**The American Journal of Human Genetics, Volume *102***

## Supplemental Data

# Bi-allelic Mutations in the Mitochondrial Ribosomal Protein MRPS2 Cause Sensorineural Hearing Loss, Hypoglycemia, and Multiple OXPHOS Complex Deficiencies

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**Supplemental Material and Methods**

**Whole exome sequencing**

Whole-exome sequencing (WES) was performed on genomic DNA extracted from patient lymphocytes using standard salting out methods on two different platforms. Details on exome capture and sequencing are available upon request. Potential pathogenicity of the identified genetic variations was evaluated using literature, evolutionary conservation, SIFT (http://sift.jcvi.org) and PolyPhen 2 (http://genetics.bwh.harvard.edu/pph2/). Earlier reporting of identified genetic variations was checked using data from our in house database and the Genome Aggregation Consortium, gnomAD (Cambridge, MA; http://gnomad.broadinstitue.org). All databases were accessed July 2017. Sequence validation and segregation analysis of the candidate variants were performed with Sanger sequencing (reference sequences GenBank: M_016034.3]). Variant nomenclature adheres to the Human Genome Variation Society (HGVS) guidelines.

**Northern blot analysis**

RNA for Northern blot analysis was extracted from cells grown in 25cm$^2$ flasks using Trizol (Thermo Fisher Scientific, Courtaboeuf, France). 1 μg RNA was fractionated through 1.2% Agarose gel and transferred onto a Nylon membrane (Merck Millipore, Fontenay sous Bois, France) using capillary transfer. After UV crosslinking, the membrane was blocked at 55$^o$C with blocking buffer (Sigma Aldrich, Lyon, France). To generate specific DNA probes against 12S, 16S and 18S rRNA, we cloned the corresponding PCR-amplified DNA fragments into pCRII-TOPO vector (Thermo Fisher Scientific Courtabeuf, France). Prior to labeling, the DNA constructs were digested with EcoRI restriction enzyme and the fragments were gel-purified. Purified DNA (50 ng) probe was radiolabeled using the Random Primers DNA Labeling System (Thermo Fisher Scientific Courtabeuf, France) and 50 μCi of [ $\alpha$-$^{32}$ P]-dCTP. Membranes were incubated with the probes for at least 2 hours at 55$^o$C followed by washing with 2xSSC-0.1%SDS buffer at room temperature. Membranes were exposed to Hyperfilm MP multipurpose films (GE Healthcare, Succursale, France) at room temperature for at least 1 hr.

**SDS-PAGE and immunodetection**

Samples for SDS-PAGE were prepared either from isolated mitochondria or whole-cell extracts. Mitochondria, isolated as described previously [1], were recentrifuged at 12,000 g for

10 min in a cooled benchtop centrifuge, and pellets were resuspended in SDS PAGE sample loading buffer at concentration of 2 μg/μl. 30 μg were fractionated through 12% Mini-Protean TGX precast gels and transferred onto low fluorescent PVDF membranes (Bio-Rad, Marnes-la Coquette, France). Mitoribosomal proteins were detected using the following specific antisera: anti-MRPS2 (Abcam, Paris, France), anti-MRPS18B (Proteintech Europe, Manchester, UK), anti-MRPS5 (Abcam, Paris, France), anti-MRPS28 (Proteintech Europe, Manchester, UK), anti-MRPL37 (Sigma-Aldrich, Lyon, France) and anti-MRPL44 (Proteintech Europe, Manchester, UK). Anti-SDHA, used as a loading control was from Abcam.

Whole-cell extracts were prepared as described in Analysis of Mitochondrial Translation. 20 μg were fractionated as above, transferred on PVDF membranes and probed with some of the specific antisera mentioned above and in addition: anti-NDUFB8 (Abcam, Paris, France), NDUFA13 (Abcam, Paris, France), anti-COXIV (Proteintech Europe, Manchester, UK ) and anti-COXI (MTCO1, Abcam, Paris, France).

IR Dye-conjugated, anti-mouse (780 nm) or anti-rabbit (680 nm) secondary antibodies were used at a dilution of 1:5000 and were purchased from Li-Cor Biosciences (Bad Homburg, Germany). Membranes were scanned with Odyssey CLX infrared scanner.

**BN-PAGE and immunodetection**

Isolation of mitochondria and Blue native gradient (5%–15%) gel electrophoresis (BN-PAGE) were performed as described previously by Calvaruso, et al. [2] Lanes were loaded with equal amounts of solubilized (mitochondrial) protein (80 or 120 μg). Gels were blotted to nitrocellulose transfer membranes (Whatman, ‘s-Hertogenbosch, the Netherlands). Membranes were incubated with the following specific antibodies: mouse anti-NDUFS3 (Invitrogen, Leek, the Netherlands), mouse anti-SDHA (Mitosciences, Eugene, USA), mouse anti-UQCRFS1 (Proteintech, Rosemont, USA), mouse anti-COXIV(Mitosciences, Eugene, USA)) and mouse anti-ATP5A (Abcam, Cambridge, UK). Goat anti-(mouse Ig) Ig peroxidase (1:10,000; GAMPO; Invitrogen, Leek, the Netherlands) was used as a secondary antibody for detection. Signal was generated using the ECL Prime Western Blotting reagent (Biosciences, Roosendaal, the Netherlands).

