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Mutation of *OPA1* causes dominant optic atrophy with external ophthalmoplegia, ataxia, deafness and multiple mitochondrial DNA deletions: a novel disorder of mtDNA maintenance

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Mutations in nuclear genes involved in mitochondrial DNA (mtDNA) maintenance cause a wide range of clinical phenotypes associated with the secondary accumulation of multiple mtDNA deletions in affected tissues. The majority of families with autosomal dominant progressive external ophthalmoplegia (PEO) harbour mutations in genes encoding one of three well-characterized proteins—polγ, Twinkle or Ant 1. Here we show that a heterozygous mis-sense mutation in *OPA1* leads to multiple mtDNA deletions in skeletal muscle and a mosaic defect of cytochrome c oxidase (COX). The disorder presented with visual failure and optic atrophy in childhood, followed by PEO, ataxia, deafness and a sensory-motor neuropathy in adult life. COX-deficient skeletal muscle fibres contained supra-threshold levels of multiple mtDNA deletions, and genetic linkage, sequencing and expression analysis excluded *POLG1*, *PEO1* and *SLC25A4*, the gene encoding Ant 1, as the cause. This demonstrates the importance of *OPA1* in mtDNA maintenance, and implicates *OPA1* in diseases associated with secondary defects of mtDNA.

Keywords: mitochondria; mitochondrial DNA; mitochondrial encephalomyopathy; autosomal dominant progressive external ophthalmoplegia; autosomal dominant optic atrophy; multiple mtDNA deletions

Abbreviations: ATP = adenosine triphosphate; COX = cytochrome c oxidase; DGUOK = deoxyguanosine kinase gene; LOD = log of the odds ratio; mtDNA = mitochondrial DNA; PEO = progressive external ophthalmoplegia; polγ = polymerase gamma; POLG1 = polymerase gamma gene

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Introduction

The synthesis of ATP by mitochondrial oxidative phosphorylation is dependent upon the coordinated expression and interaction of both nuclear and mitochondrial-encoded gene products. Mutations in nuclear genes involved in mitochondrial DNA (mtDNA) maintenance are increasingly associated with a variety of clinical phenotypes ranging from severe encephalopathy and liver failure in childhood to late-onset progressive external ophthalmoplegia (PEO), ataxia, myopathy and parkinsonism (Zeviani and Di Donato, 2004; Schapira, 2006). These include recessive mutations in *POLG1*, encoding the catalytic alpha-subunit of DNA polymerase γ, polγ or *POLGA* (Van Goethem *et al.*, 2001); *TK2*, encoding thymine kinase (Saada *et al.*, 2001); *DGUOK*, encoding dexyguanosine kinase (Mandel *et al.*, 2001), *SUCLA2* (Elpeleg *et al.*, 2005); *MPV17* (Spinazzola *et al.*,

2006); and *RRM2B* (Bourdon *et al.*, 2007), which lead to a mtDNA depletion. Alternatively, dominant mutations in *POLG1*, (Van Goethem *et al.*, 2001); *POLG2*, encoding the accessory beta-subunit of polγ, *POLGB* (Longley *et al.*, 2006); *PEO1*, previously known as *C10Orf2*, encoding the mitochondrial helicase Twinkle (Spelbrink *et al.*, 2001); *SLC25A4*, encoding adenine nucleotide translocator 1, Ant 1 (Kaukonen *et al.*, 2000); and recessive mutations in *TP*, encoding thymidine phosphorylase (Nishino *et al.*, 1999), which lead to a secondary accumulation of multiple deletions in mtDNA in affected tissues and an associated respiratory chain defect.

The majority of patients with an apparent autosomal dominant (ad) family history of mitochondrial disease and demonstrable multiple mtDNA deletions in muscle harbour a mutation in *POLG1*, *PEO1* or *SLC25A4* (Lamantea *et al.*, 2002), but in many cases it is not possible to reach a molecular diagnosis (Hudson *et al.*, 2006). Investigating these pedigrees can be revealing, identifying novel disease genes leading to the detailed dissection of critical biochemical pathways which maintain mtDNA and cause human disease (Longley *et al.*, 2006).

