Supporting Information

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ID	M/F	Age (yrs)	Clinical Presentation	Biochemical Features	Molecular Diagnosis
Cohort 1					
1018	M	57	Chronic progressive external ophthalmoplegia and myopathy	c. I,III,IV, RRF, SDH+/COX- (muscle)	Large mtDNA deletion
1024	F	51	Stroke, migraine, SNHL and expressive aphasia	Elevated CK	mt A3243G
1031	F	46	Exercise intolerance, dystonia	c. IV (muscle)	
1033	F	44	TIA, complex migraine, Crohn's disease, hearing loss, muscle weakness and cramps, exercise intolerance	c. III (muscle)	
1043	M	42	Myopathy, chronic fatigue, exercise intolerance and hemochromatosis	c. III,IV (muscle)	
1046	F	46	Myopathy, neuropathic pain, exercise intolerance and stroke	c. I (muscle), elevated CK	
1047	M	54	Exercise intolerance, myopathy, myoclonus, neuropathy, diabetes, dysphagia, lipomatosis and SNHL	Lactic acidemia	mt A8344G
1050	F	35	Neurodegenerative encephalopathy with spasticity and involuntary movements	c. I (muscle), lactic acidemia, elevated CK	
1064	M	22	Seizures, myoclonus, cardiomyopathy, mild dementia	Lactic acidemia	mt A3243G
1070	F	17	Headaches, GI dysmotility, hypotonia, muscle cramps	c. I (muscle)	
1072	M	51	Myopathy, exercise intolerance, myalgias with fasciculations, SNHL, complex migraine and atrial fibrillation	c. I,III,IV (muscle), elevated CK	mtDNA depletion
1073	M	29	Myopathy, chronic headache, sleep apnea, exercise intolerance, GI dysmotility	c. I,III (muscle)	
1075	F	25	Muscle cramps, exercise intolerance, TIA and complex migraine	Lactic acidemia	mt A3243G
1079	M	62	Encephalomyopathy	c. I,II,III,IV (muscle)	Multiple mtDNA deletions
1080	F	52	Cerebellar ataxia, dysarthria, peripheral neuropathy and SNHL	c. I,II,IV (muscle)	
1086	F	54	Exercise intolerance, GI dysmotility		mt A3243G
Cohort 2					
1058	M	53	Myopathy, exercise intolerance, sleep disorder, cardiomyopathy, GI dysmotility	c. II (muscle)	
1062	F	12	Developmental delay, language delay, learning delay, dystonia, seizure, exercise intolerance, temperature intolerance, precocious puberty	c. III (muscle)	
1074	F	48	Myoclonic seizures,dystonia, exercise intolerance, arrhythmia, GI dysmotility, myocardial infarction, affective disorder, diabetes mellitus	c. I,III,IV, SDH+/COX- (muscle)	
1088	M	24	Infantile spasms, seizure disorder, autonomic dysfunction, ataxic movements, abnormal motor movements		mt 8993
1093	F	66	Exercise intolerance, myopathy, affective disorder, GI dysmotilty, overweight	CoQ10 deficiency, c. II (muscle)	
1113	M	9	Seizure disorder,malignant hyperthermia, developmental delay, developmental regression	c. I (muscle)	
1137	F	45	Exercise intolerance, tremors, myoclonus, migraines, affective disorder, panic attacks	c. III,IV (muscle)	
1139	M	17	Hypertrophic cardiomyopathy, myopathy, diabetes mellitus, headache	c. IV (muscle), elevated CK	
1140	F	30	Exercise intolerance, dypsnea on exertion, stomach pain, bursitis both knees, carpal tunnel right hand	c. I,II,III (muscle), exercise test: elevated CvO2max	
1153	M	18	Muscle cramping, recurrent sinus infections/otitis media, hearing changes noted, GI dysmotility, sleep disorder, migraines	c. I,III (muscle)	
1154	M	7	Hypotonia, exercise intolerance, dyspnea	c. I,III (muscle)	
1157	M	11	Exercise intolerance, temperature intolerance, pervasive developmental disorder, nonverbal, overweight	c. I,II,IV, CoQ10 deficiency (muscle)	
1163	F	29	Myopathy, exercise intolerance, opthalmoplegia, visual failure, sensory cerebellar ataxia, seizures, headaches, palpitations, heat intolerance, hyperhydrosis		POLG1 A467T (homozygous)
1167	F	65	leg cramps, fatigue, bi-lateral hearing loss, retinitis pigmentosa, tremor right hand (Parkinsonism), GI reflux, dysarthria, migraines	c. I (muscle)	

Table S1. Demographic, clinical, biochemical and molecular characteristics of RCD patients in two cohorts. Abbreviations: M/F, Male/Female; CK, creatine kinase (serum); CvO2max, Venous oxygen content at maximal exercise; GI, Gastrointestinal; RRF, ragged-red fibers; SDH+/COX-, succinate dehydrogenase positive / cytochrome C oxidase negative; SNHL, sensorineural hearing loss; TIA, transient ischemic attack.

