Mitophagy and mitochondrial morphology in patients with the m.13051G>A mitochondrial DNA mutation

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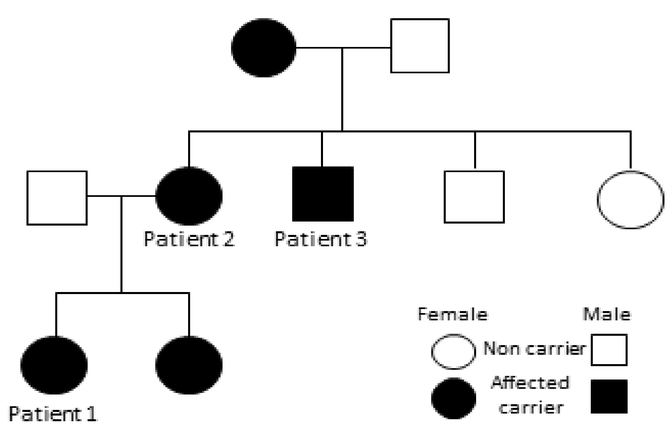
Introduction

Mitochondria have diverse functions within the cell, from supplying cellular energy, to signalling cellular differentiation and cell death. Mitochondrial diseases that result from maternally transmitted mitochondrial DNA (mtDNA) mutations occur in 1/400 individuals. Mitophagy is a form of selective autophagy, a specialized cellular mechanism for the recycling of redundant or dysfunctional mitochondria. Defects in mitochondrial quality control and mitochondrial dynamics have been reported in various neurological disorders.

Methodology

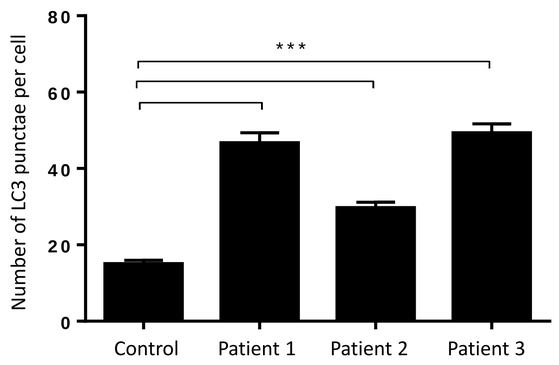
Here we used a previously developed high throughput imaging method for quantifying mitophagy and investigating mitochondrial morphology in cultured primary fibroblasts derived from three members of a family harbouring the homoplasmic m.13051G>A mitochondrial genetic mutation. Patient derived fibroblasts were cultured either in standard glucose media (25mM glucose) or galactose media (no glucose). Cells were immunostained for the autophagy marker LC3 and the mitochondrial protein TOM20 and analysed with INCell 1000.

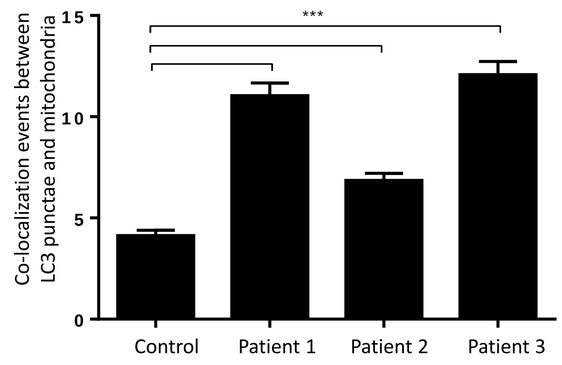
Family harbouring the homolasmic m.13051G>A mtDNA mutation



Cells were available from three members of the family (Patient 1, 2 and 3). The m.13051G>A mitochondrial genetic mutation has previously been associated with Leber's Hereditary Optic Neuropathy (LHON) (Howell et al., 2003).
Patient 1 presented at the age of 4 with a Leigh disease phenotype (auditory verbal agnosia, selective language regression, right divergent squint associated with amblyopia and bilateral sensorineural hearing loss).
Patient 4 is a single affected from an unrelated family, having a classical LHON phenotype with decline of vision over 6 days.

