,800 mg/g creatinine; RR <125) showed a large peak of 2-methyl-2,3-dihydroxybutyrate at 12.1 min (Fig. 2A). Branched-chain α-ketoacids (2-ketoisovaleric, 2-ketomethylvaleric and 2-ketoisocaproic acids) were also detected in significant amounts (Fig. 2A). On DOL #19, when lactic acidosis was significantly reduced (UOA lactate 137 mg/g creatinine; RR <125), the 2-methyl-2,3-dihydroxybutyrate peak was noted to be much smaller as were the various branched-chain α-ketoacid peaks (Fig. 2B). Of note, the tiglylglycine (derived from tiglyl-CoA) peak remained essentially

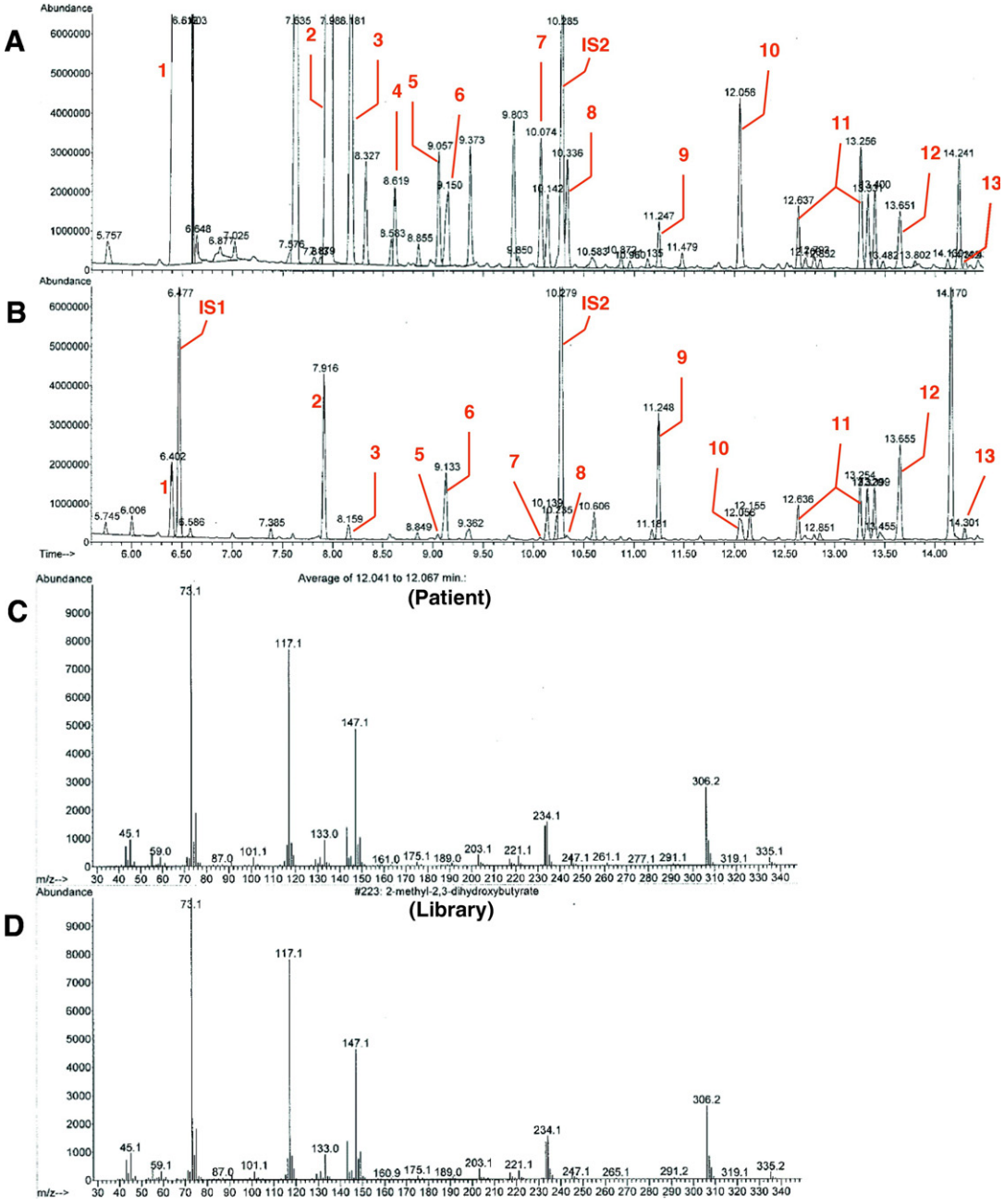

Fig. 2. Urine organic acids profiles of patient. Total ion chromatograms (A and B from DOL #3 and #19, respectively) and mass spectra (C and D). A, blood lactate 10.6–12.8 mM and UOA lactate 15,800 mg/g creatinine (RR <125); B, UOA lactate 137 mg/g creatinine; C, Average of 12.1 min, identifying the 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.