Mutations in nuclear genes that maintain mtDNA cause a diverse spectrum of overlapping phenotypes. Visual failure and optic atrophy have been described, but rarely dominate the phenotype (Hakonen *et al.*, 2005; Horvath *et al.*, 2006). By contrast, visual failure may be the major phenotype in patients with mutations in autosomal dominant optic atrophy (ad-OA). Mutations in *OPA1*, which codes for a dynamin-related guanosine triphosphatase (GTPase), are the most common cause of ad-OA (Alexander *et al.*, 2000; Delettre *et al.*, 2000). Other loci have been implicated in X-linked optic atrophy (*OPA2*; Katz *et al.*, 2006), autosomal recessive (ar) optic atrophy with cataract (*OPA3;* Anikster *et al.*, 2001), dominant optic atrophy with or without deafness (*OPA4;* Kerrison *et al.*, 1999) and *OPA5* (Barbet *et al.*, 2005) and early onset ar-OA (*OPA6*)(Barbet *et al.*, 2003). Additional neurological features have been described in these disorders, including deafness (Amati-Bonneau *et al.*, 2005) or ophthalmoplegia (Payne *et al.*, 2004) in patients with *OPA1* mutations, and spasticity, extrapyramidal dysfunction and cognitive impairment in *OPA3* (Costeff's syndrome, 3-methylglutaconic aciduria type III)(Costeff and Elpeleg, 1995). Here we describe a family with a different phenotype, presenting with profound visual failure and optic atrophy developing in childhood followed by PEO, ataxia and deafness in later life. The biochemical and molecular characterization of multiple family members revealed multiple deletions of mtDNA in skeletal muscle and a heterozygous mutation of *OPA1*, implicating this gene in the maintenance of mtDNA and multiple mtDNA deletions in the pathophysiology of ad-OA.

Materials and Methods

Patients

The family pedigree is shown in Fig. 1, with the index case (II:5) highlighted (arrow).

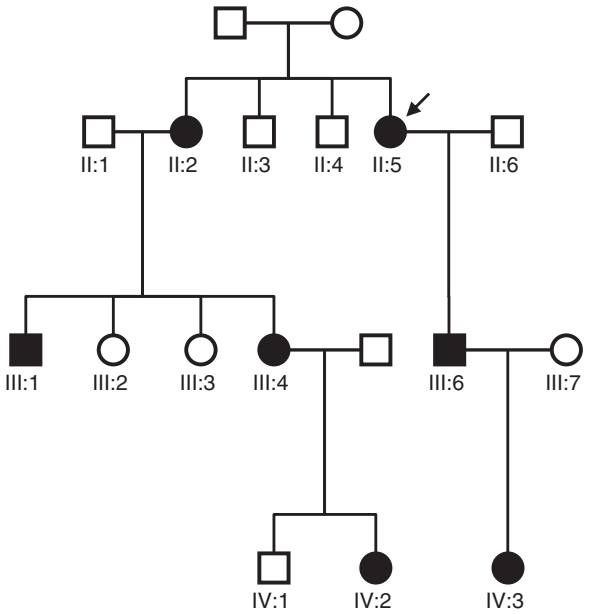

Fig. 1 Pedigree showing males (squares) and females (circles) in a family with autosomal dominant external ophthalmoplegia with optic atrophy and ataxia. Solid symbols = clinically affected as described in the text.

II:2. A 60-year-old woman, who was registered blind at the age of 6 years, presented at 50 years of age with progressive deafness and a gait disturbance. The deafness had a history of 8 years. On examination, she had an ataxic gait and Romberg test was positive. She could just detect hand movements, had bilateral ptosis, external ophthalmoplegia and bilateral optic atrophy but no retinopathy. In the limbs, she had mild symmetric wasting of quadriceps, and mild symmetric proximal muscle weakness (MRC grade 4+). She was areflexic and had impaired sensation to all modalities in a glove and stocking distribution. Clinical investigations revealed impaired glucose tolerance, an elevated serum creatine kinase (493 U/l, normal <190 U/l). A fasting blood lactate was 2.0 mM/l. Nerve conduction studies revealed an axonal sensorimotor neuropathy, with additional myopathic features on electromyography. An electrocardiogram, echocardiogram, pulmonary function tests and overnight oximetry were all within normal limits. Brain MR imaging revealed a number of small high-signal lesions in the frontal and temporal white matter on T2 imaging, thought to represent focal ischaemic lesions.

II:5. A 66-year-old woman had had visual impairment since childhood and had attended a special school for the blind. She presented with a further slowly progressive deterioration in her visual acuity, a progressive gait disturbance of 20 years duration, and a 5-year history of deteriorating hearing. Shortly after presentation she developed acute paranoid delusions, low mood and visual hallucinations which resolved spontaneously. On examination, she was just able to perceive light and had bilateral optic atrophy. There was a complete external ophthalmoplegia, bilateral ptosis and bifacial weakness. Her gait was ataxic, tendon reflexes depressed and plantar responses flexor. Clinical investigations revealed an elevated serum creatine kinase (417 U/l, normal <190 U/l) with a normal fasting blood glucose and lactate. An electrocardiogram showed left axis deviation but an

echocardiogram was normal. A chest X-ray and pulmonary function tests were normal. An EEG revealed evidence of a mild cortical disturbance. Brain MRI revealed mild cortical and cerebellar atrophy, and prominent small-vessel ischaemic lesions in the cerebral white matter bilaterally.

