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OPA1 MUTATIONS INDUCE mtDNA PROLIFERATION IN LEUKOCYTES OF PATIENTS WITH DOMINANT OPTIC ATROPHY

OPA1 mutations cause autosomal dominant optic atrophy (DOA), a debilitating mitochondrial optic neuropathy characterized by irreversible loss of retinal ganglion cells (RGCs) and progressive visual failure starting in early childhood.¹ Interestingly, ~20% of *OPA1* carriers will develop a more severe form of the disease, DOA+, where the optic atrophy is compounded by additional neurologic features.¹ *OPA1* is a multifunctional mitochondrial inner membrane protein, and rather unexpectedly, high levels of cytochrome *c* oxidase (COX)-negative muscle fibers were recently identified in biopsy specimens from patients manifesting both the pure and syndromal phenotypes.^{2,3} These COX-negative muscle fibers harbored high levels of somatically acquired mitochondrial DNA (mtDNA) deletions and marked mtDNA proliferation was also observed, clearly revealing *OPA1* to be a novel disorder of mtDNA maintenance.⁴ To further investigate this key pathologic mechanism, mtDNA copy number was quantified in blood leukocytes from 3 independent patient cohorts with molecularly confirmed *OPA1* mutations.

Methods. Using different extraction protocols (Method e-1 on the *Neurology*[®] Web site at www.neurology.org), total genomic DNA was isolated from the leukocyte fraction of venous blood from 3 groups of patients harboring pathogenic *OPA1* mutations: 1) British cohort (n = 61), 2) German cohort (n = 40), and 3) Danish cohort (n = 49). Relative mtDNA copy number was determined using the MyiQ[™] real-time PCR detection system (BioRad, Hercules, CA).⁵ The presence of multiple mtDNA deletions was investigated in a subgroup of 22 British *OPA1*-positive DNA samples using long-range PCR.¹ Visual acuity was measured using a standard Snellen vision chart (Method e-2).

Standard protocol approvals, registrations, and patient consents. This study had the relevant institutional ethical approval and complied with the Declaration of Helsinki.

Results. Blood leukocytes from British *OPA1*-positive patients had significantly higher mtDNA copy numbers compared with controls (figure 1A). In the British cohort, there was no significant differ-

ence in total mtDNA content between patients with pure DOA and DOA+ phenotypes (figure 1B). There was no significant correlation between patients' visual acuity and mtDNA copy number in blood leukocytes (figure 1C). There was evidence of significant mtDNA proliferation in blood leukocytes from German *OPA1*-positive patients (figure 1D) and Danish *OPA1*-positive patients (figure e-1). On subgroup analysis, there was no significant difference in total mtDNA content between patients with different *OPA1* mutation subtypes (figure e-2). Multiple mtDNA deletions were not detected in blood leukocytes.

Discussion. This study has revealed significant mtDNA proliferation in blood leukocytes harboring pathogenic *OPA1* mutations in 3 independent patient cohorts. The degree of mtDNA proliferation in blood was comparable to the 2- to 4-fold increase in mtDNA content observed at the single fiber level in skeletal muscle biopsies from *OPA1*-positive patients.⁴ The *OPA1* protein plays a key role in the assembly and stability of the respiratory chain complexes and these pathologic findings, in both blood and skeletal muscle, are consistent with the detrimental effect of *OPA1* mutations on mitochondrial oxidative phosphorylation, the increased mtDNA copy number probably acting as a compensatory bioenergetic mechanism.⁴ *OPA1* mediates mitochondrial membrane fusion in tandem with mitofusin 2 (MFN2), which is dysfunctional in the most common, autosomal dominant, axonal form of Charcot-Marie-Tooth disease (CMT2A).⁶ It is therefore noteworthy that *MFN2* mutations also triggered a significant mtDNA proliferative response in blood, supporting our observation for *OPA1* mutations.⁶ Unlike skeletal muscle, multiple mtDNA deletions were not identified in blood leukocytes from patients with *OPA1* mutations. This is likely related to the relatively short half-life of those cells in the circulation, their rapid turnover precluding the formation and subsequent clonal expansion of somatic mtDNA abnormalities.

The mtDNA proliferative response in *OPA1*-positive blood leukocytes was not statistically different between patients with pure optic atrophy and

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Supplemental data at
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Supplemental Data