unchanged with different blood lactate levels (Fig. 2A and B). In urine of other patients with lactic acidosis but without SCEH deficiency and following ion extraction for masses 306.2 and 234.1, we find only trace amounts of 2-methyl-2,3-dihydroxybutyric acid (data not shown). This supports the notion that identification of a large peak of 2-methyl-2,3-dihydroxybutyric acid is specific for primary deficiency of SCEH [5,10,11,14].

5. Discussion

5.1. Primary SCEH deficiency

We report a patient compound heterozygous for *ECHS1* c.836T>C and c.8C>A mutations with functional deficiency of SCEH whose clinical and biochemical presentation is consistent with other reported severe cases of SCEH deficiency. The pathogenic c.836T>C mutation is novel, but the c.8C>A variant (in a compound heterozygous state with *ECHS1* c.389T>A) was described in two siblings with severe lactic acidosis and congenital dilated cardiomyopathy with neonatal demise at ≤2 days of life, without functional studies of SCEH [13]. Although the Omicia score for the *ECHS1* c.8C>A mutation was just below the range for a "potentially pathogenic" designation and could have been easily dismissed as not significant in our analysis, the markedly reduced SCEH activity and immunoreactivity supports that the c.8C>A mutation is indeed pathogenic.

Our patient exhibited elevation in all three BCKA metabolites in urine when tested on DOL #2 although plasma BCAAs (leucine, isoleucine and valine) were within their respective reference ranges. CSF BCAAs were elevated on DOL #25. Others have reported elevation of plasma BCAAs in patients with SCEH deficiency [15,16]. The small amount of tiglylglycine (from isoleucine catabolism) in urine remained essentially unchanged with the degree of lactic acidosis (compare Fig. 2A and B). This is consistent with the observation that only a small amount of tiglyl-CoA is hydrated by SCEH [8] and the suggestion by others that there must be another hydratase that is more active towards tiglyl-CoA (and crotonyl-CoA) which helps maintain flux through the isoleucine and leucine catabolic pathways [11] in contrast to the valine catabolic pathway.

The slightly low cystine noted on DOL #2 PAAs could reflect depletion of cysteine by conjugation with methacrylyl-CoA and acryloyl-CoA. We did not measure S-(2-carboxypropyl)-L-cysteine, S-(2-carboxypropyl)cysteamine, S-(2-carboxyethyl)-L-cysteine, or S-(2-carboxyethyl)cysteamine in this patient but these derivatives have been noted to be elevated in other cases of SCEH deficiency. Although CPK was slightly high in our patient, CPK may be normal or moderately elevated in patients with SCEH deficiency [10,11] and the reason for this biochemical variability is unclear.

Our patient died from his disorder within 10 days from starting a ketogenic diet when his primary diagnosis was not yet established. Others have also reported lack of success with use of ketogenic diet for this disorder [11]. Use of ketogenic diets is currently the main therapeutic intervention in PDC deficiency, and has been reported to be beneficial: 1) in boys with primary mutations of *PDHA1*, 2) by parental report in a clinical survey, 3) in other sporadic cases, and 4) in a zebra fish model [23–26]. Blood β-hydroxybutyrate was infrequently monitored to establish whether "ketosis" was actually established in this case. After 4 days on the ketogenic diet, blood β-hydroxybutyrate increased from 0.27 to 2.28 mM (RR 0.01–0.29) while blood lactate decreased from 3.6 to 1.4 mM. 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 (TCA) cycle including succinyl-CoA synthetase deficiency [18]), or 3) combined defects of PDC and KDC (e.g., E3, thiamine pyrophosphate, or lipoate deficiencies).

