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Supplemental Data

Riboflavin-Responsive and -Non-responsive Mutations in FAD Synthase Cause Multiple Acyl-CoA Dehydrogenase and Combined Respiratory-Chain Deficiency

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Supplemental Note: Case Reports

Family 1 (F1): Subject S1a, a boy of Turkish consanguineous parents presented with cardiorespiratory collapse at 32 hours of life and died 3 days later. A metabolic post-mortem analysis, done immediately after his death, showed elevated C5-, C8-, C14-carnitines and slightly elevated C5-DC/glutaryl-carnitine in blood spots. Urine organic acids revealed markedly elevated ethylmalonic acid and adipic acid, elevated suberic and dehydro-sebacic acids and slightly elevated hexanoylglycine levels. ETF and ETFDH activities in cultured fibroblasts were within control range. Muscle and liver respiratory chain complexes activities were within control range. Family history revealed an older sister (individual S1b), who had been treated for cardiomyopathy in the first year of her life. Screening of this girl showed elevated C4-carnitine on a blood acylcarnitine profile, and urine organic acids showed elevation of ethylmalonic acid and trace methylsuccinic acid. After treatment with riboflavin all metabolites normalized. Compliance with treatment was variable; in periods without treatment high urinary ethylmalonic acid was found, accompanied by episodes of supraventricular tachycardia. She was treated with riboflavin again and urinary metabolites decreased to control range. She is currently 22 years old and doing well with a pacing/defibrillator device. There were four miscarriages between individual S1a and his sister, S1b.

Family 2 (F2): Subject S2's developmental milestones were normal. During childhood she was able to run but was slower than her peers and experienced symptoms of illness during long-lasting exercise. First evident muscle weakness was observed at age 20 years as she carried heavy bags. Thereafter, she experienced muscle discomfort and weakness during physical activity, and after strenuous exercise she had occasional muscle pain, vomiting and loss of weight. She had tachycardia and used beta blocking medication. During her pregnancy at age 30, one month before the delivery, her muscle weakness drastically deteriorated with a raise in creatine kinase (CK) activities ranging between 5000-6612 U/l (control range 35-210 U/l). It was difficult for her to control her head. She had swallowing and speech difficulties and a prominent scoliosis. No hearing or visual impairments were found. She was advised to

follow a high carbohydrate, moderate protein and low fat diet (dietary fat max. 20 g/day), which ameliorated her symptoms. Blood analysis revealed increases in C8- and C10-acylcarnitine species, and urine analysis showed increased amount of lactate, mildly increased ethylmalonic acid, an increased 2-hydroxyglutarate combined with a low/normal 2-ketoglutarate excretion that was consistent with MADD. Fibroblast fatty acid oxidation flux studies and western blot analysis of ETF and EFTDH protein were found to be within control range. Multiple respiratory chain deficiency with lipid storage myopathy was detected in a muscle biopsy. Riboflavin therapy (100 mg daily) was introduced, and she continued the dietary therapy. This resulted in drastic amelioration of muscle symptoms, and increase in muscle strength. A more comprehensive description of subject S2 with long-term treatment follow-up studies will be published elsewhere.

Family 3 (F3): Subject S3 is a 56 year-old woman, who was born at term after an uneventful pregnancy from non-consanguineous Italian parents. Early psychomotor development was normal. In the teens a mild scoliosis was diagnosed that did not required surgical or medical correction. At 45 years the affected individual presented with exercise intolerance, gait difficulties, weakness in both arms and muscle hypotrophy prevalent in the shoulder girdle. Her symptoms progressed since then and prompted neurological evaluation at age 46. Neurological examination revealed a mild waddling gait with bilateral foot drop. Standing or walking on heels was impossible. Scapular winging with hypotrophy of upper limbs, calf pseudohypertrophy and scoliosis were noticed. She was not able to lift her arms to the horizontal level, but was able to rise from the floor with Gowers maneuver. Lower limb weakness, graded using the medical research council (MRC) scale, involved iliopsoas and tibialis anterior muscles (4/5 MRC). In the upper limb, deltoid and teres muscles were the weakest (3+/5 MRC), while biceps and triceps were mildly affected (4+/5 MRC). A mild weakness in the orbicularis oris was observed. A needle EMG showed mild myopathic changes in the proximal muscles. CK was mildly elevated at 380 U/L (control range <170 U/L). Acylcarnitine profile showed increased C5-, C8-, C10-, C10:1-, and C14-

