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LETTER TO THE EDITOR

Glial mitochondropathy in infantile neuroaxonal dystrophy: pathophysiological and therapeutic implications

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Sir,

We read with great interest the article by Kinghorn *et al.* (2015) reporting that loss of phospholipase A2 group 6 (PLA2G6) leads to mitochondrial dysfunction and elevated mitochondrial lipid peroxidation in *Drosophila* neurons and patient fibroblast cultures (Kinghorn *et al.*, 2015). Importantly this work suggests administration of deuterated polyunsaturated fatty acids, serving to lower lipid peroxidation, may be beneficial in patients with pathogenic variants in *PLA2G6*, which is associated with infantile neuronal dystrophy (INAD). This extends findings of animal studies that have demonstrated PLA2G6 has an important role in neurons, with mutations producing significant changes in brain lipid metabolism and phospholipid fatty acid content (Cheon *et al.*, 2012). Disturbances in lipid metabolism are emerging as an important theme in motor neuron degeneration (Goizet *et al.*, 2009).

PLA2G6 is widely distributed in various tissues and it has been suggested that the cell-specific mitochondrial abnormalities may relate to variations in lipid and protein components of the mitochondrial membrane (Beck *et al.*, 2011). While understanding the pathophysiological mechanisms of PLA2G6-associated neurodegeneration is providing promising therapeutic strategies, the involvement of other cell types within the nervous system is not yet established and may be of pathophysiological and therapeutic relevance.

We report radiological, pathological and biochemical data obtained as part of the diagnostic work-up in a 4-year-old male patient with a clinical diagnosis of INAD secondary to homozygous pathogenic variants in *PLA2G6*, NM_003560.1:c.2070_2072del NP_001004426.1:[p.Val691del]. Respiratory chain enzymology studies were undertaken according to previously described techniques by Dr Thorburn's diagnostic laboratory (Frazier and Thorburn, 2012). This study was performed with informed consent according to the Declaration of Helsinki with approval by the Human Ethics Committees of South Eastern Sydney Local Health District (HREC/13/POWH/203) Australia. While limited to one patient affected with a *PLA2G6*-associated disorder, the case uniquely demonstrates mitochondrial abnormalities in glia surrounding cutaneous nerves. In addition, secondary abnormalities in respiratory chain enzyme activity in skeletal muscle were observed. We also describe possible dietary interventions based on disease biology.

