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LETTER TO THE EDITOR

OPA1 mutations impair mitochondrial function in both pure and complicated dominant optic atrophy

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Sir, Autosomal dominant optic atrophy (DOA) is a common cause of inherited visual failure affecting at least 1 in 35 000 of the general population (Yu-Wai-Man *et al*., 2010*a*). Pathogenic *OPA1* mutations account for about 60% of all cases, causing bilateral, symmetrical optic atrophy secondary to the highly tissue-specific loss of one cell type - the retinal ganglion cell (Lenaers *et al*., 2009). Although optic nerve degeneration remains the defining feature of DOA, we recently reported in *Brain* that up to 20% of patients with *OPA1* mutations will also develop additional neuromuscular complications including deafness, ataxia, myopathy, peripheral neuropathy and progressive external ophthalmoplegia (Yu-Wai-Man *et al*., 2010*b*).

In two earlier studies, also published in *Brain* (Amati-Bonneau *et al*., 2008; Hudson *et al*., 2008), we described for the first time the intriguing association of these syndromal DOA+ variants with multiple mitochondrial DNA deletions and cytochrome *c* oxidase (COX)-negative skeletal muscle fibres (Zeviani, 2008). Interestingly, these mitochondrial defects were subsequently identified in *OPA1* patients with pure optic nerve involvement, but at levels four times lower compared with the DOA+ group (Yu-Wai-Man *et al*., 2010*b*). The involvement of other tissue types in DOA+ could therefore be a direct consequence of the greater accumulation of these secondary mitochondrial DNA abnormalities, the latter potentiating an already compromised mitochondrial oxidative reserve due to the mutant OPA1 protein. To determine whether this was the case, we used *in vivo* phosphorus magnetic resonance spectroscopy (^{31}P-MRS) to specifically measure mitochondrial oxidative function in a subgroup of *OPA1* patients from our original reports, correlating our findings with the histochemical and mitochondrial DNA defects identified in skeletal muscle biopsies, and thus extending our previous observations (Yu-Wai-Man *et al*., 2010*b*).

For this ^{31}P-MRS study, we selected 17 patients harbouring 12 different pathogenic *OPA1* mutations [mean age = 47.7 years, standard deviation (SD) = 10.6 years, range = 30.0–65.0 years): nine patients with isolated optic atrophy and eight patients with complex neuromuscular phenotypes (Tables 1 and 2). This patient group was compared with 17 newly identified, age-matched, normal controls with no evidence of ocular or neuromuscular pathologies (mean age = 48.3 years, SD = 8.5 years, range 37.0–63.0 years, $P = 0.8457$). This study had the relevant institutional ethical approval and informed consent was obtained in accordance with the Declaration of Helsinki.

Resting ^{31}P-MRS parameters were within the normal range for the entire *OPA1* group (Table 3). Similarly, *OPA1* patients did not exhibit abnormalities in proton handling at rest (pH), during or following exercise (maximum proton efflux rate). The overall recovery of both phosphocreatine ($P = 0.0218$) and adenosine diphosphate (ADP) ($P = 0.0274$) to basal levels, as measured by the half time $\tau_{1/2}$, was significantly delayed in all *OPA1* mutational carriers. Subgroup analysis showed a significant difference for patients with both pure DOA and DOA + phenotypes compared with controls, but there was no significant difference in $\tau_{1/2}$ phosphocreatine and $\tau_{1/2}$ ADP between these two disease subgroups (Fig. 1A and B). The frequency of COX-negative fibres in skeletal muscle biopsies (mean = 1.9%, SD = 2.4%, range = 0–10.0%, $n = 16$) did not correlate with either $\tau_{1/2}$ phosphocreatine (Spearman rank correlation coefficient = 0.0310, $P = 0.9094$) or

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Table 1 Clinical features of our *OPA1* patient cohort

