Succinyl-CoA synthetase deficiency in siblings with impaired pyruvate dehydrogenase complex (PDC) and other oxidative enzymes in skeletal muscle without mtDNA depletion

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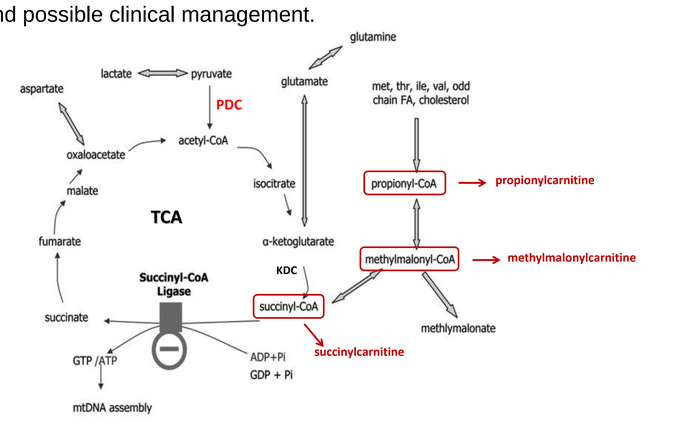
SUMMARY

Mutations in *SUCLA2* result in succinyl-CoA ligase (ATP-forming) or succinyl-CoA synthetase (ADP-forming) (A-SCS) deficiency, a mitochondrial tricarboxylic acid cycle disorder (**Fig. 1**). The phenotype associated with this gene defect is largely encephalomyopathy.

We describe two siblings compound heterozygous for *SUCLA2* mutations, c.985A>G (p.M329V) and c.920C>T (p.A307V), with parents confirmed as carriers of each mutation. We a) developed a new LC-MS/MS based enzyme assay to demonstrate the decreased SCS activity in the siblings (**Table 1** and **Fig. 2**), b) show low immunoreactivity of SUCLA2 (**Fig. 3**), and c) developed a whole exome sequencing (WES) pipeline (**Fig. 4** and **Table 2**) in order to determine the genetic etiology of subjects with well-defined pyruvate dehydrogenase complex (PDC) deficiency. Both siblings shared bilateral progressive hearing loss, encephalopathy, global developmental delay, generalized myopathy, and dystonia with choreoathetosis. Prior to diagnosis and because of lactic acidosis and low activity of skeletal muscle (SM) PDC, sibling 1 (S1) was placed on dichloroacetate (DCA), while sibling 2 (S2) was on a ketogenic diet. S1 developed severe cyclic vomiting refractory to therapy, while S2 developed Leigh syndrome, severe GI dysmotility, intermittent anemia, hypogammaglobulinemia and eventually succumbed to his disorder (**Table 3**). The mitochondrial DNA (mtDNA) contents in SM were normal in both siblings; 62% and 149% relative to tissue and age-matched controls for patient S1 and S2, respectively. PDC, ketoglutarate dehydrogenase complex, and several mitochondrial electron transport chain (ETC) complex activities were low or at the low end of the reference range in frozen SM from S1 and/or S2 (**Tables 4 and 5**). In contrast, activities of PDC, other mitochondrial enzymes of pyruvate metabolism, ETC and integrated oxidative phosphorylation, in skin fibroblasts were not significantly impaired (**Tables 4 and 5**). Although we show that propionyl-CoA inhibits PDC (**Figs. 5 and 6**), it does not appear to account for decreased PDC activity in SM (**Table 6**). A-SCS deficiency which causes a block in the TCA cycle, may cause a secondary upstream increase of acetyl-CoA that could inhibit PDC, but this would not explain low PDC activity noted in S1 (**Table 6**).

A better understanding of the mechanisms of phenotypic variability and the etiology for tissue-specific secondary deficiencies of mitochondrial enzymes of oxidative metabolism, and independently mitochondrial DNA depletion (common in other cases of A-SCS deficiency), is needed given the implications for control of lactic acidosis and possible clinical management.



Heterodimer:
SUCLA1 + **SUCLA2** ⇒ Succinyl CoA + Pi + **ADP** ↔ Succinate + CoA + **ATP**
SUCLA1 + SUCLG2 ⇒ Succinyl CoA + Pi + GDP ↔ Succinate + CoA + GTP

Fig. 1. Interrelationship of PDC, tricarboxylic acid (TCA) cycle intermediates such as A-SCS, and the methylmalonic pathway as it relates to impairment of SCS. Adopted and modified from Van Hove et al. (2010) *Pediatr Res* **68**:159.