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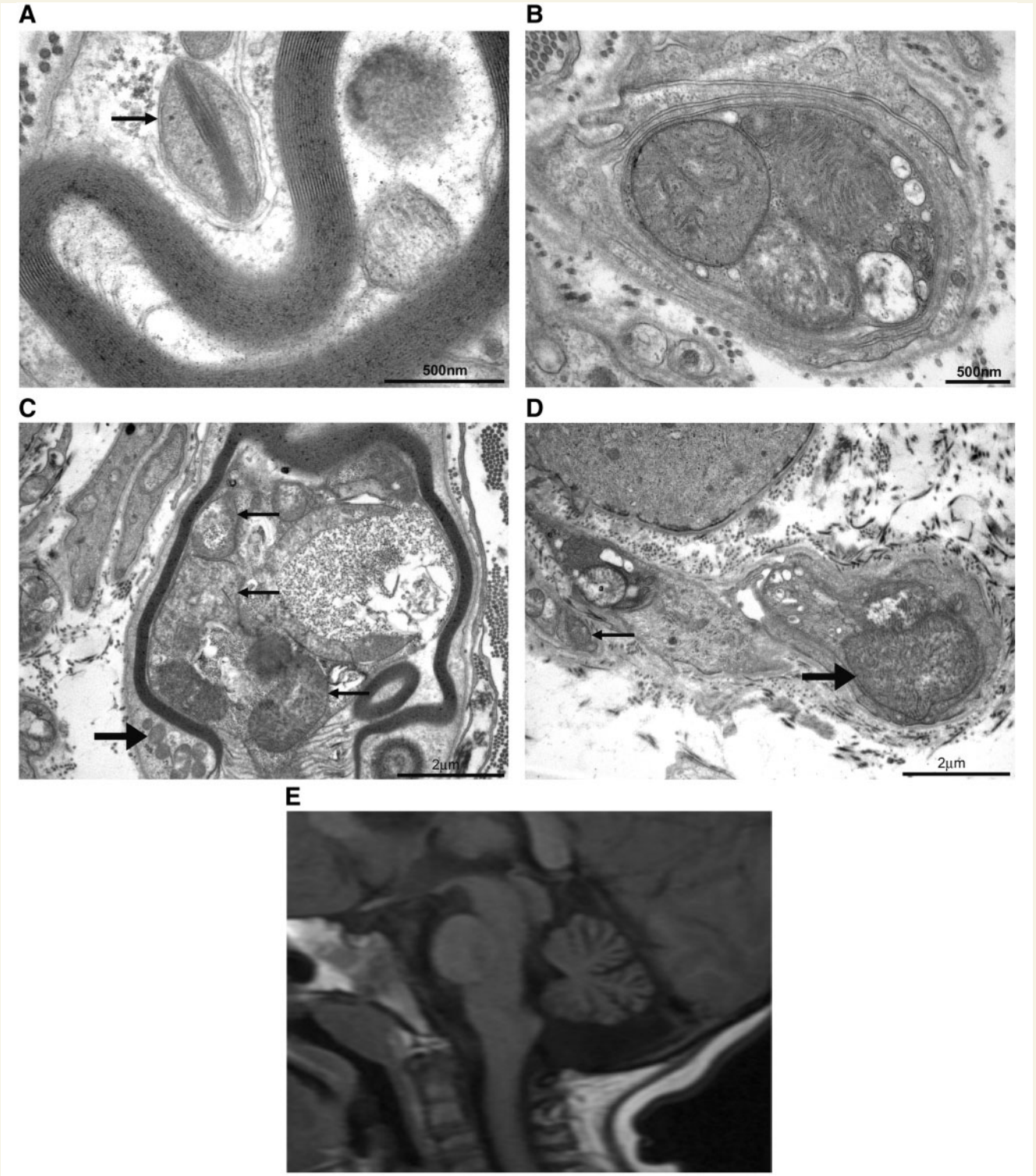

Figure 1 Neuroimaging and electron microscopy of dermal nerves in a patient with infantile neuroaxonal dystrophy (INAD). (**A**) Myelinated nerve from skin biopsy. Lying within the Schwann cell cytoplasm is an abnormal mitochondrion (arrow) showing loss of cristae and also containing a fine paracrystalline inclusion. The characteristic outer double membrane and matrix granules establish that the organelle is a mitochondrion. (**B**) Three grossly enlarged mitochondria fill an unmyelinated dermal nerve. (**C**) Grossly enlarged, pleomorphic mitochondria, some containing glycogen, are shown inside the myelin sheath (thin arrows) and a group of more normal mitochondria can be seen in the Schwann cell cytoplasm outside the myelin sheath (thick arrow). (**D**) An unmyelinated nerve demonstrates the difference in mitochondrial size—grossly enlarged on the right (thick arrow) compared with normal on the left (thin arrow). (**E**) MRI brain of patient demonstrating characteristic cerebellar atrophy.

