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LETTER TO THE EDITOR

MFN2 mutations cause compensatory mitochondrial DNA proliferation

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Sir, We read with great interest the report of a Tunisian family by Rouzier *et al*. (2011) describing the neurological disorder linked to a novel heterozygous missense mutation in *MFN2* (1p36.2) (Rouzier *et al*., 2011). *MFN2* mutations typically cause autosomal dominant axonal Charcot–Marie–Tooth disease (CMT2A, OMIM 609260), with peripheral nerve degeneration occasionally associated with visual failure and optic atrophy (Zuchner *et al*., 2004, 2006). Interestingly, the clinical manifestations among mutational carriers in this Tunisian family were even more variable, ranging from asymptomatic subclinical disease to an axonal sensorimotor neuropathy complicated by optic atrophy, deafness, cerebellar ataxia and proximal myopathy. Furthermore, the intriguing finding of cytochrome *c* oxidase (COX)-deficient fibres and multiple mitochondrial DNA deletions in skeletal muscle biopsies suggest that *MFN2* mutations can result in disturbed mitochondrial DNA maintenance and an overt respiratory chain defect, in addition to marked fragmentation of the mitochondrial network. These deleterious consequences are strikingly reminiscent of the pathological features recently highlighted in *Brain* for autosomal dominant optic atrophy due to *OPA1* mutations (Amati-Bonneau *et al*., 2008; Hudson *et al*., 2008; Yu-Wai-Man *et al*., 2010*a*). Here, we provide additional evidence that *MFN2*-associated neuropathy is a novel disorder of mitochondrial DNA maintenance in a study of 58 probands with CMT2A and confirmed *MFN2* mutations (Table 1), compared with 131 age-matched normal controls.

Total genomic DNA was extracted from the leucocyte fraction of venous blood samples. The average cellular mitochondrial DNA content was quantified with a SYBR Green™ quantitative polymerase chain reaction assay on a MyiQ™ real-time polymerase chain reaction detection system (Biorad), with *MTND1* as the mitochondrial template and *GAPDH* as the nuclear-encoded housekeeping template (Yu-Wai-Man *et al*., 2010*a*). Relative mitochondrial DNA copy number was derived from the difference in threshold cycle (C_t) values obtained for *MTND1* and *GAPDH* using the $2(2_t^{-\Delta}C)$ equation to account for two copies of *GAPDH* per cell nucleus.

Mitochondrial DNA levels in the *MFN2* group [mean mitochondrial DNA copy number = 195.7, standard deviation (SD) = 126.6, $n = 58$] were significantly higher compared with controls (mean mitochondrial DNA copy number = 60.9, SD = 42.3, $n = 131$,

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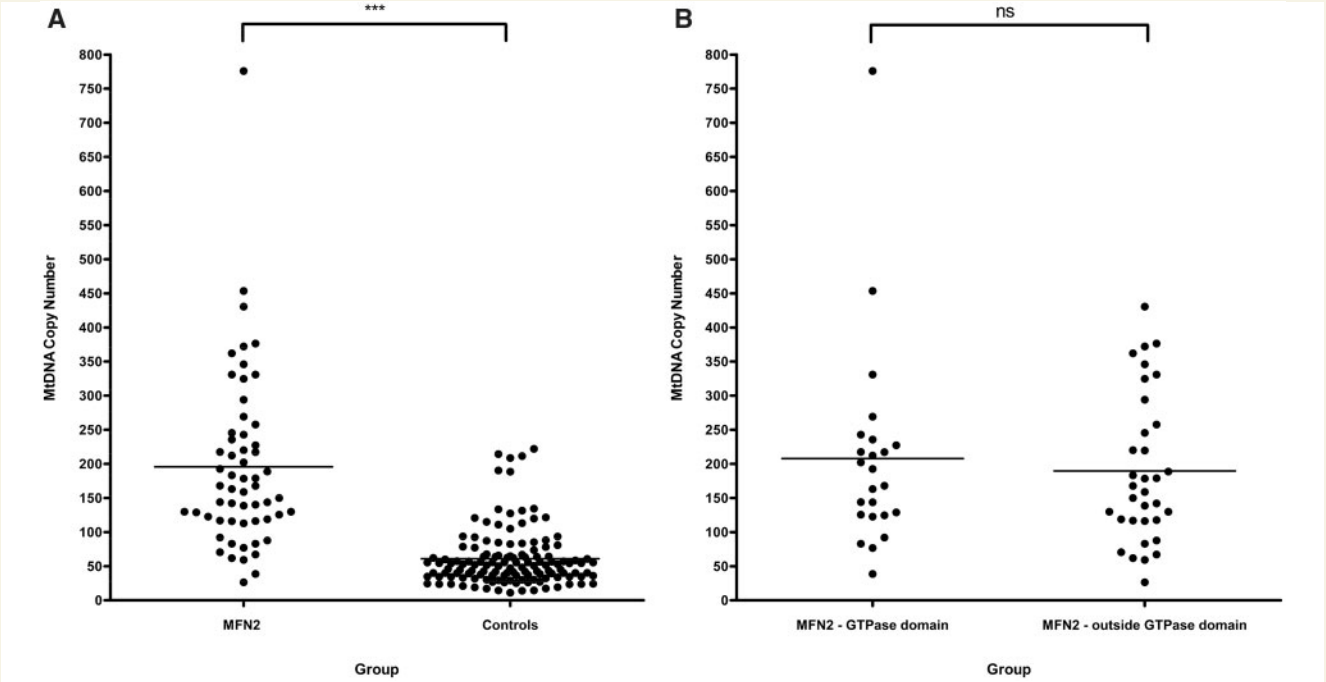

Figure 1 Comparison of mitochondrial DNA (MtDNA) blood copy number: (**A**) *MFN2* mutational carriers compared with age-matched normal controls; (**B**) patients harbouring *MFN2* mutations within the GTPase domain (mean mitochondrial DNA copy number = 207.9, SD = 150.2, *n = 0 24*) compared with those located outside this region (mean mitochondrial DNA copy number = 189.8, SD = 108.2, *n* = 34); ***$P < 0.0001$; ns = not significant at $P = 0.5957$.