III:1 A 24-year-old man had normal early motor development but developed a left hemiplegia following a road traffic accident at 5 years of age. His visual acuities progressively deteriorated throughout childhood, and he developed mild ataxia in his early 20s. On examination, his visual acuity was reduced to counting fingers in both eyes. He had a mild right-sided ptosis, bilateral optic atrophy and a partial external ophthalmoplegia predominantly affecting down-gaze. He had bifacial muscle weakness, and distal muscle wasting below the knees bilaterally, with bilateral pes cavus. He had subtle spasticity in the left leg, with mild symmetric proximal muscle weakness (MRC grade 4+) and profound bilateral distal lower limb weakness (ankle dorsiflexion MRC grade 2). His tendon reflexes were generally depressed, and absent at both ankles. Plantar responses were extensor. There was a marked gait ataxia with prominent positive Romberg sign. Clinical investigations revealed an elevated serum creatine kinase (594 U/l, normal <190 U/l), and a normal fasting blood glucose and lactate (1.0 mM/l). An electrocardiogram revealed a sinus bradycardia and an echocardiogram was normal. Nerve conduction studies and an electromyogram revealed an axonal sensorimotor neuropathy. An electroencephalogram was normal. Overnight oximetry was normal.

III:4 A 32-year-old woman was noted to have optic atrophy in her second year of life. Her gait deteriorated from the age of 18 years, associated with mild proximal muscle weakness. At 23 years of age she was diagnosed with irritable bowel syndrome. From 28 years of age she noted a slowly progressive hearing loss. On examination, she had bilateral optic atrophy, pendular nystagmus and mild gait ataxia. Routine blood tests revealed a normal creatine kinase and a mildly elevated fasting blood lactate (2.5 mM/l). An electrocardiogram and echocardiogram were normal. Respiratory function studies were normal. Peripheral neurophysiological studies identified a mild sensorimotor neuropathy with additional myopathic features on electromyography.

III:6 A 38-year-old man developed progressive visual failure in childhood and was registered as partially sighted at age 11, and registered blind at the age of 18 years. On examination he had an ataxic gait, had bilateral optic atrophy and pendular nystagmus in all positions of gaze. Up gaze and adduction was limited symmetrically for both eyes, but there was no ptosis. Peripheral tone, power and tendon reflexes were normal. Routine haematological and biochemical blood tests, including a serum creatine kinase, were normal.

IV:1 Clinical data not available. He has never been brought to medical attention with visual or neuromuscular symptoms.

IV:2 A 5-year-old girl was noted to have optic atrophy at 18 months of age, but otherwise had normal motor, verbal and social development. On examination she was noted to have gaze-evoked horizontal nystagmus on lateral gaze and bilateral optic atrophy, and walked with a broad-based unsteady gait. The cranial nerve and peripheral neurological examination was otherwise normal.

IV:3 A 6-year-old girl who was brought to medical attention at 1 year of age with a squint associated with progressive bilateral visual failure and fine pendular nystagmus. Bilateral optic atrophy was noted at 3 years of age. Her visual acuity deteriorated from 0.9 LogMAR (6/48 Snellen) bilaterally at 6 years. She was otherwise asymptomatic.

Muscle histochemistry and biochemistry

Left quadriceps needle muscle biopsy was performed in three patients (II:2, II:5 and III:1) under local anaesthetic. Histological and histochemical analyses of mitochondrial enzyme activities including a sequential reaction for cytochrome *c* oxidase (COX) and succinate dehydrogenase (SDH) activities were performed on 10 µm-thick serial cross-sections of biopsy tissue according to standard procedures. The activities of the individual respiratory chain complexes were measured in post 600 g_{av} skeletal muscle supernatants and expressed relative to the activity of the matrix marker enzyme citrate synthase (Taylor and Turnbull, 1997).

mtDNA molecular genetic analysis

Total genomic DNA was extracted from skeletal muscle biopsies and whole blood by standard procedures. Large-scale rearrangements of the mitochondrial genome were screened by Southern blot analysis of total DNA linearized with *Pvu* II and probed with a PCR generated probe (nucleotides 15782–1289) that hybridizes to the non-coding control region. A long-range PCR assay was employed to amplify muscle mtDNA across the major arc, using a pair of primers [L6249 (nucleotides 6249–6265) and H16215 (nucleotides 16225–16196)], which amplify a 9.9 kb product in wild-type mtDNA. The level of deleted mtDNA in individual COX-deficient and COX-positive staining muscle fibres isolated by laser micro-dissection was determined by quantitative real-time PCR as described (He *et al.*, 2002). This assay uses primers and fluorogenic probes to two different regions of the mitochondrial genome, one which is rarely deleted in patients (*MTND1*) and one which is located in the major mtDNA arc and is frequently deleted (*MTND4*). PCR and fluorescence analyses were performed using an ABI PRISM® 7000 sequence detection system (Foster City, CA).

The entire mitochondrial genome-coding region was amplified using a series of 36 overlapping M13-tailed oligonucleotide primer pairs. PCR products were purified (ExoSapIT, Amersham Biosciences, Bucks, UK), sequenced using BigDye Terminator cycle sequencing chemistries (v3.1, Applied Biosystems) on an ABI3100 Genetic Analyser (Applied Biosystems) and directly compared to the revised Cambridge Reference Sequence using SeqScape software (Applied Biosystems).