carnitines in blood and glutaryl-carnitine in urine. Urine organic acid revealed elevated ethylmalonic acid and presence of tiglylglycine. A muscle biopsy showed a vacuolar myopathy with fiber size variation. No muscle fiber necrosis or regeneration was observed. Oil Red O staining showed lipid storage myopathy. Several cytochrome c oxidase (COX) negative fibers were also observed. Fatty acid oxidation analysis in a fresh muscle biopsy showed a reduced oxidation of C8 and C16 but not of C4. Activity of CI+CIII and CII+CIII complexes were reduced compared to control, whereas citrate synthase activity was augmented. Heart and respiratory evaluations were normal. Lipid storage myopathy was diagnosed and supplementation with riboflavin (100 mg/day) and carnitine (2 g/day) was initiated. At follow up evaluation, 7 months later, the affected individual reported a complete resolution of exercise intolerance and improvement of muscle strength. She was able to walk on her heels, to lift her arm above the horizontal level, and to rise from the floor with minimal support of one hand. Muscle weakness was limited to deltoid, teres, and iliopsoas muscles and graded 4+/5 MRC. Muscle hypotrophy and scapular winging did not improve. An acylcarnitine/free carnitine ratio within control range, and normal respiratory chain and citrate synthase activities were found in a post-treatment muscle biopsy, but with reduced total carnitine (7.6 nM/mg protein; control range: 10.5-29.5 nM/mg protein). Currently, 10 years after initial diagnosis, she is stable and still on riboflavin and carnitine supplementation.

Family 4 (F4): S4a, a girl of consanguineous Turkish parents, presented after term delivery and uneventful neonatal period with increasing muscle hypotonia within the first months of life. Metabolic work up showed moderate elevation of acylcarnitines from C4 - C18:2 without the full pattern of MADD. Urine organic acids revealed variable ketosis and excretion of adipic and suberic acids in early age and moderate ethylmalonic acid and methylsuccinic acid excretion later. Activities of muscle respiratory chain enzymes were reduced, mainly for complexes I, II. Pyruvate dehydrogenase was also reduced. Family history revealed an older sister (affected individual S4b), who also suffered from severe muscle

hypotonia, which was diagnosed in the first year of life after term delivery and uneventful neonatal period. Metabolic follow-up showed either normal acylcarnitines, but also a measurement with an increase of multiple acylcarnitines (C4-OH, C5, C6, C8, C10, C14:1, C16:1). Variable mild ketosis was seen in organic acid evaluation. Both girls presented with severe muscle hypotonia and weakness, and S4b developed massive scoliosis. Tube feeding was necessary to avoid aspiration. Both sisters had reduced speech capacity. S4b was mentally able to finish elementary school training, whereas her sister S4a attended special school due to global developmental delay. Brain MRIs performed at the age of 6 years (S4a) or 16 years (S4b) revealed no abnormalities. Investigations of nerve conduction velocity (NCV) of peripheral nerves (tibial nerve, median nerve) were normal. No hearing or visual impairments were found, however, eyelid ptosis was observed on both eyes. Riboflavin (3x 60 mg daily) was introduced in the treatment of S4a, but could not correct biochemical abnormalities such as acylcarnitine profiles. Clinical improvement of hypotonia was however obvious. The sister was not treated with riboflavin. Unfortunately, S4b died at the age of 16 years after pneumonia and subsequent septicaemia. The family also had one older son, who died at the age of 4 years with similar symptoms and suspicion of mitochondrial disorder as well, but no detailed clinical or biochemical data are available (S4c). There is also one healthy son in this family, who is heterozygous for the *FLAD1* mutation.

Family 5 (F5): Subject S5 is a boy, who was born at 31st week of gestation following a twin pregnancy from Turkish consanguineous parents. He presented with poor sucking and hypotonia in the first few months of life, and was hospitalized due to pulmonary infection at age 6 months. During the hospital course, he developed sudden cardiac arrest few times. Holter monitoring revealed pause and pacemaker was implanted. Multiple respiratory chain deficiency with lipid storage myopathy and faint COX staining was seen in skeletal muscle (Figure S2). Blood analysis revealed increase in C3, C5, C6 and C8:1 acylcarnitine species with a low free carnitine, and urine analysis showed increased amount of adipic acid. He died of multiorgan failure at 7 months of age. Post-mortem examination showed mild

macrovascular steatosis in the liver, acute lung injury, nephrolithiasis and hemorrhagic necrosis of the kidney and spleen. There were no features of cardiomyopathy and COX staining was normal in heart (Figure S2).

