Reply: *DGUOK* recessive mutations in patients with CPEO, mitochondrial myopathy, parkinsonism and mtDNA deletions

Dario Ronchi,[1] Daniela Piga,[1] Stefano Lamberti,[1] Monica Sciacco,[2] Stefania Corti,[1] Maurizio Moggio,[2] Nereo Bresolin[1] and Giacomo Pietro Comi[1]

1 Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), University of Milan, Neurology Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
2 Neuromuscular and Rare Disease Unit, Department of Neuroscience, Fondazione IRCCS Ca' Granda Ospedale Policlinico Milano, Italy

Correspondence to: Dario Ronchi
University of Milan
Department of Pathophysiology and Transplantation, Neuroscience Section
IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35,
20122 Milan, Italy
E-mail: dario.ronchi@unimi.it

Sir,

We read with interest the letter from Caporali *et al.* (2017) reporting three novel patients from two independent families displaying adult onset mitochondrial disorders due to recessive *DGUOK* mutations, confirming the findings of our original paper (Ronchi *et al.*, 2012). Since then, the molecular screening of *DGUOK* in our cohort of patients featuring the accumulation of multiple mitochondrial DNA (mtDNA) deletions in skeletal muscle has not detected additional cases. However, the use of traditional and next generation sequencing (NGS) has expanded the number of variants in canonical and novel genes (Kornblum *et al.*, 2013; Ronchi *et al.*, 2013, 2015; Reyes *et al.*, 2015) associated with impaired muscle mtDNA maintenance (Table 1). *DGUOK* mutations now represent the 4.7% cause of disease in our cohort of patients and, considering the novel cases described by Caporali *et al.* (2017), they account for 6.9% of adult patients with mtDNA maintenance disorders in which a molecular diagnosis has been established (the third most frequent molecular defect after mutations in *POLG* and *PEO1*/*TWNK* encoding, respectively, for the mtDNA polymerase POLG and the helicase TWINKLE).

Clinical features of the novel probands include progressive bilateral ptosis followed by muscle weakness in upper and lower limbs. Serum creatine kinase levels were found to be increased in two patients and reached large amounts episodically in Patient 3, similar to one of our cases. Interestingly the same patient displayed visual, auditory and CNS involvement. Neuroimaging demonstrated a diffuse hypometabolism in basal ganglia and extending bilaterally to the occipital cortex, as observed in other mitochondrial encephalomyopathies (Martikainen *et al.*, 2016). DATscan showed a bilateral reduction of basal ganglia uptake. Consistently, clinical features of this patient included rigidity and bradykinesia but parkinsonism seems an isolated finding since it was not observed in other *DGUOK* patients. Nevertheless, mutations in *DGUOK* add to the group of defects impairing mtDNA maintenance found to segregate with parkinsonism, which include *POLG* (Miguel *et al.*, 2014), *POLG2* (Van Maldergem *et al.*, 2016), *PEO1*/*TWNK* (Kiferle *et al.*, 2013), *OPA1* (Carelli *et al.*, 2015), *MPV17* (Garone *et al.*, 2012). Nigrostriatal dysfunction has been observed *in vivo* (Tzoulis *et al.*, 2016) and *ex vivo* (Tzoulis *et al.*, 2013) in patients harbouring *POLG* and *PEO1* mutations as well as in transgenic mice selectively expressing mutant forms of these enzymes in striatal midbrain neurons (Song *et al.*, 2012; Perier *et al.*, 2013). In this regard, the massive accumulation of mtDNA deletions in the proband presenting parkinsonism supports the hypothesis that mtDNA homeostasis is essential for dopaminergic survival.

The authors evaluated mtDNA content and integrity by using standard techniques as well as digital PCR, an

Table 1 Relative proportion of genetically diagnosed patients harboring muscle mtDNA deletions

	Patients	Independent probands	Familial cases	Sporadic cases
POLG	35 (27.6%)	24 (24.2%)	7 (22.6%)	17 (25.0%)
TWNK/PEO1	18 (14.2%)	18 (18.2%)	13 (41.9%)	5 (7.4%)
SLC25A4/ANT1	6 (4.7%)	6 (6.1%)	6 (19.4%)	0
DGUOK	6 (4.7%)	5 (5.1%)	1 (3.2%)	4 (5.9%)
DNA2	4 (2.7%)	3 (2.3%)	1 (3.2%)	2 (2%)
TYMP	4 (3.1%)	2 (2.0%)	1 (3.2%)	1 (1.5%)
OPA1	3 (2.4%)	3 (3.0%)	0	3 (4.4%)
TK2	2 (1.6%)	2 (2.0%)	0	2 (2.9%)
MGME1	2 (1.6%)	1 (1.0%)	1 (3.2%)	0
RNASEH1	1 (0.8%)	1 (1.0%)	0	1 (1.5%)
CHCHD10	1 (0.8%)	1 (1.0%)	0	1 (1.5%)
Undiagnosed	45 (35.4%)	64 (33.3%)	1 (3.2%)	63 (47.1%)
Total	127	99	31	68

