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A novel *OPA1* mutation in a Chinese family with autosomal dominant optic atrophy

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ABSTRACT

A large four-generation Chinese family with autosomal dominant optic atrophy (ADOA) was investigated in the present study. Eight of the family members were affected in this pedigree. The affected family members exhibited early-onset and progressive visual impairment, resulting in mild to profound loss of visual acuity. The average age-at-onset was 15.9 years. A new heterozygous mutation c.C1198G was identified by sequence analysis of the 12th exon of the *OPA1* gene. This mutation resulted in a proline to alanine substitution at codon 400, which was located in an evolutionarily conserved region. This missense mutation in the GTPase domain was supposed to result in a loss of function for the encoded protein and act through a dominant negative effect. No other mutations associated with optic atrophy were found in our present study. The c.C1198G heterozygous mutation in the *OPA1* gene may be a novel key pathogenic mutation in this pedigree with ADOA. Furthermore, additional nuclear modifier genes, environmental factors, and psychological factors may also contribute to the phenotypic variability of ADOA in this pedigree.

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1. Introduction

Autosomal dominant optic atrophy (ADOA), also known as Kjer's disease (OMIM ID: 165500), is clinically characterized by variable level of visual impairment, temporal optic disc pallor, color vision defects, and centrocecal scotoma [1,2]. Visual impairment begins in early childhood, and can lead to a severe decrease in visual acuity, which results in legal blindness in some cases [3,4]. Pathological examination has indicated that ADOA is caused by a loss of retinal ganglion cells followed by ascending atrophy of the optic nerve [5]. ADOA is one of the most common forms of dominantly inherited optic neuropathy [6]. The estimated prevalence of ADOA is 1:50,000 worldwide [7]. The characteristics of ADOA are similar to Leber's hereditary optic atrophy (LHON) (OMIM ID: 535000), and it is difficult to distinguish LHON from ADOA without a family history. However, LHON patients suffer from a sudden onset of visual impairment in both eyes asynchronously, and the age of onset (18–35 years) is later than in ADOA patients. Most importantly, LHON is maternally transmitted and is associated with mutations in the mitochondrial DNA (mtDNA) [8]. In addition, ADOA is reported to have greater penetrance than LHON [9].

The major locus associated with ADOA is the *OPA1* gene, which was first mapped to chromosome 3q28-qter in three Danish pedigrees. This locus was located on a 1.4 cM interval flanked by the markers D3S3669 and D3S3562 in a subsequent linkage study [6]. Another three loci, *OPA4* (18q12.2–q12.3), *OPA3* (19q13.2–q13.3) and *OPA5* (22q12.1–q13.1) have also been identified [10–12]. The *OPA1* gene consists of 31 exons that span more than 100 kb of genomic DNA, of which 28 are coding exons. Alternative splicing of three additional exons 4, 4b and 5b, leads to eight transcript variants [13]. *OPA1* is widely expressed throughout the body, especially in the cells of the retinal ganglion cell layer, the inner and outer plexiform layers and the inner nuclear layer [3]. *OPA1* encodes a mitochondrial dynamin-related guanosine triphosphatase (GTPase) protein with 960 amino acids. The protein is composed of five regions: a mitochondrial leader sequence, a GTPase domain, a central dynamin domain, and two coil-coiled regions

Abbreviations: ADOA, autosomal dominant optic atrophy; GTPase, guanosine triphosphatase; LHON, Leber's hereditary optic atrophy.

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[14]. Mutations in the *OPA1* gene have been recognized to be associated with ADOA. Till now, there are 230 mutations that have been reported, including missense mutations, nonsense substitutions, deletions, insertions and complex rearrangements [15]. Most of the mutations are located in the GTPase domain encoded by exons 8–16 and in the C-terminal coil-coiled domain encoded by exons 27–28 [13]. The mutations cause protein truncation and functional loss of one allele, thereby resulting in haploinsufficiency of *OPA1* [16]. In our present study, a large four-generation Chinese family with ADOA was investigated, and the investigation included clinical examination, *OPA1* mutation analysis and mtDNA analysis. Patients were found to be heterozygous for the c.C1198G mutation in the *OPA1* gene, which indicated that this may be a novel key pathogenic mutation for ADOA in this pedigree.

2. Materials and methods

2.1. Subjects

This study was approved by Wenzhou Medical College Ethics Committee, Wenzhou, Zhejiang, China. The Chinese family was evaluated at the ophthalmic clinic of Wenzhou Medical College. Each subject had signed the informed consent form and was treated in accordance with the tenets of the Declaration of Helsinki. Thirteen family members (5 females and 8 males) participated in the study, and their ages ranged from 15 to 74 years.

