SHORT REPORT

Fatal infantile mitochondrial encephalomyopathy, hypertrophic cardiomyopathy and optic atrophy associated with a homozygous OPA1 mutation

Ronen Spiegel,1,2 Ann Saada,3 Padraig J Flannery,4 Florence Burté,4 Devorah Soiferman,3 Morad Khayat,2 Verónica Eisner,5 Eugene Vladovski,6 Robert W Taylor,4 Laurence A Bindoff,7 Avraham Shaag,3 Hanna Mandel,8 Ora Schuler-Furman,9 Stavit A Shalev,2 Orly Elpeleg,3 Patrick Yu-Wai-Man4,10

ABSTRACT

Background Infantile-onset encephalopathy and hypertrophic cardiomyopathy caused by mitochondrial oxidative phosphorylation defects are genetically heterogeneous with defects involving both the mitochondrial and nuclear genomes. Objective To identify the causative genetic defect in two sisters presenting with lethal infantile encephalopathy, hypertrophic cardiomyopathy and optic atrophy. Methods We describe a comprehensive clinical, biochemical and molecular genetic investigation of two affected siblings from a consanguineous family. Molecular genetic analysis was done by a combined approach involving genome-wide autozygosity mapping and next-generation exome sequencing. Biochemical analysis was done by enzymatic analysis and Western blot. Evidence for mitochondrial DNA (mtDNA) instability was investigated using long-range and real-time PCR assays. Mitochondrial cristae morphology was assessed with transmission electron microscopy.

Results Both affected sisters presented with a similar cluster of neurodevelopmental deficits marked by failure to thrive, generalised neuromuscular weakness and optic atrophy. The disease progression was ultimately fatal with severe encephalopathy and hypertrophic cardiomyopathy. Mitochondrial respiratory chain complex activities were globally decreased in skeletal muscle biopsies. They were found to be homozygous for a novel c.1601T>G (p. Leu534Arg) mutation in the OPA1 gene, which resulted in a marked loss of steady-state levels of the native OPA1 protein. We observed severe mtDNA depletion in DNA extracted from the patients’ muscle biopsies. Mitochondrial morphology was consistent with abnormal mitochondrial membrane fusion.

Conclusions We have established, for the first time, a causal link between a pathogenic homozygous OPA1 mutation and human disease. The fatal multisystemic manifestations observed further extend the complex phenotype associated with pathogenic OPA1 mutations, in particular the previously unreported association with hypertrophic cardiomyopathy. Our findings further emphasise the vital role played by OPA1 in mitochondrial biogenesis and mtDNA maintenance.

Mitochondrial disorders are genetically and phenotypically highly heterogeneous. Due to the limited coding capacity of the mitochondrial genome, the majority of structural and accessory proteins required for the proper assembly and functioning of the mitochondrial respiratory chain are encoded by the nuclear genome. Mutations in an increasing number of these critical nuclear-encoded genes have been identified over the last decade with next-generation sequencing. These advances have allowed the elucidation of the underlying genetic diagnosis in patients with complex phenotypes and also broadened the spectrum of clinical manifestations associated with specific disease-causing genes. Syndromes characterised by infantile-onset hypertrophic cardiomyopathy and encephalopathy are genetically heterogeneous, but mitochondrial dysfunction with impairment of mitochondrial oxidative phosphorylation has emerged as an important pathophysiological mechanism. Accordingly, these specific syndromes may be caused by mutations in various genes involving both the mitochondrial or nuclear genomes. Unsurprisingly, a number of nuclear genes causing isolated or combined mitochondrial respiratory chain defects have been identified in patients with infantile hypertrophic cardiomyopathy and variable forms of encephalopathy, of which recent examples include NDUFS2, COX6B1, and SCO2.1–3

Heterozygous OPA1 mutations cause autosomal dominant optic atrophy (DOA), which is the most common form of inherited mitochondrial blindness with a minimum prevalence of 1 in 25 000 in the general population.4 Here, we report on two siblings from a consanguineous family who presented with a fatal, infantile-onset, global encephalopathy and progressive hypertrophic cardiomyopathy due to a novel homozygous mutation in the OPA1 gene. Homozygous OPA1 mutations have not been reported previously and the unexpected association with cardiac involvement further broadens the genotypic and phenotypic spectrum associated with syndromic DOA.