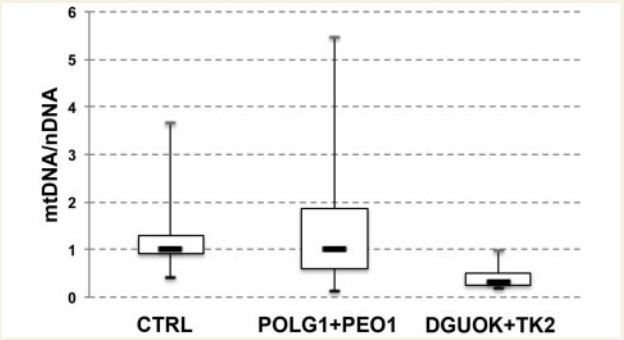

Figure 1 Box plot showing muscle mtDNA content in adult patients featuring mitochondrial myopathy with muscle mtDNA deletions associated with mutations in *POLG* or *TWNK/PEO1* ($n = 33$) and *DGUOK* or *TK2* ($n = 7$) compared with healthy controls ($n = 27$). Solid bars represent median values. mtDNA quantification, normalized to nuclear DNA (nDNA) content, was performed by quantitative real-time PCR as previously described (Spinazzola *et al.*, 2006). Mitochondrial DNA content in *DGUOK/TK2* patients was significantly reduced compared to *POLG1/PEO1* patients (*t*-test, $P \leq 0.05$) and control subjects (*t*-test, $P \leq 0.01$).

approach that combines superior sensitivity and absolute quantification of mtDNA molecules. These assays disclosed the co-occurrence of mtDNA depletion and deletions in patients' samples. This finding is consistent with the fundamental role of deoxyguanosine kinase in the mitochondrial supply of dNTPs in post-mitotic tissues. Similarly, in our cohort of patients with mtDNA instability, a reduction in muscle mtDNA content was observed in subjects harbouring mutations in DGUOK and TK2 (two key enzymes of the dNTPs salvage pathway) but not in patients with mutations in POLG or TWINKLE (mtDNA replisome machinery) or in a cohort of age-matched controls without neurological involvement (Fig. 1). Age at biopsy, the type of muscle sampled, the different patterns of inheritance and the number of patients analysed restricts the range of this finding.

In conclusion, the study from Caporali *et al.* (2017) confirms and expands the pathogenetic role of DGUOK dysfunction in adult-onset mitochondrial presentations. The application of next generation sequencing in adult patients with mitochondrial phenotypes is expected to identify novel *DGUOK* cases in the future. In parallel, paediatric patients who underwent liver transplantation because of *DGUOK*-related hepatocerebral mtDNA depletion syndrome are candidates to develop some of the neurological symptoms here described. These findings reinforce the paradigm according to which defects in the same gene might result in a spectrum of clinical conditions ranging from severe early-onset syndromes to milder adult-onset presentations according to the behaviour of the causative mutations (and the relative residual enzymatic activities in affected tissues) (Viscomi and Zeviani, 2017). The existence of common mechanisms for such heterogeneous conditions lays the basis for the development of shared therapies. For *DGUOK* patients, the effects of nucleosides supplementation in symptomatic patients, or even before symptoms onset, should be considered in view of the encouraging results obtained in preclinical studies addressing other forms of mtDNA instability (Cámara *et al.*, 2014; Lopez-Gomez *et al.*, 2017).

Funding

The financial support of Fondazione Cariplo to Dario Ronchi (Project number: 2014-1010) is gratefully acknowledged.

References

Cámara Y, González-Vioque E, Scarpelli M, Torres-Torronteras J, Caballero A, Hirano M, et al. Administration of deoxyribonucleosides

or inhibition of their catabolism as a pharmacological approach for mitochondrial DNA depletion syndrome. Hum Mol Genet 2014; 23: 2459–67.

Caporali L, Bello L, Tagliavini F, La Morgia C, Maresca A, Di Vito L, et al. *DGUOK* recessive mutations in patients with CPEO, mitochondrial myopathy, parkinsonism and mtDNA deletions. Brain 2017; 141: e3.