**Analysis of mitochondrial protein synthesis**

In vitro pulse labeling of mitochondrial translation products was performed essentially as described before [3]. Briefly, near-confluent fibroblasts were incubated for 30 minutes at 37 °C

in methionine- and cysteine-free DMEM media (Thermo Fisher Scientific, Courtaboeuf, France) supplemented with dialyzed FBS (Thermo Fisher Scientific, Courtaboeuf, France) and 400 μCi of EasyTag Express35S protein labeling mix (Perkin Elmer SAS, Cortaboeuf, France) in the presence of 100$\mu$g/ml of the cytosolic translation inhibitor emetine. At the end of the pulse, labelling media was substituted with standard DMEM growth media followed by a 10 min incubation during which synthesis of labelled translation products is completed. Cells were washed twice with ice-cold PBS (Thermo Fisher Scientific, Courtaboeuf, France) and harvested by scraping in 1ml PBS. Cells were reisolated by centrifugation at 2000xg for 10min in a cooled centrifuge and resuspended in 50 μl PBS. Bradford assay (Sigma-Aldrich, Lyon, France) was performed on the cell suspensions, which were then centrifuged as before and cell pellets were resuspended in 2x SDS PAGE loading buffer at a concentration of 1μg/μl. 10 μg of each sample were fractionated through a 17% SDS polyacrylamide gel (Protean II XL, Bio-Rad, Marnes-la Coquette, France) for overnight at 110V. The gel was stained with Coomassie blue colloidal dye and incubated for 20 min in Amplify fluorographic reagent (GE Healthcare, Succursale, France) prior to drying. Exposures were for up to 5 days at -80$^{o}$C using Hyperfilm MP multipurpose films (GE Healthcare, Succursale, France).

**Complexome profiling**

In-gel tryptic digestion, mass spectrometry and data analysis were performed as described by Huynen, et al[7]. In summary a mitochondrial enriched fraction of solubilized protein was loaded on a blue native gradient gel. Lanes were sliced into 60 even pieces, proteins were digested with trypsin. Peptides were extracted and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) in a Q-exactive mass spectrometer (Thermo Fisher Scientific, Whalthma, MA, USA). All raw files were analyzed by MaxQuant software (version 1.4.1.2[8]). Unique plus razor peptides were considered for protein quantification. Protein abundance migration profiles were normalized considering multiple profiles, that is, taking into account iBAQ values from all slices. Profiles were hierarchically clustered using Cluster 3.0 software [9,10] by distance measures based on Pearson correlation coefficient (uncentered) and the average linkage method.

**Cloning of human MRPS2 and lentiviral transduction**

Wild type MRPS2 was amplified from cDNA using primers designed according to the recommendations for cloning using the Electra cloning technology (Atum, Basel, Switzerland): Forward primer: 5'- TACACGTACTTAGTCGCTGAAGCTCTTCT**ATGGC**

**GACATCCTCGGCC–3’** and reverse primer: AGGTACGAACTCGATTGACGGCTCTTC TACC**TCACAGGGAATGGCTCATGTCA**-3’; the sequence complementary to the MRPS2 cDNA is indicated in bold) and cloned in a lentiviral vector pD2109-CMV (Atum, Basel, Switzerland) carrying a puromycin resistance gene, which enables the generation of stably transduced cell lines. The corresponding control vector pD2109-CMV-03 expressing GFP was also purchased from Atum. Production of lentiviral particles was performed in HEK293FT cells transfected with either of the above lentiviruses and viral packaging constructs expressing REV, VSV-G and PAX2. Cells were incubated for 72 hours and culture supernatants were collected, filtered through 0.2 μm sterile filters and used for transduction of near confluent control and patient fibroblasts. Puromycin (Thermo Fisher Scientific, Courtaboeuf, France) was added 72 hours after transduction at a final concentration of 3 μg/ml and selection was performed for 2 weeks during which media was changed every two days. Under these conditions, about 10-20% of the initially transduced cells survived the puromycin selection. At the end of this procedure, surviving cells carry stably integrated MRPS2- or GFP-expressing vectors.

