Supplementary Data

e-Methods-1: Cybrid studies (m.8782G>A mutation)

Transmitochondrial cybrids were generated using polyethylene glycol (PEG) fusion, followed by selection in a uridine-free medium, as previously described.[1] Cytoplasts derived from cytochalasin-treated controls and patient's fibroblasts were fused with a mtDNA-less (ρ°) derivative of the human osteosarcoma 143B cell line. The absence of mtDNA in ρ° cell lines, and its presence in cybrids, was confirmed by PCR analysis using pairs of primers to amplify the D-loop region.[2] The m.8782G>A stop mutation creates a DdeI-specific restriction site which was used for restriction fragment length polymorphism (RFLP) analysis on a 400-bp PCR fragment encompassing np 8540 to 8940 of the human mtDNA Cambridge sequence.[3]

In the absence of the mutation the fragment was cleaved into 340- and 60-bp fragments for the presence of a natural DdeI-specific restriction site; in the presence of the mutation, the fragment was cleaved into 240-, 100- and 60-bp fragments. RFLP analysis was performed as described.[4] The cleaved fragments were separated from the corresponding uncut fragments by agarose-gel electrophoresis. The proportion of mutant versus total mtDNA was calculated by densitometry. This method allowed to obtain several clones harbouring mutation proportions ranging from 0% to 95% (Figure e-1). Spectrophotometric assays of CI-CV mitochondrial RC complexes, normalized for CS activity, was subsequently undertaken in 0%, 10% and 95% mutated patient's transmitochondrial cybrids.

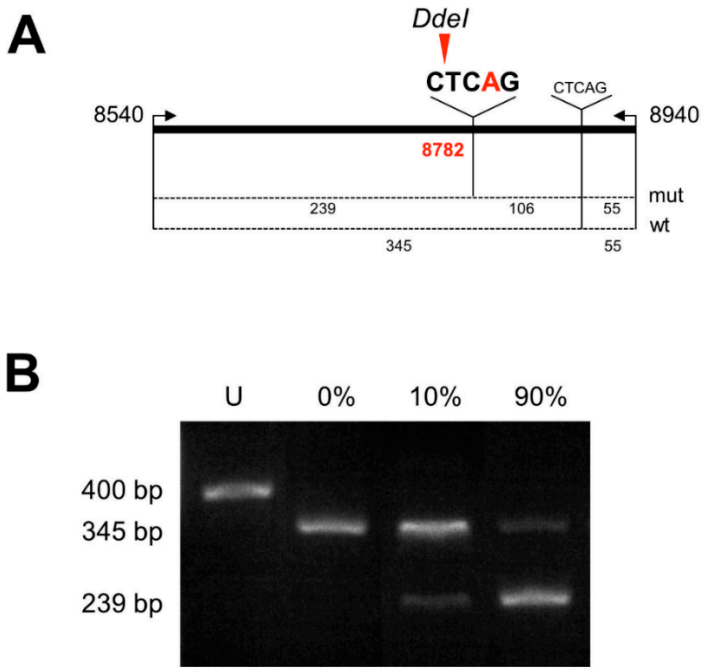

Figure e-1: Restriction fragment length polymorphism analysis in cybrids

(A) Restriction fragment length polymorphism (RFLP) analysis strategy. The 400 bp-PCR fragment amplified from np 8540 to 8940 was cut with DdeI enzyme cleaving the sequence 5-C/TNAG-3'. The 400 bp-fragment contains a natural DdeI site resulting two fragments 345 bp and 55 bp both in wt and mutated molecules. The m.8782G>A mutation inserts in 345 bp-fragment another digestion site for DdeI resulting further two fragments 239 bp and 106 bp respectively. (B) The 2% agarose gel allows to resolve the pool of fragments from the DdeI digestion, to distinguish the wt mtDNA from the mutated mtDNA and to quantify the mutation percentage. The image shows PCR-RFP analysis on cybrids in a clone with 0% mutation, 10% and 95% heteroplasmy. U indicates the uncut PCR fragment.

e-Methods-2 and Results: Mitochondrial morphology

Since there is a relation between mitochondrial morphology alterations and OXPHOS defects, mitochondrial morphology was evaluated in the cultured fibroblasts of Patient 1 and Patient 3. Approximately 2 x 10^4 cells (previously grown either in glucose or galactose medium for 36 hours) were seeded on coverslips, washed in PBS, fixed (3.7% formaldehyde) and permeabilised in PBS with 0.1% Triton X-100, then stained with anti-TOMM20 (Santa Cruz Biotechnology, sc-11415) and anti-568 Alexa Fluor (Invitrogen) in blocking solution (5% goat serum). Coverslips were mounted with Prolong Gold with DAPI and optical sections were acquired using a Zeiss LSM880 confocal system. There was no alteration in mitochondrial shape or structure in both patients when fibroblasts were cultured in both glucose and galactose medium (Figure e-2).

