

Exome sequencing identified a novel *de novo* *OPAI* mutation in a consanguineous family presenting with optic atrophy

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Summary

Inherited optic neuropathies are a heterogeneous group of disorders characterized by mild to severe visual loss, colour vision deficit, central or paracentral visual field defects and optic disc pallor. Optic atrophies can be classified into isolated or non-syndromic and syndromic forms. While multiple modes of inheritance have been reported, autosomal dominant optic atrophy and mitochondrial inherited Leber's hereditary optic neuropathy are the most common forms. Optic atrophy type 1, caused by mutations in the *OPAI* gene is believed to be the most common hereditary optic neuropathy, and most patients inherit a mutation from an affected parent. In this study we used whole-exome sequencing to investigate the genetic aetiology in a patient affected with isolated optic atrophy. Since the proband was the only affected individual in his extended family, and was a product of consanguineous marriage, homozygosity mapping followed by whole-exome sequencing were pursued. Exome results identified a novel *de novo* *OPAI* mutation in the proband. We conclude, that though *de novo* *OPAI* mutations are uncommon, testing of common optic atrophy-associated genes such as mitochondrial mutations and *OPAI* gene sequencing should be performed first in single individuals presenting with optic neuropathy, even when dominant inheritance is not apparent.

Introduction

Inherited optic neuropathies are a heterogeneous group of disorders characterized by mild to severe visual loss, colour vision deficit (dyschromatopsia), central or paracentral visual field defects and optic disc pallor. Optic atrophy can be isolated or associated with multi-systemic involvement (syndromic; Allen *et al.*, 2015) such as in Costeff's syndrome (methyl-glutaconic aciduria type 3; OMIM 258501; Ho *et al.*, 2008), Behr's syndrome (OMIM 210000; Pyle *et al.*, 2014) or Wolfram syndrome (OMIM 598500; Safarpour *et al.*, 2016). While multiple modes of inheritance have been reported, autosomal dominant optic atrophy (ADOA) and mitochondrial

inherited Leber's hereditary optic neuropathy (LHON) are the most common forms (Pfeffer *et al.*, 2013; Skidd *et al.*, 2013). Optic atrophy type 1, caused by mutations in the *OPAI* gene is believed to be the most common hereditary optic neuropathy, and most patients inherit a mutation from an affected parent. Both ADOA and LHON are characterized by selective involvement of the retinal ganglion cells. Optic atrophy type 1 is characterized by bilateral and symmetric optic nerve pallor, associated with a childhood-onset decrease in visual acuity, visual field defects and colour vision defect (often tritanopia, blue–yellow loss). The estimated prevalence of *OPAI*-associated optic atrophy is 1:10 000–1:50 000. Up to 10% of individuals with *OPAI* mutations have additional extra-ophthalmologic abnormalities such as sensorineural hearing loss, ataxia and myopathy, termed 'OPAI plus' (Amati-Bonneau *et al.*, 2005; Yu-Wai-Man *et al.*, 2010; Skidd *et al.*, 2013). In this study we used whole-exome sequencing to investigate

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the genetic aetiology in a patient affected with isolated optic atrophy.

Patients and methods

We applied whole-exome sequencing to a consanguineous family in which the proband presented with isolated optic atrophy. Local approval for this study was provided by the Rabin Medical Center Institutional Review Board, and all participants signed an informed consent.

Whole-exome sequencing

Genomic DNA extraction, exome enrichment, sequencing and analysis have been completed as described before (Reinstein *et al.*, 2015). Genomic DNA was extracted from peripheral leukocytes following standard protocols. Exome enrichment was achieved by the Nextera Rapid Capture Expanded Exome Kit (FC-140-1006) following manufacturer's guidelines. Sequencing was performed using the Illumina HiSeq 2500 machinery to generate paired end reads of 150 bp with average coverage of 80X. The analytic pipeline available at the genetics and genomic medicine laboratory (Gene by Gene, Houston, Texas) was used to map the obtained fragments to the human reference genome (hg19) with the Burrows–Wheeler Alignment (BWA MEM) algorithm and genetic variants were identified using SAM tools. The variants were annotated using SnpEff (Li *et al.*, 2009; Cingolani *et al.*, 2012). The results were analysed based on all modes of inheritance. Variants were filtered to generate a final list of rare functional variants only (missense, nonsense, splice site variants and indels). Variants with minor allele frequency >0.01 in the Exome Variant Server (release ESP6500) or that have allele count >150 in the ExAC database of Europeans (NFE) were removed. For validation and segregation analyses PCR primers were designed to amplify the regions flanking the two mutations. PCR products were purified using magnetic-particle technology (Seradyn, Inc.). After purification, all fragments were sequenced using forward and backward internal primers to determine the noted regions. Sequencing was performed on a 3730xl DNA Analyser (Life Technologies), and the resulting sequences were analysed with the Sequencher software (Gene Codes Corporation). The Illumina HumanOmniExpress-24 v1.0 BeadChip was used to detect copy number variations and for homozygosity mapping.