Patient	Age (years)	Sex	FHx	Onset[a] (years)	Snellen BCVA		Clinical phenotype						
					Left	Right	Optic Atrophy	Deafness	Ataxia	Myopathy	Neuropathy	PEO	Others
1	50	M	+	8	CF	CF	+						
2	58	F	+	–	20/30	20/30	+		+				MS-like illness
3	30	M	+	5	CF	CF	+		+	+	+	+	
4	38	F	+	1	CF	CF	+	+	+	+	+	+	Migraine
5	43	M	+	5	20/200	20/200	+						
6	59	M	+	5	20/200	20/200	+						
7	43	M	+	13	CF	CF	+		+	+	+	+	
8	65	M	+	5	20/200	20/200	+						
9	60	F	+	16	CF	CF	+						
10	40	F	+	15	20/60	20/120	+						
11	44	F	+	15	20/120	20/200	+		+		+		
12	54	M	+	5	CF	CF	+	+					
13	43	M		5	20/200	20/200	+	+		+		+	
14	54	M	+	–	20/20	20/30	+						
15	39	M	+	15	20/200	20/120	+						
16	59	M	+	5	CF	CF	+				+		HSP, Migraine
17	31	M	+	11	20/40	20/60	+						

[a]Age of onset of visual failure.

The clinical and molecular characteristics of the eight patients with DOA+ phenotypes have been detailed previously (Yu-Wai-Man *et al*., 2010*b*): Patient 2 (Pedigree UK-6), Patient 3 (Pedigree UK-11), Patient 4 (Pedigree UK-11), Patient 7 (Pedigree UK-12), Patient 11 (Pedigree UK-8), Patient 12 (Pedigree UK-1), Patient 13 (Pedigree UK-13) and Patient 16 (Pedigree UK-3). BCVA = best corrected visual acuity; CF = counting fingers; F = female; FHx = family history; HSP = hereditary spastic paraparesis; M = male; MS = multiple sclerosis; PEO = progressive external ophthalmoplegia.

Table 2 *OPA1* mutations and mitochondrial abnormalities identified in skeletal muscle biopsies

Patient	*OPA1* mutations				Skeletal muscle biopsy	
	cDNA change	Location	Consequence	Domain	COX-negative fibres (%)	Mitochondrial DNA deletions
1	c.2613+1g>a	Intron 25	Splicing defect		0.0	–
2	c.2613+1g>a	Intron 25	Splicing defect		1.4	+
3	c.1635C>A	Exon 17	p.S545R	Dynamin	0.4	+
4	c.1635C>A	Exon 17	p.S545R	Dynamin	N/A	N/A
5	c.2708_2711delTTAG	Exon 27	p.V903fsX3	GED	0.6	+
6	Exons 1-5b deletion		p.M1fsX208		2.0	+
7	c.1294A>G	Exon 13	p.I432V	GTPase	10.0	+
8	c.2818+5g>a	Intron 27	Splicing defect		3.0	+
9	c.2713C>T	Exon 27	p.R905X	GED	3.0	+
10	c.2713C>T	Exon 27	p.R905X	GED	0.5	+
11	c.32+1g>a	Intron 1	Splicing defect		2.0	+
12	c.1212+3a>t	Intron 12	Splicing defect		0.3	+
13	c.1334G>A	Exon 14	p.R445H	GTPase	3.1	+
14	c.1516+1g>t	Intron 15	Splicing defect		1.8	+
15	c.1516+1g>t	Intron 15	Splicing defect		0.0	+
16	c.876-878delTGT	Exon 9	p.V294fsX667		2.1	+
17	c.876-878delTGT	Exon 9	p.V294fsX667		0.1	+

COX = cytochrome *c* oxidase; GED = GTPase effector domain; GTP = guanosine triphophate; N/A = not available.

$\tau_{1/2}$ ADP (Spearman rank correlation coefficient = −0.1636, $P = 0.5449$) (Fig. 1C and D). The correlation between COX deficiency and both $\tau_{1/2}$ phosphocreatine and $\tau_{1/2}$ ADP remained non-significant after exclusion of two outlying data points (Fig. 2). For three DOA+ patients (Patients 3, 7 and 16), citrate synthase and mitochondrial respiratory chain enzyme activities were determined in mitochondrially enriched suspensions obtained from homogenized muscle specimens (Taylor *et al*., 2004). All measurements were within the normal assay range.