The subjects of this study are two sisters of Arab Muslim origin and their parents are entirely healthy first-degree cousins with no visual or neurological complaints. Patient I-1 was born prematurely at 35 weeks gestation and failure to thrive was a problem since birth. At 2 months of age, she was also noted to have truncal hypotonia that was replaced by multiple episodes of hypertonia and
Genotype-phenotype correlations

opisthotonic posturing. Furthermore, she had a weak cry and abnormal eye pursuits. An initial echocardiography and ophthalmological examinations performed at that time were unremarkable. Metabolic investigations revealed elevated serum alanine and lactate levels, but a normal cerebrospinal fluid (CSF) lactate concentration. Disease progression was marked by a persisting failure to thrive with severe neurodevelopmental delay, but brain MRI was normal. The patient subsequently developed a progressive, non-obstructive, generalised hypertrophic cardiomyopathy, in the absence of overt heart failure. The patient died at 10 months of age following an apnoeic episode.

Patient I-2 was born at term following a caesarean section due to breech presentation. Two days later, she developed generalised hypertonia and opisthotonic posturing, and feeding was inadequate due to poor sucking. Initial echocardiography showed mild thickening of the left ventricular myocardium. Serum and CSF lactate concentrations were both elevated. Brain MRI performed at 1.5 months of age was normal and no abnormal lactate peaks were detected on functional spectroscopic imaging. Due to gastro-oesophageal reflux and persisting failure to thrive, a gastrostomy tube and Nissen funduplication procedures were performed at 3 months of age. The disease course was further complicated by progressive, hypertrophic, non-obstructive cardiomyopathy, profound neurodevelopmental retardation, hypotonia with significant muscle wasting and sensorineural deafness. There was no nystagmus, ptosis or limitation of eye movements. Ophthalmological examination at 6 months of age revealed mild pallor of both optic nerves. Visual electrophysiology revealed severely attenuated visual evoked potentials, consistent with primary retinal ganglion cell pathology, and the electroretinogram responses were significantly reduced, indicative of more global retinal degeneration. The patient died at 11 months of age following an apnoeic episode while sleeping.

A muscle biopsy was performed at 8 months of age (patient I-1) and at 1 month of age (patient I-2). Routine mitochondrial immunohistochemical staining was apparently normal with no cytochrome c oxidase-deficient muscle fibres. Enzymatic activities of the five respiratory chain complexes and citrate synthase (CS) activity, a mitochondrial matrix enzyme marker, were determined in isolated muscle mitochondria by spectrophotometric assays. This analysis revealed global decrease in all the respiratory chain complexes when normalised to CS activity (% of control). Metabolic investigations revealed elevated serum alanine and lactate concentrations, both patients, with complexes I and IV being the most affected when normalised to CS activity in metric assays. This analysis revealed global decrease in all the respiratory chain complexes and citrate synthase (CS) activity, a mitochondrial matrix enzyme marker, were determined in isolated muscle mitochondria by spectrophotometric assays. This analysis revealed global decrease in all the respiratory chain complexes when normalised to CS activity (% of control). Metabolic investigations revealed elevated serum alanine and lactate concentrations, both patients, with complexes I and IV being the most affected when normalised to CS activity (% of control).

<table>
<thead>
<tr>
<th>Assay</th>
<th>Patient I-1 (%)</th>
<th>Patient I-2 (%)</th>
<th>Controls±SD (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>0.047 (24%)</td>
<td>0.050 (25%)</td>
<td>0.199±0.043</td>
</tr>
<tr>
<td>Complex I+II</td>
<td>0.087 (40%)</td>
<td>n.d.</td>
<td>0.217±0.599</td>
</tr>
<tr>
<td>Complex II</td>
<td>0.099 (64%)</td>
<td>0.091 (59%)</td>
<td>0.154±0.024</td>
</tr>
<tr>
<td>Complex II+III</td>
<td>0.100 (65%)</td>
<td>0.053 (34%)</td>
<td>0.153±0.039</td>
</tr>
<tr>
<td>Complex III</td>
<td>0.934 (46%)</td>
<td>n.d.</td>
<td>2.01±0.40</td>
</tr>
<tr>
<td>Complex IV</td>
<td>0.270 (21%)</td>
<td>0.230 (22%)</td>
<td>1.03±0.24</td>
</tr>
<tr>
<td>Complex V</td>
<td>0.151 (44%)</td>
<td>n.d.</td>
<td>0.34±0.096</td>
</tr>
</tbody>
</table>

* mU/U citrate synthase (% of control), n.d. not determined.

