

Multiethnic involvement in autosomal-dominant optic atrophy in Singapore

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CLINICAL STUDY

Abstract

Purpose Autosomal-dominant optic atrophy (ADOA), often associated with mutations in the *OPA1* gene (chromosome 3q28-q29) is rarely reported in Asia. Our aim was to identify and describe this condition in an Asian population in Singapore.

Patients and methods Preliminary cross-sectional study at the Singapore National Eye Centre, including patients with clinical suspicion of ADOA, who subsequently underwent genetic testing by direct sequencing of the *OPA1* gene.

Results Among 12 patients (10 families) with clinically suspected ADOA, 7 patients (5 families) from 3 different ethnic origins (Chinese, Indian, and Malay) carried a heterozygous pathogenic variant in the *OPA1* gene. The *OPA1* mutations were located on exons 8, 9, 11, and 17: c.869G > A (p.Arg290Glu), c.892A > G (p.Ser298Gly), c.1140G > A (splicing mutation), and c.1669C > T (p.Arg557*), respectively. One splicing mutation (c.871-1G > A) was identified in intron 8. We also identified a novel mutation causing optic atrophy and deafness (c.892A > G (p.Ser298Gly)). Among the phenotypic features, colour pupillometry disclosed a dissociation between low vision and preserved pupillary light reflex in ADOA.

Conclusion We report the first cases of genetically confirmed *OPA1*-related ADOA from Singapore, including a novel mutation causing 'ADOA plus' syndrome. Further epidemiological studies are needed in order to determine the prevalence of ADOA in South-East Asia.

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characterized by bilateral visual loss, typically occurring in early childhood, associated with central or cecentral visual field defects, colour vision deficits, and temporal or diffuse pallor of the optic disc.^{1,2} *OPA1* mutations are responsible for about 60–80% of genetically confirmed ADOA cases.^{3–5} To date, the locus-specific database dedicated to *OPA1* (<http://opa1.mitodyn.org/>), under our curation, has listed a total of 377 *OPA1* gene variants, of which 65% are considered pathogenic.^{6,7} The *OPA1* gene, localized on 3q28-q29, has 30 coding exons including three alternative exons. It encodes a mitochondrial dynamin-related GTPase critical to mitochondrial function.^{3,4} Despite the reported autosomal-dominant inheritance, there is incomplete penetrance of the condition⁸ and almost half of the *OPA1* mutations have been detected in apparently sporadic cases with no demonstrable family history.⁹ As a result of the wide spectrum of mutations, there is considerable inter and intrafamilial variability in the ADOA phenotype ranging from minimal to significant visual loss to severe phenotypes affecting young adults with systemic associations such as sensorineural deafness, ataxia, external ophthalmoplegia, peripheral neuropathy, and myopathy, also known as the ADOA 'plus' phenotypes.¹⁰ Severe syndromes affecting young children, due to recessive *OPA1* inheritance, were also recently reported.^{5,11,12}

Although ADOA is an ubiquitous condition,^{13–18} several studies have reported its possibly lower incidence in Asia.^{19,20} Our aim was to investigate, for the first time, its occurrence in the multiethnic population of Singapore.

Materials and methods

Patients with unexplained optic neuropathies, clinically suggestive of ADOA, seen at Singapore National Eye Centre (January 2013 to August

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Introduction

Autosomal-dominant optic atrophy (ADOA) is the most common inherited optic neuropathy,

2014) were genetically tested for *OPA1* mutations. Inclusion criteria were: bilateral visual loss, dyschromatopsia, optic disc pallor or atrophy, and visual field loss, irrespective of the presence of ophthalmic family history. Other causes of optic neuropathies were ruled out in all included patients. The study was approved by our central institutional review board ethics committee.

A blood sample was taken from 12 included patients after informed consent. The 30 coding *OPA1* exons and the exon–intron junctions were sequenced using Sanger technique. The *OPA1* variants are described according to the isoform 1 (RefSeq: NM_015560.2). Phenotyping of genetically confirmed carriers included monocular chromatic pupillometry, described in detail elsewhere.²¹ In brief, one eye of each subject was exposed to blue (469 nm) and red (631 nm) light (order of light exposure randomized) of gradually increasing intensity from 6.8 to 13.8 log photons/cm²/s¹ at the level of the cornea, over 2 min, preceded and followed by 1 min of darkness. The dark pupil diameter before light exposure was used to convert the rest of the pupillary constriction data into constriction ratio. The pupillary constriction during the light exposure was binned in bins of 0.5 log units between 7 and 14 log photons/cm²/s¹, generating 14 data points per light exposure recording. Pupillometry results were compared with those of 54 healthy control subjects that underwent the same protocol.