OPA1 mutations exert a deleterious effect on *in vivo* mitochondrial function, irrespective of mutational subtypes and disease severity. Our *a priori* hypothesis was that the additional

Table 3 Resting and post-exercise ^{31}P-MRS measurements for *OPA1* patients and controls

	Patients ($n = 17$) Mean (95% CI)	Controls ($n = 17$) Mean (95% CI)	*P*-value
Age (years)	47.7 (42.2–53.1)	48.3 (43.9–52.7)	0.8457
Resting			
PCr (mM)	30.9 (29.8–32.0)	31.9 (30.9–32.8)	0.1539
Pi (mM)	2.90 (2.40–3.39)	3.02 (2.81–3.24)	0.6279
PCr/Pi ratio	11.6 (9.7–13.5)	10.7 (9.9–11.6)	0.3803
ADP (μM)	9.79 (9.48–10.09)	9.82 (9.39–10.25)	0.9029
pH	7.05 (7.04–7.06)	7.05 (7.03–7.07)	0.7598
Post-exercise			
Initial PCr resynthesis rate (mM/min)	11.8 (7.9–15.6)	10.5 (8.5–12.5)	0.5366
$\tau_{1/2}$ PCr (s)	39.4 (28.0–50.8)	25.9 (22.7–29.1)	0.0218*
$\tau_{1/2}$ ADP (s)	30.1 (21.3–38.8)	20.2 (17.9–22.4)	0.0274*
Maximum proton efflux rate (mmol/l/min)	2.75 (2.09–3.42)	2.55 (2.01–3.09)	0.6157

Phosphorus spectra were acquired from the gastrocnemius and soleus muscles using a 3-T Intera Achieva scanner (Philips, Best, The Netherlands) during: (i) a 1-minute period of rest; (ii) a 3-minute period of plantar flexion at 25% of the maximum voluntary contraction; and (iii) a 6-minute recovery period (Trenell *et al*., 2006; Hollingsworth *et al*., 2008). Spectral quantification was performed with the Java-based magnetic resonance user interface (jMRUI v.3.0), using AMARES with custom prior knowledge appropriate to skeletal muscle (Naressi *et al*., 2001). ADP = adenosine diphosphate; PCr = phosphocreatine; Pi = inorganic phosphate. *Significant *P*-value.