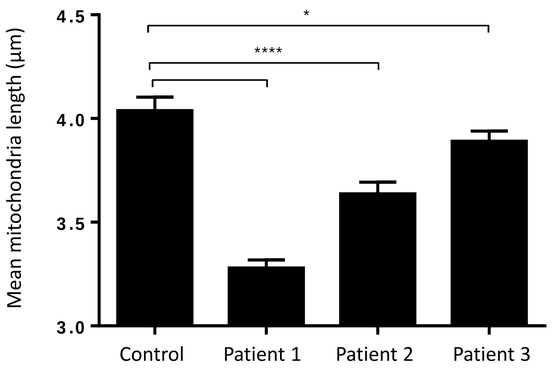
Levels of autophagy and mitophagy

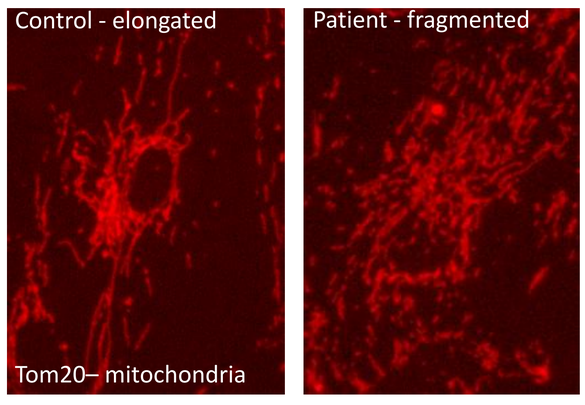




Autophagy was measured by counting the LC3 positive punctae in the cells. Fibroblasts derived from all three patients showed an increase in autophagic activity when compared to control.
Mitophagy was measured by counting the co-localization events between LC3 punctae and mitochondria. Patient derived fibroblasts harbouring the m.13051G>A mtDNA mutation showed significantly elevated levels of mitophagy when compared to control. ***=p<0.001

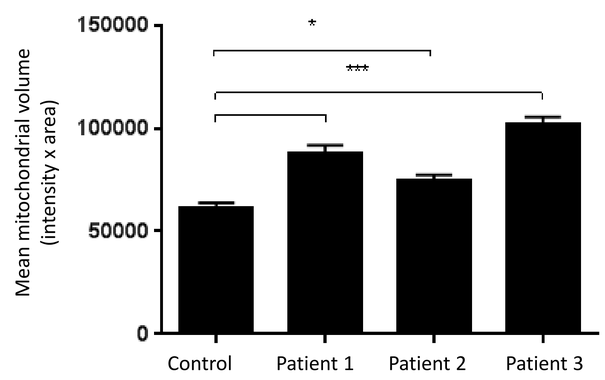
Fragmented mitochondrial network

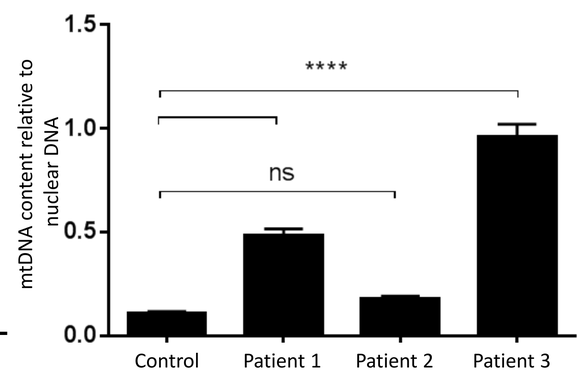




Mean mitochondrial length is significantly shorter in patient cells when compared to control. Mitochondrial dynamics play an important role in mitochondrial quality control. Mitochondrial fission is believed to be a pre-requisite for mitophagy resulting in the presence of a fragmented mitochondrial network (Gomes and Scorrano, 2013). *=P<0.05, ****=p<0.0001

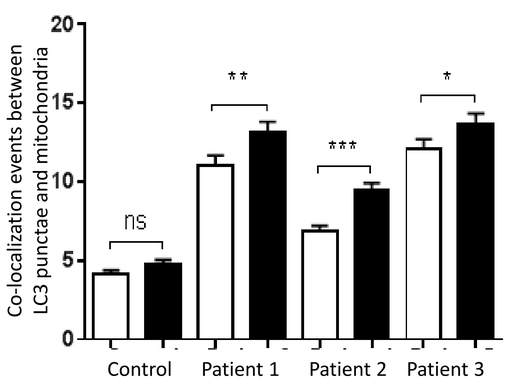
Increased mitochondrial volume

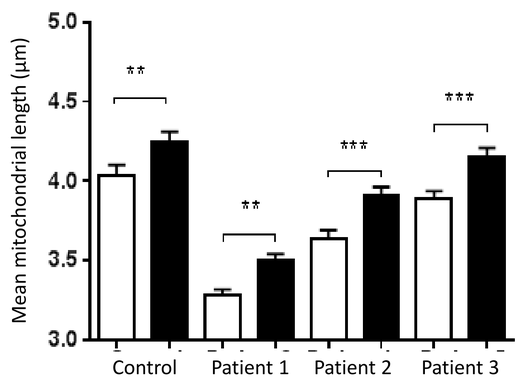


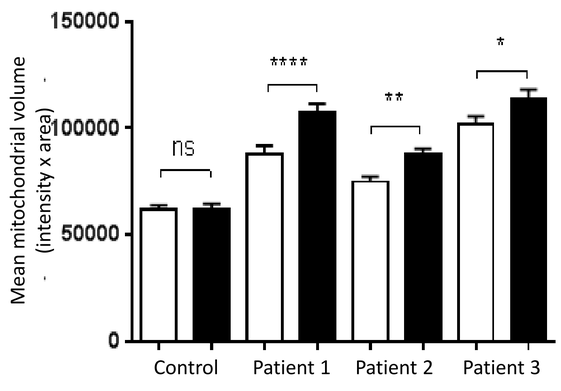


Mitochondrial volume is increased in all patient cells when compared to control. Proliferation of mitochondria and increased mtDNA levels are hallmarks of mitochondrial dysfunction seen in patients with mitochondrial disease. *=p<0.05, ***=p<0.001, ****=p<0.0001