Results

Patient 1 A 27-year-old Malay man, with a family history of poor vision, was evaluated for unexplained poor vision and recent, bilateral hearing loss. He admitted having poor vision since primary school as well as a family history of poor childhood vision (in a second-degree relative). Best corrected visual acuity (BCVA) was 6/45 OU and colour vision was 13/15 on Ishihara plates OU. Fundoscopy disclosed bilateral temporal disc pallor and normal appearance of the retina. MRI brain was normal. Genetic testing revealed a novel pathogenic heterozygous missense variant c.892A>G (p.Ser298Gly) in exon 9 of *OPA1* gene.

Patient 2 A 28-year-old Indian male with a family history of unexplained, poor vision, was evaluated for longstanding visual loss. His BCVA was 6/24 RE and 6/30 LE, and fundoscopy disclosed pale, cupped discs. Work-up for toxic, nutritional, and compressive causes of optic atrophy (including MRI brain and anterior visual pathway) was negative. Genetic testing disclosed a pathogenic missense pathogenic variant c.869G>A (p.Arg290Glu) in exon 8 of *OPA1*.⁴

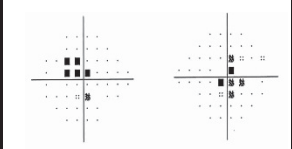
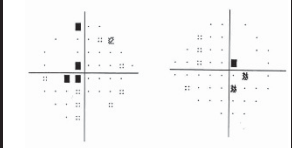
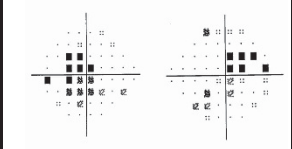
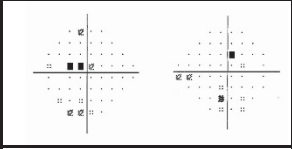
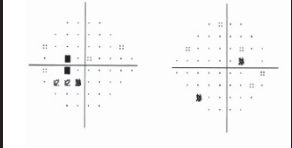
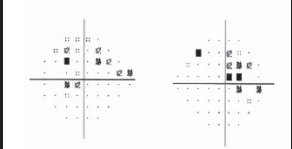
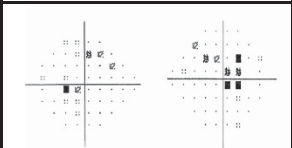
Patient 3 A 70-year-old male Chinese patient with no significant ophthalmic family history had unexplained visual loss since his youth. BCVA was 6/120 in both eyes, associated with bilaterally pale, cupped discs; the remainder of the neuro-ophthalmic examination was normal. A dedicated CT of brain, orbits, and pituitary fossa ruled out compression of the afferent pathways and the remainder of the evaluation did not disclose a nutritional, toxic, inflammatory, or infectious cause of this bilateral optic neuropathy. Genetic testing disclosed a pathogenic splicing variant in the intron 8 of the *OPA1* gene (c.871-1G>A).²²

Patient 4 A 24-year-old Chinese man with a family history of visual loss presented unexplained bilateral progressive visual loss since childhood. BCVA was 6/45 OU, and fundoscopy revealed bilateral temporal disc pallor. Genetic testing revealed a pathogenic heterozygous non sense variant in the exon 17 of the *OPA1* gene (c.1669C>T (p.Arg557*)).²²

Patients 5, 6, and 7 This family, originally from Myanmar, was composed of three affected male patients: two brothers (patients 5 and 6) and their father (patient 7). No other family members were affected. The proband, age 25, (patient 5), was independently, initially diagnosed with Leber's hereditary optic neuropathy (LHON) in a context of poor, unexplained visual loss (6/60 OU), and a mitochondrial point mutation (m.11253T>C (p.Ile165Thr)) in the *MT-ND4* gene.²³ At that time, no nuclear DNA testing was performed. His brother (patient 6), also suffered of unexplained visual loss, thought to be related to LHON. The LHON diagnosis was later challenged, when the father, age 61 (patient 7), was evaluated for longstanding visual loss (6/45 OU) and centrocaecal scotomas. Genetic testing in all three patients disclosed a pathogenic, heterozygous c.1140G>A splicing variant in exon 11 of *OPA1* gene. This mutation is responsible for exon 11 skipping and has been previously reported to be pathogenic in ADOA.²⁴

