Rescue of primary ubiquinone deficiency due to a novel COQ7 defect using 2,4–dihydroxybensoic acid

Christoph Freyer[1,2], Henrik Stranneheim[1,3], Karin Naess[1,4], Arnaud Mourier[5], Andrea Felser[4], Camilla Maffezzini[2], Nicole Lesko[1,4], Helene Bruhn[1,4], Martin Engvall[1,6], Rolf Wibom[1,4], Michela Barbaro[1,6], Yvonne Hinze[5], Måns Magnusson[3], Robin Andeer[3], Rolf H Zetterström[1,6], Ulrika von Döbeln[1,4], Anna Wredenberg[1,2], Anna Wedell[1,2,3]

Supplemental information:

Materials and Methods

Biochemistry

Mitochondrial ATP production rate (MAPR) in skeletal muscle samples and respiratory chain enzyme activities were determined as previously described[1]. Mitochondria were prepared from fresh biopsies of the Tibialis anterior muscle and either used directly for MAPR measurements, or snap frozen for respiratory chain enzyme activities. Mitochondrial oxygen consumption measurements of fibroblasts were performed on permeabilised cells, using glutamate, malate, pyruvate (GMP), ADP, with and without succinate for complex I+II or I respiration, using an Oroboros oxygraph. Maximal respiration was measured by titration of mitochondrial uncoupler CCCP.

Sanger sequencing

Sanger sequencing was performed on genomic DNA extracted from blood from the patient, unaffected sibling and both parents, using COQ7-f (tgtaaaacgacggccagtcgatgtctggtgcagagg) and COQ7-r (caggaaacagctatgacctgaggcactgacctgagc) M13-tagged (underlined) primers to cover exon 4 of COQ7 with the BigDye version 3.1 sequencing kit (LifeTechnologies). Sequences were analysed on a 3130xl capillary sequencer (LifeTechnologies) and aligned to reference sequence NM_016138.

Next generation sequencing and MIP analysis

Patient and parents were sequenced to an average 61-101-fold coverage of the exome (Supplementary Table 1), with sufficient coverage at 95.7% of the bases according to our cut-off limits. 120267 variants were called after alignment to GRCh37/hg19. 86% (103082 variants) were single nucleotide variants (SNV) and 14% (17185 variants) were indels. Sequencing data from exome-enriched libraries were aligned, variant called, annotated, scored and ranked using MIP[2 3]. MIP aligned the sequence data using MOSAIK[4] and called variants with GATK[5 6]. MIP annotates the variants with information from external and in-house databases as well as the genetic inheritance pattern (including compound mutations). A weighted sum model based on a subset of these annotations was applied to generate a rank score for each variant, with most weight applied on the inheritance pattern, minor allele frequency, gene coding annotation, functional annotation and protein predictions. Thus, MIP retains all variants but priorities them according to disease-causing potential based on the rank score. In the top 20 scored variants, only the c.422T>A transition in CoQ7 assumed homozygosity and a mitochondrial association.

Measuring absolute ubiquinone levels

Ubiquinone quantification was adopted from previously described methods[7 8]. Protein content of all samples was measured by standard Bradford analysis (BioRad). Quinones were extracted by suspending pellets of isolated human mitochondria or fibroblasts in 200µL of cupric sulfate 1mM, followed by addition of 200µL ethanol. After sonication, the homogenate was vortexed in the presence of 400µL hexane. The hexane-soluble upper fraction was collected and dried, using a SpeedVac apparatus, followed by resuspension in 50-200µL ethanol/methanol (9:1), vortexed, incubated for 2min in an ultrasonication bath and filtered through a 0.2 µm modified nylon centrifugal filter (VWR) by centrifugation at 6°C with 21000xg. Absolute CoQ10 concentrations were measured in positive ESI MRM mode (multi reaction monitoring), using an Acquity UPLC (Waters) connected to a XevoTM TQ (Waters). An Acquity UPLC BEH C18 1.7µm, 2.1 x 50mm column was used at 40°C. Solvent A was 90% methanol + 10% propanol + 0.1% FA, and solvent B was 45% acetonitril/acetone + 10%