2.2. Ophthalmological examinations

All participating family members were interviewed at length to identify both personal and family medical histories of visual impairment and other clinical abnormalities. The clinical examinations of the proband and other participants were conducted in a consulting room, including visual acuity, visual field (Humphrey Visual Field Analyzer IIi, SITA Standard; Carl Zeiss, Inc., Dublin, CA), and visual evoked potentials (VEP) (Roland Consult RETI port gamma, flash visual evoked potential; Brandenburg, Germany), as well as fundus photography (Canon CR6-45NM fundus camera; Tokyo, Japan). The degrees of visual impairment were defined according to the visual acuity as follows: normal > 0.3, mild = 0.3–0.1, moderate < 0.1–0.05, severe < 0.05–0.02, and profound < 0.02 [8]. After examination, blood samples were obtained from each participant. In addition, 102 unrelated control DNA samples that were used for the *OPA1* mutation screening were obtained from a panel of unaffected individuals with Chinese ancestry.

2.3. Mutational analysis of OPA1

Genomic DNA was isolated from the whole blood of participants (anti-coagulated with EDTA-K_2) using the phenol/chloroform extraction method. First, four exons of the *OPA1* gene (exons 8, 9, 12 and 27) were screened. Then, 28 coding exons were screened using 25 pairs primers located in flanking intron and Untranslated Regions (UTR) sequences were amplified by standard 40 μl PCR (in 10× E× Taq buffer, 5 pM of each primer, 2 μM Mg^{2+} and 200 μM each dNTP including 25–50 ng DNA and 1.25 U Takara E× Taq DNA polymerase) [17]. The cycling parameters were 5 min at 95 °C, 35 cycles of 30 s at 95 °C, 40 s at 58 °C, 50 s at 72 °C, and a final 10 min extension at 72 °C. The PCR products were purified by ultrafiltration (Centricon-100 cartridges, Amicon) and the samples were sequenced using Big Dye Terminator chemistry. Then, the sequences were edited and aligned using the Lasergene Software package (DNAStar). Protein sequence alignments were performed using the seqweb program GAP (GCG). Subsequently, we analyzed the conservation of *OPA1* with ClustalX. PCR/RFLP analysis of the c.C1198G mutation indicated a loss of the *Sex*A I site in this pedigree. Then, we separated the samples on a 2.5% agarose gel stained with ethidium bromide using electrophoresis.

2.4. Mutational analysis of the mitochondrial genome

Genomic DNA was isolated from whole blood of the participants (anti-coagulated with EDTA-K2) with phenol/chloroform extraction method. The entire mitochondrial genome of the proband III-11 was amplified in 24 overlapping fragments using sets of the light (L) strand and the heavy (H) strand oligo nucleotide primers, as described previously [18]. Each fragment was purified by ultrafiltration (Centricon-100 cartridges, Amicon) and subsequently analyzed by direct sequencing (model 3730 automated DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit; Applied Biosystems, Inc. [ABI], Foster City, CA). These sequence results were compared with the updated consensus Cambridge sequence (GenBank Accession No. NC_012920). DNA and protein sequence alignments were performed using the seqweb program GAP (GCG). At last, we analyzed the conservation of the mitochondrial genome from *Drosophila* to human using ClustalX.

3. Results

3.1. Clinical presentation

The proband (III-11) came to the ophthalmology clinic of Wenzhou Medical College at the age of 24. She had been experiencing painless, progressive, synchronously visual impairment in both eyes over the past 10 years. The best corrected visual acuity was 0.1 in each eye. Visual field test indicated diffuse sensitivity descending in both eyes. Color vision examination showed that there was a little color weakness. Fundus examination found no other abnormality except for diffuse optic disc pallor (Fig. 1). No other abnormalities were found in radiological and neurological examinations. Therefore, she exhibited the typical clinical features of ADOA.

The family originated from Zhejiang province in eastern China, and most of the family members lived in the same area. All participants underwent detailed clinical assessment. The family members had no history of ophthalmic disease, trauma, surgery, or any other known cause to account for visual impairment. Comprehensive family medical history revealed no systematic abnormalities. The familial history was consistent with the characteristics of autosomal dominant inheritance (Fig. 2). Of the ten relatives carrying this mutation, four males (II-9, II-11, II-15, II-17) and four females (I-2, II-6, III-10, III-11) were affected, and exhibited early-onset, bilateral, symmetric and progressive visual impairment as the sole clinical symptom. The other two relatives (one male and one female) had normal vision (Table 1).

3.2. Mutation analysis of OPA1 and mitochondrial genome

It was essential to analyze the *OPA1* gene because it was apparent that the visual dysfunction in this family was transmitted through autosomal dominant transmission. First, we examined exon 12 using PCR amplification and direct sequencing, and found that the patients were heterozygous for the c.C1198G mutation (Fig. 2A). In contrast, this mutation was not found in the controls.

To further examine the genotype of the family, the fragment spanning exon 12 was PCR amplified and subsequent restriction enzyme digestion analysis of the PCR fragments was performed for five of the relatives carrying the mutation (proband III-11, affected males II-9, II-11, II-15 and asymptomatic carrier III-15) and two controls (II-5, III-12) (Fig. 2B). The c.C1198G mutation

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