Supplementary material

Clinical evaluation

Physical examination and compilation of medical history was performed as part of the clinical workup.

Muscle and skin biopsy samples and establishment of cell cultures

The patient was subjected to two percutaneous skeletal muscle biopsies taken from *m. tibialis anterior* under local anaesthesia using a conchotome, in the years 2008 and 2016.

Cultures of primary skin fibroblasts, from a skin biopsy performed year 2008, and myoblasts, from the muscle biopsy performed year 2016, from the patient and controls were established. Skin fibroblasts were cultured at 37°C/5% CO_2 atmosphere in standard high-glucose DMEM-GlutaMAX media (Gibco Life Technologies), supplemented with 10% FBS (Gibco Life Technologies). Myoblasts were cultured at 37°C/5% CO_2 atmosphere in Ham´s F10 Nutrient Mix (Gibco Life Technologies) supplemented with 20% FBS (Gibco Life Technologies) and 2.5 ng/mL growth factor bFGF (Gibco Life Technologies). Prior to the investigations described below cells were harvested by trypsination using TrypLE Express 1x (Gibco Life Technologies) and washed twice in Phosphate Buffered Saline (1xPBS) without calcium and magnesium (Gibco Life Technologies). Immunocytochemistry on primary fibroblasts and myoblasts was performed as described earlier[e1]. Primary antibodies used were alpha-tubulin (Merck T6199), desmin (Cell Signaling #5332) and secondary antibodies Alexa Fluor® 488 (Thermo Fisher Scientific, A-11017, mouse), Alexa Fluor® 568 (Thermo Fisher Scientific, A-11036, rabbit) and 0.01 µg/ml DAPI (Merck, D9542).

Morphological investigation of skeletal muscle

Morphological examinations of skeletal muscle, including enzyme histochemical staining and electron microscopy, were performed on both biopsies, from 2008 and 2016, as described earlier[e2].

Genetic analysis

Genomic DNA from the patient was isolated from EDTA-blood, cultured fibroblasts and cultured myoblasts using the QIAamp DNA Midi Kit (Qiagen), from skeletal muscle using QIAamp DNA Mini Kit (Qiagen) and from urinary epithelial cells using QIAamp DNA Micro Kit (Qiagen).

The entire mtDNA sequence from the patient was determined from total DNA isolated from skeletal muscle and cultured myoblasts. MtDNA was amplified by PCR in 28 overlapping M13-tailed fragments as described before[e3]. Subsequent sequencing of PCR products was carried out with M13 primers, using the BigDye version 3.1 sequencing kit (Applied Biosystems) on a 3500xl Genetic Analyzer (Applied Biosystems). Sequence data were compared with the revised Cambridge reference sequence for human mtDNA (GenBank NC_012920.1).

Mutation levels were quantified by last cycle hot restriction fragment length polymorphism (LCH-RFLP) analysis, where PCR products (forward primer 5´-TATCAATCACCTGAGCTCAC and reverse primer 5´-TGTAAATGAGGGGCATTTGG to amplify an 855 bp fragment) were labelled with ^{32}P-dCTP (GE Healthcare) in the last cycle, followed by digestion with endonuclease *HphI* (New England Biolabs) and separation by electrophoresis in non-denaturing polyacrylamide gels. Quantification was performed by phosphor-imaging (BioRad) and corrected for GC-content of the wild type and mutant fragments.

Whole genome sequencing (WGS) on skeletal muscle DNA from the patient was performed to a sequencing depth of 30x mean coverage using a NovaSeq 6000 sequencing instrument (Illumina) after library preparation with NxSeq AmpFREE Low DNA Library Kit (Lucigen). This was followed by in-house bioinformatics analysis, using the mutation identification pipeline (MIP) as earlier described[e4]. The entire mtDNA, all disease associated genes in OMIM (3715 genes) and all genes in Human Mitocarta 2.0 (1158 genes) were analyzed in the genomic data.

