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LETTER TO THE EDITOR

Reply: Mitochondrial DNA copy number differentiates the Leber's hereditary optic neuropathy affected individuals from the unaffected mutation carriers

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Sir,

We read the interesting letter by Bianco and colleagues (2015) on their assessment of mtDNA copy number, as a surrogate measure of mitochondrial biogenesis, in families affected with Leber's hereditary optic neuropathies (LHON) belonging to two independently collected cohorts from southern Italy (Apulia) and Spain. We are very pleased by their confirmatory results of our observations published recently in *Brain* (Giordano *et al.*, 2014). Similar to our original study, these authors found that unaffected mutation carriers from their Italian and Spanish LHON families present the highest mtDNA copy number in blood-derived total DNA, compared with their affected maternal relatives and controls. These results represent an important independent confirmation that cells carrying the LHON primary mutations orchestrate a compensatory response, which is significantly more efficient in those individuals who remain unaffected, possibly life-long. These data consolidate the notion that mitochondrial biogenesis is a compensatory strategy that successfully influences penetrance in LHON, leaving a large number of the mutation carriers spared by blindness. Both Bianco *et al.* in their letter, and us in the original paper, envisage a possible application of a standardized and validated test assessing mtDNA copy number in blood cells and in other accessible tissues (buccal mucosa, urinary tract epithelium) to predict the fate of LHON mutation carries, together with other relevant information such as environmental exposure to tobacco smoking (Kirkman *et al.*, 2009), the anatomical conformation of optic nerve head (Ramos *et al.*, 2009) and other clinical (Barboni *et al.*, 2010) and biological markers (Guy *et al.*, 2008). Standardization and validation of such a predictive test will be necessary, given the intrinsic variability of mtDNA amount (individual variation, tissue specificity, training, age, etc.) and the high number of technical factors that may interfere with its accurate evaluation (storage-dependent degradation, PCR inhibitors, assay variations), as highlighted in Bianco *et al.* (2015). The relative evaluation (ratio) of mtDNA copy number as function of nuclear gene copy will be overcome soon by upcoming new technical approaches providing an absolute assessment of mtDNA copy number, for example as performed by digital PCR (Manoj, 2014).

A further implication of mitochondrial biogenesis, as an efficient compensatory mechanism to overcome

mitochondrial dysfunction in LHON, is the possible exploitation of this mechanism for therapeutic purposes. We reported in 2011 that oestrogens ameliorate mitochondrial dysfunction in LHON cybrids by activating mitochondrial biogenesis, proposing this as a possible explanation for the lower penetrance of LHON in females (Giordano *et al.*, 2011). Different successful approaches aimed at activating mitochondrial biogenesis as therapy have been published (Wenz *et al.*, 2008; Cerutti *et al.*, 2014). We also studied the effects phyto-oestrogens, natural oestrogen-like molecules present in plants, on LHON cybrids, administered singularly or in combination, as possible therapeutic agents successfully activating mitochondrial biogenesis in cells (Giordano and Carelli, unpublished data).

Many issues remain to be clarified. Mitochondrial biogenesis balances removal of damaged mitochondria operated by mitophagy, and the two processes possibly respond to the same master regulation. Furthermore, the retrograde signalling pathway generated by mitochondrial dysfunction in LHON is poorly understood. Finally, what is the genetic basis for the successful compensation operated by those individuals remaining unaffected despite carrying a LHON mutation? Obviously more work is needed to truly understand and cure LHON, the first human disease to be associated with a maternally inherited mtDNA point mutation more than 25 years ago (Wallace *et al.*, 1988), and currently quoted as the most frequent mitochondrial disease (Yu-Wai-Man *et al.*, 2003).

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