5.2. Secondary PDC deficiencies associated with SCEH deficiency

Decreased PDC activity in cases with *ECHS1* mutations (with a few also confirmed to be functionally SCEH deficient) has been reported in fibroblasts as well as in liver and skeletal muscle (SM) tissues (Table 2). Here we report for the first time decreased PDC activity in lymphocytes from a patient with confirmed biochemical, functional and molecular diagnosis of SCEH deficiency. In all, 42% (5/12) of cases with *ECSH1* mutations had low PDC activity in fibroblasts. Functional PDC deficiency can be generalized to all tissues tested or appear isolated to one (or a few) specific tissue/cell type(s) tested, with variances observed even between siblings (Table 2). For example, tissue/cell type variability of PDC deficiency is observed in sibs homozygous for *ECHS1* c.817A>G and c.88+5G>A (Table 2). In cases with mutations in *ECHS1* and with either lactate and/or MDHB elevation, 50% (4/8) of cases showed low PDC activity in fibroblasts, while low PDC activity in SM is noted in 33% (2/6) of cases to date. In those cases where functional SCEH deficiency was noted in fibroblasts, 60% (3/5) also exhibited concurrent functional PDC deficiency in fibroblasts. Therefore, it appears that about half of cases with primary SCEH deficiency also exhibit secondary PDC deficiency.

The mechanism for decreased PDC activity and the reason for this variability are unclear. It is possible that secondary PDC deficiency is only present in severely affected patients irrespective of the degree of functional SCEH deficiency because of additional genetic modifiers of PDC function. Comparative analysis of WES or whole genome sequencing data of patients who underwent both SCEH and PDC functional testing may be informative in identifying potential genetic modifiers that could explain the functional PDC heterogeneity.

The slight reduction in oxygen consumption observed in permeabilized patient fibroblasts in the presence of pyruvate, malate and ADP would be consistent with slight impairment of pyruvate oxidation in intact mitochondria, although assay of PDC activity in disrupted cultured fibroblasts was normal. Because the respiratory chain in intact mitochondria was normal (Results 4.2.1), there is uncertainty about the cause of the observed slight reduction in oxygen consumption from malate oxidation (i.e., decreased NADH production by malate dehydrogenase) in the presence of glutamate.

The pathogenic mechanism for secondary functional PDC deficiency with primary SCEH deficiency could also be due to inhibitory metabolites. It is hypothesized that toxic enoyl-CoAs such as methacrylyl-CoA and acryloyl-CoA from valine catabolism, at high concentrations could react with lipoyl domain(s) of the E2 subunit or other specific amino acids of PDC, thereby reducing its activity [11,27]. However, a concurrent reduction of activity of: 1) other dehydrogenase complexes with lipoyl moieties such as KDC, branched-chain ketoacid dehydrogenase complex (BCKDC), and α-ketoadipate dehydrogenase complex (KADC); or 2) the H-protein of the glycine cleavage enzyme (GCE), have either not been observed (in case of KDC and GCE; as in this report for KDC and others for KDC and GCE, [11]) or have not been investigated to our knowledge (in case of BCKDC and KADC).

Others have also found mild to moderate elevations of glycine in patients with SCEH deficiency [16], implying possible intermittent reduction of GCE activity, but this also remains to be determined. Reduced immunoreactive E2 in liver and muscle tissues and less so in cultured fibroblasts has been noted as cause of functional PDC deficiency in some cases [11]. 3-Hydroxyisobutyryl-CoA hydrolase (HIBCH), the next distal enzyme to SCEH in the valine catabolic pathway, with secondary functional PDC deficiency has also been reported with primary HIBCH deficiency [27,28]. Secondary functional PDC deficiency is also reported with primary succinyl-CoA synthetase deficiency and the reason for this also remains unexplained [18]. Therefore, secondary functional PDC deficiency is observed in certain primary defects of valine

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				PDC				Reference
				Tissue	Activity*	Mean ± SD	RR	Tissue	Activity* (%mean)	Mean ± SD	RR	
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	11
								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			10
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****			12
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	5
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	16
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. Control mean ± SD with RR noted.

Table 3
Currently known and potential etiologies of impaired pyruvate oxidation.

Enzyme/complex/function/pathway	Gene	
	Known	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 [18].
[c] Bedoyan et al., unpublished data.

catabolism, fatty acid β-oxidation and TCA pathway, and the pathophysiologic reason(s) remain to be determined. In all these disorders, there is accumulation of acyl-CoA esters in the mitochondrial matrix where some acyl-CoA esters are known to readily react with certain amino acid groups (e.g., lysine) of some of these complexes, which may affect activity. Table 3 summarizes the currently known genetic etiologies for impaired pyruvate oxidation.

6. Summary and 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. 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.

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