In order to further assess the pathogenicity of this c.1601T>G OPA1 variant, western blot analysis was performed on homogenate muscle tissue from patient I–1 using standard monoclonal antibodies that bind to the C-terminus region of the OPA1 protein. There was a significant reduction in steady-state OPA1 protein level in patient I–1 compared with age-matched controls (figure 1A,B). This observation was consistent with the reduced OPA1 protein levels observed in another patient (NO-1) with a complicated syndromic DOA phenotype characterised by optic atrophy and marked generalised neurodegenerative features, who has been confirmed to harbour compound heterozygous OPA1 missense mutations in exon 5b (c.768C>G, p.Ser256Arg) and exon 8 (c.854A>G, p.Gln285Arg; figure 1C). To further investigate how a homozygous missense OPA1 mutation could result in markedly reduced OPA1 protein level, in silico protein modelling was carried out. The c.1601T>G OPA1 variant replaces a highly hydrophobic and evolutionary conserved leucine residue with an arginine residue. Our modelling work suggests that this amino acid substitution, in the close vicinity of the protein’s critical GTPase domain, could destabilise the protein, accounting for the significantly reduced OPA1 protein level observed on western blot analysis (see online supplementary figure S2).

Long-range PCR performed on homogenate DNA extracted from the muscle biopsy specimens of the two affected sisters did not reveal any multiple mitochondrial DNA (mtDNA) deletions (see online supplementary figure S3). However, there was evidence of significant mtDNA depletion with a 78% decrease for patient I-1 and a 78% decrease for patient I-2 compared with

Table 1 Enzymatic activities of mitochondrial respiratory chain complexes
control values (figure 1D). Transmission electron microscopy (TEM) assay of muscle biopsy from patient I-2 disclosed large mitochondria with incomplete fusion of the inner mitochondrial membrane (see online supplementary figure S4).

**OPA1** encodes for a large multimeric dynamin-like GTPase protein, which localises to the inner mitochondrial membrane where it plays a crucial role in mediating mitochondrial fusion.4 In addition, **OPA1** is essential for the assembly and stability of the mitochondrial respiratory chain supercomplexes,5 mitochondrial cristae organisation,6 the sequestration of pro-apoptotic cytochrome *c* molecules within these tight junctions and, importantly, mtDNA maintenance.7 Heterozygous **OPA1** mutations account for the majority of cases of non-syndromic DOA, which classically presents in early childhood with progressive visual loss. Although optic atrophy is the defining feature of DOA, non-penetrance has been reported in 10%–20% of **OPA1** mutation carriers.4 Furthermore, several papers and recent reports highlighted the fact that a significant proportion of **OPA1** mutation carriers, up to 20% in a large case series, can develop a more severe syndromic form of the disease (termed DOA plus) where the visual loss is compounded by a cluster of neurological deficits that include chronic progressive external ophthalmoplegia, sensorineural hearing loss, ataxia, myopathy and peripheral neuropathy.8 Interestingly, the extracocular manifestations of pathogenic **OPA1** mutations can sometimes develop in the absence of overt optic atrophy and visual failure.9 10 As expected, bi-allelic **OPA1** mutations result in a more severe phenotype characterised by an earlier age of onset and with a more aggressive neurological course—referred to by some investigators as Behr syndrome.10–12 More recently, a deep intronic **OPA1** mutation was shown to behave as an intralocus modifier that results in DOA plus phenotypes when it occurs in conjunction with an exonic **OPA1** missense variant (c.1146A>G, p. -Ile382Met; NM_015560.2)—the latter being insufficient to cause disease on its own.13 When present in the heterozygous state, the c.1601T>G **OPA1** variant in our family apparently does not result in clinical or subclinical disease. Taken together, these observations further add to the complexity of genotype–phenotype correlations and it demonstrates the spectrum of **OPA1** mutations from the classical dominant mutations (which accounts for the majority of cases), to compound heterozygous mutations causing more severe syndromes (including Behr syndrome), and our current report with a homozygous mutation.

