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Leber's hereditary optic neuropathy is associated with mitochondrial ND6 T14502C mutation

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ABSTRACT

We report here the clinical, genetic, and molecular characterization of three Chinese families with Leber's hereditary optic neuropathy (LHON). There were variable severity and age of onset in visual impairment among these families. Strikingly, there were extremely low penetrances of visual impairment in these Chinese families. Sequence analysis of complete mitochondrial genomes in these pedigrees showed the homoplasmic T14502C (I58V) mutation, which localized at a highly conserved isoleucine at position 58 of ND6, and distinct sets of mtDNA polymorphisms belonging to haplogroups M10a, F1a1, and H2. The occurrence of T14502C mutation in these several genetically unrelated subjects affected by visual impairment strongly indicates that this mutation is involved in the pathogenesis of visual impairment. Here, mtDNA variants I187T in the ND1, A122V in CO1, S99A in the A6, and V254I in CO3 exhibited an evolutionary conservation, indicating a potential modifying role in the development of visual impairment associated with T14502C mutation in those families. Furthermore, nuclear modifier gene(s) or environmental factor(s) may play a role in the phenotypic manifestation of the LHON-associated T14502C mutation in these Chinese families.

Introduction

Leber's hereditary optic neuropathy (LHON) is a maternally inherited eye disease that generally affects young adults with the rapid, painless, bilateral loss of central vision [1–3]. Mutations in mitochondrial DNA (mtDNA) are the molecular bases for this disorder [4–7]. Up to date, more than 30 mtDNA mutations have been associated LHON worldwide [7]. Of these, the *ND1* G3460A, *ND4* G11778A, and *ND6* T14484C mutations, in the genes encoding the subunits of respiratory chain complex I, are the most commonly LHON-associated mtDNA mutations, accounting for more than 50% of LHON pedigrees in different ethnic origins worldwide [2,7–10]. Typical features in LHON pedigrees are incomplete penetrance and male bias among the affected subjects, reflecting the complex etiology of this disease [11,12]. These primary LHON-associated mtDNA mutations such as *ND4* G11778A mutation by themselves are insufficient to produce a clinical phenotype. Therefore, other modifier factors including the nuclear modifier genes, mitochondrial haplotypes, and environmental factors modify the risk of optic neuropathy [12,13].

With the aim of investigating the molecular basis of LHON in the Chinese population, a systematic and extended mutational screening of mtDNA has been initiated in the large clinical population of Ophthalmology Clinic at the Wenzhou Medical College, China [11–20]. In the previous investigations, we showed that the LHON was associated with the *ND4* G11778A mutation in 15 Chinese families with variable penetrance and severity and age-at-onset of visual impairment [11–16]. Furthermore, *ND6* T14484C mutation and *ND1* G3460A mutation were identified in three Han Chinese families and one Chinese family, respectively [17,18]. In addition, we showed that LHON is associated with the *ND4* G11696A and *ND1* T3394C mutations in Chinese families with extremely low penetrances of visual loss [19,20]. In the present study, we performed the clinical, genetic, and molecular characterization of the other three Chinese families with suggestively

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maternally transmitted LHON. Molecular analysis has led to identification of the known T14502C mutation in *ND6* gene in these Chinese families. To elucidate the role of mitochondrial haplotype in the phenotypic manifestation of the T14502C mutation, we performed a PCR-amplification of fragments spanning the entire mitochondrial genome and subsequent DNA sequence analysis in the matrilineal relatives of those Chinese families.

Materials and methods

Patients. As a part of genetic screening program for visual impairment, three Chinese families (Fig. 1) were ascertained through the School of Ophthalmology and Optometry, Wenzhou Medical College, and Ophthalmology Clinic, Beijing Dongfang Hospital, respectively. Informed consent, blood samples, and clinical evaluations were obtained from all participating family members, under protocols approved by the Cincinnati Children's Hospital Medical Center Institute Review Board and the Wenzhou Medical College Ethics Committee. Members of those pedigrees were interviewed at length to identify both personal and family medical histories of visual impairments, and other clinical abnormalities. The 167 control DNA samples used for screening for the presence of mtDNA mutations were obtained from a panel of unaffected individuals from Chinese ancestry.

Ophthalmological examinations. The ophthalmologic examinations of probands and other members of these families were conducted, including visual acuity, visual field examination (Humphrey Visual Field Analyzer II*i*, SITA Standard), visual evoked potentials (VEP) (Roland Consult RETI port gamma, flash VEP), and fundus photography (Canon CR6-45NM fundus camera). The degree of visual impairment was 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.