To assess m.8839G>A heteroplasmy in tissues and individual muscle fibres, a 250 bp fragment of mtDNA encompassing the mutation site was amplified (30 cycles) using a forward primer L8777 (nucleotides 8777–8794) and the reverse primer H9026 (nucleotides 9026–9008). Following the addition of 30 pmol of each primer, 5 µCi [α-^{32}P] dCTP (3000Ci/mmol) and 1 U of *Taq* polymerase, PCR reactions were subjected to an additional cycle of amplification. Labelled products were precipitated, digested at 37°C with 7.5 U *Hae* III (Roche Biochemicals). Restriction fragments were separated through a 12% non-denaturing polyacrylamide gel, dried onto a support and exposed to a PhosphorImager cassette. The level of mtDNA heteroplasmy was quantified using ImageQuant software (Molecular Dynamics). Two *Hae* III recognition sites in the wild-type product generates fragments of 156, 63 and 31 bp; the m.8839G>A transition results in the loss of a recognition site, yielding two products of 219 and 31 bp.

Nuclear DNA molecular genetic analysis

Both sense and anti-sense strands of the coding region and intron–exon boundaries for *POLG1, POLG2, PEO1* and *SLC25A4* were sequenced using published primer sequences (Beckman–Coulter Quickstart and CEQ 8000 fluorescent DNA analyzer) (Hudson *et al.*, 2006). The 30 coding exons and intron–exon junctions of the *OPA1* gene were sequenced using a set of 30 primer pairs (sequences available on request). Purified PCR products were sequenced using a Ceq2000 DNA sequencer (CEQ DTCS-Quick Start Kit, Beckman Coulter, Fullerton, CA, USA).

Genetic linkage analysis of the chromosomal regions flanking *POLG1, PEO1* and *SLC25A4*, was performed using selected markers from the Applied Biosystems 10 cM screening set and the 9 cM Cooperative Human Linkage Centre (CHLC) Weber Human Screening set (Invitrogen, version 10aRG). The predicted LOD score for the pedigree was obtained using Simlink v 2.52 based on a dominant inheritance model for a rare mutated allele (100% penetrant, 0.001 frequency). Two- and multi-point linkage analyses were performed using the EasyLinkage (v. 4.00beta) interface operating Fastlink (v1.4) and Genehunter (v2.1r5) algorithms assuming the disorder was dominantly transmitted, fully penetrant and had a gene frequency of 0.001 in the background population. Marker frequencies were derived from the pedigree data.

Nuclear gene expression studies

Patient (III:1 and II:2) and control primary fibroblasts were cultured in DMEM containing 4.5 g/l glucose and 0.58 g/l L-glutamine (Sigma) supplemented with 10% FBS (Gibco) and maintained in humidified incubator at +37°C with 5% CO_2. Cells were cultured to reach 90% confluency in fresh medium, and collected using 0.25% Trypsin (Invitrogen). Total cellular RNA was extracted utilizing RNeasy mini kit (QIAgen). cDNA synthesis was performed with 500 ng of total RNA, 0.25 µg of random primers, 300 µM dNTPs, 25 U of RNasin ribonuclease inhibitor and 200 U of M-MLV reverse transcriptase in 40 µl of its buffer (Promega). Measurements of transcript levels were performed with quantitative PCR application, utilizing Applied Biosystem (ABI PRISM® 7000 Sequence Detection System). The assay-on-demand primers were as follows: Twinkle (Hs00222440_m1), Ant 1 (Hs00154037_m1) and β-actin (Hs00181698_m1). Primers were cDNA specific, not amplifying genomic DNA. PCR reactions were made using 1 µl of cDNA, 1.5 µl assay mix and 15 µl TaqMan Universal PCR Master Mix (Applied Biosystem) in total volume of 30 µl. Every sample was analysed as duplicates or triplicates. Results were analysed with the aid of 7000 SDS 1.2 Sequence Detection Software (Applied Biosystems).

Results

Muscle histochemistry and biochemistry

Muscle biopsy of the index case (II:5) revealed features that were diagnostic of mitochondrial myopathy. Approximately 10% of all fibres were deficient in histochemical COX activity, with several fibres showing evidence of subsarcolemmal accumulation of abnormal mitochondria (2% ragged-red fibres, Fig. 2a). Similar findings were apparent in the biopsy from her sister (Patient II:2), whereas the histochemical COX defect in Patient III:1 was less pronounced with only 2% COX-deficient cells with no ragged-red fibres.