Family 6 (F6): Subject S6 is a girl born to Italian non-consanguineous parents at 33 weeks after pregnancy complicated with uterine contractions. The mother had a previous miscarriage at 10 weeks. Soon after birth she presented with hypotonia, poor sucking, frequent vomiting episodes, and gastro-esophageal reflux. She was repeatedly hospitalized because of bronchiolitis and severe respiratory insufficiency. Due to persistent hypotonia with no head control, a suspicion of muscle disease was raised at age 5 months, and the girl underwent muscle biopsy. The spectrophotometric determination of respiratory chain complex activities in the muscle biopsy displayed a severe reduction of complex II and complex II+III activities, with a marked increase of citrate synthase, suggesting mitochondrial proliferation. She was treated with riboflavin, but with bad compliance and without response. She died aged 9 months due to septic shock following pneumonia.

Family 7 (F7): Subject S7, a girl of Turkish consanguineous parents, presented with severe neonatal hypotonia and muscle weakness with elevated plasma CK activity (717 U/L – control range < 240). When she was 2 months old she was hospitalized for swallowing difficulties, requiring nasogastric feeding. Outcome was severe with a sudden respiratory deterioration at 4 months of age, requiring non-invasive ventilation and leading to death. Acylcarnitine profile in plasma revealed increase in C10-, C12-, C14:1-, C16:1-, C16- and C18:1-carnitines and to a lesser extent in C4-, C6-, C8- and C10:1-carnitines. Urine organic acids revealed markedly elevated ethylmalonic and 2-hydroxyglutaric acid. Muscle biopsy revealed major lipid accumulation and a faint SDH staining with normal COX.

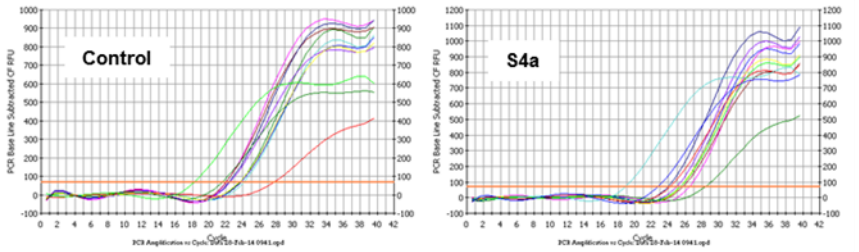
Figure S1. Quantitative Real Time PCR of *FLAD1*

A

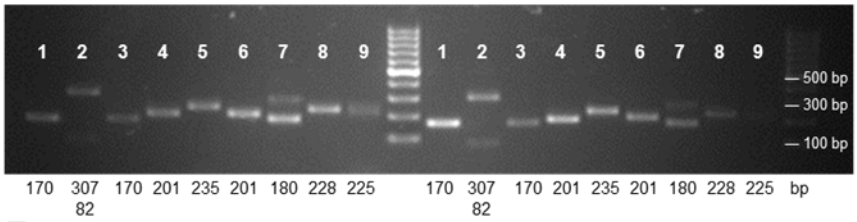
Reaction	Size [bp]	ct control	ct patient	Δct control	Δct patient	ΔΔct control-patient
1-FLAD1	170	23,6	25,4	3,8	4,7	-0,9
2-FLAD1	82 (307)	24,0	26,4	4,2	5,7	-1,5
3-FLAD1	170	23,7	25,6	3,9	4,9	-1,0
4-FLAD1	201	22,0	23,9	2,2	3,2	-1,0
5-FLAD1	235	22,0	24,9	2,2	4,2	-2,0
6-FLAD1	201	22,4	25,3	2,6	4,6	-2,0
7-FLAD1	180	22,5	24,1	2,7	3,4	-0,7
8-FLAD1	228	23,6	25,3	3,8	4,6	-0,8
9-FLAD1	225	27,7	28,3	7,9	7,6	0,3
RPL27	179	18,2	18,8			
HPRT	137	21,5	22,7			

