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Supplemental Data

Mutations in *CYC1*, Encoding Cytochrome c_1 Subunit of Respiratory Chain Complex III, Cause Insulin-Responsive Hyperglycemia

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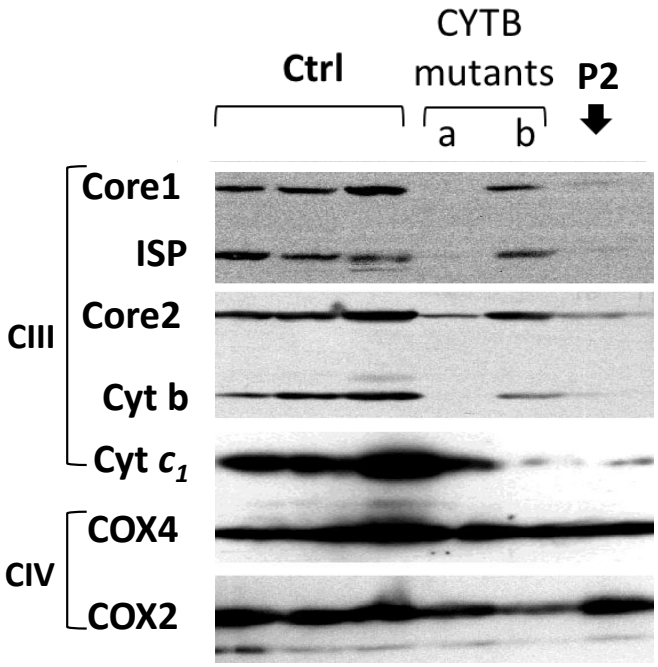

Figure S1. Complex III Composition Studied on P2 Muscle Homogenates

Western blot analyses performed at the time of initial evaluation showed a global decrease of all tested complex III subunits coexisting with normal amounts of complex IV subunits. For the sake of comparison data from two patients harbouring *CYTB* mutations (nonsense mutation, lane a, or missense mutation, lane b are presented. P2: Patient 2 with *CYT1* mutation.