Biochemical investigation of respiratory chain activities in muscle homogenates did not show any obvious defect in complex IV activity, although there was some suggestion that complex I activity was subtly decreased in both Patient II:5 and Patient III:1 to 45% and 65% of controls, respectively (Table 1).

mtDNA analysis

Total muscle DNA was analysed for possible mtDNA rearrangements by Southern blotting, long-range PCR and real-time PCR. Southern blotting of total muscle DNA

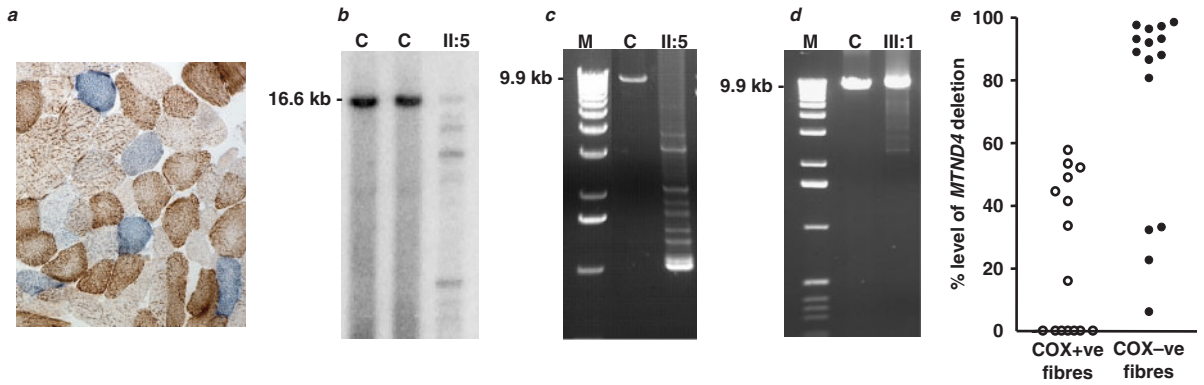

Fig. 2 Histochemical and molecular genetic analysis of muscle biopsy. **(a)** Histochemical demonstration of combined COX and SDH activities in muscle biopsy from Patient II:5, revealing ~10% COX-deficient fibres, some showing subsarcolemmal accumulation of abnormal mitochondria. **(b)** Southern blot and **(c)** long-range PCR analysis of muscle DNA demonstrating multiple mtDNA deletions in Patient's II:5 muscle biopsy. **(d)** Long-range PCR of muscle DNA from another affected family member (Patient III:I) also reveals detectable, multiple mtDNA deletions but to a lesser extent than observed Patient II:5. **(e)** Single-fibre, real-time PCR showing the low levels of *MTND4* gene deletion in individual COX-positive fibres whilst the majority of COX-deficient fibres contain high levels of mtDNA deletion, confirming the diagnosis of a multiple mtDNA deletion disorder.

revealed a characteristic laddering of smaller, deleted mtDNA molecules following *Pvu*II digestion, suggestive of multiple mtDNA deletions (Fig. 2b). This was confirmed by long-range PCR across the major arc region (Fig. 2c), although the defect was less pronounced in muscle from Patient III:1 (Fig. 2d). Real-time PCR of individual COX-deficient and COX-positive muscle fibres showed high levels of deleted mtDNA in the majority, but not all, of the COX-deficient fibres (Fig. 2e), a pattern typical in patients with multiple mtDNA deletion disorders (He *et al.*, 2002).

Sequencing of the entire mtDNA coding region in muscle from Patient II:5 revealed a number of known polymorphic variants and a previously unreported change (m.8839G>A) in the *MTATP6* gene. Hot-last cycle PCR–RFLP analysis demonstrated heteroplasmy at this site in muscle from this patient (39% mutated mtDNA in muscle) and Patient II:2 (81% mutated mtDNA in muscle), and also showed that the m.8839G>A mutation was present at higher levels in post-mitotic tissues than circulating lymphocytes (2% in Patient II:5, 2% in Patient III-6 and 6% in II:3), a common trait of pathogenic mtDNA point mutations (Fig. 3a). Single-fibre PCR–RFLP analysis of COX-positive and COX-deficient fibres from both Patients II:2 and II:5 did not however demonstrate any segregation of the mutant genotype with the histochemical defect, inferring that although heteroplasmic, this sequence variant was a polymorphism (Fig. 3b and c).

Table 1 Activities of the mitochondrial respiratory chain complexes in patient muscle

	II:5	III:1	Controls ($n = 20$)
Complex I	0.072	0.104	0.166 ± 0.047
Complex II	0.137	0.281	0.208 ± 0.070
Complex IV	2.600	1.147	1.805 ± 0.550

All respiratory chain activities are expressed relative to the activity of the mitochondrial matrix marker, citrate synthase. Complex I is expressed as nmols NADH oxidized min^{-1} unit citrate $synthase^{-1}$. Complex II is expressed as nmols DCPIP reduced min^{-1} unit citrate $synthase^{-1}$. Complex IV is expressed as $K\ s^{-1}$ unit citrate $synthase^{-1} \times 10^3$.