mean ΔΔct
-1,1

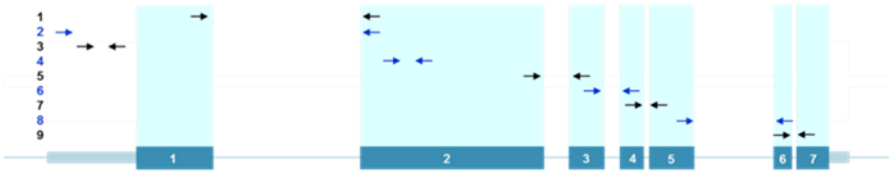
B



C

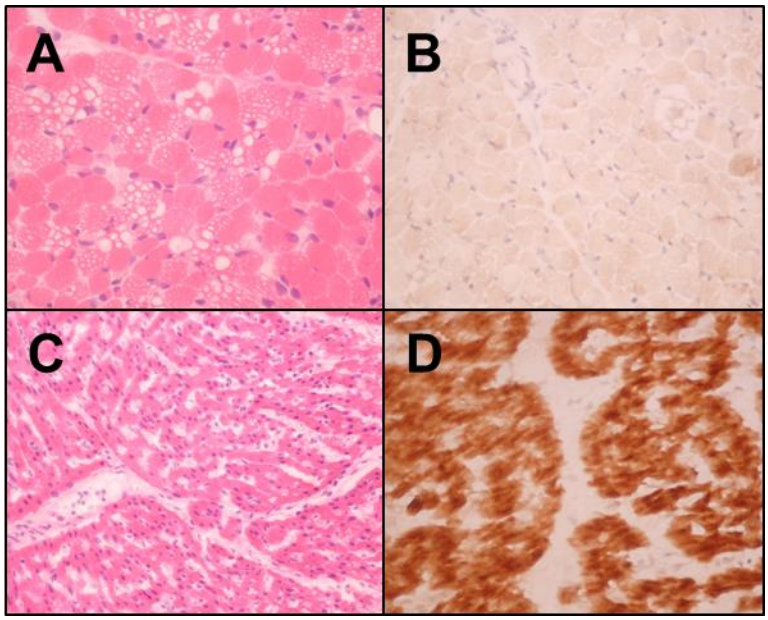


D



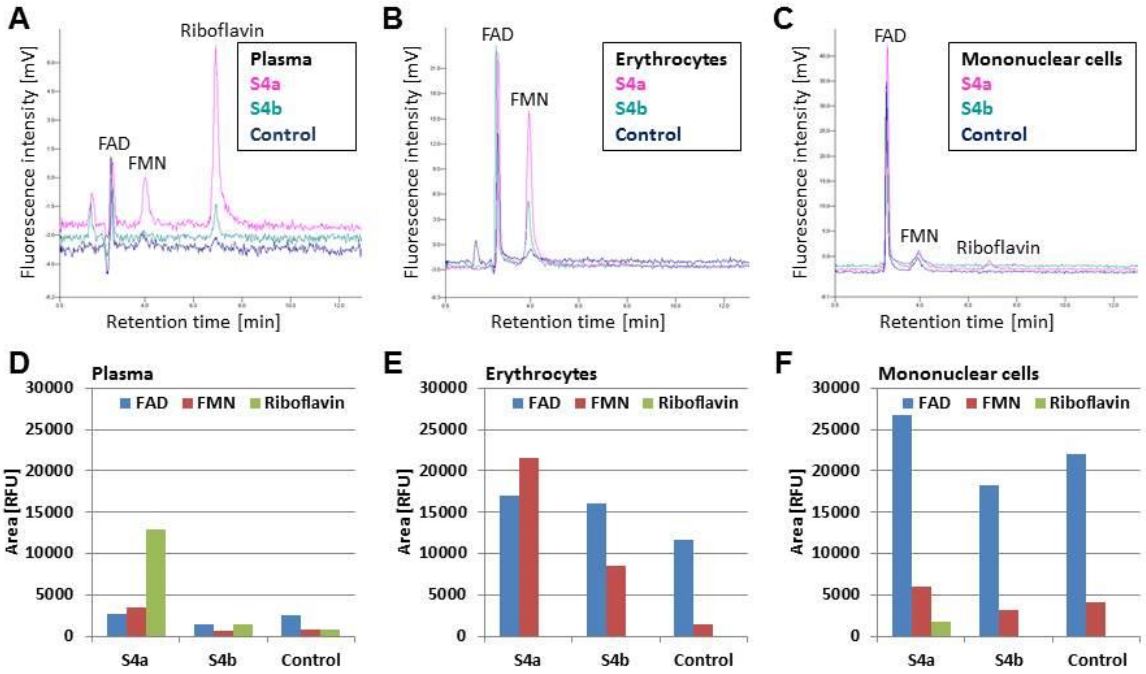
(A) Quantitative real time PCR (qRT-PCR) of fibroblast cDNA from control and affected individual S4a identified reduction of *FLAD1* mRNA in samples from S4a compared to the control. qRT-PCR was analyzed using the comparative CT method. ct is the PCR cycle at which the PCR fluorescent signal crosses the threshold. Δct = ct(FLAD1) – ct(mean of reference genes). ΔΔct = Δct(affected individual)-Δct(control). RPL27 and HPRT are stably expressed reference genes.
(B) All single qRT-PCR products were amplified later in S4a in respect to the control.
(C) qRT-PCR products visualized on the agar gel.
(D) Schematic representation of primer bindings.

