Reply: Glial mitochondropathy in infantile neuroaxonal dystrophy: pathophysiological and therapeutic implications

Kerri J. Kinghorn, Jorge Iván Castillo-Quan, Li Li, Kailash P. Bhatia, Andrey Y. Abramov, John Hardy, Linda Partridge

Sir,

Mitochondrial dysfunction is increasingly being recognized as a therapeutic target in PLA26G-associated neurodegeneration (PLAN). We demonstrated in *Drosophila* and in human *PLA2G6* mutant fibroblasts that loss of normal *PLA2G6* activity is associated with increased mitochondrial lipid peroxidation and mitochondrial dysfunction. Furthermore, we identified the therapeutic benefits of deuterated polyunsaturated fatty acids (D-PUFAs) in PLAN, through inhibition of lipid peroxidation. D-PUFAs ameliorated the locomotor deficits in flies lacking the fly orthologue of *PLA2G6* (*iPLA2-VIA*), and reversed the mitochondrial abnormalities in patient fibroblasts harbouring pathogenic mutations in *PLA2G6* (Kinghorn *et al.*, 2015).

In their Letter to the Editor, Farrar *et al.* (2016) report abnormal mitochondrial morphology at the ultrastructural level in muscle and cutaneous nerves, as well as in cutaneous Schwann cells of a patient with infantile neuroaxonal dystrophy (INAD) associated with homozygous *PLA2G6* mutations. They also observed a selective loss of complex IV of the respiratory chain in skeletal muscle, and they report elevated urine catecholamine levels and serum neuronal specific enolase in their patient. This study is thus supportive of the abnormal mitochondrial morphology observed in the CNS of *Drosophila* and mouse models of PLA2G6 deficiency (Beck *et al.*, 2011; Kinghorn *et al.*, 2015; Kinghorn and Castillo-Quan, 2016). It also provides evidence for the involvement of peripheral nerves outside of the CNS, and that mitochondrial pathology extends beyond neurons to glia. The next step will be to confirm these findings in both central and peripheral neurons and glia in additional patients with PLAN, not only in those with INAD, but also in patients with later-onset disease such as adult-onset dystonia-parkinsonism.

PLAN comprises a group of disorders that are rare and usually rapidly progressive. To date there are no clinical trials or even anecdotal evidence to help guide treatment in this set of patients. The current management of individuals with neurodegenerative disorders linked to mutations in *PLA2G6* is therefore aimed at palliation and symptom control. Our study highlighted the potential therapeutic benefits of lipid peroxidation-lowering strategies in reversing the mitochondrial defects in PLAN. To our knowledge D-PUFAs are the only specific treatment that has been shown to ameliorate mitochondrial pathology in a disease model of *PLA2G6* deficiency (Kinghorn *et al.*, 2015). Future work is required to test the beneficial properties of D-PUFAs, and other compounds that reduce mitochondrial lipid peroxidation, in order to develop disease-modifying agents for PLAN. Farrar *et al.* (2016) treated their patient affected by INAD with a fish-derived supplement containing DHA and EPA as well as vitamin E, antioxidants that have not been tested in PLAN. Their report

raises a number of issues, in addition to the urgent need for disease-modifying therapies, such as the necessity to promptly diagnose patients early so that they can benefit from treatment prior to irreversible neuronal loss. Farrar *et al.* (2016) also highlight the importance of being able to effectively monitor therapeutic efficacy in PLAN, especially in INAD, which is an unrelenting progressive disorder, with death usually occurring before puberty (Nardocci *et al.*, 1999). They suggest that urinary catecholamines and serum-specific neuronal enolase may prove to be useful biomarkers for assessing the clinical efficacy of disease-modifying therapies. These markers now require validation in patients with PLAN. Furthermore, a recent study observed an elevated AST/ALT ratio and LDH in patients with PLAN, detectable from the early stages of disease and persistent with time. Interestingly AST exists as a mitochondrial isoform, in addition to a cytosolic one, and may reflect release from damaged mitochondria (Kraoua *et al.*, 2016). Future work is now required to identify other possible biomarkers, and to create the optimal multi-parameter biomarker set, to monitor disease progression and therapeutic efficacy.

Even if reliable clinical biomarkers are identified and we are able to enrol patients into trials at an early stage of disease, the best possible disease-modifying therapies must be available. Conventional antioxidants have not been tested in PLAN, although a number of antioxidant moieties such as vitamin C and E, creatine and co-enzyme Q_{10} have shown benefit in a range of *in vitro* and *in vivo* models of Parkinson's disease. Furthermore, testing of these antioxidants in clinical trials has by and large been inconclusive, with co-enzyme Q_{10} demonstrating the most promising results in Parkinson's disease patients (Jin *et al.*, 2014). It may be that for antioxidants to be sufficiently effective, they will require direct targeting to the mitochondria, the site where most of the oxidizing species are generated. A number of mitochondria-targeted antioxidants have been developed, including those mediating the transfer across the mitochondrial phospholipid bilayer through conjugation of the antioxidant moiety to the lipophilic triphenylphosphosphomnium cation. Such compounds based on the natural antioxidants include MitoQ® (mitoquinone), MitoVitE (mitotocopherol) and MitoApocynin, and have been shown to protect against mitochondrial lipid peroxidation in both *in vitro and in vivo* studies (Jauslin *et al.*, 2003; Smith and Murphy, 2010; Jin *et al.*, 2014; Finichiu *et al.*, 2015; Jameson *et al.*, 2015). Human studies have also demonstrated that MitoQ® can be administered orally for at least 1 year safely and, although the benefits have yet to be confirmed in patients with a neurodegenerative condition, it was shown to reduce mitochondrial oxidative damage in chronic hepatitis C infection (Gane *et al.*, 2010; Smith and Murphy, 2010). Recent work has also demonstrated that another antioxidant, tiron, permeabilizes the mitochondrial membrane, exhibiting not only profound antioxidant effects but also iron chelating properties *in vitro* (Oyewole and Birch-Machin, 2015). Such dual-functioning antioxidants may therefore be particularly beneficial in neurodegenerative conditions such as PLAN, in which, likely as a downstream effect of mitochondrial dysfunction, iron is deposited within brain structures such as the basal ganglia (Morgan *et al.*, 2006). Future work targeting mitochondrial lipid peroxidation in PLAN will involve testing the existing compounds, as well as optimizing the delivery of antioxidants to mitochondria and, in the case of PLAN, across the blood–brain barrier, perhaps using novel

biological nanomaterials. Furthermore, it is likely that combination therapies will be more effective than single antioxidant treatments with, for example, dual targeting of oxidation and iron chelation. Lastly, as PLAN is a genetically inherited disease, with an age of onset much earlier than in adult-onset sporadic neurodegenerative conditions such as Parkinson's disease, treatments will need to be suitable for administration before clinical disease onset, and in the early stages of disease, therefore requiring the treatment to be safe and well tolerated in infants and children.

In conclusion, increasing evidence points towards lipid peroxidation and mitochondrial dysfunction as being the main toxic events in PLAN. A concerted effort is now required to identify and validate the best biomarkers in PLAN, and to develop optimal anti-oxidant drug combinations. As PLAN is considered rare, clinical trials will require prompt early diagnosis and involve multi-centre collaboration. Given the neuropathological similarities between PLAN and more common neurodegenerative diseases such as Parkinson's disease, effective strategies in protecting mitochondria against oxidative stress may also be relevant to the treatment of this much larger group of patients.

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