

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

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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/TWINK* 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/TWINK* ([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 |
|---------------------|------------|----------------------|----------------|----------------|
| <i>POLG</i> | 35 (27.6%) | 24 (24.2%) | 7 (22.6%) | 17 (25.0%) |
| <i>TWNK/PEO1</i> | 18 (14.2%) | 18 (18.2%) | 13 (41.9%) | 5 (7.4%) |
| <i>SLC25A4/ANT1</i> | 6 (4.7%) | 6 (6.1%) | 6 (19.4%) | 0 |
| <i>DGUOK</i> | 6 (4.7%) | 5 (5.1%) | 1 (3.2%) | 4 (5.9%) |
| <i>DNA2</i> | 4 (2.7%) | 3 (2.3%) | 1 (3.2%) | 2 (2%) |
| <i>TYMP</i> | 4 (3.1%) | 2 (2.0%) | 1 (3.2%) | 1 (1.5%) |
| <i>OPA1</i> | 3 (2.4%) | 3 (3.0%) | 0 | 3 (4.4%) |
| <i>TK2</i> | 2 (1.6%) | 2 (2.0%) | 0 | 2 (2.9%) |
| <i>MGME1</i> | 2 (1.6%) | 1 (1.0%) | 1 (3.2%) | 0 |
| <i>RNASEH1</i> | 1 (0.8%) | 1 (1.0%) | 0 | 1 (1.5%) |
| <i>CHCHD10</i> | 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 |

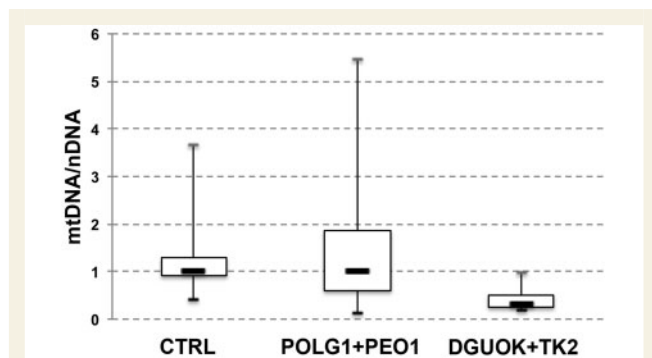


Figure 1 Box plot showing muscle mtDNA content in adult patients featuring mitochondrial myopathy with muscle mtDNA deletions associated with mutations in *POLG* or *TWNK/PEO1* ($n = 33$) and *DGUOK* or *TK2* ($n = 7$) compared with healthy controls ($n = 27$). Solid bars represent median values. mtDNA 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 *POLG/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 pre-clinical studies addressing other forms of mtDNA instability (Cámara *et al.*, 2014; Lopez-Gomez *et al.*, 2017).

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