Table 1. Fibroblast ATP- and GTP-Succinyl-CoA synthetase (SCS) Analysis

	Activity					
	Control 1	Control 2	Control 3	S1	S2-1	S2-2
ATP	1.02 ± 0.45 (n=15)	0.98 ± 0.32 (n=13)	1.04 ± 0.50 (n=10)	0.10 ± 0.00 (n=2)	0.09 ± 0.05 (n=12)	0.18 ± 0.06 (n=7)
Mean ± SD of 3 control lines	1.01 ± 0.03					
% control				8.8%	17.5%	9.5%
GTP	8.58 ± 1.90 (n=13)	7.60 ± 2.45 (n=9)	10.91 ± 3.85 (n=7)	12.07 ± 6.92 (n=3)	13.91 ± 3.42 (n=9)	10.51 ± 2.41 (n=9)
Mean ± SD of 3 control lines	9.03 ± 1.70					
% control				154.04%	116.34%	133.70%

Activity, nmol/min/mg protein; Rxn [succinate] = 10 mM

† **These authors contributed equally to this work.**

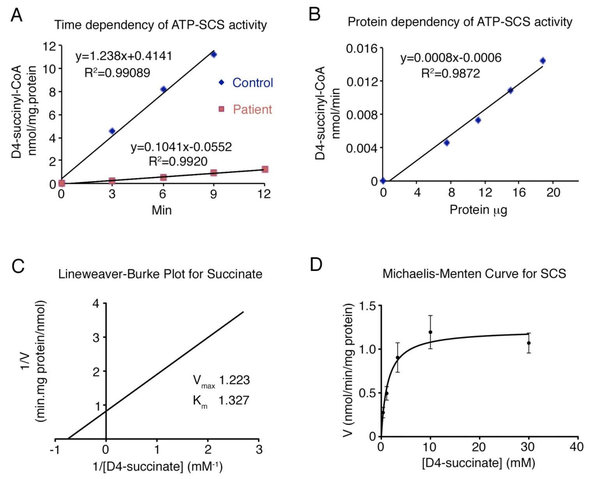


Fig. 2. A-SCS activity measurement by LC-MS/MS. (A) Time dependency was measured in control line and patient line at 37°C for 0, 3, 6, 9 and 12 min with 50 mM Tris buffer pH 8.0, 5 mM $MgCl_2$, 10 mM D4-succinate, 1 mM ATP, 1 mM CoA, oligomycin 2 µg/ml and homogenate protein concentration 1mg/ml. Activity linearity is shown within 9min. (B) Protein dependency was measured in control line for 6 min at different protein concentrations, 0, 0.5, 0.75, 1 and 1.25 mg/ml. Linear for protein concentrations <1.25 mg/ml. (C) and (D) Apparent K_m and V_{max} of succinate were measured from two control lines with different D4-succinate concentrations, 0.37, 1.11, 3.33, 10 and 30 mM, and 1mg/ml homogenate incubated at 37°C for 6min. Other conditions were same as (A).

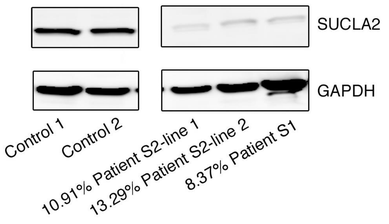


Fig. 3. Western blot analyses result of decreased SUCLA2 in patient lines. SUCLA2 protein detected by Western Blot and normalized to total GAPDH levels as loading controls. Relative SUCLA2 amounts were calculated using mean value of controls (as %).

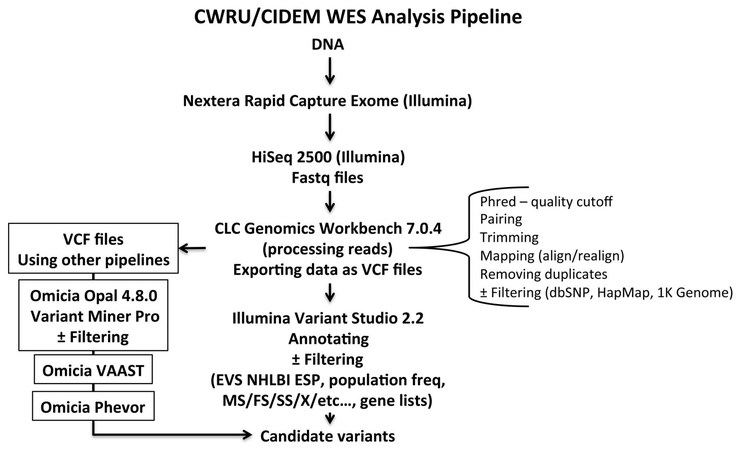


Fig. 4. Summary of the WES pipeline used in this work. MS, missense; FS, frameshift; SS, splice site; and X, stop.