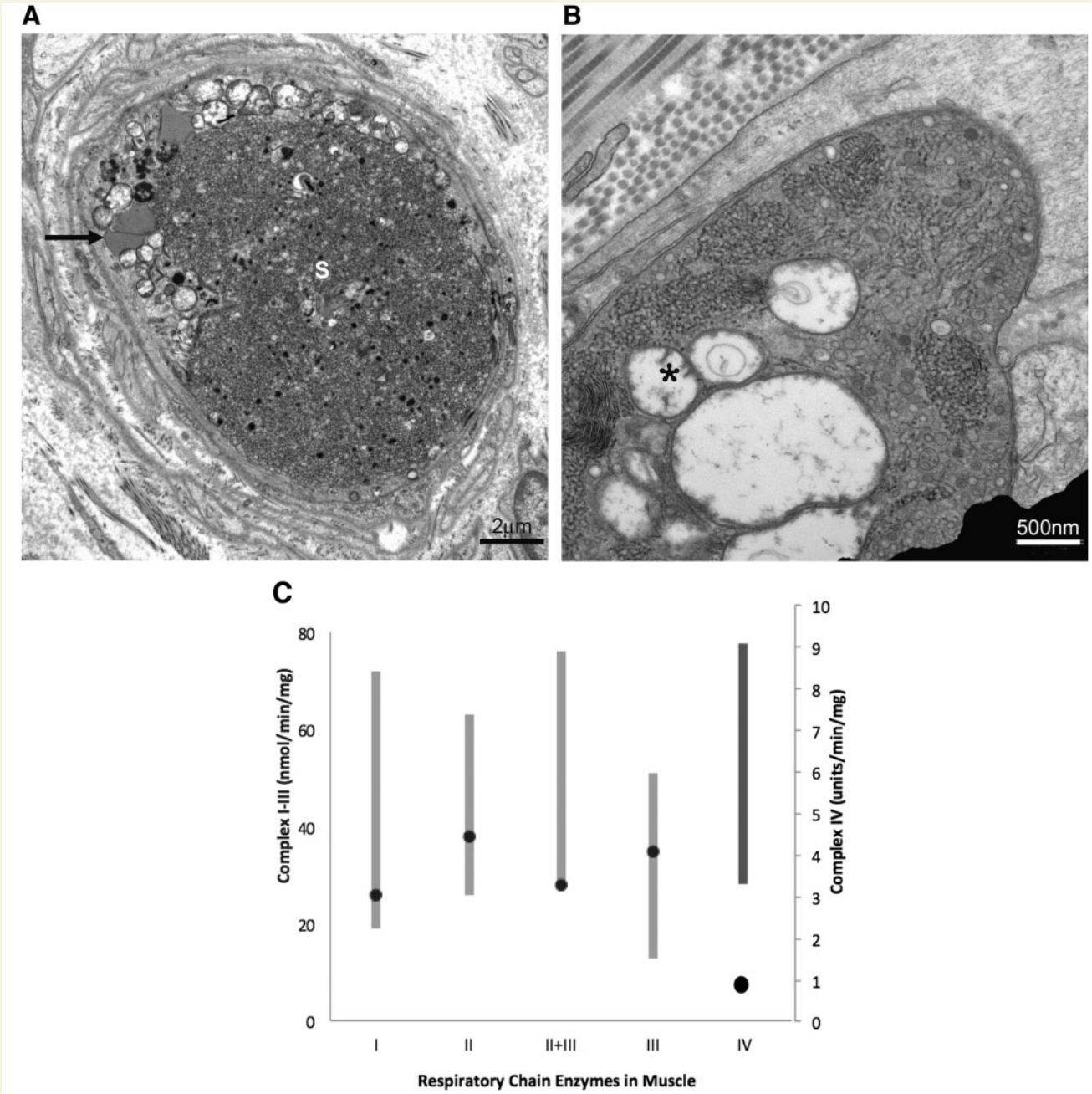

Figure 2 Electron microscopy and respiratory chain enzyme findings of skeletal muscle in a patient with infantile neuroaxonal dystrophy (INAD). (**A**) Large spheroid (S) in an unmyelinated axon in skeletal muscle biopsy. Arrow indicates lipid droplets. (**B**) Intramuscular unmyelinated nerve containing a spheroid and enlarged, swollen mitochondrion with loss of matrix and cristae. The outer double membranes are present and residual cristae can be seen next to the asterisk. (**C**) Respiratory chain enzyme levels from skeletal muscle represented as absolute enzyme activity values. Reference citrate synthase activity was 138 (85–179) nmol/min/mg, therefore percentage activity as a citrate synthase ratio was 59% for complex I, 78% for complex II, 58 % for complexes II and III, 107% for complex III and 13% for complex IV (Methodology as per Frazier and Thorburn, 2012). Circles represent patient values and grey vertical bars represent laboratory reference values with light grey ranges corresponding to values on the left *y*-axis and dark grey ranges corresponding to values on the right *y*-axis.