Figure S2. Muscle Histology in S5



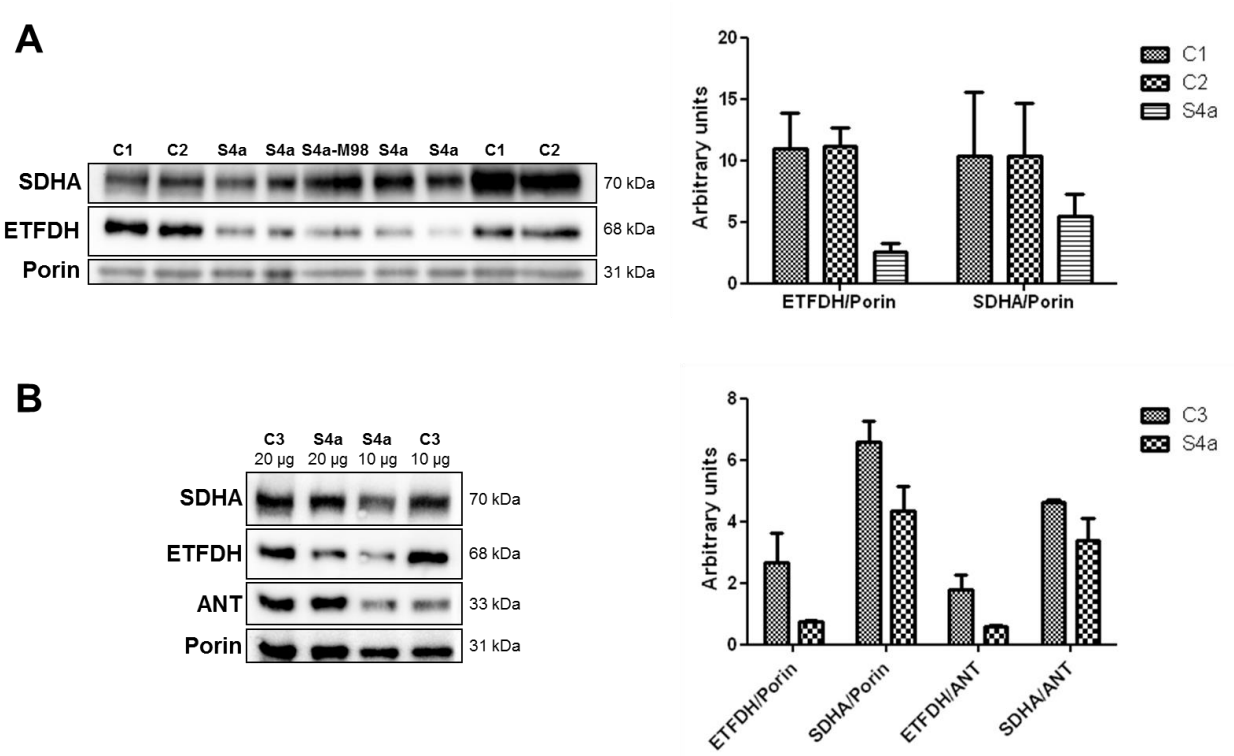
Investigations of muscle histopathology in individual S5. Skeletal muscle biopsy detected vacuolar changes (A) and faint COX staining (B). Heart muscle showed no cardiomyopathy (C) and normal COX staining (D).

Figure S3. Flavin Content in Blood Cells from S4a and S4b



HPLC quantification of riboflavin, FMN and FAD in (A, D) plasma, (B, E) enriched erythrocytes, or (C, F) mononuclear cells from Ficoll gradient centrifugation of EDTA blood derived from a control, subject S4a (under riboflavin treatment) or S4b. Areas in relative fluorescence units (RFU).

Figure S4. Decreased ETFDH and Complex II Protein Levels in Fibroblast Mitochondria from S4a



Two immunoblot experiments, (A) and (B) were performed to measure ETFDH and SDHA protein levels in enriched fibroblast mitochondria from three controls (C1, C2, C3) and four independently grown cells at different passage numbers of subject S4a. Protein extracts (10 µg in A, and 10-20 µg in B) were separated by SDS-PAGE and immunoblotted with antibodies against the proteins indicated to the left of the blots. ETFDH and SDHA protein intensities were quantified relative to porin or adenine nucleotide translocator (ANT). Data are presented as mean of two independent protein extracts (C1 and C2 in A, C3 and S4a in B), or four independent protein extracts (S4a in A). The error bars represent the standard error of the mean. In (A), there are also loaded protein extracts from enriched fibroblast mitochondria of a cell culture of S4a, which was transduced with a lentiviral construct bearing the full length wild-type construct starting at methionine-98 (S4a-M98). However, no normalization of ETFDH and SDHA was seen in these cells, probably due to insufficient *FLAD1* expression or mitochondrial targeting.