Table 2. Summary of Key Prediction Parameters for the SUCLA2 Variants Identified by WES

Variant	Polyphen-2 (0 to 1)	PhyloP-vertebrate (-11.764 to +6.924)	CADD-Phred (1 to 99)	CADD-raw	Mutation Taster (0 to 1)	Omicia score (0 to 1)	Allele Freq
M329V	0.999	4.56	20.10	3.94	1	0.94	<0.0002
A307V	0.446	4.33	14.45	2.54	1	0.79	0.0000

CADD-Phred; ≥20 or ≥10 means within 1% or 10% of most deleterious, respectively.

Table 3. Phenotypic and management heterogeneity of the two siblings

	Sibling 1	Sibling 2
Onset of disease	Neonatal	Neonatal
Course	Less severe	Lethal by 9 years old
Lactic acidosis	3 months old	2 months old
Muscle biopsy PDC deficiency	6 months old	16 months old
DCA therapy	Yes, 6 months old (still treated now at 14 years old)	No
Ketogenic diet	No	Yes, 16 months old (stopped at 21 months old)
Diagnosis of SUCLA2	7 years old	3 years old

Table 4. Summary of other mitochondrial enzyme assays on the two siblings

		Patient			Controls		
		S1		S2			
	Cell/Tissue	Activity (% mean)	Average (% mean)	Activity (% mean)	Mean ± SD	Range	n
PDC	Lymph	1.95 (120)		3.24 (199)	1.63 ± 0.48	0.98-2.72	596
	FB	1.57 (65)		1.31 (54)	2.42 ± 0.88	1.26-4.42	329
	SM	0.28 (9) 1.37 (43) 0.94 (30)	27%	0.73 (23)	3.17 ± 1.49	1.20-6.52	340
KDC	FB	2.07 (96)		1.52 (72)	2.10 ± 1.03	0.73-4.58	42
	SM	NA		0.68 (22)	3.14 ± 1.44	0.82-6.70	71
PC	FB	1.72 (121) 1.21 (85)	103%	2.74 (193)	1.42 ± 0.79	0.56-3.22	338
PEPCK	FB	0.64 (14)		3.28 (71)	4.60 ± 2.56	1.09-10.04	309

Activity, nmol/min/mg protein; PDC, pyruvate dehydrogenase complex; KDC, α-ketoglutarate dehydrogenase complex; PC, pyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; Lymph, lymphocytes; FB, fibroblasts; SM, skeletal muscle; NA, not available; and SD, standard deviation.

Table 5. Electron transport chain (ETC) activity in fibroblasts and skeletal muscle*

		Complexes; enzymatic activity, units below (% of mean)						
Tissue	Patient	I-III	"I"	II-III	"II"	III	IV	CS
FB	S1	10.5 (50%)		25.9 (101%)	5.8 (181%)	72.0 (90%)	1.3 (65%)	43.0 (82%)
FB control	Mean ± SD (n=144)	20.9 ± 10.9		25.6 ± 4.5	3.2 ± 0.8	79.8 ± 17.7	2.0 ± 0.3	52.2 ± 8.8
	Range	6.1-50.8		17.1-33.6	1.9-4.3	49.7-116.7	1.5-2.6	36.3-69.9
FB	S2	ND	ND	16.7 (246%)	ND	80.1 (46%)	1.0 (91%)	52.5 (122%)
FB control	Mean ± SD (29)			6.8 ± 3.7		173.2 ± 76.7	1.1 ± 0.4	42.9 ± 10.4
	Range			3.6-20.3		37.5-311.0	0.6-2.5	22.9-63.3
SM	S1	0.3 (25%)	48.5(162%)	1.1 (52%)	1.2 (150%)	16.9 (111%)	64.5 (43%)	12.5 (67%)
	S2	0.4 (33%)	25.3 (85%)	0.4 (19%)	0.5 (63%)	5.8 (38%)	61.6 (41%)	24.5 (132%)
SM control	Mean ± SD (n=49)	1.2 ± 1.1	29.9 ± 12.9	2.1 ± 1.2	0.8 ± 0.4	15.2 ± 6.8	148.9 ± 67.2	18.6 ± 4.7
	Range	0.2-4.7	11.5-60.1	0.4-4.9	0.1-2.0	6.8-35.2	57.3-373.0	9.4-30.0