Blue Native Polyacrylamide Gel electrophoresis (BN-PAGE) and in silico analyses

Mitochondria isolated from muscle were analyzed by BN-PAGE. Mitochondria corresponding to 20 µg protein were pelleted, resuspended in 23 µl N-dodecyl b-D-maltoside buffer (1% N-dodecyl b-D-maltoside, 1.75 M 6-aminocaproic acid, 7.5 mM Bis-Tris, 2 mM EDTA, pH 7.0) and incubated for 15-30 min on ice. Following centrifugation at 20,000xg for 10 minutes, supernatants were mixed with sample buffer (750 mM 6-aminocaproic acid, 5% Coomassie Blue G250) and loaded onto a 5-15% gradient blue native gel. Gel preparation and electrophoresis were carried out as described earlier[e5]. The gel was blotted to PVDF membrane (Millipore Immobilon-P PVDF 0,45 µM). After blocking in 5% dry milk the blots were incubated with antibodies from Abcam detecting mitochondrial respiratory chain complex I (Mouse Anti-NDUFA9 antibody, ab14713), complex II (Mouse Anti-SDHA antibody, ab14715), complex III (Mouse Anti-UQCRC2 antibody, ab14745), complex IV (Mouse Anti-COX4 + COX4L2 antibody, ab110261) and secondary HRP-conjugated antibody (Goat Anti-Mouse IgG H&L, ab97023).

Multiple sequence alignment was performed with Clustal Omega at standard settings[e6]. Structural representations of ND3 were generated on the basis of the PDB electron

microscopic structure 5XCT[e7] in PyMOL v2.3.4. Models were built with ProMod3 v3.1.1 in SWISS-MODEL[e8] based on the full length 5XCT structure (1-115) at standard settings.

e-References

e1. Calvo-Garrido J, Maffezzini C, Schober FA, et al. SQSTM1/p62-Directed Metabolic Reprogramming Is Essential for Normal Neurodifferentiation. *Stem Cell Reports*. 2019;12(4):696-711. doi:10.1016/j.stemcr.2019.01.023

e2. Larsson N-G. Mitochondrial myopathies. *Acta physiol Scand*. 2001;171:385-393.

e3. Taylor RW. The determination of complete human mitochondrial DNA sequences in single cells : implications for the study of somatic mitochondrial DNA point mutations. *Nucleic Acids Res*. 2001;29(15):1-8.

e4. Stranneheim H. Rapid pulsed whole genome sequencing for comprehensive acute diagnostics of inborn errors of metabolism. *BMC Med Genomics*. 2014;15:1-10.

e5. Calvaruso MA, Smeitink J, Nijtmans L. Electrophoresis techniques to investigate defects in oxidative phosphorylation. *Methods*. 2008;46(4):281-287. doi:10.1016/j.ymeth.2008.09.023

e6. Sievers F, Wilm A, Dineen D, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol*. 2011;7(539). doi:10.1038/msb.2011.75

e7. Guo R, Zong S, Wu M, Gu J, Yang M. Architecture of Human Mitochondrial Respiratory Megacomplex I2III2IV2. *Cell*. 2017;170(6):1247-1257.e12. doi:10.1016/j.cell.2017.07.050

e8. Waterhouse A, Bertoni M, Bienert S, et al. SWISS-MODEL: Homology modelling of protein structures and complexes. *Nucleic Acids Res*. 2018;46(W1):W296-W303. doi:10.1093/nar/gky427

Table e-1. First and second electroneurography assessment

	Electroneurography, 2007		*Electroneurography, 2009*	
Motor nerves	**CMAP (mV)**	**MCV (m/s)**	**CMAP (mV)**	**MCV (m/s)**
Median (l)	4.0	50	not done	
Median (r)	5.3	50	8.3	53
Tibial (l)	no response		not done	
Tibial (r)	0,2	30	0.7	14
Peroneal (l)	1.2	37	not done	
Peroneal (r)	0.6	40	0.1	30
Sensory nerves	**SNAP**	**SCV**	**SNAP**	**SCV**
Median (l)	4	52	not done	
Median (r)	4	49	2	46
Radial superficial (l)	6	46	not done	
Radial superficial (r)	7	52	5	59
Sural (l)	no response		not done	
Sural (r)	1	31	no response	

CMAP; compound muscle action potential, MCV; motor conduction velocity, SNAP; sensory nerve action potential, SCV; sensory conduction velocity, r; right, l; left

Table e-2. Muscles assessed and EMG patterns identified in first vs. second investigation

Muscles assessed	***EMG, 2007***	***EMG, 2009***
Abductor pollicis brevis (r)	not done	myopathic
First interosseous dorsalis I (l)	normal	not done
Biceps brachii (r)	not done	myopathic
Iliopsoas (r)	not done	myopathic
Vastus lateralis (r)	not done	myopathic
Vastus lateralis (l)	chronic neurogenic	not done
Tibialis anterior (r)	chronic neurogenic	myopathic
Tibialis anterior (l)	chronic neurogenic	not done
Gastrocnemius (r)	acute neurogenic with spontaneous activity	myopathic
Gastrocnemius (l)	acute neurogenic with spontaneous activity	not done

EMG; electromyography, r; right, l; left