Clinical report

The nuclear family is of Arab Muslim ancestry, and consists of parents and four children. The parents are first-degree cousins and report no medical problems. The proband has three healthy siblings, aged

Patient	A	P	D	T	E	E	T	I	F	S
Human	A	P	D	T	K	E	T	I	F	S
Rhesus	A	P	D	T	K	E	T	I	F	S
Mouse	A	P	D	T	K	E	T	I	F	S
Dog	A	P	D	T	K	E	T	I	F	S
Chicken	A	P	D	T	K	E	T	I	F	S
Frog	A	P	D	T	K	D	T	I	F	N
Zebrafish	A	A	D	T	K	E	T	I	F	S
Fruit fly	A	S	D	D	K	D	S	I	H	Q
Lamprey	A	P	D	T	K	D	A	I	F	A
C. elegans	A	R	E	T	K	D	D	I	I	R

K397

Fig. 1. Evolutionary conservation analysis of the *OPAI* mutated domain. Lys 397 is marked.

13, 12 and 4 years, with normal vision. The proband is a 7 year old male, with no significant past medical history. He was admitted to the pediatric ward because of headache, nausea, vomiting and decrease in visual acuity (VA) in both eyes over the past few months. The parents reported regression in his learning ability as well as in his fine motor skills recently and he was diagnosed with attention deficit disorder by a child neurologist. Physical examination revealed normal findings with no facial dysmorphism. Ophthalmology examination revealed reduced VA, 6/30 both eyes on first exam, with mild deterioration to 6/60 both eyes (right eye [RE] 6/60 and left eye [LE] 6/90) 2 years later. Near vision was Jeger 16 for both eyes together at the time of diagnosis and remained stable until the end of the follow up. Refraction was +2.25 RE and +2.50 LE with no improvement in vision with this correction. No relative afferent pupillary defect (normal pupillary response to rapid shift of light from one eye to the other indicates equal optic nerve function) was detected. He had normal colour vision (Ishihara colour plate), 12/12 for each eye at diagnosis, and 8/12 for each eye on last exam. Orthoptic evaluation revealed exotropia of -30 prism diopters (PD) for distance and -10 PD for near. Fundoscopic examination showed no papilloedema or other abnormal findings except for diffuse optic disc pallor. Visual field test was considered as unreliable. Additional work up including full-field rod–cone electroretinogram and flash visual evoked potential were normal. Optical coherence tomography measured severe reduction in average nerve fibre layer thickness around the optic disc. Initial metabolic work up including plasma amino acids, very long chain fatty acids, carnitine, acylcarnitine and urinary organic acids was normal. Serum vitamin E and vitamin A levels were within normal limits. Serologic test results were negative for aquaporin-4 (AQP4) antibodies (detected in neuromyelitis optica), anti-proteinase 3 and anti-myeloperoxidase (detected in vasculitides-associated optic neuropathies). Serology for Bartonella henselae (cat-scratch disease) was also negative. Levels of C3

Table 1. *Main forms of non-syndromic optic atrophy.*

Autosomal dominant	OPA1 (Kjer-type)	OPA3 ^a	OPA4 (locus only)	OPA5 (locus only)
Chromosomal locus	High penetrance rate ~90% 3q28-q29	19q13	18q12 ^b	~100% 22q11-q12
Prevalence	1:50 000 worldwide (1:10 000 in Denmark, 1:25 000 in UK)	Rare		
Mutations	>200 mutations are known (see MITOchondrial DYNamics variation portal ^c)			
Clinical characteristics	Childhood-onset bilateral vision loss (2–10 years) Slow deterioration Temporal pallor of optic discs Visual acuity 6/6-6/120 Visual field defect (central, paracentral scotoma) Colour vision defect often blue–yellow loss (tritanopia) <u>Up to 10% of patients have dominant optic atrophy plus:</u> Auditory neuropathy Peripheral neuropathy Ataxia Myopathy Late-onset chronic progressive external ophthalmoplegia	Childhood onset of optic atrophy Cataract May have neurologic symptoms – extrapyramidal signs, tremor	Childhood to adolescence onset vision loss Slow deterioration Visual acuity 6/6-6/120 Visual field defect (central, paracentral scotoma)	Bilateral vision loss Pallor of optic discs Visual field defect (central scotoma) Colour vision defect
Autosomal recessive	TMEM126A (OPA7)	ACO2 (OPA8)	OPA6 (locus only)	
Chromosomal locus	11q14	22q13	8q21-q22	
Prevalence	Rare (described in north African consanguineous families) Founder mutation in north Africa – c.163C > T; p.R55X	Very rare (one family described)	Rare	
Clinical characteristics	Juvenile-onset optic atrophy Optic atrophy and mild auditory neuropathy	Childhood-onset bilateral vision loss (first decade) Pallor of optic discs Paracentral scotoma Colour vision defect (often red–green)	Early childhood-onset bilateral vision loss Photophobia Dyschromatopsia for red–green confusion) Visual acuity 1/10-2/20	
Mitochondrial	Leber's hereditary optic neuropathy (LHON)			
Prevalence	1:15 000–50 000			
Mutations	95% have one of three mutations: m.11778G > A m.14484 T > C m.3460G > A			
Clinical characteristics	Adulthood-onset vision loss (18–35 years) Painless sub-acute visual impairment in both eyes asynchronously Males are more affected Few reports for spontaneous recovery Postural tremor, loss of ankle reflexes Multiple sclerosis like illness			

OPA1 and LHON are the most common forms of hereditary optic neuropathies.

