Mitochondrial compensation in patients with m.4300A>G mutation

Nynke van Polanen[1], Xiao Liang[1], Eszter Dombi[1], Ana Victoria Lechuga-Vieco[2], Betty Raman[3], Jo Poulton[1]

[1] Nuffield Department of Women's and Reproductive Health, University of Oxford, Oxford, United Kingdom

[2] Kennedy Institute of Rheumatology, University of Oxford, Oxford, United Kingdom

[2] Cardiovascular Medicine Division, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom

Background: Mitochondrial diseases may originate from maternally inherited mutations in mitochondrial DNA (mtDNA). The homoplasmic (100% mutant mtDNA) m.4300A>G mtDNA mutation (Figure 1) can result in hypertrophic cardiomyopathy (HCM) and a respiratory chain defect that is confined to the heart[1]. Activating mitophagy, a cellular control mechanisms for turning over damaged mitochondria might benefit m.4300A>G patients. 2-Deoxy-D-glucose (2DG) is a drug that improves mitochondrial quality in heteroplasmic m.3243A>G cultures via mtDNA replication but potentially also by activating mitophagy[3].

Methods: Primary fibroblasts from controls and m.4300A>G patients and were cultured in low (3mM) and high (25mM) glucose medium. Mitophagy and mitochondrial morphology were visualized using high-content fluorescent microscopy. Colocalization of mitochondria (Tom20) and autophagosomes (LC3) was used as a readout for mitophagy. Intracellular oxygen consumption, reactive oxygen species (ROS) and mitochondrial membrane potential were measured with a CLARIOstar monochromator (BMG Labtech). In addition, an ELISA was performed on serum collected from both controls and m.4300A>G patients to determine interleukin 6 (IL-6) levels.

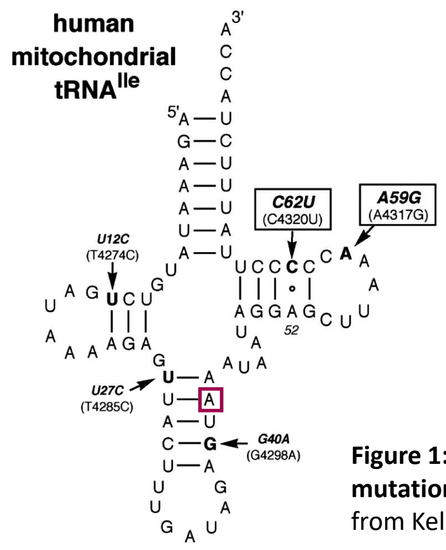

Figure 1: m.4300A>G mutation. Figure adapted from Kelley *et al.*[2]

Results mitochondrial length and mitophagy: Mitochondrial length was 12% (p=0.003) and mitophagy 41% (p=0.013) greater in fibroblasts from m.4300A>G patients compared to controls.

