Brain Advance Access published July 23, 2015



BRAIN
A JOURNAL OF NEUROLOGY

# LETTER TO THE EDITOR

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

**Angelica Bianco,[1]* Iñigo Martínez-Romero,[2]* Luigi Bisceglia,[3] Leonardo D'Agruma,[3] Paola Favia,[1] Eduardo Ruiz-Pesini,[2,4] Silvana Guerriero,[1] Julio Montoya[2] and Vittoria Petruzzella[1]**

*These authors contributed equally to this work.

1 Dipartimento di Scienze Mediche di Base, Neuroscienze e Organi di Senso - Università degli Studi "Aldo Moro", Piazza G. Cesare, 70124, Bari, Italy
2 Departamento de Bioquímica, Biología Molecular y Celular; Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER); Instituto de Investigación Sanitaria de Aragón. Universidad de Zaragoza. 50013-Zaragoza, Spain
3 IRCCS Casa Sollievo della Sofferenza, UOC Genetica Medica, San Giovanni Rotondo, Italy
4 Fundación ARAID, Universidad de Zaragoza, 50013-Zaragoza, Spain

**Correspondence to:** Vittoria Petruzzella,
Dipartimento di Scienze Mediche di Base,
Neuroscienze e Organi di Senso,
Università degli Studi "Aldo Moro",
70124 Bari, Italy
E-mail: vittoria.petruzzella@uniba.it

Correspondence may also be addressed to: Julio Montoya,
Departamento de Bioquímica,
Biología Molecular y Celular,
Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER);
Instituto de Investigación Sanitaria de Aragón,
Universidad de Zaragoza,
50013-Zaragoza, Spain
E-mail: jmontoya@unizar.es



Sir,

We read with great interest the article by Giordano and colleagues (2014) reporting that cellular activation of compensatory mitochondrial biogenesis, as measured by mitochondrial DNA (mtDNA) copy number, is a major determinant of incomplete penetrance in Leber's hereditary optic neuropathy (LHON), a mitochondrial disease characterized by bilateral subacute loss of central vision due to optic atrophy (Carelli *et al.*, 2004; Yu-Wai-Man *et al.*, 2011). In most known mitochondrial diseases due to mtDNA mutations the penetrance or the severity of the condition depends on the level of heteroplasmy of the mutation (Schon *et al.*, 2012). However, LHON is due to mtDNA mutations that exemplify a different paradigm. The three primary mutations, namely the m.3460G>A/ND1, m.11778G>A/ND4 and m.14484T>C/ND6, are generally found as homoplasmic. One of the three primary mtDNA mutations is necessary but not sufficient to cause optic neuropathy and disease penetrance can vary in different families harbouring the same mutation, and even within different branches of the same family (Howell and Mackey, 1998). The study by Giordano and colleagues (2014) highlights how in large LHON cohorts of individuals of European descent, higher mtDNA content in blood cells

discriminates the unaffected mutation carriers from LHON affected and control subjects. A significantly higher mtDNA copy number was observed in asymptomatic maternal relatives, hereafter called 'Carriers', moving progressively towards lower values from carriers to affected subjects to controls. Previously, different data were reported on this issue. The mtDNA copy number was significantly higher in blood cells of affected and carrier Chinese subjects harbouring the m.11778A-LHON compared to control subjects, but no difference was found between affected and carrier subjects (Yen *et al.*, 2002). In another study, using blood samples from a Japanese LHON pedigree harbouring the m.14484C allele, a significantly higher mtDNA level was found in carriers with respect to both affected and control subjects (Nishioka *et al.*, 2004).

Herein, we add our observations on Italian and Spanish populations harbouring the LHON homoplasmic mutations. The Italian cohort, from Apulia, includes 16 affected and 11 carrier subjects from six different pedigrees, and 39 controls. Including both affected and unaffected, 18 subjects (67%) harboured the m.11778G>A mutation and nine (33%) the m.3460G>A mutation. The Spanish cohort includes 24 affected and 28 carriers from 13 different pedigrees, including 25 (48%) and 27 (52%) subjects who harboured the m.3460A and m.11778A alleles, respectively, and 27 controls. Quantification of mtDNA copy number was performed on total DNA extracted from blood samples by real-time PCR using the relative method (Zoccolella *et al.*, 2012) for the Italian population and the absolute method (Marcuello *et al.*, 2005) for the Spanish cohort. We found significantly higher mtDNA content in carriers compared with affected and control subjects both in the Italian and Spanish populations (Fig. 1). Thus, our results reproduce in two independent cohorts of subjects the Giordano *et al.* (2014) findings showing that mtDNA content in carriers' blood samples is higher than in affected subjects. Specifically, in the Italian sample the frequency distribution of the mtDNA copy number showed that the peak of mtDNA content shifted progressively towards higher values from controls to affected subjects to carriers assessing a threshold value estimated at ~400 mtDNA copies, very similar to the ~500 copies estimated by Giordano and colleagues, presumably for having used a similar experimental approach. According to the male prevalence of LHON (Man *et al.*, 2003), we also found in the overall Italian and Spanish population an unbalanced gender distribution between affected and unaffected LHON mutation carriers. Males are more susceptible to suffer from LHON; consequently our patients' samples could be enriched in male individuals. Moreover, because LHON mutations are transmitted along the maternal lineage, mothers but not fathers, are usually included in genetic analysis of mtDNA diseases and thus carriers can be enriched in female individuals. In the overall population, male patients represented 62.5% and female carriers represented 68%. This bias in sex distribution was also found in other reports (Yen *et al.*, 2002; Nishioka *et al.*, 2004; Giordano *et al.*, 2014).