Table S1. Sequenced Genes in Subjects S1a, S2, S3, S6 and S7

Gene	Genbank	MIM	S1a	S2	S3	S6	S7
ETFA	NM_000125	608053	+	+	+	+	+
ETFB	NM_001985	130410	+	+	+	+	+
ETFDH	NM_004453	231675	+	+	+	+	+
SLC52A1	NM_017986.3	607883	+	+	-	-	+
SLC52A2	NM_024531	607882	+	+	-	-	+
SLC52A3	NM_033409.3	613350	+	+	-	-	+
RFK	NM_018339.5	613010	+	+	-	-	+
FLAD1	NM_025207.4	610595	+	+	+	+	+
ACADM	NM_000016.5	607008	-	-	-	-	+
ACADS	NM_000017.3	606885	-	-	-	-	+
ACADVL	NM_000018.3	609575	-	-	-	-	+
CPT1a	NM_001876.3	600528	-	-	-	-	+
CPT2	NM_000098.2	600650	-	-	-	-	+
HADHA	NM_000182.4	600890	-	-	-	-	+
HADHB	NM_000183.2	143450	-	-	-	-	+
SLC22A5	NM_001308122.1	603377	-	-	-	-	+
SLC25A20	NM_000387.5	613698	-	-	-	-	+
SLC25A32	NM_0300780	6108156	-	-	-	-	+

Table S2. Mass Spectrometry Detection of Peptides from Human FADS (Uniprot codes Q8NFF5|FAD1_HUMAN)

Peptide Analysis no.	FADS Domain Location	Peptide Amino Acid Sequence	Precursor	Transitions	m/z of Product Ions
P1	MPTb	VSVVPDEVATIAAEVTSFSNR	1096.0657++	A - y10+	0.1877
				A - y9+	0.015
				T - y6+	12.51
				S - y5+	0.057
				P - y17++	903.95
P1-heavy	MPTb	VSVVPDEVATIAAEVTSFSNR-	1101.0698++	A - y10+	1091.54
				A - y9+	1020.5
				T - y6+	721.35
				S - y5+	620.3
				P - y17++	908.95
P2	MPTb	NVYLFPGIPELLR	765.9376++	F - y9+	1041.61
				P - y8+	894.54
				P - y5+	627.38
				Y - b3+	377.18
				I - b8+	904.49
P2-heavy	MPTb	NVYLFPGIPELLR-heavy	770.9417++	F - y9+	1051.62
				P - y8+	904.55
				P - y5+	637.39
				Y - b3+	377.18
				I - b8+	904.49
P3		GLFQNPAVQFHSK	736.8859++	N - y9+	1027.53
				P - y8+	913.49
				Q - y5+	646.33
				F - y4+	518.27
				F - y11++	651.83
P3-heavy		GLFQNPAVQFHSK-heavy	740.8930++	N - y9+	1035.55
				P - y8+	921.5
				Q - y5+	654.34
				F - y4+	526.29
				F - y11++	655.84
P4		LGLGSYPDWGSNYYQVK	973.9678++	P - y11+	1356.62
				W - y9+	1144.54
				G - y8+	958.46
				P - y11++	678.81
P4-heavy		LGLGSYPDWGSNYYQVK-heavy	977.9749++	P - y11+	1364.64
				W - y9+	1152.56
				G - y8+	966.48
				P - y11++	682.82
P5	FADS synthase	LPDVPNPLQILYIR	825.9825++	P - y10+	1226.73
				P - y8+	1015.63
				P - y13++	769.44
				P - y10++	613.87
				V - b4+	425.24
P5-heavy	FADS synthase	LPDVPNPLQILYIR-heavy	830.9866++	P - y10+	1236.73
				P - y8+	1025.64
				P - y13++	774.44
				P - y10++	618.87
				V - b4+	425.24

Abbreviations are as follows: MPTb, Molybdopterin binding

Table S3. Plasma Acylcarnitines and Urine Organic Acids in Individuals with *FLAD1* Variants