Our case report provides a number of new insights into the molecular and phenotypic manifestations of pathogenic **OPA1** mutations. First, the two affected siblings harboured a novel homozygous **OPA1** variant, which have not been reported previously in association with human disease. These two patients developed a rapidly progressive lethal phenotype beginning in early infancy and characterised by marked multisystemic involvement due to generalised mitochondrial respiratory chain dysfunction. This ultimately fatal clinical presentation is

---

**Figure 1** (A) Densitometric analysis of **OPA1** in muscle tissue from patient I-1. The level of the **OPA1** protein was normalised against GAPDH. Four different control muscle samples were used. Error bars represent SE of the mean. An unpaired Student’s t test was used in statistical comparison between patient and controls (p=0.0144). (B) Western blot illustrating **OPA1** protein levels in muscle controls and muscle tissue of patient I-1. (C) Densitometric analysis of **OPA1** in fibroblasts from patient NO-1 who carries compound heterozygous, c.768C>G (p.Ser256Arg in exon 5b) and c.854A>G (p.Gln285Arg in exon 8).8 The level of **OPA1** protein was normalised against GAPDH. Four different control muscle samples were used. An unpaired Student’s t test was used in statistical comparison between patient and controls (p=0.0039). (D) mtDNA copy number in skeletal muscle from the two affected sisters compared with controls. The analysis was performed using a SYBR Green real-time PCR assay with the mtDNA gene *MTND1* corrected against the nuclear gene *B2M* to calculate mtDNA copy number. Error bars represent SE of the mean. Statistical analysis performed with Student’s unpaired t test (p<0.0001). *Level of statistical significance.

consistent with the rather striking observations made in three Opal mouse models. Homozygous mutant mice died in utero during embryogenesis due to severe abrogation of Opal protein levels, confirming a critical role for this pro-fusion mediator in early life and development. In silico modelling indicates that the c.1601T>G Opal missense mutation affects the integrity of the protein structure in the vicinity of the catalytic GTPase domain, providing a plausible explanation for the significant reduction in Opal level confirmed by western blot analysis. Opal also forms oligomeric complexes in vivo and the lack of any wild-type protein due to the homozgyous mutations could further exacerbate protein stability due to impaired oligomerisation.

Carriers harbouring missense Opal mutations affecting the catalytic GTPase region have a significantly increased risk of developing the more severe DOA plus phenotype, which is again consistent with the rapidly fatal clinical progression observed for the two affected sisters. Second, hypertrophic cardiomyopathy has not been reported previously to be associated with human Opal mutations. Interestingly, histopathological evidence of cardiomyopathy was apparent in a heterozygous mutant Opal mouse model as early as at 5 months of age and the affected cardiac tissues exhibited a combined complex I and IV respiratory chain defect with reduced ATP production. Importantly, previous reports have noted cardiac manifestations in a number of patients with DOA plus including symptomatic tachycardia, early-onset myocardial infarction and sinus bradycardia. These potentially life-threatening associations are important and further investigations of a larger cohort of patients with DOA will clarify whether this patient group is at risk of clinical or subclinical cardiac involvement, and if so, the need for prophylactic screening and intervention in at-risk mutation carriers.

Although the precise disease mechanisms by which Opal mutations lead to human disease is still under intense investigation, a growing body of evidence points towards a synergy between disturbed mitochondrial dynamics, mtDNA instability and impaired mitochondrial oxidative phosphorylation, which eventually precipitates an irreversible molecular cascade towards cell death. Several studies have demonstrated reduced activities of various mitochondrial respiratory chain complexes, in particular complex I and complex IV in muscle biopsies and fibroblasts obtained from patients with DOA and confirmed Opal mutations. In keeping with these findings, muscle biopsies from both affected sisters showed a marked global deficiency in mitochondrial respiratory chain activity, which was relatively more pronounced for complexes I and IV. It is therefore biologically plausible that such a severe impairment in mitochondrial oxidative phosphorylation would have a catastrophic consequence for tissues with a high basal level of energy consumption such as skeletal muscle, cardiac muscle and the central nervous system, in addition to the retina.