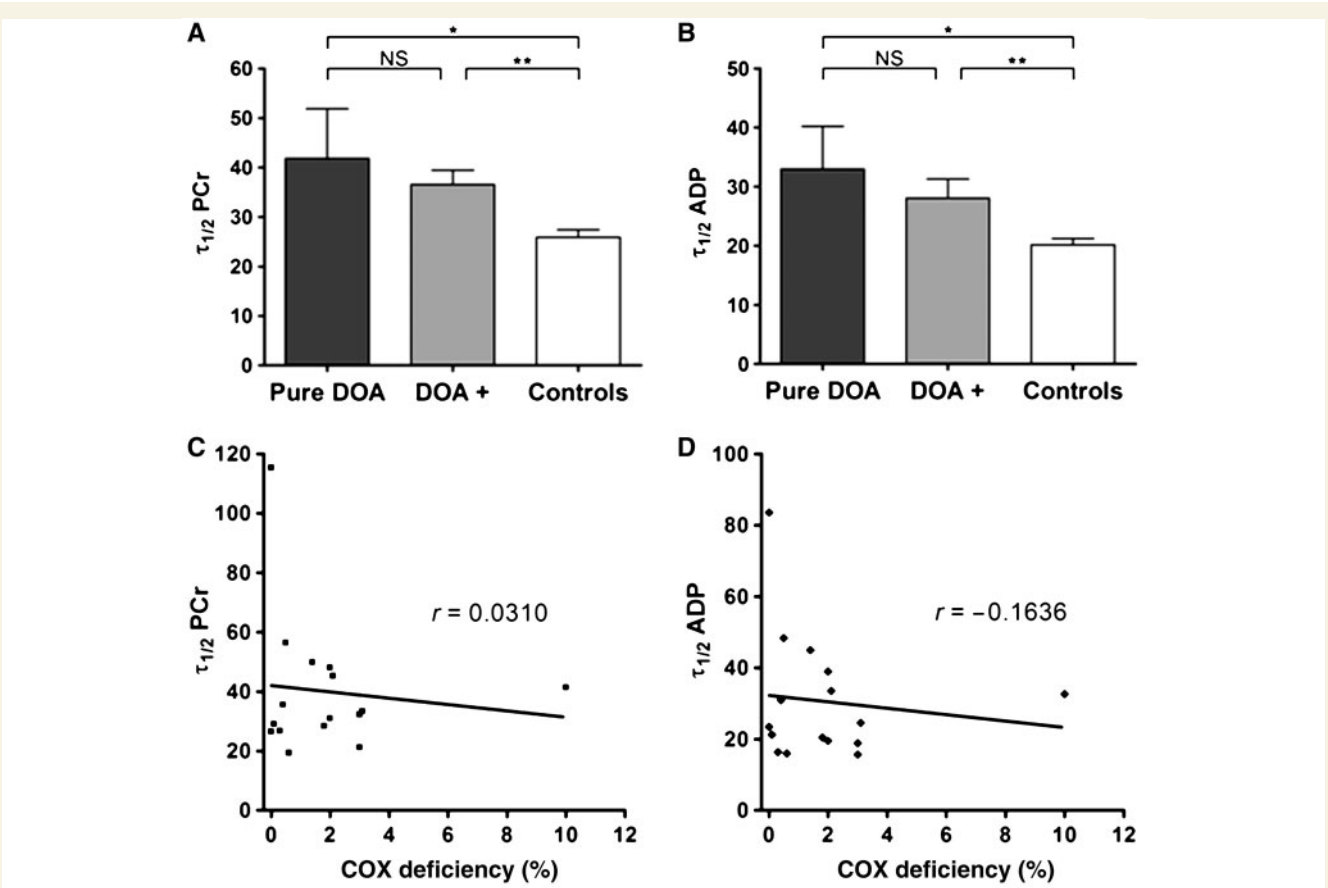

Figure 1 Subgroup comparison of post-exercise ^{31}P-MRS parameters between patients with pure DOA ($n = 9$), DOA+ ($n = 8$) and age-matched normal controls ($n = 17$), for (**A**) $\tau_{1/2}$ phosphocreatine (PCr): *$P = 0.0434$, ** $P = 0.0014$, non-significant (NS) at $P = 0.6406$, and (**B**) $\tau_{1/2}$ adenosine diphosphate (ADP): *$P = 0.0256$, **$P = 0.0079$, NS at $P = 0.5627$. Correlation of *in vivo* markers of metabolic recovery with the frequency of COX-negative fibres identified in skeletal muscle biopsies: (**C**) $\tau_{1/2}$ phosphocreatine: Spearman rank correlation coefficient = 0.0310, $P = 0.9094$, and (**D**) $\tau_{1/2}$ ADP: Spearman rank correlation coefficient = −0.1636, $P = 0.5449$. The error bars represent the standard error of the mean.

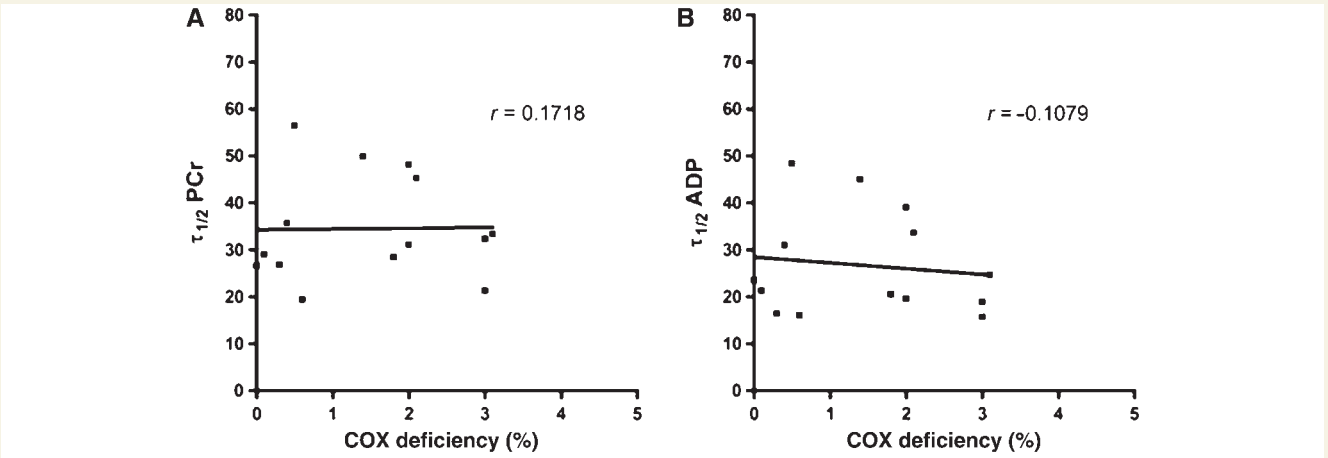

Figure 2 Correlation of *in vivo* markers of metabolic recovery with the frequency of COX-negative fibres identified in skeletal muscle biopsies, after exclusion of two outlying data points: (**A**) $\tau_{1/2}$ phosphocreatine (PCr): Spearman rank correlation coefficient = 0.1718, $P = 0.5570$, and (**B**) $\tau_{1/2}$ ADP: Spearman rank correlation coefficient = −0.1079, $P = 0.7134$.