Humphrey visual fields were variably abnormal in all included patients (Table 1). Chromatic pupillometry was performed in five of the seven subjects with genetically confirmed ADOA and compared with those obtained in 54 healthy control subjects. Dose–response curves for pupillary constriction were generated for controls and patients with ADOA for both blue 469 nm light and red 631 nm light. The mean constriction amplitude of pupils, both to blue and red light, at each point of the curve was comparable in the ADOA group and in the healthy control group (Figure 1).

Table 1 Summary of included patients

Pt ID	Mutation	Reference	Age at diagnosis (age at onset) and ethnicity	Right BCVA	Left BCVA	Humphrey visual field (HVF) 24-2 PSD	HVF Mean Deviation (dB)
1	c.892A>G (p.Ser298Gly) Exon 9	Present study	27 (9) Malay	6/45	6/45		RE -6.57 <0.5% LE -5.69 <0.5%
2	c.869 G>A (p.Arg290Glu) Exon 8	Alexander et al ⁴	31 (18) Indian	6/24	6/30		RE -8.04 <0.5% LE -9.80 <0.5%
3	c.871-1G>A Intron 8 Splice variant	Tooms et al ²²	70 (20) Chinese	6/120	6/120		RE -7.64 <0.5% LE -6.70 <0.5%
4	c.1669 C>T (p.Arg557*) Exon 17	Tooms et al ²²	24 (9) Chinese	6/45	6/45		RE -4.33 <1% LE -5.28 <1%
5	c.1140G>A Exon 11 Splice Variant	Schimpf et al ²⁴	33 (6) Burmese	6/60	6/60		RE -3.84 <1% LE -5.04 <1%
6	c.1140G>A Exon 11 Splice Variant	Schimpf et al ²⁴	30 (7) Burmese	6/24	6/24		RE -5.64 <1% LE -5.47 <1%
7	c.1140G>A Exon 11 Splice Variant	Schimpf et al ²⁴	61 (20) Burmese	6/45	6/30		RE -4.18 <1% LE -3.22 <1%

Underlying genetic mutation, references of first report of the mutation,^{4,22,24} patient's age at diagnosis and at onset, ethnicity, visual acuity, visual fields (pattern SD-PSD), and mean deviation (MD).

Discussion

This is the first report of genetically confirmed ADOA in Singapore and in South-East Asia. ADOA is rare in Asia, occurring in only 6.3% of the Chinese patients with hereditary optic neuropathies.²⁰ This is in contrast to a European study, which reported 30% positivity for *OPA1*

mutations in 980 patients from France and Spain with suspected hereditary optic neuropathy.⁹ It is not clear though, whether ADOA is less prevalent in Asia, or merely less frequently detected, due to unfamiliarity with the condition. Indeed, milder phenotypes and limited access to genetic testing may explain, partly, the reduced

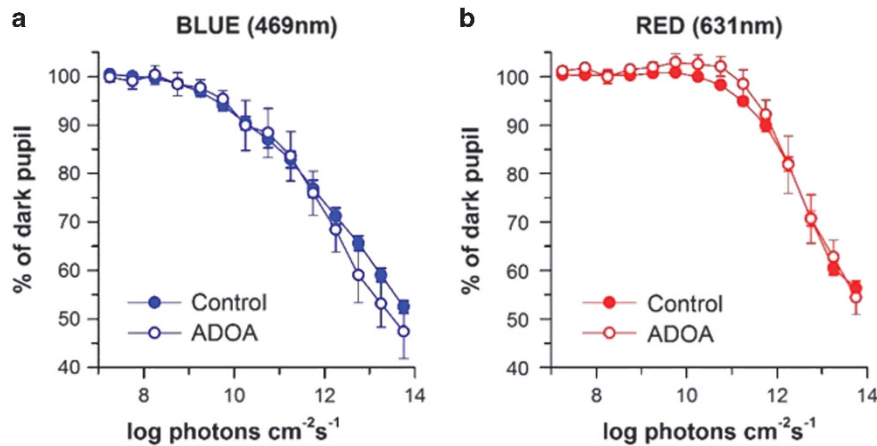


Figure 1 Colour pupillometry in genetically confirmed ADOA patients. Dose–response curves for pupillary constriction are shown for controls ($n=54$, filled circles) and patients with ADOA ($n=5$, open circles), who were exposed to blue 469 nm light (a, left, blue trace) and red 631 nm light (b, right, red trace). There was no significant difference of the pupil responses in ADOA patients, compared with controls. Pupil diameter is expressed as a percentage of the dark pupil measured prior to each light exposure. The mean \pm SEM is shown in the graphs.