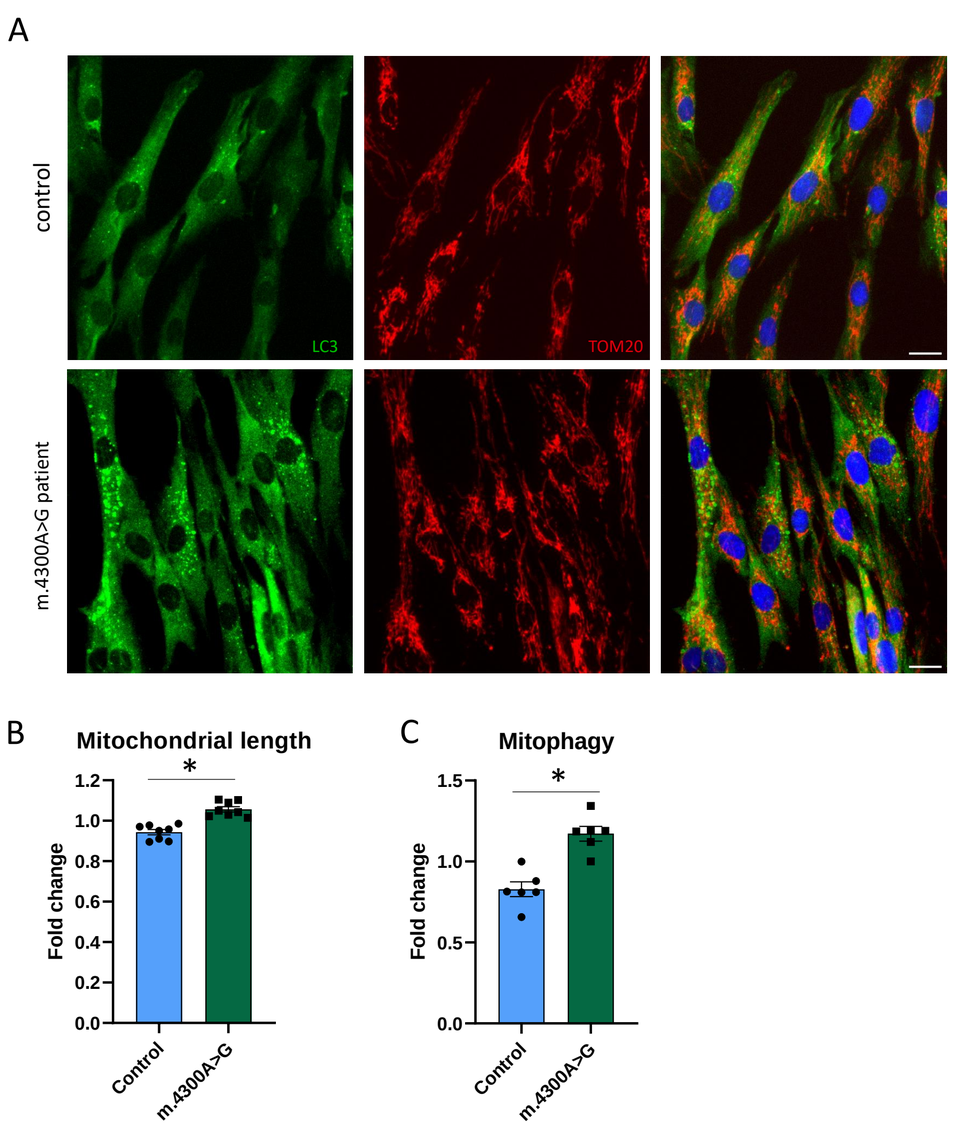

Figure 2: Mitochondrial length and mitophagy is increased in m.4300A>G patients. A) Example fluorescent images of control and m.4300A>G patient fibroblasts. Autophagosomes and mitochondria were stained with an antibody against LC3 (green) and Tom20 (red) respectively. Nuclei are stained with DAPI. Scale bar = 20 µm. B) Mean mitochondrial length determined by Tom20 staining in fixed fibroblasts from controls and patients with the m.4300A>G mutation, cultured in high glucose medium. Mean±SEM (n=8 technical replicates) C) Mitophagy as percentage of mitochondrial area. Mitophagy was determined by colocalization of Tom20 and LC3. Mean ± SEM (n=6).

Results serum IL-6: Acute exposure to IL-6 may increase mitochondrial biogenesis and respiration[4]. Therefore serum IL-6 levels were measured in controls and m.4300A>G patients. Surprisingly, serum IL-6 levels were higher in patients compared to controls (p=0.004, Figure 4).

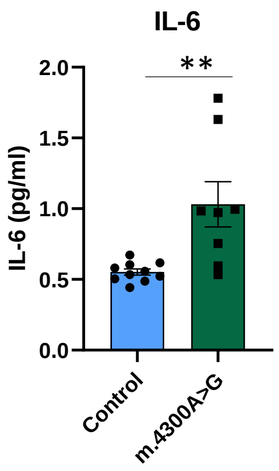

Figure 4: Serum IL-6 levels are higher in m.4300A>G patients compared to controls. IL-6 levels were determined by an ELISA. Bar graphs represent the mean ± SEM (ten controls and eight patients).

Results mitochondrial respiration: Intracellular oxygen concentration (Figure 3A) and ROS production (Figure 3B) were similar between control and patient fibroblasts. Mitophagy was further activated by 2DG (all p<0.05) which also increased mitochondrial elongation (Figure 3D). 2DG tended to increase mitochondrial membrane potential in control but not in patient cells (p=0.007, control vs patient with 2DG, Figure 3C).