A skin biopsy taken for fibroblast culture and standard transmission electron microscopy showed cutaneous nerves containing grossly enlarged and pleomorphic mitochondria with altered cristae and a rare paracrystalline inclusion within Schwann cell cytoplasm and also in axons (Fig. 1). While tubulovesicular structures consistent with spheroids were rare in cutaneous nerves, most contained enlarged mitochondria, which ranged in size from 1.1 to 2.7 µm (normal ~ 0.25–0.50 µm). This broadens the findings of abnormal mitochondrial morphology identified within

neurons by Kinghorn *et al.* (2015). Glia are emerging as critical in maintaining axonal function and integrity, providing axons with trophic substances and metabolic support, with primary Schwann cell dysfunction implicated in axonal degeneration (Beirowski, 2013; Menezes *et al.*, 2016). The present pathological data indicate that Schwann cells contain abnormal mitochondria, contributing to the pathogenesis of INAD, and suggest that glia may be an additional therapeutic target with the potential to stabilize axons in INAD.

The overall ultrastructural appearance of the muscle biopsy was consistent with a neuropathic process, characterized by marked variation in fibre size and grouped atrophic fibres. No abnormal mitochondria were seen within muscle fibres, although these may not be evident in a patient aged 4 years. Many of the intramuscular nerves examined showed spheroids and some had enlarged, swollen mitochondria with loss of matrix and cristae (Fig. 2). Respiratory chain enzymology of skeletal muscle also demonstrated mitochondrial dysfunction, with complex 4 deficiency [0.89 (normal: 3.3–9.1) units/min/mg] and borderline low levels of complex II and III. The functional alterations in muscle respiratory chain enzymology with differential loss of complex IV is likely to be a secondary event, generated by abnormal oxidation of the inner mitochondrial membrane. In support of this concept, each monomer of complex IV binds to two molecules of cardiolipin, and therefore oxidation of cardiolipin in the inner mitochondrial membrane is probably responsible for the loss of complex IV activity (Pope *et al.*, 2008).

Separately, very high urine catecholamines were identified in our patient, with elevated catecholamine/creatinine ratio of 486 ($n < 80$), a DOPA/creatinine ratio of 1583 ($n < 1400$), a VMA/creatinine ratio of 21 ($n < 21$) and an HVA/creatinine ratio 19 ($n < 15$). Serum neuronal-specific enolase was also elevated at 84 µg/l (normal 0–12 µg/l). Together, these were considered markers of neuronal membrane instability and probable neuronal loss (Marangos *et al.*, 1987). INAD has followed a rapid neurodegenerative course for the patient. There is as yet poor evidence for any successful treatment in humans in the literature (Morgan *et al.*, 2006). Based on preclinical studies (Green *et al.*, 2008) and safety profile (Kris-Etherton *et al.*, 2009), the patient received a child-friendly, salmon-derived supplement containing 500 mg DHA + EPA and 100 mg/kg/day vitamin E that was safe and well tolerated. Vitamin E was added as it also plays a putative role in lipid peroxidation pathways in humans (Guggenheim *et al.*, 1982; Muller *et al.*, 1983; Meagher *et al.*, 2001). It is difficult to measure clinical responses in neurodegenerative conditions such as INAD, highlighting the necessity to understand the natural history of this disorder, develop biomarkers and build clinical trial readiness. As such, further studies understanding the effect of PLA2G6 loss in Schwann cells may be instructive.

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