Figure 2 Evolutionary conservation analysis of the OPA1 p.(Ser298Gly) mutation (patient id #1). Eight species were aligned using the multiple sequence alignment tool CLUSTAL Omega³⁰ (version 1.2.2) to analyze the evolutionary conservation of the OPA1 proteins. Sequences of *Caenorhabditis elegans* (CAA87771.3), *Drosophila melanogaster* (NP_610941.1), *Danio rerio* (NP_001007299.1), *Gallus gallus* (NP_001034398.1), *Mus musculus* (NP_001186106.1), *Bos taurus* (NP_001179890.1 2), *Pan troglodytes* (XP_003310226.1), and *Homo sapiens* (NP_056375.2) were retrieved from GenBank.

prevalence of the disease in Asia. It has nevertheless been suggested that screening for OPA1 mutation is needed in Chinese populations suspected with hereditary optic neuropathies.²⁵

Our patients encompassed all major ethnic groups in Singapore, including Chinese, Indian, and Malay, presenting with classical clinical findings. Phenotypically, our patients had similar findings with those disclosed in Caucasian ADOA populations, that is, early onset of a slowly progressive optic neuropathy. The pupillometry results were also similar to those reported in Caucasian cohorts.²⁶ It has been postulated that the ipRGCs expressing melanopsin, mediating blue light responses, are relatively resistant to the intracellular metabolic dysfunction induced by the various genetic defects in mitochondrial optic neuropathies.²⁷ In all five patients tested among our group, there was no significant

difference in the pupil responses to red and blue light, when compared with healthy controls, suggesting resistance of ipRGCs in ADOA.

The detected mutations were classical, spanning across exons 8, 9, 11, and 17 and in intron 8.⁶ We also report an ADOA ‘plus’ patient due to a novel missense mutation c.892A > G (p.Ser298Gly) in exon 9, not previously reported.^{6,7,28} This variant is predicted by the state of the art *in silico* methods to be disease causing with high confidence; (1) the substituted nucleic acid is highly conserved²⁹ (phyloP score: 4.88) leading to the substitution of a serine amino acid, which is highly conserved in the eight species aligned³⁰ in this study (Figure 2), (2) the main prediction tools for predicting damaging effects of missense mutations predict this mutation to affect the protein function with high confidence^{31–33} (Mutation Taster probability: 0.99;

PolyPhen-2 probability: 1.00; SIFT score: 0.00, with sequences used for prediction diverse enough), (3) we submitted the first report of this variant in the reference *OPA1* locus-specific database^{6,7} (DB-ID: OPA1_00306), and this mutation is unknown in the catalog of genome variations³⁴ and both in exome^{35,36} or genome³⁷ databases of healthy individuals.

One family (patients 5, 6, and 7) was carrying a pathogenic, heterozygous *OPA1* mutation, c.1140G>A. Interestingly, one of the affected male members in this family (patient 5) was also carrying the mitochondrial DNA variant m.11253T>C, detected by an initial, limited genetic work-up. The m.11253T>C variant is currently referenced both as a polymorphism and as a candidate LHON mutation in the Mitomap reference database, indicating that the pathogenic status of this variant is not definitive. It is, therefore, well possible that a synergistic effect might have played a role, between the *OPA1* and the mtDNA variant, given also that patient 5 had a lower visual acuity than his affected father.

In conclusion, we report here the first seven cases of genetically confirmed ADOA, in a multiethnic population from Singapore. Although ADOA may be a rare condition in Asia, it should be part of the differential diagnosis in unexplained optic neuropathies, even in the absence of family history.

Summary

What was known before

- ADOA is well known in the western world. There are very few reports of genetically confirmed ADOA from Asia/Singapore.

What this study adds

- First case series of genetically confirmed ADOA from Singapore with report of a novel mutation causing ADOA plus phenotype and genetic analysis to support its pathogenicity.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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