$P < 0.0001$) (Fig. 1A). Given the suggestion by Rouzier *et al.* (2011) that *MFN2* mutations involving the functional GTPase domain were more likely to precipitate the severe multi-system phenotype documented in their family, we performed a subgroup analysis based on whether or not patients in our cohort harboured mutations within the highly conserved GTPase gene region. No significant difference was found between these two distinct mutational subgroups ($P = 0.5957$) (Fig. 1B).

Although we have previously shown that variation in the differential blood cell count can affect blood-derived mitochondrial DNA copy number (Pyle *et al.*, 2010), the 3-fold increase in mitochondrial DNA content detected in our *MFN2* cohort was substantially greater than the error attributable to this possible confounding factor. Mitochondrial proliferation is a well-recognized important diagnostic feature in skeletal muscle of patients with a range of mitochondrial cytopathies (Taylor *et al.*, 2004; Aure *et al.*, 2006). This compensatory mechanism is thought to occur in response to an underlying cellular bioenergetic crisis (the 'sick mitochondrion hypothesis'), which leads to the classical 'ragged-red fibre' appearance on Gomori Trichrome or succinate dehydrogenase staining (Chinnery and Samuels, 1999; Capps *et al.*, 2003; Durham *et al.*, 2007). In this study, we have demonstrated the same phenomenon in blood leucocytes derived from patients with *MFN2* mutations. Although Rouzier *et al.* (2011) determined mitochondrial DNA copy number in four skeletal muscle biopsies, they only report on the absence of mitochondrial DNA depletion in homogenate muscle extracts (Table 1). Pathologically increased mitochondrial DNA levels have been detected in laser-microdissected single skeletal muscle fibres from patients with *OPA1* mutations, this effect being particularly marked in COX-deficient muscle fibres (Yu-Wai-Man *et al.*, 2010*b*). Given the overlapping clinical, histological and molecular characteristics observed in these two primary disorders of mitochondrial dynamics, it would be of great interest to know whether mitochondrial DNA proliferation was also present in muscle fibres from patients with *MFN2* mutations, especially in the two biopsies noted to have ragged-red fibres.

MFN2 is the newest member of an expanding group of nuclear mitochondrial disorders characterized by disturbed mitochondrial DNA maintenance, a process which, increasingly, seems to be intrinsically related to the state of the mitochondrial network (Chen *et al.*, 2010; Elachouri *et al.*, 2011). Our observation that *MFN2* mutations cause mitochondrial proliferation in blood adds weight to the novel disease mechanism reported by Rouzier *et al.* (2011). Future work is needed to disentangle the complex interplay between disturbed mitochondrial fusion and fission, mitochondrial DNA instability and the eventual development of both neurological and visual deficits in patients with CMT2A and *MFN2* mutations.

Table 1 ***MFN2*** **mutations identified in our patient cohort**

Mutation	Type	Functional domain	Number of patients
p.Glu65Stop	Nonsense	–	3
p.Arg94Trp	Missense	–	8
p.Arg94Gln	Missense	–	2
p.Ala100Gly	Missense	GTPase	1
p.Arg104Leu	Missense	GTPase	3
p.Arg104Trp	Missense	GTPase	1
p.Thr105Ala	Missense	GTPase	1
p.His165Tyr	Missense	GTPase	6
p.Gly202Ala	Missense	GTPase	4
p.Thr206Ile	Missense	GTPase	2
p.Thr232Asn	Missense	GTPase	1
p.Arg250Gln	Missense	GTPase	1
p.Arg259Cys	Missense	GTPase	1
p.Arg280His	Missense	GTPase	1
p.Gly298Arg	Missense	GTPase	1
p.Glu308Stop[a]	Nonsense	GTPase	1
p.Arg364Trp	Missense	Coiled-Coil 1 (CC1)	1
p.Arg364Pro	Missense	Coiled-Coil 1 (CC1)	1
p.Arg364Gln	Missense	Coiled-Coil 1 (CC1)	1
p.Met376Val	Missense	Coiled-Coil 1 (CC1)	1
p.Met376Ile	Missense	Coiled-Coil 1 (CC1)	1
p.Ala383Val	Missense	Coiled-Coil 1 (CC1)	1
p.Arg468His	Missense	–	2
p.Asp496Gly	Missense	–	2
p.Arg519Pro[a]	Missense	–	1
p.Leu673Pro	Missense	–	3
p.Val705Ile	Missense	Coiled-Coil 2 (CC2)	2
p.Arg707Trp	Missense	Coiled-Coil 2 (CC2)	1
p.Arg707Pro	Missense	Coiled-Coil 2 (CC2)	1
p.Ala716Thr	Missense	Coiled-Coil 2 (CC2)	1
p.His750Pro	Missense	–	1
p.Gln751Stop	Nonsense	–	1
p.Tyr752Stop	Nonsense	–	1

[a]One patient harboured two heterozygous *MFN2* mutations.

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