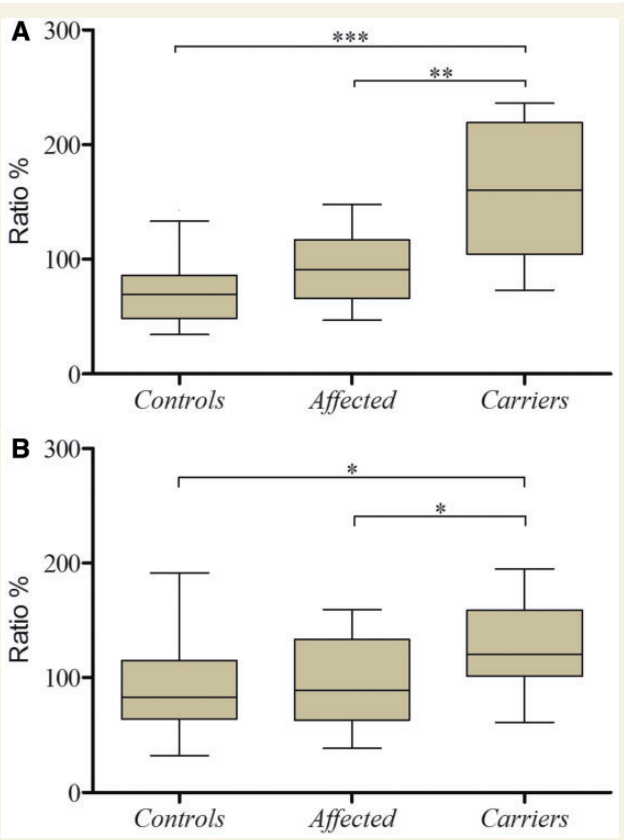


**Figure 1 Analysis of mtDNA content in affected subjects and carriers versus controls.** Mitochondrial DNA copy number (mtDNA/nDNA) with means ± SD was evaluated for affected subjects, carriers with respect to the controls whose mean was set as 100%. Experiments were performed in triplicate for all samples. Data were analysed by using GraphPad Prism package applying the ANOVA test. One-way analysis of variance was significant for both Italian ($P < 0.0001$) and Spanish ($P = 0.0025$) cohorts. Tukey's Multiple Comparison Test was applied to evaluate the statistical significance of differences of affected subjects, carriers and control data both in Italian (**A**) and Spanish (**B**) samples. Statistical significance was set at $P < 0.05$. Carriers showed increased mtDNA content compared with affected individuals (Italian, $P < 0.0001$; Spanish, $P < 0.05$). Graphical representation is corrected by Whiskers: Tukey. *$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$.

The results herein point to an increase of mitochondrial proliferations as the outcome of subtle and non-severe damaging mutations affecting mitochondrial activity, which is compensated by the proliferation of mitochondria that are underperforming due to the LHON mutation. In several mitochondrial diseases, a compensatory strategy to mitochondrial dysfunction is commonly observed as massive proliferation of mitochondria in skeletal muscle fibres known as ragged red fibres (DiMauro and Schon, 2003). Subsarcolemmal accumulation of defective mitochondria is probably triggered by energy depletion due to heteroplasmic pathogenic mtDNA mutations affecting the mitochondrial protein synthesis apparatus, while it is usually not

reported for mutations either homoplasmic or associated to defects in protein-coding genes such as exemplified by LHON mutations (De Vivo, 1993; DiMauro and Schon, 2003). It has been demonstrated that all three LHON mutations decrease complex I-driven ATP synthesis rate and lead to chronic increase of reactive oxygen species production that predispose cells to apoptosis (Vergani *et al*., 1995; Ghelli *et al*., 2003; Floreani *et al*., 2005). Probably, the overall weak pathogenic potential of LHON mutations leads to a general increase of mitochondrial mass accompanying mtDNA replication that can overcome the complex I defect and compensate the ATP deficit, suggesting a possible molecular explanation for the variable penetrance of the LHON primary mutations.