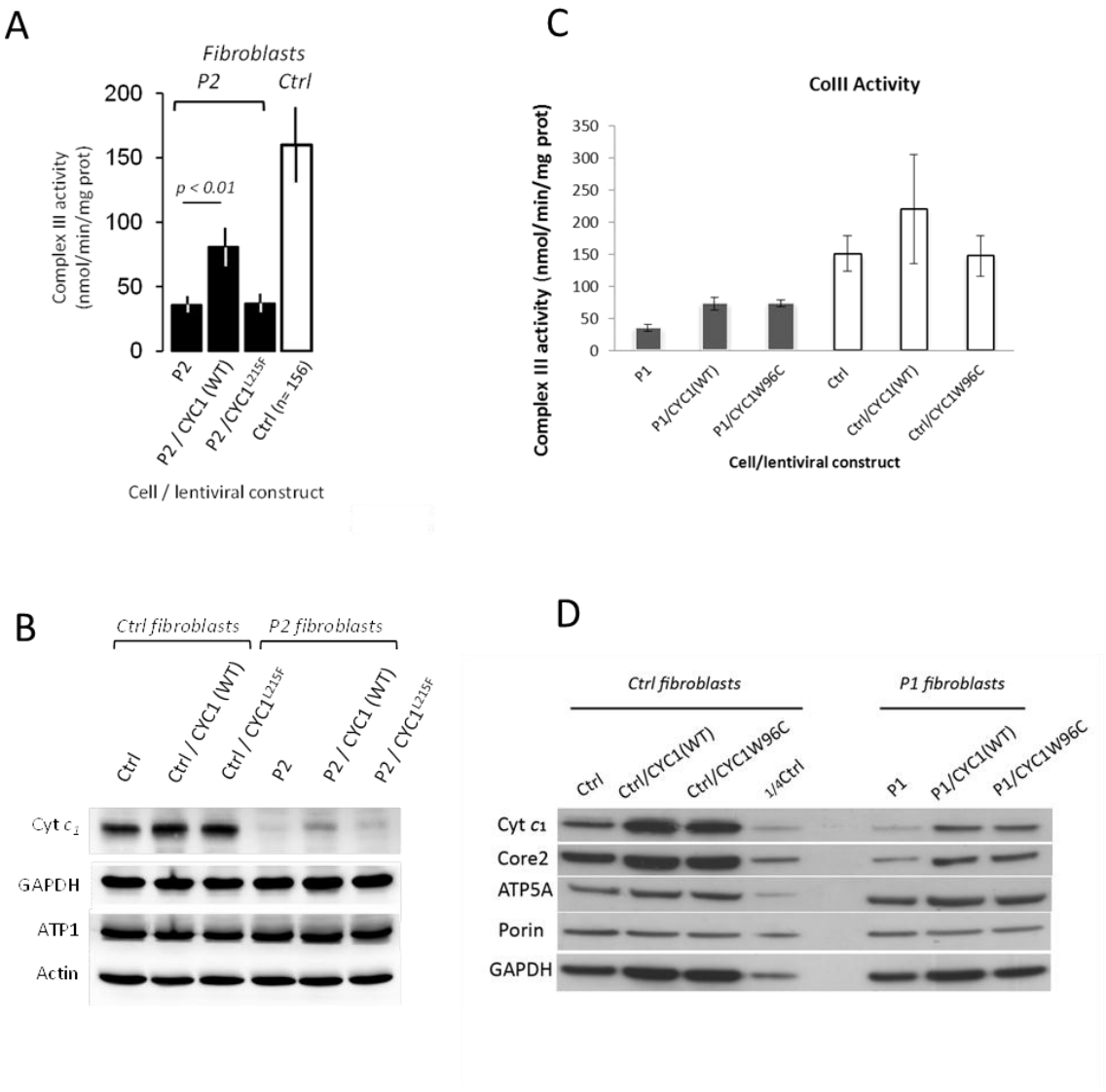

Figure S2. Lentiviral Rescue of Patient Fibroblasts

Control or patient fibroblasts were transduced with lentivirus expressing wild-type (WT) or corresponding mutant (L125F or W96C) CYC1 cDNA, and harvested after growth in selection media for RC enzyme and Western blot analyses. Mitochondrial RC activity (A) and Western blot (B) analyses in lentiviral-infected P2 fibroblasts under standard infection conditions (MOI=5). Human wild-type Cyt1 (Cyt1-WT) and mutant P2 Cyt1 (Cyt1-Mut L215F) cDNAs were synthesized and subcloned into a lentiviral expression vector (AMS Biotechnology (Europe) Limited, UK) as a C-terminal 6HIS-Tag fusion protein under the control of a CMV promoter. Lentiviral particles were produced by co-transfection with packaging vectors allowing expression of gag, pol, rev and VSVG (HT-pack, AMS Biotechnology, UK) in 293T cells. Primary skin fibroblasts from P2 were plated in 10 cm culture dishes at a cell density of 10^6 and infected by lentiviral constructs using a MOI=5. After incubation overnight at 37°C in 5% CO2, transduction media were changed to complete growth media

without antibiotic. After 24h incubation, Blasticidin (Life Technologies) was added in complete culture media to a final concentration of 2 μg/ml. Transduced clones were selected for 2 weeks and were then expanded in complete growth medium with antibiotic for analyses. Western blot conditions as in Figure 2. Mitochondrial RC activity (C) and Western blot (D) analyses in lentiviral-infected P1 fibroblasts using high infection conditions (MOI=120). Mitochondrial CIII activity (antimycin 278 sensitive duroquinol cytochrome *c* reductase) in patient P1 fibroblasts was ~25% of levels in control fibroblasts. Transduction of P1 fibroblasts with either WT or mutant W96C CYC1 partially rescued levels of CIII to ~50% of control levels. Transduction of Control fibroblasts with WT or mutant W96C CYC1 slightly increased CIII activity. Activity of RC mirrors results from Western blotting (D). Whole cell lysate (35 μg) from fibroblasts was separated by SDS-PAGE on a 4-13% polyacrylamide gel. Transduction of either WT or W96C CYC1 results in increased expression of CYC1 in all cell lines, with accompanying increase in Core 2 (CIII), ATP5A (Complex V) and GAPDH, with no effect on mitochondrial protein content inferred by Porin. Overexpression of either the WT or W96C CYC1 induced comparable increases in CIII Core 2 subunit, consistent with yeast studies (Figure 2), where growth of CYC1 null yeast was also rescued by overexpression of either WT or W96C CYC1.