Carelli V, Musumeci O, Caporali L, Zanna C, La Morgia C, Del Dotto V, et al. Syndromic parkinsonism and dementia associated with OPA1 missense mutations. Ann Neurol 2015; 78: 21–38.

Garone C, Rubio JC, Calvo SE, Naini A, Tanji K, Dimauro S, et al. MPV17 Mutations causing adult-onset multisystemic disorder with multiple mitochondrial DNA deletions. Arch Neurol 2012; 69: 1648–51.

Kiferle L, Orsucci D, Mancuso M, Lo Gerfo A, Petrozzi L, Siciliano G, et al., Twinkle mutation in an Italian family with external progressive ophthalmoplegia and parkinsonism: a case report and an update on the state of art. Neurosci Lett 2013; 556: 1–4.

Kornblum C, Nicholls TJ, Haack TB, Schöler S, Peeva V, Danhauser K, et al. Loss-of-function mutations in MGME1 impair mtDNA replication and cause multisystemic mitochondrial disease. Nat Genet 2013; 45: 214–9.

Lopez-Gomez C, Levy RJ, Sanchez-Quintero MJ, Juanola-Falgarona M, Barca E, Garcia-Diaz B, et al. Deoxycytidine and deoxythymidine treatment for thymidine kinase 2 deficiency. Ann Neurol 2017; 81: 641–52.

Martikainen MH, Ng YS, Gorman GS, Alston CL, Blakely EL, Schaefer AM, et al., Clinical, genetic, and radiological features of extrapyramidal movement disorders in mitochondrial disease. JAMA Neurol 2016; 73: 668–74.

Miguel R, Gago MF, Martins J, Barros P, Vale J, Rosas MJ. POLG1-related levodopa-responsive parkinsonism. Clin Neurol Neurosurg 2014; 126: 47–54.

Perier C, Bender A, Garcı´a-Arumı´ E, Melia` MJ, Bove´ J, Laub C, et al. Accumulation of mitochondrial DNA deletions within dopaminergic neurons triggers neuroprotective mechanisms. Brain 2013; 136: 2369–78.

Reyes A, Melchionda L, Nasca A, Carrara F, Lamantea E, Zanolini A, et al. RNASEH1 Mutations impair mtDNA replication and cause adult-onset mitochondrial encephalomyopathy. Am J Hum Genet 2015; 97: 186–93.

Ronchi D, Garone C, Bordoni A, Gutierrez Rios P, Calvo SE, Ripolone M, et al. Next-generation sequencing reveals DGUOK mutations in adult patients with mitochondrial DNA multiple deletions. Brain 2012; 135: 3404–15.

Ronchi D, Di Fonzo A, Lin W, Bordoni A, Liu C, Fassone E, et al. Mutations in DNA2 link progressive myopathy to mitochondrial DNA instability. Am J Hum Genet 2013; 92: 293–300.

Ronchi D, Riboldi G, Del Bo R, Ticozzi N, Scarlato M, Galimberti D, et al. CHCHD10 mutations in Italian patients with sporadic amyotrophic lateral sclerosis. Brain 2015; 138(Pt 8): e372.

Song L, Shan Y, Lloyd KC, Cortopassi GA. Mutant Twinkle increases dopaminergic neurodegeneration, mtDNA deletions and modulates Parkin expression. Hum Mol Genet 2012; 21: 5147–58.

Spinazzola A, Viscomi C, Fernandez-Vizarra E, Carrara F, D'Adamo P, Calvo S, et al. MPV17 encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial DNA depletion. Nat Genet 2006; 38: 570–5.

Tzoulis C, Tran GT, Schwarzlmü ller T, Specht K, Haugarvoll K, Balafkan N, et al., Severe nigrostriatal degeneration without clinical parkinsonism in patients with polymerase gamma mutations. Brain 2013; 136: 2393–404.

Tzoulis C, Schwarzlmü ller T, Biermann M, Haugarvoll K, Bindoff LA. Mitochondrial DNA homeostasis is essential for nigrostriatal integrity. Mitochondrion 2016; 28: 33–7.

Van Maldergem L, Besse A, De Paepe B, Blakely EL, Appadurai V, Humble MM, et al. POLG2 deficiency causes adult-onset syndromic sensory neuropathy, ataxia and parkinsonism. Ann Clin Transl Neurol 2016; 4: 4–14.

Viscomi C, Zeviani M. MtDNA-maintenance defects: syndromes and genes. J Inherit Metab Dis 2017; 40: 587–99.