Nuclear genetic analysis

No sequence variants were identified in the coding region or flanking exon–intron boundaries for *POLG1*, *PEO1*, *POLG2* and *SLC25A4*. The expected maximum LOD score at zero recombination (Θ) for the dominant model was 2.06 (SD = 0.79). Microsatellite analysis excluded linkage to *POLG1* and *PEO1*. (Supplementary Figs 1a and b, and Supplementary Tables 1 and 2). Positive LOD scores were obtained for the region of chromosome 4 including *SLC25A4* (Zmax = 2.11 at $\theta = 0$, Supplementary Fig. 1c, Supplementary Table 3). All affected subjects and one

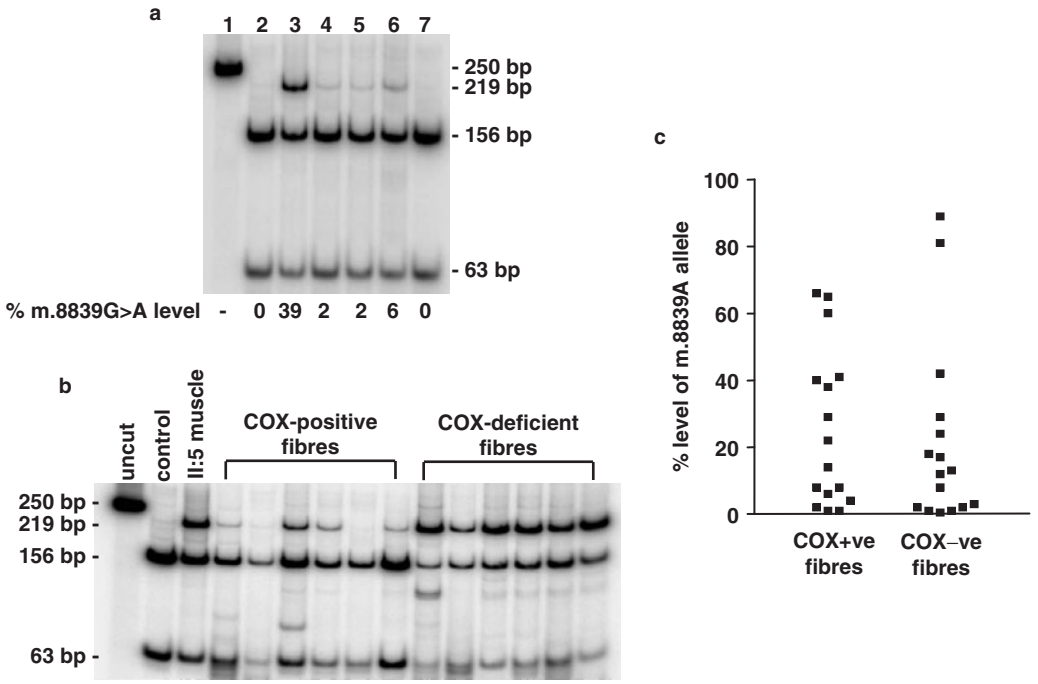

Fig. 3 Analysis of m.8839G>A *MTATP6* substitution. **(a)** Quantification of the relative amounts of mutant and wild-type mtDNA in available patient samples by hot-last cycle PCR–RFLP analysis. Lane 1, uncut sample; lanes 2 and 7, control DNA; lane 3, skeletal muscle DNA (Patient II:5); lane 4, blood (Patient II:5); lane 5, blood (Patient III:6); lane 6, blood (II:3). The proportion of mutant mtDNA (%) is shown beneath each lane. **(b)** Single-fibre PCR–RFLP analysis of the mutation in individual skeletal muscle (COX-positive and COX-deficient) fibres. **(d)** Graphical representation of the data presented in (b) confirming a lack of segregation of high levels of the m.8839G>A mtDNA mutation in individual COX-deficient fibres.

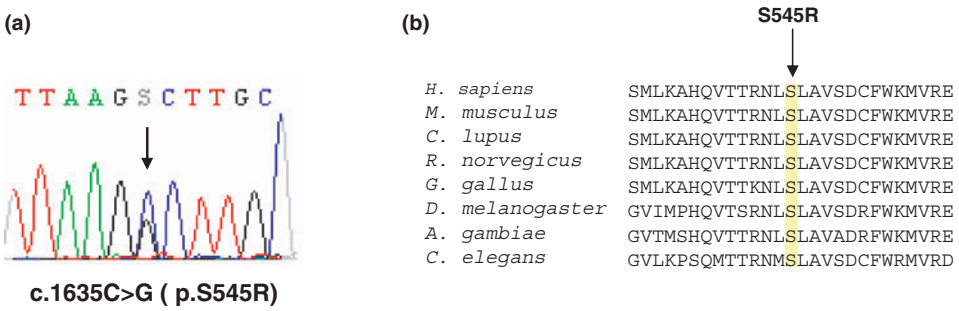

Fig. 4 Heterozygous *OPA1* mutation c.1635C>A (p.S545R). (**a**) Sequence chromatogram, arrow indicates mutation. (**b**) Amino acid sequence conservation in the opal protein flanking the mutation.

unaffected individual (II:4) shared all chromosome 4 marker alleles apart from the 4p telomeric marker D4S425 (Supplementary Fig. 2).

