OPA1 mutations result in a deficit of in vivo mitochondrial ATP production in patients with autosomal dominant optic atrophy

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Introduction
Autosomal dominant optic atrophy (ADOA) is the most common form of hereditary optic neuropathy with a disease prevalence ranging from 1:10,000 to 1:50,000 in different populations (1). The disease, clinically characterised by an insidious onset of variable visual loss, is due to a progressive degeneration of retinal ganglion cells (2, 3). In most patients a mutation in the OPA1 gene on the chromosome 3q28 is detected (2, 3). OPA1 encodes a ubiquitously expressed large GTPase related to dynamins, anchored to the mitochondrial inner membrane and implicated in the formation and maintenance of mitochondrial network and morphology (4). A mitochondrial dysfunction has been implicated as a central pathogenic mechanism of ADOA (4, 5). The aim of our study was to assess, using phosphorous MR spectroscopy, the rate of mitochondrial ATP synthesis in the skeletal muscle of ADOA patients with different mutations in the OPA1 gene and of ADOA patients where a mutation in the OPA1 gene was excluded.

Methods
We studied 15 patients with ADOA (9 females, 6 males, mean age 47 ± 16, range 21-71) from 5 Italian families with ADOA and 11 age- and sex-matched controls. Ten ADOA patients carried mutations in the OPA1 gene (c.2708-2711delTTAG in six, c.948+3A>T in three and 1516+1G>C in one) while in five mutations were absent. Ophthalmologic examination showed in all patients a pale optic disc with reductions of visual acuity and central vision defect. Informed consent was obtained from each patient and normal volunteer.

The study used a 1.5T General Electrics Medical Systems (Milwaukee, Wisconsin) Signa Horizon LX whole-body scanner. Subjects lay supine with a 6 cm diameter surface coil centred on the maximal circumference of the right calf muscle. Spectra were acquired, with a repetition time of 5 s, at rest (128 FIDs), during an aerobic incremental exercise of plantar flexion (time resolution of 1 min = 12 FIDs per spectrum), and the following recovery. Sixty-four recovery spectra with a time resolution of 10 sec (2 FIDs) were collected. Spectra were post-processed by a time-domain fitting routine AMARES/MRUI (http://carbon.uab.es/mrui) and the concentrations of inorganic phosphate (Pi), phosphocreatine (PCr), ADP and the inverse phosphorylation potential (1/PP = [ADP]x[Pi]/[ATP]) were calculated (5). Intracellular pH was calculated from the chemical shift of Pi relative to PCr. The rate of PCr recovery was calculated from the mono-exponential equation best fitting the experimental points, reported as time constants (TC_PCr) in relationship to the minimum cytosolic pH and then normalized to pH = 7 (5). Data are presented as mean ± SD. Statistical significance, determined by the Student t test for unpaired data, was taken as p<0.05. Individual results were taken as abnormal when they fell outside the entire range of the control values.

Results
The resting 1/PP was significantly increased in OPA1 patients carrying the c.2708-2711delTTAG (16±4, x10^6 mM, mean±SD, vs controls 11±3), the c.948+3A>T (18±5) and the 1516+1G>C mutation (19; normal range 5-16) (Figure 1A). TC_PCr was significantly increased in OPA1 patients carrying the c.2708-2711delTTAG (28±7 s vs controls 19±4), the c.948+3A>T (39±15) and the 1516+1G>C mutation (29; normal range 11-27) (Figure 1B). Resting 1/PP (13±4) and TC_Pcr (18±4) were not significantly different in non-OPA1 ADOA patients and controls (Figure 1).

Discussion
We have shown that different mutations in the OPA1 gene consistently result in a deficit of mitochondrial ATP synthesis not detected in non-OPA1 ADOA patients. Our in vivo results support the central role of mitochondrial dysfunction in the pathophysiology of ADOA in patients with OPA1 gene mutations. Defective OPA1 may interfere with the assembly and function of the respiratory complexes and ultimately lead to cytochrome c release and caspase-dependent apoptotic cell death (6).

References

Figure. A) 1/PP at rest and B) post-exercise TC_Pcr in ADOA patients with or without OPA1 mutations and controls. * p<0.05 vs controls/outside the control range.