*Oxidative phosphorylation (OxPhos, integrated function) assayed in intact and permeabilized fibroblasts in both sibs were normal (data not shown); Enzyme activity: FB, nmol/min/mg protein and SM, µmol/min/g wet weight. I-III, NADH-cytochrome *c* reductase (rotenone sensitive); "I", NADH-ferricyanide reductase; II-III, succinate-cytochrome c reductase (antimycin sensitive); "II", succinate dehydrogenase; III, decylubiquinol-cytochrome *c* reductase; IV, cytochrome c oxidase; CS, citrate synthase; ND, not done; and SD: standard deviation. Low CIII in SM of S2 is shown in bold and red. Other SM ETC activities at the low end of the reference range in S1 and S2 are shown in bold and blue. FB ETC assays of S1 and S2 were as previously described [Ye and Hoppel (2013) *Anal Biochem* **437**:52 and Hoppel et al. (1987) *J Clin Invest* **80**:71], respectively.

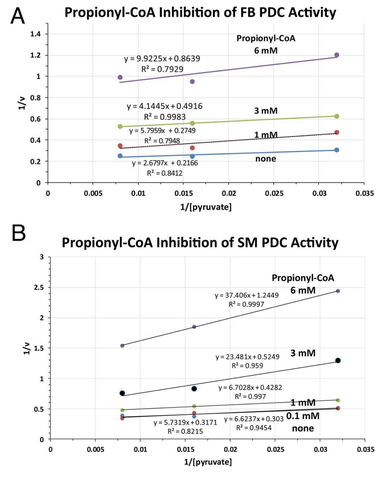


Fig. 5. Double-reciprocal (Lineweaver-Burk) plots from which apparent K_i was determined for propionyl-CoA inhibition of PDC. (A) FB, fibroblast extract and (B) SM, skeletal muscle homogenate shown only.

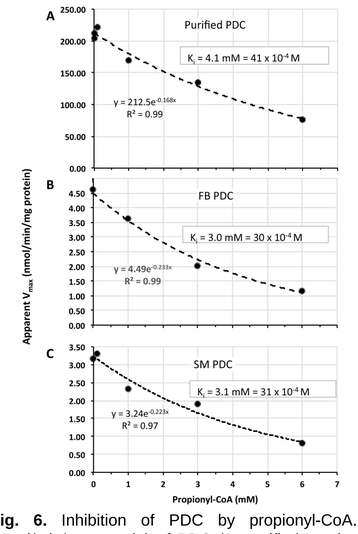


Fig. 6. Inhibition of PDC by propionyl-CoA. V_{max} (nmol/min/mg protein) of PDC (A, purified porcine heart complex); or apparent V_{max} (B, complex in disrupted cultured fibroblasts; and C, complex in SM homogenate) vs propionyl-CoA concentration (mM) and their respective determined K_i or apparent K_i.

Table 6. Summary of Kinetic Parameters of PDC and A-SCS

		Measured (pyruvate as substrate)				Extrapolated concentration*** (patient)	
Enzyme	Tissue (species)	V_{max} (nmol/min/mg protein)	K_m (mM)	K_i (Acetyl-CoA)	K_i (Propionyl-CoA)	Acetyl-CoA	Propionyl-CoA
PDC	Purified complex (porcine heart)	212.8	23.2	13 µM* 5-10 µM**	4.1 mM		
	FB (human)	4.6	12.4		3.0 mM		
	SM (human)	3.2	18.1		3.1 mM	2 µM (S1) 13 µM (S2)	0 µM (S1) 1 µM (S2)
A-SCS	FB (human)	1.2	1.3				

FB, fibroblasts and SM, skeletal muscle.
*Garland and Randle (1964) *Biochem J* **91**:6c.
Behal et al. (1993) *Annu Rev Nutr* **94:490.
***Extrapolated from measured acetyl- and propionyl-carnitine concentrations in SM from the two siblings. **Note**: In human SM, the content of total carnitine is normally approximately 100-fold higher than total CoA; carnitine *vs* CoA, 3120 ± 720 *vs* 31.1 ± 6.3 nmol/gm wet weight; Friolet et al. (1994) *JCI* **94**:1490.
Note: Kanzaki et al. (1969) *JBC* **244**:1183 – K_i of porcine heart PDC in over-all oxidation of 2-oxo-3-methylvalerate (isoleucine) and 2-oxoisovalerate (valine) acids are 21 mM and 3.7 mM, respectively.

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