The expression levels of Twinkle and Ant 1 were normalized against a ubiquitously expressed housekeeping gene, β-actin. The expression levels of Twinkle (PEO1) showed to be similar in all the cell lines. The expression level of Ant 1 in the cells of Patient II:2 was similar compared to control, whereas the cells of Patient III:1 showed increased expression up to 2-fold compared to control (Supplementary Fig. 3). This is unlikely to have any affect on the phenotype and is considered as normal variation.

Sequence analysis revealed a heterozygous mis-sense mutation in exon 17 of *OPA1*: c.1635C>G, predicted to alter the amino acid sequence (p.S545R, Fig. 4a). The mutation was present in all eight affected individuals, and not in three unaffected individuals, nor 280 British control chromosomes.

Discussion

Affected individuals in the family described here initially presented with bilateral visual failure and optic atrophy of childhood onset, followed by hearing impairment, ophthalmoplegia, and a combination of cerebellar and sensory ataxia in adulthood, partly related to the sensory-motor neuropathy. Histochemical and biochemical analysis revealed a defect affecting respiratory chain complex I in a muscle homogenate, and a mosaic defect of complex IV (COX) affecting specific muscle fibres. These findings pointed towards a defect involving mtDNA.

At the time of the initial study, IV:3 was clinically unaffected, and the inheritance pattern was therefore consistent with a maternally inherited mtDNA defect (Fig. 1). After excluding common mtDNA point mutations, we therefore sequenced the entire mitochondrial genome, and identified a previously unreported nucleotide transition (m.8839G>A) in *MTATP6*. This mutation was heteroplasmic, suggesting that it was of recent onset, it was present in higher percentage levels in post-mitotic tissues than blood, it was not present in the mtDB Human Mitochondrial Genome Database (http:www.genpat.uu.se/mtDB/) of >2700 mtDNA sequences, and it altered a highly conserved Alanine residue at position 105 of the ATPase6 protein to a Threonine residue (A105T). All of these features point towards a causative pathogenic mtDNA mutation. However, although different mutations in *MTATP6* can cause disease, a defect in the ATP synthetase would not explain the complex I and histochemical complex IV deficiencies present in multiple family members, as demonstrated by a lack of segregation of the m.8839G>A mutation in individual COX-deficient muscle fibres. This prompted further mtDNA analysis, which identified multiple deletions of mtDNA in skeletal muscle, indicating a generalized disorder of mtDNA maintenance.

The heterozygous *OPA1* mutation, c.1635C>G (p.S545R), segregated with the disease phenotype in six patients, is predicted to alter a highly conserved neutral amino acid residue to an acidic residue in the GTPase domain of the protein (Fig. 4b). Although the *OPA1* base substitution is novel, a mutation affecting the same codon resulting in the same amino acid substitution has previously been described in three members of a Japanese family affected with ad-OA (Nakamura *et al.*, 2006). Moreover, the Japanese substitution was not detected in 100 Japanese (Nakamura *et al.*, 2006), and c.1635C>G was not detected in 140 British controls. Given that linkage, mutation and expression analysis effectively excluded a coding or promoter region mutation of *POLG1, POLG2, PEO1* or *SLC25A4*, we conclude that the clinical phenotype affecting this family is due to the c.1635C>G (p.S545R) *OPA1* mutation. Further evidence to support this is offered by the finding of the same mutation in a French patient who presented with bilateral optic atrophy, an axonal sensory-motor neuropathy and gait ataxia whose muscle biopsy reveals COX-deficient fibres and multiple mtDNA deletions (Patrizia Amati-Bonneau and Valerio Carelli, manuscript submitted).

Dominant optic atrophy, ptosis, ophthalmoplegia and sensorineural deafness has previously been described in a Belgian and North American family with the p.R445H

OPA1 GTPase-domain mutation (Payne *et al.*, 2004; Li *et al.*, 2005). Intriguingly, p.R445H is one of the most common *OPA1* mutations, but the phenotype usually only involves the visual system (*eOPA1 database*). It remains to be established why the same mutation can cause an organ-specific phenotype in some families, but a multi-system disorder in another. The family we describe here provide a clue as to the underlying disease mechanism, with broader implications for the pathophysiology of ad-OA caused by mutations in *OPA1*.

