

De novo *DNM1L* mutation associated with mitochondrial epilepsy syndrome with fever sensitivity

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Catastrophic epileptic encephalopathy of unclear etiology following a mild metabolic insult generally has a poor outcome. Here, we present 2 such unrelated individuals in whom whole-exome sequencing identified the same de novo recurrent mutation (c.1207C>T p.Arg403Cys) in the gene encoding the guanosine triphosphatase (GTPase) Dynamin-1 like Protein (*DNM1L*) (reference sequence NM_012062.4).

The dynamic fission and fusion of the intracellular mitochondrial network are essential to facilitate mitophagy and thus mitochondrial quality and function.¹ During mitochondrial division, the GTPase *DNM1L* forms multimeric collars at specific fission sites, constricting portions of the mitochondrial reticulum and generating fragments for engulfment and degradation.²

DNM1L has been implicated in several presentations of refractory epilepsy.³ Both of our patients exhibited signs of preexisting developmental delay and presented with epilepsy during, or recently following, a febrile illness or exercise. Elevated lactate levels, epilepsy partialis continua, nonspecific imaging, and evidence of lipid storage myopathy all support mitochondrial dysfunction (See table for presentation summary and e-case report for details, links.lww.com/NXG/A63). This evidence supports an etiological role for *DNM1L* in mitochondrial epilepsy syndrome with fever sensitivity (MEFS).

Methods

We used IN Cell Analyzer 1000 (IN Cell 1000), a previously validated high-throughput imaging method for quantifying mitophagy and mitochondrial DNA (mtDNA) in cultured fibroblasts from patients compared with cultures derived from karyotypically normal controls. Cells were immunostained for the autophagy marker Light Chain 3 (LC3) and the mitochondrial import receptor, translocase of outer membrane 20 (TOM20) and analyzed with IN Cell1000. The readout for mitophagy was colocalization of LC3 puncta with TOM20-positive mitochondria.

Results

We showed that the mitochondria in fibroblasts from both patients are lengthened and hyperpolarized (figure e-1, A and B, links.lww.com/NXG/A62). Total mitophagic flux was increased, showing that mitophagy is activated (figure e-1C, i). This is consistent with the

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Table Comparison of clinical presentations and investigations

	Case 1	Case 2
Prepresentation development	Moderate language delay	Mild general developmental delay
Seizure features	Age 3: focal status epilepticus; continuous partial seizures and a right hemiparesis	Age 5: asymmetric generalized tonic-clonic seizures provoked by febrile illness. Age 6: focal and convulsive status epilepticus
Etiological insult	5 days after influenza vaccination; concurrent mild febrile illness	Shortly after febrile illness or exercise
Biochemical findings	Increased lactate level (2.7 mmol/L)	Increased lactate level (2.5 mmol/L)
EEG findings	Left frontotemporal epileptogenic focus	Focal right-sided clonic seizures associated with left frontal ictal onset; nonconvulsive status epilepticus with bilateral slow spike waves
MRI findings	Transient left thalamic lesion with progression of T2 white matter hyperintensity	Normal morphology and myelination; global bilateral cerebral volume loss
Muscle biopsy	Variable muscle fiber size and type II atrophy	Type II atrophy and cytochrome oxidase negative (COX-negative) succinate dehydrogenase-positive (SDH-positive) fibers
Muscle lipid	Increased	Prominent in type I fibers
Muscle mtDNA	33% of expected (borderline low)	71% of expected (normal)
Mitochondrial appearance	Prominent—consistent with long mitochondria in fibroblasts	Prominent—consistent with long mitochondria in fibroblasts
Mitochondrial activity	Borderline low complex IV	Normal
Whole mitochondrial genome sequencing	No pathologic variants	No pathologic variants
Genes implicated in autosomal mitochondrial disorders	No abnormalities in 17 genes	Normal <i>NPC1</i> , <i>NPC2</i> , and <i>POLG</i>
Whole-exome sequencing	c.1207C>T p.Arg403Cys in <i>DNM1L</i>	c.1207C>T p.Arg403Cys in <i>DNM1L</i>
Treatment	Moderate response to carbamazepine, clobazam, and coenzyme Q	Moderate response to levetiracetam, carbamazepine, and clobazam

mtDNA depletion documented in fibroblasts (figure e-1C, iii) and the borderline low mtDNA content in skeletal muscle from patient 1 (table).