In conclusion, mtDNA copy number may be an important factor in determining conversion to disease in mutation carriers. It is conceivable that assessing mtDNA content may become a predictive genetic biomarker and, in conjunction with other clinical and metabolic markers, assist in the prognosis. Even more importantly, the activation of mitochondrial biogenesis could be exploited as a therapeutic strategy for LHON (Viscomi *et al*., 2015).

## Funding

This work was supported by 2010 grants from the University of Bari (Fondi ex-60%, 2009-2011) and Petruzzella00724113Prin – Finanziamento Università Prin 2009 and grants from the Instituto de Salud Carlos III (FIS-PI14/00005 and PI14/00070); Departamento de Ciencia, Tecnología y Universidad del Gobierno de Aragón y Fondo Social Europeo (Grupos Consolidados B33) and FEDER Funding Program from the European Union. CIBERER is an initiative of the ISCIII.

## References

Carelli V, Ross-Cisneros FN, Sadun AA. Mitochondrial dysfunction as a cause of optic neuropathies. Prog Retin Eye Res 2004; 23: 53–89.

De Vivo DC. The expanding clinical spectrum of mitochondrial diseases. Brain Dev 1993; 15: 1–22.

DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. N Engl J Med 2003; 348: 2656–68.

Floreani M, Napoli E, Martinuzzi A, Pantano G, De Riva V, Trevisan R, et al. Antioxidant defences in cybrids harboring mtDNA mutations associated with Leber's hereditary optic neuropathy. FEBS J 2005; 272: 1124–35.

Ghelli A, Zanna C, Porcelli AM, Schapira AH, Martinuzzi A, Carelli V, et al. Leber's hereditary optic neuropathy (LHON) pathogenic mutations induce mitochondrial-dependent apoptotic death in transmitochondrial cells incubated with galactose medium. J Biol Chem 2003; 278: 4145–50.

Giordano C, Iommarini L, Giordano L, Maresca A, Pisano A, Valentino ML, et al. Efficient mitochondrial biogenesis drives incomplete penetrance in Leber's hereditary optic neuropathy. Brain 2014; 137: 335–53.

Howell N, Mackey DA. Low-penetrance branches in matrilineal pedigrees with Leber hereditary optic neuropathy. Am J Hum Genet 1998; 63: 1220–4.

Man PY, Griffiths PG, Brown DT, Howell N, Turnbull DM, Chinnery PF. The epidemiology of Leber hereditary optic neuropathy in the North East of England. Am J Hum Genet 2003; 72: 333–9.

Marcuello A, Gonzalez-Alonso J, Calbet JA, Damsgaard R, Lopez-Perez MJ, Diez-Sanchez C. Skeletal muscle mitochondrial DNA content in exercising humans. J Appl Physiol 2005; 99: 1372–7.

Nishioka T, Soemantri A, Ishida T. mtDNA/nDNA ratio in 14484 LHON mitochondrial mutation carriers. J Hum Genet 2004; 49: 701–5.

Schon EA, DiMauro S, Hirano M. Human mitochondrial DNA: roles of inherited and somatic mutations. Nat Rev Genet 2012; 13: 878–90.

Vergani L, Martinuzzi A, Carelli V, Cortelli P, Montagna P, Schievano G, et al. MtDNA mutations associated with Leber's hereditary optic neuropathy: studies on cytoplasmic hybrid (cybrid) cells. Biochem Biophys Res Commun 1995; 210: 880–8.

Viscomi C, Bottani E, Zeviani M. Emerging concepts in the therapy of mitochondrial disease. Biochim Biophys Acta 2015; 1847: 544–57.

Yen MY, Chen CS, Wang AG, Wei YH. Increase of mitochondrial DNA in blood cells of patients with Leber's hereditary optic neuropathy with 11778 mutation. Br J Ophthalmol 2002; 86: 1027–30.

Yu-Wai-Man P, Griffiths PG, Chinnery PF. Mitochondrial optic neuropathies - disease mechanisms and therapeutic strategies. Prog Retin Eye Res 2011; 30: 81–114.

Zoccolella S, Artuso L, Capozzo R, Amati A, Guerra F, Simone I, et al. Mitochondrial genome large rearrangements in the skeletal muscle of a patient with PMA. Eur J Neurol 2012; 19: e63-4.