neuromuscular manifestations seen in DOA+ patients were the result of a more pronounced biochemical defect. What are the reasons therefore for the lack of a significant difference in bioenergetic impairment between patients with pure DOA and DOA+ phenotypes? Our results are statistically robust and consistent for both phosphocreatine and ADP recovery parameters. It is therefore unlikely that the inclusion of additional patients would influence the outcome of this ^{31}P-MRS study. The level of COX deficiency was determined in quadriceps or tibialis anterior biopsies, whereas phosphorus spectral data were acquired from the gastrocnemius and soleus muscles. It is possible that structural and functional differences exist between these various muscle groups, which could have influenced our measurement parameters and comparisons. Notwithstanding this caveat, our observations overall indicate that impaired oxidative phosphorylation is only part of the problem, and different pathways must be mediating retinal ganglion cell loss and cellular dysfunction in other organ systems. It is also intriguing that the same *OPA1* mutation can lead to both isolated optic nerve involvement and a more severe form of the disease, with the development of neuromuscular complications (Yu-Wai-Man *et al*., 2010*b*). Additional factors are clearly modulating the pathogenic expression of the *OPA1* mutation resulting in markedly variable clinical phenotypes. Future studies are required to determine whether DOA+ is related to other pathological consequences triggered by the greater accumulation of these mitochondrial DNA deletions; such as the induction of mitochondrial proliferation, which is known to have a pro-apoptotic effect (Aure *et al*., 2006; Yu-Wai-Man *et al*., 2010*c*), or other unrelated mechanisms linked with mitochondrial network instability, OPA1 being a critical pro-fusion protein (Lenaers *et al*., 2009).

Our ^{31}P-MRS study supports an earlier report of impaired *in vivo* mitochondrial function in six affected individuals from two families, segregating isolated optic neuropathy and the c.2708_2711del(TTAG) deletion (p.V903fsX3) (Lodi *et al*., 2004). We have extended this observation to 11 additional pathogenic *OPA1* mutations, including for the first time eight patients with DOA+ phenotypes. Previous ^{31}P-MRS studies have also confirmed a respiratory chain complex defect in Leber hereditary optic neuropathy (LHON), the classic paradigm of a primary mitochondrial optic neuropathy (Yu-Wai-Man *et al*., 2009). There seemed to be a mutational hierarchy; the m.11778G>A LHON mutation exhibiting the most pronounced effect on mitochondrial ATP synthesis, followed by m.14484T>C and m.3460G>A (Lodi *et al*., 1997, 2002). The latter only led to a subtle metabolic deficit, highlighting the vulnerability of retinal ganglion cells to even mild energetic imbalances. Given the pathological similarity shared by LHON and DOA, it is likely that the bioenergetic defect revealed by ^{31}P-MRS is contributing to selective retinal ganglion cell loss and visual failure in both disorders.

In three DOA+ patients, biochemical studies performed on skeletal muscle mitochondrial fractions were normal, despite the fact that both their $\tau_{1/2}$ phosphocreatine and $\tau_{1/2}$ ADP measurements exceeded the upper 95% control values. These findings suggest that ^{31}P-MRS could be a useful diagnostic adjunct in patients with suspected mitochondrial disease, when histochemistry and *in vitro* biochemical studies are inconclusive. Furthermore, mitochondrial oxidative physiology can be inferred both at rest and crucially following a period of exercise, the post-recovery kinetics being particularly sensitive in detecting a tissue's capacity to respond to energy demands (Barbiroli *et al*., 1998).

There is a complex, and still poorly defined, interplay between the multiple cellular functions regulated by the OPA1 protein. However, irrespective of the combination of factors involved, the ultimate loss of mitochondrial membrane potential and the release of cytochrome *c* molecules are likely to be key final common events triggering apoptotic cell death. Unravelling these intricately linked mechanisms will hopefully contribute to the long-term goal of developing therapeutic interventions, not only for DOA, but also for other mitochondrial optic neuropathies.

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