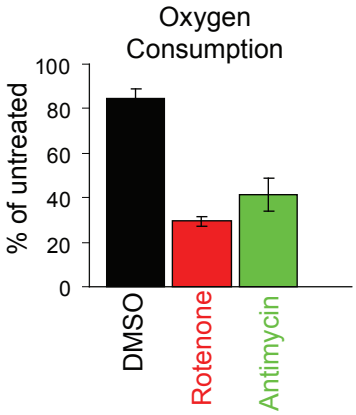

Figure S1. Decrease in oxygen consumption following respiratory chain inhibition with rotenone and antimycin. Oxygen consumption was measured minutes before and after addition of inhibitors. Data presented as mean +/− s.d. (n=3).

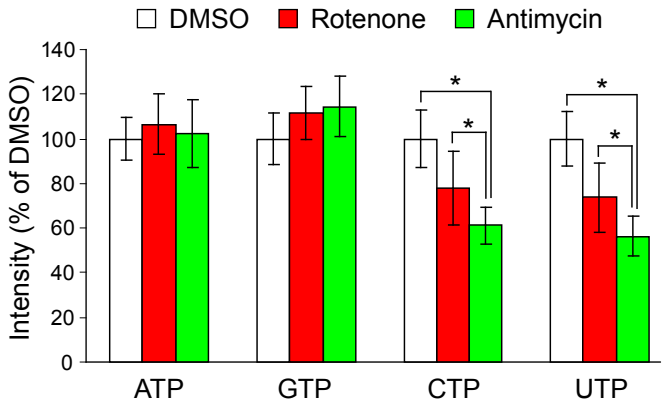

Figure S2. Intracellular levels of nucleotides in myotubes treated with RC inhibitors. Levels of purine and pyrimidine nucleotides were measured in cell extract following 8 hours of inhibition with rotenone or antimycin, and compared to treatment with vehicle (DMSO). Data is presented as mean +/- s.d. over 8 biological replicates per condition. *denotes p-value < 0.05. Abbreviations: ATP, adenine triphosphate; GTP, guanine triphosphate; CTP, cytidine triphosphate; UTP, uridine triphosphate.

Materials and Methods

LC-MS/MS Measurement of Plasma Metabolites in Cohort 2

Measurement of plasma metabolites in Cohort 2 was done using LC-MS/MS with the same equipment as in Cohort 1 (Materials and Methods) but different liquid chromatography methods. A hydrophobic interaction chromatography (HILIC) method was used to profile metabolites in the positive ion mode (e.g. amino acids, biogenic amines) and modification of the ion paring chromatography (IPC) method described by Luo et al (1) was used to profile metabolites is the negative ion mode (e.g. organic acids). Plasma samples (10 μL) were extracted for the HILIC method using nine volumes of acetonitrile/methanol (75:25, v/v) and a separate plasma aliquot (150 μL) was extracted with 80% methanol and reconstituted in water for the IPC method. HILIC separations were achieved using an Atlantis HILIC column (150 x 2.1 mm; Waters, Milford, MA) that was eluted at 250 μL/min with a 10 min linear gradient, initiated with 95% mobile phase B (acetonitrile with 0.1% formic acid, v/v) and concluding with 60% mobile phase A (10 mM ammonium formate and 0.1% formic acid, v/v). The modified IPC method was performed using an Atlantis T3 column (150 x 2.1 mm; Waters, Milford, MA). IPC mobile phases were 10 mM tributylamine/15 mM acetic acid (mobile phase A) and methanol (mobile phase B), and the column was eluted at a flow rate of 300 μL/min using the following program: 100% mobile phase A at initiation, 100% A at 4.0 min, 2% A at 34.0 min, and held at 2% mobile phase A to 39.0 min. Multiple reaction monitoring (MRM) was used to acquire MS data for targeted metabolites. The scheduled MRM algorithm in the Analyst 1.5 software (Applied Biosystems/Sciex; Foster City, CA) was used to automatically set dwell times for each transition.

Proton NMR Measurement of Intracellular Creatine and Phosphocreatine

Quantitative NMR analyses of cellular metabolites were performed on a Varian ^{UNITY}Inova 700 MHz NMR (Palo Alto, CA) as described before (2). The 1D proton NMR spectra were acquired using the WET (Water suppression Enhanced through T1 effects) pulse sequence. The 1D proton NMR covered a spectral window of -2.37 ppm to 11.91

ppm. The acquisition time was set to 3 seconds, corresponding to a digital resolution of 0.33 Hz. The delay between acquisitions was set to 15 seconds. The selective excitation profile in WET was based on a sinc wave function of 50 Hz coverage around the HDO peak. Each NMR spectrum was collected from accumulation of 512 scans. Identification of endogenous metabolites and calculation of their quantitative levels were carried out using dataChord (One Moon Scientific, Inc., Newark, NJ) based on a 600-compound reference library. Metabolite quantification was based on 2.0 mM internal reference standard DSS-d_6.

References

1. Luo, B., Groenke, K., Takors, R., Wandrey, C., & Oldiges, M. (2007) Simultaneous determination of multiple intracellular metabolites in glycolysis, pentose phosphate pathway and tricarboxylic acid cycle by liquid chromatography-mass spectrometry. *J Chromatogr A* 1147:153-164.
2. Xu, E. Y., *et al.* (2008) Integrated pathway analysis of rat urine metabolic profiles and kidney transcriptomic profiles to elucidate the systems toxicology of model nephrotoxicants. *Chem Res Toxicol* 21:1548-1561.