Discussion

In 2 patients, we identified a de novo dominant mutation in *DNM1L*, the same mutation having now been identified in 4 unrelated patients with refractory epilepsy.³ Presentation features and investigation findings supported an underlying mitochondrial pathology. Altered mitochondrial dynamics are now a well-established cause of disease (see supplementary information for reference, links.lww.com/NXG/A63), and defective mitochondrial fission may both cause synaptic dysfunction¹ and impair responses to infection.⁴

In mice, constitutive homozygous knockouts of *Drp1* (the murine homolog of *DNM1L*) do not survive embryogenesis, while conditional ablation leads to developmental defects, both associated with abnormal mitochondrial fission.⁵ Individuals heterozygous for *DNM1L* p.Arg403Cys display

a milder phenotype, both in terms of mitochondrial structural and functional abnormalities and symptom severity.

Previously described cases similarly display several years of relatively normal development followed by severe, refractory epilepsy following a mild metabolic insult, vaccinations, or low-grade fever, resulting in profound global developmental delay.³ As in case 1, nonspecific thalamic hyperintensities were seen on MRI scans on the 2 previously reported probands with the p.Arg403Cys mutation.³ Case 2 demonstrated diffuse cerebral volume atrophy. These changes are in keeping with previously reported nonspecific T2 MRI hyperintensities and cerebral or cerebellar atrophy seen in other mitochondrial disorders. It remains to be seen whether MEFS is part of the same clinical spectrum of other conditions associated with status epilepticus related to febrile illnesses, i.e., new-onset refractory status epilepticus or febrile illness–related epilepsy syndrome.⁶

DNM1L is required for division of mitochondria and peroxisomes, interacting with receptor Mif and endoplasmic reticulum (ER) components. The missense mutation shared in

our cases lies in the middle domain of DNMI1L,³ impairing oligomerization and recruitment to mitochondria³ consistent with our findings of elongated mitochondria in fibroblasts from both patients (figure e-1A, links.lww.com/NXG/A62). Furthermore, knockdown of the DNMI1L ortholog Drp1 in mouse cells and its ligand Mff can each cause mtDNA depletion and mitochondrial dysfunction,⁷ consistent with the borderline low mtDNA content and COX activity in skeletal muscle we demonstrated in case 1. This is likely due to increased mitophagic flux (figure e-1C, i).

Our findings suggest that *DNMI1L* is implicated as a genetic contributor to MEFS. *DNMI1L* p.Arg403Cys mutation screening could therefore be useful in patients with similar presentations and in identifying impaired mitochondrial fission causing synaptic dysfunction¹ and defective response to infection.

Author contributions

E. Ladds wrote the initial case reports and manuscript draft, was part of the editing and submissions process, and prepared the cases for presentation at the British Paediatric Neurology Association Conference 2018. A. Whitney was the pediatrician responsible for identifying SP and summarizing his case. E. Dombi and M. Hofer performed the laboratory tests and analysis of the data. G. Anand was the pediatrician responsible for initially summarizing MN's clinical presentation with the key learning points, bringing together the coauthors, and initiating and supervising case presentation at the National British Paediatric Neurology Association Conference 2018. He also contributed to the final editing process. V. Harrison initiated the laboratory tests and analysis of the data. C. Fratter, J. Carver, and I.A. Barbosa performed the laboratory tests and analysis of the data. M. Simpson performed the whole-exome sequencing. S. Jayawant was the pediatric neurology consultant responsible for MN's clinical care and identifying the case for publication. J. Poulton performed

conceptualization of the study and analysis of the data and revised the manuscript.

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Disclosure

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