		S1a (-)	S1b (-)	S1b (+)	S2 (-)	S2 (+)[a]	S2 (+)[b]	S3 (-)	S4a (-)[c]	S4b (-)[c]	S5 (-)	S7 (-)
Acylcarnitines µmol/L (control range)	C4	NR	2.2↑ (<1.7)	UD	0,68 (≤0,68)	0.31 (≤0.68)	0.23 (0.14-0.66)	NR	0.72↑ (<0.56)	0.53 (<0.56)	NR	0.85↑ (<0,6)
	C3	NR	NR	NR	NR	NR	NR	NR	NR	NR	3.89↑ (0.28-2.90)	NR
	C5	elevated↑	NR	NR	0,39 (≤0,64)	0.29 (≤0.64)	0.33 (0.12-0.43)	1.45↑ (<0.85)	0.32 (<0.35)	0.41↑ (<0.35)	1.20↑ (≤0.52)	0.28 (<0.3)
	C5-DC	elevated↑	NR	NR	0,06 (≤0,13)	UD	0.05 (0.04-0.46)	NR	0.22 (<0.23)	0.13 (<0.23)	NR	0.20 (<0,2)
	C6	NR	NR	NR	0,26 (≤0,29)	0.16 (≤0.29)	0.11 (0.03-0.20)	NR	0.34↑ (<0.17)	0.33↑ (<0.17)	2.32↑ (≤0.59)	0.50↑ (<0.4)
	C8	elevated↑	NR	NR	0,45↑ (≤0,19)	0.47↑ (≤0.19)	0.40↑ (0.05-0.21)	1.23↑ (<0.30)	0.63↑ (<0.54)	0.79↑ (<0.54)	NR	0.54↑ (<0.2)
	C10:2	NR	NR	NR	NR	NR	NR	NR	0.08↑ (<0.07)	0.03 (<0.07)	NR	0.10 (<0.25)
	C10:1	NR	NR	NR	0,17↑ (≤0,10)	0.26↑ (≤0.10)	0.13 (0.04-0.19)	0.76↑ (<0.17)	0.23 (<0.27)	0.19 (<0.27)	NR	0.38↑ (<0.2)
	C10	NR	NR	NR	0,76↑ (≤0,39)	1.06↑ (≤0.39)	0.69↑ (0.05-0.27)	1.86↑ (<0.30)	0.67 (<0.80)	0.85↑ (<0.80)	NR	1.70↑ (<0.2)
	C12	NR	NR	NR	0,48 (≤0,59)	0.23 (≤0.59)	0.31↑ (0.05-0.19)	NR	0.16 (<0.19)	0.13 (<0.19)	NR	0.89↑ (<0.1)
	C14:2	NR	NR	NR	NR	NR	0.05↑ (0.01-0.03)	NR	0.10↑ (<0.08)	0.05 (<0.08)	NR	0.15 (<0.15)
	C14:1	NR	NR	NR	0.46 (≤0.75)	0.18 (≤0.75)	0.24↑ (0.04-0.12)	NR	0.44↑ (<0.25)	0.35↑ (<0.25)	NR	0.60↑ (<0.10)
	C14	elevated↑	NR	NR	0.47 (≤0.96)	0.14 (≤0.96)	0.20 (0.07-0.23)	1.49↑ (<1.41)	0.16↑ (<0.09)	0.08 (<0.09)	NR	0.44 (<0.10)
	C16:1	NR	NR	NR	0.54 (≤0.54)	0.09 (≤0.54)	0.16 (0.04-0.18)	NR	0.37↑ (<0.07)	0.20↑ (<0.07)	NR	0.47↑ (<0.10)
	C16	NR	NR	NR	1.65 (0.63-5.27)	0.72 (0.63-5.27)	1.08 (0.58-2.08)	NR	0.23 (<0.24)	0.10 (<0.24)	NR	0.35↑ (<0.20)
	C18:2	NR	NR	NR	0.32 (≤0.85)	0.55 (≤0.85)	0.26↑ (0.04-0.13)	NR	0.16↑ (<0.14)	<0.05 (<0.14)	NR	0.23↑ (<0.12)
	C18:1	NR	NR	NR	2.10 (0.54-3.11)	0.89 (0.54-3.11)	1.36 (0.49-1.78)	NR	0.69↑ (<0.42)	0.42 (<0.42)	NR	0.49↑ (<0.30)
	C18	NR	NR	NR	0.89 (0.55-1.43)	0.55 (0.55-1.43)	0.68 (0.30-1.04)	NR	0.17↑ (<0.13)	0.1 (<0.13)	NR	0.08 (<0.1)
Urine Organic Acids mmol/mol Creatinine (control range)	ethylmalonic acid	41↑ (<21)	elevated↑	UD	elevated↑	2 (<10)	UD	32↑ (0,1-14,6)	elevated↑	NR	NR	248 (<15)
	adipic acid	elevated↑	NR	NR	NR	NR	NR	NR	elevated↑	NR	elevated↑	<30 (<30)
	glutaric acid	NR	NR	NR	elevated↑	4 (<20)	UD	NR	NR	NR	NR	109 (<50)
	suberic acids	elevated↑	NR	NR	NR	NR	NR	NR	elevated↑	NR	NR	<20 (<20)
	hexanoylglycine	elevated↑	NR	NR	NR	NR	NR	NR	NR	NR	NR	<5 (<5)
	dehydrosebacic acid	elevated↑	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	methylsuccinic acid	NR	elevated↑	UD	NR	NR	NR	NR	NR	NR	NR	<5 (<5)
	lactate	NR	NR	NR	elevated↑	NR	elevated↑	NR	NR	NR	NR	<150 (<150)
	tiglylglycine	NR	NR	NR	NR	NR	NR	elevated↑	NR	NR	NR	<5 (<5)