Mutational analysis of the mitochondrial genome. Genomic DNA was isolated from whole blood of participants using the Puregene DNA Isolation Kits (Gentra Systems). The presence of the G3460A, G11778A, and T14484C mutations was examined as detailed elsewhere [2]. Briefly, affected individuals' DNA fragments spanning these mtDNA mutations were amplified by PCR using oligodeoxynucleotides corresponding to mtDNA at positions 3108–3717 for the G3460A mutation, 11654–11865 for the G11778A mutation, and 14260–14510 for the T14484C mutation [20], respectively. For the detection of the G3460A mutation, the amplified PCR segments were digested with a restriction enzyme BsaHI [2], while the presence of the T14484C mutation was examined by digesting PCR products with a restriction enzyme MvaI [2]. For the examination of the G11778A mutation, the amplified PCR segments were digested with the restriction enzyme Tsp45I [11–16].

The entire mitochondrial genome of three probands was PCR amplified in 24 overlapping fragments using sets of the light (L) strand and the heavy (H) strand oligonucleotide primers as described previously [21]. Each fragment was purified and subsequently analyzed by direct sequencing in an ABI 3100 automated DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit. These sequence results were compared with the updated consensus Cambridge sequence (GenBank Accession No.: NC_012920) [22]. DNA and protein sequence alignments were carried out using seqweb program GAP (GCG). The allele frequency of T14502C mutation in *ND6* gene was determined by PCR-amplification of fragments spanning the corresponding regions, using the genomic DNA derived from Chinese controls as templates and performing subsequent sequence analysis of PCR products, as described above.

Results

Clinical presentation

In family WZ401, the proband (III-1), as shown in Table 1, came to the Ophthalmology Clinic of Wenzhou Medical College at the age of 17 years. She began experiencing bilateral visual impairment at the age of 11 years. She saw a dark cloud in the center of her vision and had problems in appreciating colors, all of which seemed dark gray. Her visual acuity was 0.1 in both eyes. Visual field testing demonstrated large centrocecal scotomata in both eyes. Fundus examination showed that both of her optic discs were abnormal: vascular tortuosity of the central retinal vessels, a circumpapillary telangiectatic microangiopathy, and swelling of the retinal nerve fiber layer. The flash VEP showed that there was a decreased amplitude with delayed latencies in the right eye. Therefore, she exhibited typical clinical features of LHON. However, other seven matrilineal relatives in this family had normal vision.

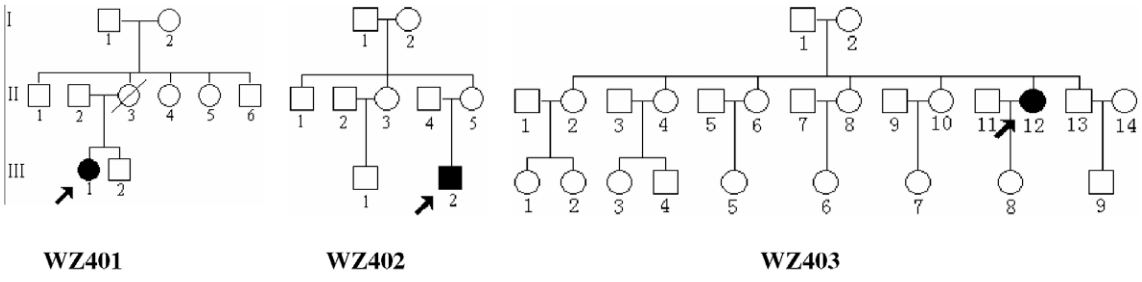

Fig. 1. Three Chinese pedigrees with Leber's hereditary optic neuropathy. Vision-impaired individuals are indicated by filled symbols.

Table 1
Summary of clinical and molecular data for affected matrilineal relatives of three Chinese families with LHON.

Subjects	Gender	Age of test (years)	Age of onset (years)	Visual acuity		Level of visual impairment	Number of matrilineal relatives	mtDNA haplogroup
				Right	Left			
WZ401-III-1	F	17	11	0.1	0.1	Mild	8	M10a
WZ402-III-2	M	4	2	0.1	0.1	Mild	6	F1a1
WZ403-II-12	F	40	30	0.3	0.2	Mild	16	H2