The opa1 protein is a 960 amino acid mitochondrial dynamin-related GTPase involved in the regulation of mitochondrial fusion (Delettre *et al.*, 2000; Olichon *et al.*, 2002). Downregulation of opa1 leads to inner mitochondrial membrane disruption, fragmentation of mitochondria and an increased propensity for apoptosis (Olichon *et al.*, 2002; Olichon *et al.*, 2003; Olichon *et al.*, 2007), but the precise function of opa1 is not known, and it is not clear how mutated opa1 causes respiratory chain dysfunction. There is emerging evidence that mitochondrial morphology is intimately linked to oxidative phosphorylation and the maintenance of mtDNA. Proteolytic processing of opa1 in patients with mitochondrial encephalopathies leads to mitochondrial fragmentation (Duvezin-Caubet *et al.*, 2006), and depletion of mtDNA has been documented in peripheral blood leucocytes from patients with *OPA1* mutations (Kim *et al.*, 2004). Our observation of age-associated somatic multiple mtDNA deletions in the skeletal muscle of patients with an *OPA1* mutation demonstrates the importance of opa1 in maintaining the structural integrity of mtDNA. Given that both opa1 and mtDNA are intimately related to the inner mitochondrial membrane (Olichon *et al.*, 2002), opa1 could be a component of the mtDNA nucleoid, which is the structural and functional unit of mtDNA expression and segregation within the cell (Garrido *et al.*, 2003). It is likely that disruption of the nucleiod would lead to enhanced mtDNA mutation, and facilitate the clonal expansion of somatic mtDNA mutations (Jacobs *et al.*, 2000). Neurons and skeletal muscle are particularly vulnerable to the accumulation of somatic mtDNA mutations (Corral-Debrinski *et al.*, 1992; Cortopassi *et al.*, 1992*a*; Cortopassi *et al.*, 1992*b*), and any process that accelerates mutation and/or clonal expansion will preferentially affect these tissues. This would explain why the family we describe here have a classical 'mitochondrial' phenotype with PEO and ataxia. A milder disruption of the same process would also explain why the extra-ocular muscle bears the brunt of the pathology in ad-PEO. Pre-myelinated retinal ganglion cells are rich in mitochondria (Andrews *et al.*, 1999), making them particularly vulnerable to subtle perturbations of mitochondrial function, as in Leber hereditary optic neuropathy (Carelli *et al.*, 2004).

Why did we also find the novel m.8839G>A *MTATP6* mtDNA mutation in this family? Heteroplasmy implies a recent mutation event. This could simply be a co-incidence, but given that this substitution has never been seen in 2600 controls, this seems unlikely. The mutation could be a consequence of the enhanced mtDNA mutation rate in subjects with the c.1635C>G (p.S545R) mutation in *OPA1*. Mutation in the female germ-line followed by random genetic drift through the mtDNA bottleneck could lead to high levels within this pedigree. Although m.8839G>A does not appear to be the primary cause of the mitochondrial disorder affecting this family, it has the potential to impair ATP synthesis, and thus modify the clinical phenotype. It was present at low levels in blood of both clinically affected and unaffected maternal relative, and was present at higher levels in skeletal muscle of one individual, implying that the mutation segregates to high levels in other post-mitotic tissues such as optic nerve.

Two animal models of ad-OA have recently been described: one with a splice site mutation in *OPA1* leading to a loss of the GTPase domain (Alavi *et al.*, 2007), and the other with a premature stop codon mutation in *OPA1* immediately before the central dynamin GTPase (Davies *et al.*, 2007). Both models mimic the human disease, with progressive visual failure due to the focal neurodegeneration of retinal ganglion cells in the papilomacular bundle. In both models, homozygous mutation are embryonic lethal, demonstrating the fundamental importance of opa1 for human development. These mice will be invaluable in elucidating the underlying disease mechanisms in ad-OA, and provide an ideal tool to study the accumulation of somatic mtDNA mutations in a range of neural and non-neural tissues. In this way we will test the hypothesis that the phenotype is related to, or is modulated by, clonally expanded mtDNA mutations.

Our observations have broader implications for other proteins involved in mitochondrial fusion and fission. Like opa 1, mitofusin (mfn) 1 and 2 are pro-fusion dynamin-related GTPases whose function is balanced by the pro-fission GTPases (Drp1) and Fis1 (Lee *et al.*, 2004; Chen *et al.*, 2005). Mutations in *MFN2* are a common cause of CMT2A (Zuchner *et al.*, 2004), occasionally with optic atrophy and deafness (Verhoeven *et al.*, 2006; Zuchner *et al.*, 2006), and recently a mutation in *DRP1* have been described in a childhood encephalopathy with lactic acidosis (Waterham *et al.*, 2007). Given the emerging relationship between mitochondrial structure and mtDNA integrity, it is possible that a disruption of mtDNA maintenance is central to the pathophysiology of these disorders. It is intriguing that a major disruption of mitochondrial morphology occurs in cells lacking Fis1, leading to phenotypic changes reminiscent of cellular senescence (Lee *et al.*, 2007). Again, somatic mutation of mtDNA may be involved here, possibly contributing to age-related neurodegeneration (Sorensen *et al.*, 2001; Bender *et al.*, 2006).

In conclusion, we have shown that mutation of *OPA1* is linked to the formation of multiple somatic mtDNA deletions, and that these contribute to the phenotype.

It is likely that *OPA1* is a gene important for mtDNA maintenance, which should be considered in patients with unexplained multiple mtDNA deletions.

Supplementary material

Supplementary material is available at *Brain* online.

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