**Web Resources**

GenBank, https://www.ncbi.nlm.nih.gov/genbank/

OMIM, http://www.omim.org

MutationTaster, http://www.mutationtaster.org

PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/

SIFT, http://sift.jcvi.org

**Figure S1**

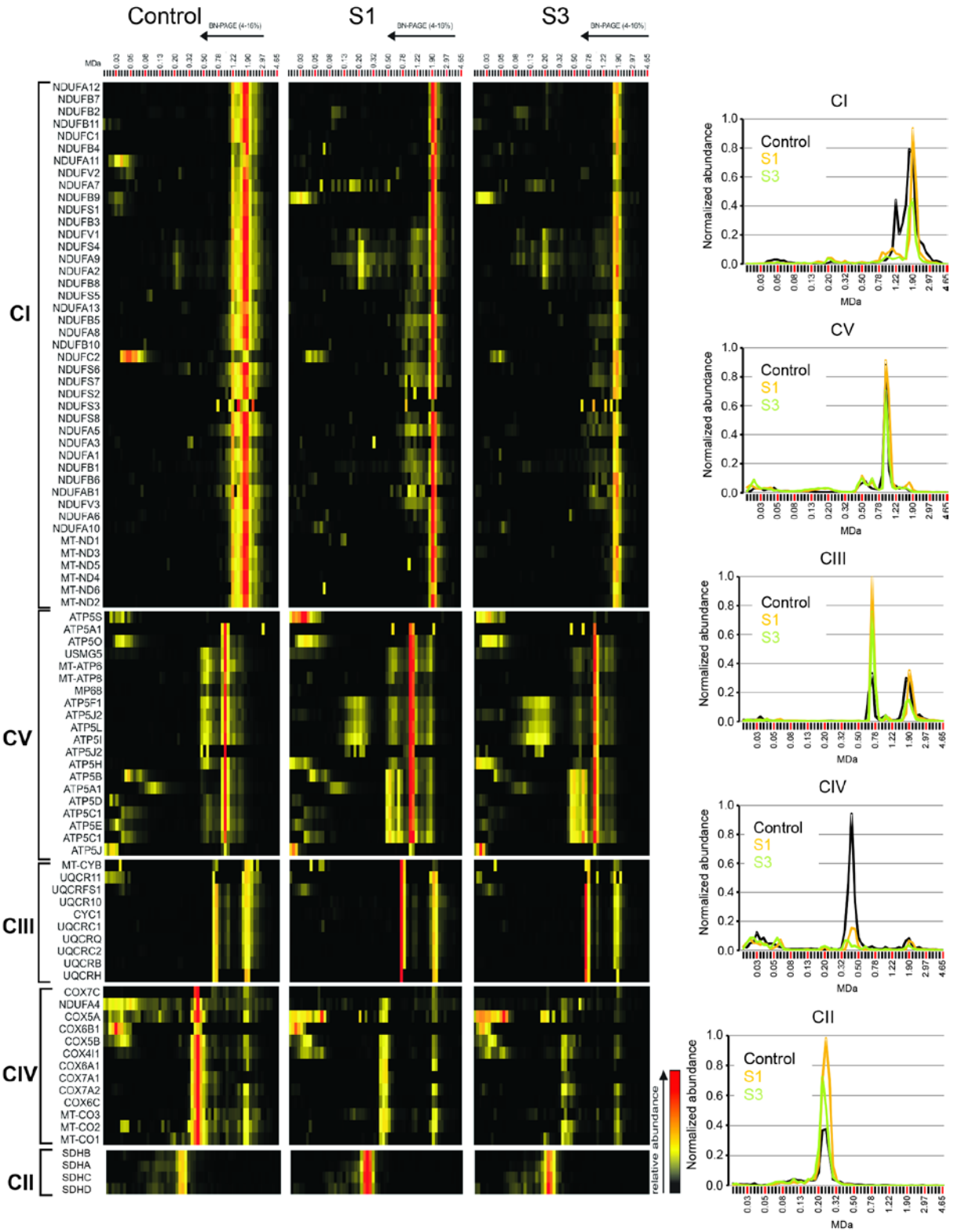


**Figure S1. Complexome profiling of OXPHOS complex assembly**

Heat maps (**left**) and migration profiles (**right**) of OXPHOS complexes in control, S1 and SS3 fibroblasts demonstrating defects in multiple OXPHOS Complexes and an accumulation of CI and CV assembly intermediates in both [11].

**Supplementary Table 1 Identified *MRPS2* variants and predictions of their pathogenicity**

| | **Subject 1** | **Subject 1** | **Subject 2** |
|---|---|---|---|
| Nucleotide change | c.328C>T | c.340G>A | c.413G>A |
| Amino Acid change | p.Arg110Cys | p.Asp114Asn | p.Arg138His |
| SIFT | Deleterious | Tolerated | Deleterious |
| PolyPhen-2 | Probably damaging | Probably damaging | Probably damaging |
| Mutation Taster | Disease causing | Disease causing | Disease causing |
| | | | |
| dbSNP | rs791334309 | rs201229537 | rs758539748 |
| gnomAD allele frequency | 0.00001450% | 0.00008318 | 0.00005282 |

Pathogenicity was predicted by SIFT. MutationTaster. and PolyPhen-2. Earlier reporting of identified genetic variations was checked using data from the Genome Aggregation Database. gnomAD. Allele frequencies for both RefSNP numbers are not reported. The *MRPS2* reference sequence used is GenBank accession code NM_016034.3

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