^a OPA3 mutations can also cause 3-methyl-glutaconic aciduria type 3 (Costeff syndrome), AR disease, early-onset bilateral optic atrophy. Later-onset spasticity, extrapyramidal dysfunction, cognitive impairment.

^b Most pedigrees showed linkage to telomeric region of 3q28-q29.

^c MITOchondrial DYNamics variation portal: http://mitodyn.org/home.php?select_db=OPA1.

and C4 were normal. Cerebrospinal fluid oligo-clonal bands (detected in optic neuritis) were negative. Lumbar puncture showed normal opening pressure. Brain MRI scan was normal except for bilateral thickening of the optic nerves with increased fluid around the optic nerve sheaths. Based on these findings, a diagnosis of pseudotumour cerebri was suspected and he was treated with oral acetazolamide and steroids with no improvement in his symptoms.

Results

Screening for the three common LHON-associated mtDNA mutations (m.11778G > A, m.3460G > A and m.14484 T > C) revealed normal results. Subsequently, a SNP-based microarray was completed and showed no copy number variations. We did not identify known optic-atrophy associated genes within the homozygous regions, which encompassed 4% of the DNA (~1300 OMIM morbid genes). Next, whole-exome sequencing was carried out on the proband, his 13 year old healthy brother, and their parents. Exome results were analysed for all modes of inheritance, and revealed a heterozygous variant in the *OPA1* gene in the proband: *OPA1* c.1189A > G, p.Lys397Glu (chr3:193361347A > G). This variant has never been reported in any public domains. The lysine residue in this position is highly conserved in evolution (Fig. 1), and prediction algorithms (SIFT, Polyphen) showed high functional effect. The variant was validated by Sanger sequencing and segregation analysis showed that the proband's sibling and parents do not carry the identified *OPA1* variant.

Discussion

We report a novel *de novo OPA1* variant in a child presenting with isolated optic atrophy. The vast majority of individuals diagnosed with optic atrophy type 1 have an affected parent. Hence, since the proband was the only affected individual in his extended family, and was a product of consanguineous marriage, a recessive inheritance was suspected and homozygosity mapping followed by whole-exome sequencing were pursued, rather than a targeted *OPA1* gene test. *De novo OPA1* mutations have been reported in the literature before. In one report, *OPA1* testing in 20 patients with ADOA and no family history revealed two cases (10%) with *de novo OPA1* mutations (Baris *et al.*, 2003). In a larger study, mutation screening for LHON, *OPA1* and *OPA3* genes was performed in 980 patients with suspected hereditary optic neuropathy (Ferré *et al.*, 2009). Molecular diagnosis was established in 440 (45%) patients, out of which 295 patients (67%) had an *OPA1* mutation and half of them (157/295) were apparently 'sporadic' cases without known family history.

However, since parental samples were not available for testing, *de novo* mutations were confirmed in only 12 (4%) of the apparently sporadic cases.

Non-syndromic recessive optic atrophy is a rare form of optic neuropathy. To date, only two genes (*TMEM126A* and *ACO2*) and one locus (8q) have been identified (Allen *et al.*, 2015). The main forms of non-syndromic optic atrophy are depicted in Table 1.

Over 200 mutations in *OPA1* have been reported hitherto (Ferré *et al.*, 2009). Most of these mutations result in the loss of function of the mutated allele, supporting haploinsufficiency as the most likely mechanism (Pesch *et al.*, 2001). The estimated penetrance is ~90% in the familial forms of the disease (Cohn *et al.*, 2007). The *OPA1* protein is composed of five regions: a mitochondrial leader sequence, a GTPase domain, a central dynamin domain and two coil-coiled regions (Alavi & Fuhrmann, 2013). Most reported mutations are located in the GTPase domain encoded by exons 8–16 or in the C-terminal coil-coiled domain encoded by exons 27–28. The identified mutation in this report also involves the GTPase domain of the *OPA1* protein, which is highly conserved in evolution. Based on yeast studies showing that mutations in the yeast protein homologue, *Mgm1*, disrupt mitochondrial fusion, it was suggested that *OPA1* mutations affect mitochondrial functions including fusion, energy metabolism, control of apoptosis and maintenance of mitochondrial genome integrity. Retinal ganglion cells are the most sensitive to apoptosis in optic atrophy type 1 patients and it still remains unclear why ADOA manifests with a restricted ocular phenotype, since the *OPA1* gene is ubiquitously expressed.

In conclusion, although *de novo OPA1* mutations are uncommon, testing of LHON associated mutations followed by *OPA1* gene sequencing should be performed first in sporadic patients presenting with optic neuropathy, even when dominant inheritance is not apparent.

Declaration of interest

Doron M. Behar and Concetta Bormans are compensated and serve as the chief medical officer and the laboratory director of Gene by Gene, respectively.

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