Figure 1 MtDNA copy number analysis

(A) Comparison of total mtDNA content in blood leukocytes from the British cohort (*OPA1*-positive patients: mean = 111.3, SD = 46.1, n = 61; controls: mean = 56.9, SD = 43.3, n = 87). (B) Phenotype subgroup analysis of the British patient cohort (pure dominant optic atrophy subgroup: mean = 101.6, SD = 39.0, n = 36; DOA+ subgroup: mean = 125.3, SD = 52.6, n = 25, NS with Bonferroni correction at *p* value = 0.0471). (C) Correlation of logMAR visual acuity (VA) with total mtDNA content in blood leukocytes from British *OPA1*-positive patients (Spearman rank correlation coefficient = 0.08, NS at *p* value = 0.4081). (D) Comparison of total mtDNA content in blood leukocytes from the German cohort (*OPA1*-positive patients: mean = 149.8, SD = 95.0, n = 40; controls: mean = 68.7, SD = 39.4, n = 44). Statistical analysis was performed using GraphPad™ v.4 statistical software (San Diego, CA). Group comparisons were made with the unpaired *t* test. The relationship between logMAR VA and total mtDNA content level was assessed with Spearman rank correlation coefficient. ****p* < 0.0001. NS refers to a nonsignificant *p* value, with *p* < 0.0167 being the threshold level for statistical significance after Bonferroni correction for multiple testing.

those who developed additional neurologic complications. Furthermore, irrespective of whether or not *OPA1* mutation carriers developed DOA+ features, there was no correlation between absolute mtDNA copy number and the severity of visual loss. Given the 3-fold increased risk of developing DOA+ with missense mutations and those mutations involving the GTPase catalytic domain, additional subgroup analysis was carried out based on these *OPA1* muta-

tional subtypes.¹ No consistent significant difference in mtDNA copy number was identified, which suggests that this parameter of mitochondrial dysfunction is not associated with clinical severity per se, at least in blood leukocytes. Paradoxically, a previous study found relative mtDNA depletion in blood samples collected from patients with DOA. However, only 8 *OPA1*-positive cases were included and the observed reduction in mtDNA copy number was relatively small,

~25% of the mean control value.⁷ Further work is needed to clarify the still poorly understood pathways linking *OPA1* mutations with mtDNA instability and ultimately RGC loss in DOA.

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1. Yu-Wai-Man P, Griffiths PG, Gorman GS, et al. Multi-system neurological disease is common in patients with *OPA1* mutations. *Brain* 2010;133:771–786.
2. Amati-Bonneau P, Valentino ML, Reynier P, et al. *OPA1* mutations induce mitochondrial DNA instability and optic atrophy plus phenotypes. *Brain* 2008;131:338–351.
3. Hudson G, Amati-Bonneau P, Blakely EL, et al. Mutation of *OPA1* causes dominant optic atrophy with external ophthalmoplegia, ataxia, deafness and multiple mitochondrial DNA deletions: a novel disorder of mtDNA maintenance. *Brain* 2008;131:329–337.
4. Yu-Wai-Man P, Sitarz KS, Samuels DC, et al. *OPA1* mutations cause cytochrome c oxidase deficiency due to loss of wild-type mtDNA molecules. *Hum Molec Genet* 2010;19:3043–3052.
5. Pyle A, Burn DJ, Gordon C, et al. Fall in circulating mononuclear cell mitochondrial DNA content in human sepsis. *Intens Care Med* 2010;36:956–962.
6. Sitarz KS, Yu-Wai-Man P, Pyle A, et al. *MFN2* mutations cause compensatory mitochondrial DNA proliferation. *Brain* 2012;135:e219.
7. Kim JY, Hwang JM, Ko HS, et al. Mitochondrial DNA content is decreased in autosomal dominant optic atrophy. *Neurology* 2005;64:966–972.

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REVISITING THE ISSUE OF MITOCHONDRIAL DNA CONTENT IN OPTIC MITOCHONDRIOPATHIES

Involvement of the visual system in mitochondrial diseases, in particular of retinal ganglion cells (RGCs) and the optic nerve, is very common and has been defined “optic mitochondriopathies.”¹ The prototype is Leber hereditary optic neuropathy (LHON, OMIM 535000), the first disease to be associated with a mitochondrial DNA (mtDNA) point mutation and now considered the most frequent mitochondrial disease.² LHON has been joined by dominant optic atrophy (DOA, OMIM 165500) Kjer type, which in a large set of cases is associated with heterozygous mutations in the nuclear gene *OPA1* encoding for a mitochondrial fusion protein.² Both disorders have features in common: clinically the preferential involvement of the papillomacular bundle and central vision, biochemically a defective

respiratory chain and an increased propensity toward apoptosis.²

Furthermore, the shared selectivity for RGCs and the associated optic nerve has been related to the peculiar arrangement of this axonal system, which is nonmyelinated and very energy dependent in the retina, and only posterior to the anatomic constrain of the lamina cribrosa becomes myelinated and less energy dependent.² It has been hypothesized that this anatomic peculiarity imposes a fine tuning of the uneven mitochondrial distribution within the system, particularly abundant in the nonmyelinated section. Thus, either through an energy failure or an impaired fusion of mitochondria, this distribution may become abnormal and lead to loss of RGCs viability.² It has been reported that the mtDNA content of blood cells was significantly lower in *OPA1* mutant cases of DOA compared to controls, providing an indirect

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