propanol + 0.1% FA. A linear gradient of solvent A, ranging from 100% to 0% in 3.5min at a flow rate of 0.45ml/min was used. 2-6µL of sample was injected, either at 6°C for standards or directly after thawing for samples. Source temperature was set to 150°C, desolvation temperature was 650°C and desolvation gas was set at 800l/h and cone gas at 50l/h. MRM transitions were: CoQ10 m/z 863.67 to 196.94 (quantifier) collision energy 36V, m/z 863.67 to 95.02 (qualifier) collision 54V, m/z 863.67 to 80.97 (qualifier) collision 66V, cone was in all cases 35V. Data management was performed by MassLynx (Waters) and data evaluation and absolute quantification was done with TargetLynx (Waters). All compounds were freshly prepared daily and dissolved in ethanol/methanol (9:1). CoQ10 standards (2, 5, 10, 25, 50, 200, 300, 500, 700, 1000ng/mL) were prepared from 100µg/ml stock solutions.

Rescue of patient fibroblasts

Human COQ7 cDNA was cloned into pIRES2-eGFP and empty vector, or pIRES2-eGFP-hCOQ7 was transfected into $2x10^6$ cells, using the P2 transfection kit and a nucleofector 4D (Lonza). After 3 days $1x10^6$ cells were used for oxygen consumption measurements on an oroboros oxygraph as described. Transfection efficiency was controlled by visualizing eGFP co-expression from the same vector.

References

1 Wibom R, Hagenfeldt L, Döbeln von U. Measurement of ATP production and respiratory chain enzyme activities in mitochondria isolated from small muscle biopsy samples. *Anal Biochem* 2002;**311**:139–51.

2 Stranneheim H. Mutation Identification Pipeline (MIP), https://github.com/henrikstranneheim/MIP. DOI:10.5281/zenodo.10023. 2014. doi:10.5281/zenodo.10023

3 Stranneheim H, Engvall M, Naess K, Lesko N, Larsson P, Dahlberg M, Andeer R, Wredenberg A, Freyer C, Barbaro M, Bruhn H, Emahazion T, Magnusson M, Wibom R, Zetterström RH, Wirta V, Döbeln von U, Wedell A. Rapid pulsed whole genome sequencing for comprehensive acute diagnostics of inborn errors of metabolism. *BMC Genomics* 2014;**15**:1090.

4 Lee W-P, Stromberg MP, Ward A, Stewart C, Garrison EP, Marth GT. MOSAIK: a hash-based algorithm for accurate next-generation sequencing short-read mapping. *PLoS ONE* 2014;**9**:e90581.

5 DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, McKenna A, Fennell TJ, Kernytsky AM, Sivachenko AY, Cibulskis K, Gabriel S, Altshuler D, Daly MJ. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* 2011;**43**:491–8.

6 McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research* 2010;**20**:1297–303.

7 Jonassen T, Larsen PL, Clarke CF. A dietary source of coenzyme Q is essential for growth of long-lived Caenorhabditis elegans clk-1 mutants. *Proc Natl Acad Sci USA* 2001;**98**:421–6.

8 Rodríguez-Acuña R, Brenne E, Lacoste F. Determination of coenzyme Q10 and Q9 in vegetable oils. *J Agric Food Chem* 2008;**56**:6241–5.

Supplemental table 1: WES report. For exome sequencing, 2-5µg of genomic DNA from patient and both parents were used to prepare paired-end libraries, using TruSeq chemistry according to standard protocols (Illumina Inc.). Exome-enriched libraries were made using the in-solution Nimblegen SeqCap EZ Exome Library (Roche Nimblegen Inc., Madison, WI), Agilent SureSelect version 5 (Agilent) or standard protocols supplied by Illumina and sequenced on a HiSeq 1000, 2000, 2500 sequencing system (Illumina Inc). Coverage and sequencing data is shown (Ts: transitions, Tv: transversion).

Metric	Patient	Parent 1	Parent 2
Uniquely aligned reads (%)	93	91	92
Duplicated reads (%)	56	61	58
Mean target coverage	101	61	77
target bases at 30x (%)	87	72	80
Number of evaluated exonic variants	21436	21742	21389
dbSNP129 concordant rate (%)	99.76	99.76	99.75
Ts:Tv ratio	3.07	3.07	3.04