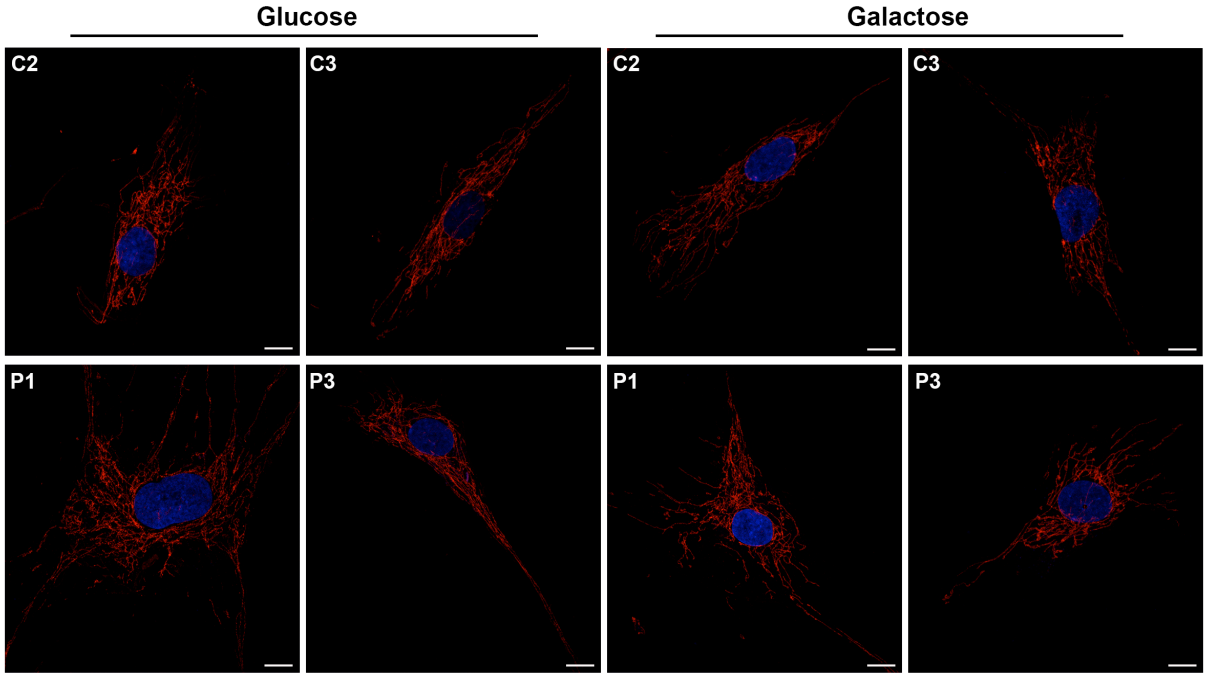

Figure e-2: Mitochondrial morphology of cultured skin fibroblasts. Abbreviations: C2 and C3, Controls; P1: Patient 1; P3, Patient 3. Confocal micrographs showing the Z-stacks maximum projection of the mitochondrial network in cells cultured either in DMEM, high glucose (left panel) or in DMEM supplemented with 5 mM galactose for 36 h (right panel). Scale bar = 10 µm.

References

1. Mariotti C, Tiranti V, Carrara F, Dallapiccola B, DiDonato S, Zeviani M. Defective respiratory capacity and mitochondrial protein synthesis in transformant cybrids harboring the tRNA(Leu(UUR)) mutation associated with maternally inherited myopathy and cardiomyopathy. J Clin Invest 1994;93:1102–1107.
2. Munaro M, Tiranti V, Sandona D, et al. A single cell complementation class is common to several cases of cytochrome c oxidase-defective Leigh's syndrome. Hum Mol Genet 1997;6:221–228.
3. Anderson S, Bankier AT, Barrell BG, et al. Sequence and organization of the human mitochondrial genome. Nature 1981;290:457–465.
4. Uziel G, Moroni I, Lamantea E, et al. Mitochondrial disease associated with the T8993G mutation of the mitochondrial ATPase 6 gene: a clinical, biochemical, and molecular study in six families. J Neurol Neurosurg Psychiatry 1997;63:16–22.