Galactose media further increases mitophagy, mitochondrial fragmentation and mitochondrial volume







In contrast to standard conditions (25mM glucose, white bars), cells cultured in glucose-free galactose media are unable to used glycolysis for energy production and are hence forced to use their mitochondria (energetic stress). Culture under these conditions for three days (black bars) caused a stress-related increase in mitochondrial length ("stress induced mitochondrial hyperfusion"). It also caused a compensatory increase in mitochondrial volume that was significant in the patients but not the controls. They also showed an increase in mitophagy when compared to cells cultured under standard conditions, consistent with increased mitochondrial damage resulting from energetic stress. This was manifest as subtle changes in low levels of mtDNA heteroplasmy on next generation sequencing of mtDNA (not shown). *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001

Treatment with idebenone

	% of cells remaining in culture after 7 days treatment			
treatment	glucose media	glucose media + idebenone	galactose media	galactose media + idebenone
Control	100%	86%	71%	65%
Patient 1	100%	130%	98%	129%
Patient 2	100%	102%	57%	67%
Patient 3	100%	108%	59%	77%

Idebenone has successfully been used to treat patients with Leigh syndrome and LHON (Heitz et al., 2012). Here we show that 7 days treatment of fibroblasts derived from patients with the m.13051G>A mitochondrial mutation increases the number of cells remaining in culture both in glucose and galactose media when compared to cells grown in standard glucose media without idebenone. Idebenone treatment also increaess the mitochondrial membrane potential (particularly patient 4) and decreased levels of mitophagy (not shown).

Conclusions

Mitochondrial fragmentation and a compensatory increase in both mitochondrial volume and mtDNA content are frequent manifestations of mitochondrial dysfunction. Here we showed that fibroblasts from all patients had a fragmented mitochondrial network and increased mitochondrial DNA and volume, consistent with mitochondrial dysfunction. They also manifested appropriately elevated levels of mitophagy.
In contrast to standard conditions (25mM glucose), cells cultured in glucose-free galactose media are unable to use glycolysis for energy production and are hence forced to use their mitochondria (energetic stress). This markedly increased mitochondrial volume in the patients but not in the control, and decreased their mitochondrial membrane potential measured by TMRM (not shown). Furthermore, these conditions elicited a stress response ("stress induced mitochondrial hyperfusion" (Tondera, 2009)) in which energetic stress elicits mitochondrial elongation (Rambold et al., 2011) and increased ATP production. This short-term response inhibits mitophagy and impairs mitochondrial quality control. We found that neutral mtDNA changes accumulated under these conditions in patients. Despite the known inhibitory effect of mitochondrial elongation on mitochondrial quality control (Gomes and Scorrano, 2013), we found that mitophagy was further increased in the patient cells. This suggests that the mitochondrial damage conferred by the energetic stress on patient cells was considerable and that mitochondrial biogenesis increased substantially in parallel with degradation.
Patients with LHON benefit from treatment with idebenone (Heitz et al., 2012) and we assayed its effects on cell growth . We found that idebenone conferred a significant growth advantage to patient cells when cultured both under standard conditions and energetic stress. However, we are unable to fully explain the behaviour of patient 1's cells in this assay and plan further studies to determine whether they are robust. Despite clear evidence that energetic stress had a substantial effect on mitochondrial volume and length in patient 1's cells, growth in galactose medium appeared to confer a growth advantage on these cells over controls. Furthermore, idebenone treatment increased this advantage. We also showed that idebenone treatment attenuated the increased mitophagy in these cells. In an earlier study of fibroblasts from patients with dominant optic atrophy, we also showed that marked fragmentation of the mitochondrial network increased levels of mitophagy (Liao et al., unpublished data). We postulated that excessive mitophagy disadvantages cell growth. Hence, we postulate that the growth advantage conferred by idebenone on our 13051 cells may be reinforced by relief from excessive mitophagy. Given that the mitophagy is most marked in patient 1, this may explain the particular benefit that idebenone confers on the growth of patient 1's cells.
In conclusion, we provide evidence of increased mitophagy in LHON cells. We show that idebenone benefits cell growth and attenuates increased mitophagy. Drug modulators of mitophagy are potentially useful treatments for mitochondrial patients.

References

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