Table S1. Respiratory Chain Enzyme Activities in Skeletal Muscle Homogenates, Liver Homogenates and Skin Fibroblast Mitochondria from Patients and Controls

Patient 1	CI*	CII	CIII	CIV
Skeletal muscle	292	578	**57**	40
Liver	**185**	2590	**11**	25
Fibroblasts	n.t.	n.t.	**42**	n.t
Controls				
Skeletal muscle	318 (222-474)	352 (280-382)	236 (72-402)	51 (39-59)
Liver	345 (267-429)	2226 (1790-2790)	279 (169-380)	25 (17-33)
Fibroblasts	-	-	167±16	-
Patient 2	**CI**	**CII**	**CIII**	**CIV**
Skeletal muscle	24	22	**58**	69
Fibroblasts	n.t.	26	**11**	41
Controls				
Skeletal muscle	24±13	31±9	128±31	69±24
Fibroblasts	-	23±9	45±14	43±13

* For patient 1, data are expressed as a ratio relative to citrate synthase activity and control data as mean *plus* observed normal range between brackets. For patient 2, enzyme data are expressed as nmol/min per mg protein with control data as mean ± SD. Bold characters indicate abnormal values. CI, NADH quinone reductase; CII, succinate quinone dichlorophenol indophenol reductase; CIII, ubiquinol cytochrome *c* reductase; CIV, cytochrome *c* oxidase; n.t., not tested.

Table S2. Patients' Clinical and Biochemical Features

	Patient 1	Patient 2
Age	3y	2.5y
Gestational age / Birth parameters	Preterm / BW 2300 g /BL 42 cm/ BOFC 32 cm	Term / BW 2110 g / BL 45 cm / BOFC 34 cm
Clinical signs	Vomiting Encephalopathy Dehydration Polypnea	Vomiting Coma Dehydration Polypnea
Blood Glucose (mM)	30	15.1
pH / $HCO3^-$ (mM)	7.04 / 4	7.16 / 4
Ketonuria	++++	++++
GOT/GPT (IU/L)	37/42	60/75
PT (sec) / V factor (%)	20 then 8/not measured	18 / 39
Blood ammonia (µM)	260	172
Blood lactate (mM) / pyruvate (mM) / L/P	12.6 / 0.19 / 66	10.6 / 0.41 / 27
Blood alanine (µM) / proline (µM)	2070/not measured	3650/1300
Urine organic acids and acylglycines	Abnormal*	

*lactate gross increase, 2-hydroxybutyrate gross increase, 2-hydroxyisovalerate slight increase, 3-hydroxybutyrate and acetoacetate gross increase, 3-hydroxyisobutyrate slight increase, hexanedioate moderate increase, octanedioate slight increase, 3-hydroxydecanedioate and 3-hydroxydodecanedioate moderate increase, 3-hydroxyhexanedioate lactone slight increase, 3-hydroxyisovalerate and 2-ethylhydracrylate slight increase, 3-hydroxyvalerate gross increase, 3-hydroxypropionate slight increase, methylcitrate not increased, propionylglycine not detected, fumarate gross increase, succinate and 2-oxoglutarate not increased. BOFC, birth occipito frontal circumference; BW, birth weight.

Table S3. Yeast Strain Characteristics and NADH-Cytochrome c Reductase Activity

Strain	Genotype	Mutation	Copy	NADH-cytochrome c reductase (μmoles/min/mg protein)
W303-1A	*yCYC1*	None	Single	1.26 ± 0.01
aW303Δ-yCYC1	*y-cyc1Δ*	NA	None	0.08 ± 0.01[1]
aW303Δ-yCYC1/ST12	*yCYC1*	None	Single	0.79 ± 0.04
aW303Δ-yCYC1/ST13	*y-cyc1*	L195F	Single	0.06± 0.00
aW303Δ-yCYC1/ST15	*y-cyc1*	L195F	Multiple	0.58 ± 0.01
aW303Δ-yCYC1/ST16	*y-cyc1*	W76C	Single	0.06 ± 0.00
aW303Δ-yCYC1/ST17	*y-cyc1*	W76C	Multiple	0.41 ± 0.01

Mitochondria at a protein concentration of 10 mg/ml were permeabilized with 0.1% lauryl maltoside. Reduction of cytochrome *c* was measured at 24^{o}C by addition of 1 μmole of NADH to 1 ml of reaction buffer containing 10 mM potassium phosphate, pH 7.5, 0.8 mg oxidized horse heart cytochrome c, 5x10^{-4}M KCN and 50 μg mitochondrial protein. [1]The low rate of cytochrome *c* reduction in the null mutant was completely insensitive to 1 μM antimycin A. All the other activities have been corrected for the antimycin-insensitive rate. The values reported are averages of duplicate assays with the ranges indicated.