In addition to the generalised cellular energy shortage, Opal is now well established as a regulatory factor involved in mtDNA maintenance and genome stability. Previous studies have clearly shown that mtDNA deletions can accumulate to high levels in postmitotic tissues of affected Opal mutation carriers. MtDNA deletions were not detected in the two affected sisters, which is not surprising given their very young age and the need for clonal expansion of somatic mtDNA abnormalities to proceed over several decades before they become detectable using routine PCR-based detection techniques. However, mtDNA depletion was clearly demonstrated in both patients—a striking finding that has not been reported before with pathogenic Opal mutations. This observation is mechanistically important and support a number of recent studies linking Opal and also its sister protein mitofusin 2 (Mfn2), with the complex process of mtDNA replication. Opal is thought to physically anchor nucleoids to the inner mitochondrial membrane via the peptide segment encoded by exon 4b, and using an elegant series of mouse models, marked mtDNA depletion was observed in the context of Opal null cells. Mfn2 is a major pro-fusion located within the outer mitochondrial membrane and it works in tandem with Opal to control the sequential steps that eventually lead to mitochondrial fusion. Supporting evidence for abnormal inner mitochondrial membrane fusion was provided by TEM of patient’s I-2 muscle biopsy. As for Opal mutations, Mfn2 mutations predominantly cause the accumulation of multiple mtDNA deletions, but a recent case report indicates that mtDNA depletion can occur as well.

It is now abundantly clear that the deleterious consequences of pathogenic Opal mutations are not limited to the optic nerve. We have further extended the mutational and phenotypic spectrum of Opal disease to include lethal infantile mitochondrial encephalomyopathy and hypertrophic cardiomyopathy secondary to a novel homozygous missense mutation that targets the key functional GTPase domain. Our case report also further reinforces the power of next-generation exome sequencing in elucidating the genetic aetiology of complex disease phenotypes when combined with rigorous phenotyping and a clear a priori list of candidate genes, in this particular family, Opal given the clear link with optic atrophy.

**Author affiliations**

1Pediatric Department B, Genetic Institute, Emek Medical Center, Afula, Israel
2Genetic Institute, Emek Medical Center, Rappaport School of Medicine, Technion, Haifa, Israel
3Monique and Jacques Roboh Department of Genetic Research, Hebrew University, Hadassah Medical Center, Jerusalem, Israel
4Wellcome Trust Centre for Mitochondrial Research, Newcastle University, Newcastle upon Tyne, UK
5Department of Cellular and Molecular Biology, School of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile
6Department of Pathology, Rambam Medical Center, Haifa, Israel
7Department of Clinical Medicine, University of Bergen, Bergen, Norway
8Metabolic Unit, Rambam Medical Center
9Department of Microbiology and Molecular Genetics, Hebrew University, Hadassah Medical Center, Jerusalem, Israel
10Newcastle Eye Centre, Royal Victoria Infirmary, Newcastle upon Tyne, UK

**Correction notice** This article has been corrected since it published Online First. The 7th and 13th author's names has been corrected and affiliations updated.

**Acknowledgements** Corinne Belaiche is acknowledged for technical assistance.

**Contributors** RS drafted the manuscript for content, included medical writing for content; designed and conceptualised the study and interpreted the data. AS drafted the manuscript for content, designed and conceptualised the study and interpreted the data. PIF drafted the manuscript for content, designed and analysed the molecular and biochemical data. FB drafted the manuscript for content and analysed the data. DS drafted the manuscript for content and analysed the biochemical data. MK drafted the manuscript for content and analysed the molecular data. VES drafted the manuscript for content, designed and analysed the TEM data. EV drafted the manuscript for content, designed and analysed the TEM data. RTW drafted the manuscript for content and analysed the data. LAB drafted the manuscript for content and analysed the data. AS drafted the manuscript for content and analysed the molecular data. HM drafted the manuscript for content and analysed the clinical data. OF drafted the manuscript for content and designed and conceptualised the study and interpreted the data. EVS drafted the manuscript for content, designed and conceptualised the study and interpreted the data. W-Y-M designed and conceptualised the study and interpreted the data. W-Y-M designed and conceptualised the study and interpreted the data. SAS drafted the manuscript for content and analysed the data. AS drafted the manuscript for content, designed and conceptualised the study and interpreted the data. TEM data. EV drafted the manuscript for content, designed and analysed the biochemical data. VES drafted the manuscript for content, designed and conceptualised the study and interpreted the data. AS drafted the manuscript for content and analysed the molecular data.
REFERENCES