Abbreviations are as follows: NR, not reported; UD, undetectable, (-), before riboflavin; (+), after riboflavin; ↑, elevated; [a], measurements 1 year after starting treatment; [b], measurements 11 years after starting treatment; [c], C3-DC/C4-OH S4a 0,27(<0,28) S4b 0,55↑(<0,28); C4, butyrylcarnitine; C4-OH, OH-butyrylcarnitine; C5, isovalerylcarnitine; C5-DC, glutarylcarnitine; C6, hexanoylcarnitine; C8, octanoylcarnitine; C10, decanoylcarnitine; C10:1, decenoylcarnitine; C10:2, decadinoylcarnitine; C12, dodecanoylcarnitine; C14, tetradecanoylcarnitine; C14:1, tetradecenoylcarnitine; C14:2, tetradecienoylcarnitine; C16, hexadecanoylcarnitine; C16:1, hexadecenoylcarnitine; C18, octadecanoylcarnitine; C18:1, octadecenoylcarnitine; C18:2, octadedienoylcarnitine.

Table S4. Muscle Respiratory Control for S2 and S4a, Pre-treatment

Substrate	**S2 (control range)**	**S4a (control range)** (nmol/h/mg protein)
Pyruvate + malate	27 (54 ±44)	121 (263-900)
Succinate + rotenone	25 (95 ±54)	NR
Ascorbate + TMPD	266 (828 ±318)	NR
Palmitoyl-CoA	NR	NR
Alpha-ketoglutarate	NR	NR
Pyruvate + carnitine	NR	174 (302-856)
Pyruvate + malate + ADP	NR	39 (32-102)
Pyruvate + malate + CCCP	NR	148 (304-889)
Pyruvate + malate + atractyloside	NR	28 (19-90)
Malate + pyruvate + malonate	NR	114 (282-874)
Malate + acetylcarn. + malonate	NR	148 (273-678)
Malate + acylcarn. + arsenite	NR	98 (156-378)
Glutamate + acylcarn.	NR	43 (86-209)

Abbreviations are as follows: NR, not reported

Table S5. Muscle Respiratory Chain Enzyme Activities for S2[a], S3, S4a, S5 and S6

	S2[a] (control range) [nmol/minmg]	S4a (control range) [mUnit/mg protein]	S5 (control range) (U/UCS)	S6 (control range) [mmol/min/g]
Citrate synthase	2223 (1316±486)	228 (150-338)	175.5 (45.00–100.00)[b]	47.38 (7.80-10.90)
CI	NR	8 (28-76)	UD (0.17-0.56)	26.61 (27.50-39.50)
CI+III	155 (324 ±180)	32 (49-218)	NR	1.39 (0.65-1.50)
CII	77 (181 ±81)	13 (33-102)	NR	0.51 (1.20-2.00)
CII+III	56 (155 ±111)	22 (65-180)	0.03 (0.08–0.45)	0.17 (0.45-0.90)
CIII	NR	238 (304-896)	NR	NR
CIV	1805 (2177 ±808)	169 (181-593)	0.145 (1.10–5.00)	2.40 (1.80-2.45)
CV	NR	68 (86-257)	NR	NR
PDHC	NR	3.6 (5.3-19.8)	NR	NR

Abbreviations are as follows: NR, not reported; UD, undetectable; UCS, unit citrate synthase. [a]measured after treatment. [b]measured in U/gNCP