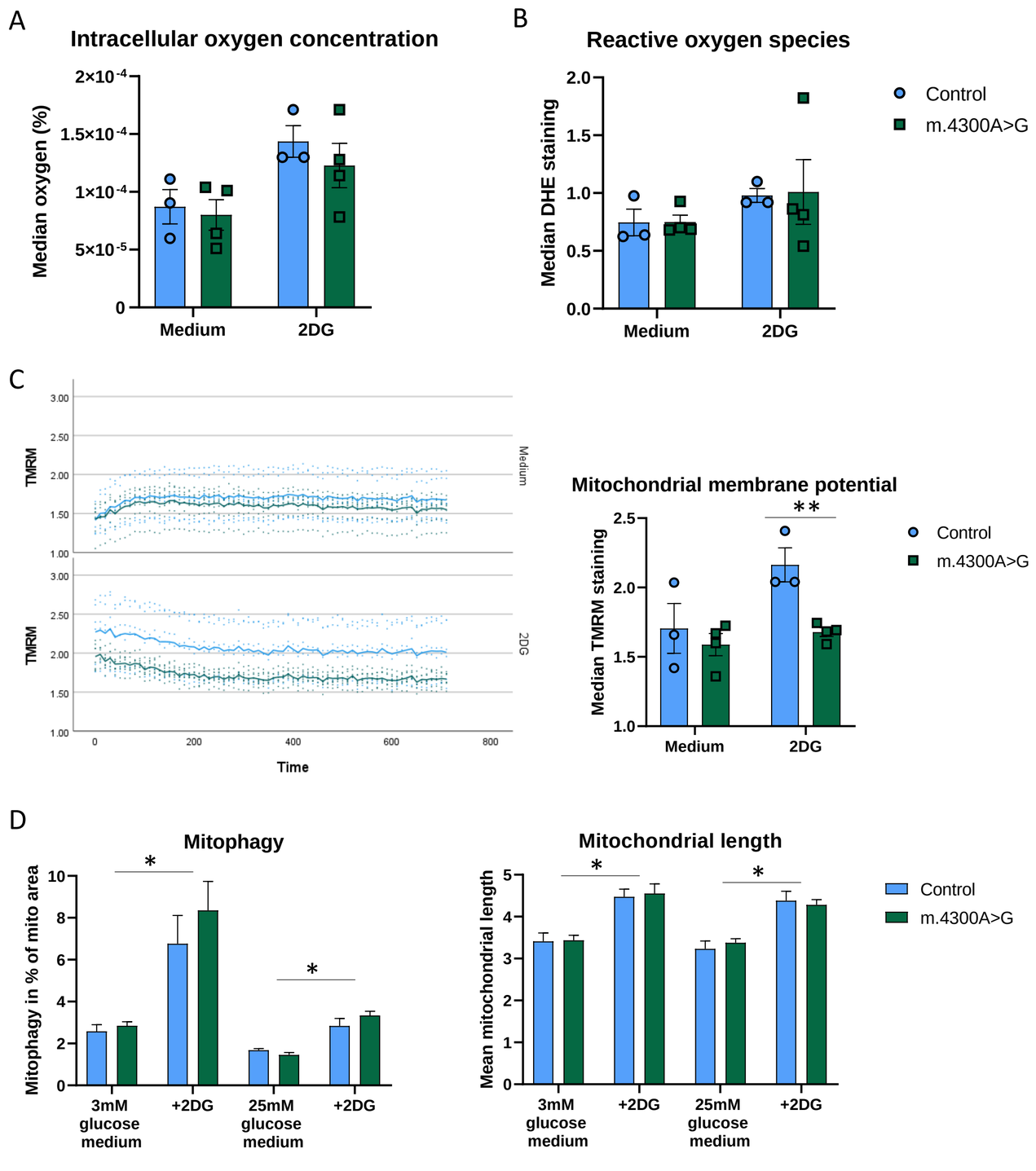

Figure 3: Mitochondrial respiration. Intracellular oxygen levels (%) (A), ROS determined by Dihydroethidium (DHE) signal (B), and mitochondrial membrane potential (C) determined by TMRM signal in live fibroblasts over time (minutes). A-C) Primary fibroblasts from controls and m.4300A>G patients were cultured in low glucose (3mM) ± 2DG (3mM) for 48 hours and measured for 12 hours under low oxygen concentrations (8%), 37°C, 5% CO_2. Bar graphs represent the median values of the stable period after 70 minutes (A), 400 minutes (B) or 300 minutes (C). Mean ± SEM (three controls and four m.4300A>G patients). D) Mitophagy (colocalization of Tom20 and LC3) and mitochondrial length (Tom20) determined in fixed fibroblasts cultured in both low (3mM) glucose medium and high (25mM) glucose medium and after the addition of 2DG (10mM) for 7 days. Mean ± SEM (three controls and five m.4300A>G patients).

Discussion:

Fibroblasts from m.4300A>G patients appear to compensate by elongating their mitochondria and increasing mitophagy, compared to controls. However, patient cells exposed to 2DG were unable to compensate by increasing their mitochondrial membrane potential. This suggests that treatment with 2DG is less likely to benefit m.4300A>G than other classes of patients. Immunology markers such as IL-6 may be a more interesting marker for m.4300A>G patients.

References

(1) Taylor RW *et al.*, A homoplasmic mitochondrial transfer ribonucleic acid mutation as a cause of maternally inherited hypertrophic cardiomyopathy. J Am Coll Cardiol. 2003 May 21;41(10):1786-96. doi: 10.1016/s0735-1097(03)00300-0.

(2) Kelley SO *et al.*, Fragile T-stem in disease-associated human mitochondrial tRNA sensitizes structure to local and distant mutations. J Biol Chem. 2001 Apr 6;276(14):10607-11. doi: 10.1074/jbc.M008320200.

(3) Pantic B *et al.*, 2-Deoxy-D-glucose couples mitochondrial DNA replication with mitochondrial fitness and promotes the selection of wild-type over mutant mitochondrial DNA. Nat Commun. 2021 Dec 6;12(1):6997. doi: 10.1038/s41467-021-26829-0.

(4) Abid H *et al.*, Extramyocellular interleukin-6 influences skeletal muscle mitochondrial physiology through canonical JAK/STAT signaling pathways. FASEB J. 2020 Nov;34(11):14458-14472. doi: 10.1096/fj.202000965RR.