Supplementary Figure 1

A

Affected patient 1-2

Mother (heterozygous)

Normal control

B

OPA1 Patients
OPA1 L. sapiens
OPA1 P. troglodytes
OPA1 A. mediterranea
OPA1 D. rerio
OPA1 C. elegans
OPA1 S. cerevisiae
OPA1 S. pseud

c.1601T>G
Supplementary Figure 2

Supplementary Figure 4:
Supplementary Figure 3
Supplementary Figure 4

- Apparently normal structure
- Mitochondria with aberrant ultrastructure
- Aberrant mitochondria, showing details of clustered matrix-carrying IMM, surrounded by OMM
The resultant 56.94 million reads were aligned, by the DNAnexus software (Palo Alto, CA, USA) with the human genome assembly hg19 (GRCh37) as reference. Of the called variants, only ten were homozygous, at a minimal depth of X6, on-target, non-synonymous, and with a MAF<0.1% at dbSNP138 and MAF<1% in the Hadassah in-house dbSNP (table).

<table>
<thead>
<tr>
<th>chr</th>
<th>nt (Hg19)</th>
<th>ref</th>
<th>mut</th>
<th>known SNP</th>
<th>gene</th>
<th>AA change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>230914853</td>
<td>C</td>
<td>T</td>
<td>rs148341318</td>
<td>CAPN9</td>
<td>T-363-M</td>
</tr>
<tr>
<td>1</td>
<td>235331875</td>
<td>C</td>
<td>T</td>
<td></td>
<td>ARID4B</td>
<td>A-1302-T</td>
</tr>
<tr>
<td>3</td>
<td>193364865</td>
<td>T</td>
<td>G</td>
<td></td>
<td>OPA1</td>
<td>L-535-R</td>
</tr>
<tr>
<td>3</td>
<td>197880164</td>
<td>GCAGCA</td>
<td></td>
<td></td>
<td>FAM157A</td>
<td>QQQ-81-Q</td>
</tr>
<tr>
<td>9</td>
<td>90501759</td>
<td>G</td>
<td>T</td>
<td>rs80294513</td>
<td>SPATA31E1</td>
<td>R-786-L</td>
</tr>
<tr>
<td>15</td>
<td>79603608</td>
<td>T</td>
<td>G</td>
<td>rs370013859</td>
<td>TMED3</td>
<td>P-6-L</td>
</tr>
<tr>
<td>15</td>
<td>89399986</td>
<td>C</td>
<td>G</td>
<td>rs201822759</td>
<td>ACAN</td>
<td>D-1390-E</td>
</tr>
<tr>
<td>17</td>
<td>38519452</td>
<td>C</td>
<td>A</td>
<td>rs201822759</td>
<td>GJD3</td>
<td>V-206-L</td>
</tr>
<tr>
<td>19</td>
<td>37487873</td>
<td>C</td>
<td>T</td>
<td>rs1667364</td>
<td>ZNF568</td>
<td>A-427-V</td>
</tr>
<tr>
<td>19</td>
<td>58967064</td>
<td>C</td>
<td>A</td>
<td></td>
<td>ZNF324B</td>
<td>H-251-Q</td>
</tr>
</tbody>
</table>
Supplementary Table 1: Homozygous regions shared by the two affected siblings.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Start Position</th>
<th>End Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>190090661</td>
<td>194028647</td>
</tr>
<tr>
<td>4</td>
<td>127448140</td>
<td>129685158</td>
</tr>
<tr>
<td>5</td>
<td>44626810</td>
<td>50328257</td>
</tr>
<tr>
<td>8</td>
<td>77570266</td>
<td>78463539</td>
</tr>
</tbody>
</table>

The start and end point coordinates are in relation to the human genome reference sequence hg19.