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Leber's hereditary optic neuropathy is associated with mitochondrial ND1 T3394C mutation

Min Liang [a,b,1], Minqiang Guan [b,1], Fuxing Zhao [a,1], Xiangtian Zhou [a,1], Meixia Yuan [a,b], Yi Tong [a,c], Li Yang [d], Qi-Ping Wei [e], Yan-Hong Sun [e], Fan Lu [a], Jia Qu [a,b,*], Min-Xin Guan [b,d,f,*]

[a] School of Ophthalmology and Optometry, Wenzhou Medical College, Wenzhou, Zhejiang 325003, China
[b] Zhejiang Provincial Key Laboratory of Medical Genetics, School of Life Sciences, Wenzhou Medical College, Wenzhou, Zhejiang 325003, China
[c] The First Affiliated Hospital, Fujian Medical University, Fuzhou, Fujian 350005, China
[d] Division of Human Genetics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229, USA
[e] Department of Ophthalmology, Dongfang Hospital, Beijing University of Chinese Medicine and Pharmacology, Beijing 100078, China
[f] Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH 45229, USA

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ABSTRACT

We report here the clinical, genetic and molecular characterization of four 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 T3394C (Y30H) mutation, which localized at a highly conserved tyrosine at position 30 of ND1, and distinct sets of mtDNA polymorphisms belonging to haplogroups D4b and M9a. The occurrence of T3394C mutation in these several genetically unrelated subjects affected by visual impairment strongly indicates that this mutation is involved in the pathogenesis of visual impairment. However, there was the absence of functionally significant mtDNA mutations in these four Chinese pedigrees carrying the T3394C mutation. Therefore, nuclear modifier gene(s) or environmental factor(s) may play a role in the phenotypic expression of the LHON-associated T3394C mutation.

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]. Since the landmark discovery of the first LHON-associated *ND4* G11778A mutation [4], more than 30 mtDNA mutations have been associated LHON among various ethnic background [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]. The primary LHON-associated mtDNA mutations such as *ND4* G11778A mutation by themselves are insufficient to produce a clinical phenotype. Therefore, other modifiers including the nuclear modifier genes, mitochondrial haplotypes and environmental factors modify the rick of visual loss [12,13].

It was anticipated that additional mutations causing LHON can be found in the mitochondrial genome in the Asian populations. To further elucidate 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 [11–19]. 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 families, respectively [18,19]. In addition, we showed that LHON is associated with the *ND4* G11696A mutation

* Corresponding authors. Addresses: School of Ophthalmology and Optometry, Wenzhou Medical College, Wenzhou, Zhejiang 325003, China (J. Qu), Division of Human Genetics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229, USA (M.-X. Guan). Fax: +1 (513) 636 3486 (M.-X. Guan).
E-mail addresses: jqu@wzmc.net (J. Qu), min-xin.guan@cchmc.org (M.-X. Guan).
[1] These authors have equally contributed to this work.

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in five Chinese families with extremely low penetrances of visual loss [17]. In the present study, we performed the clinical, genetic and molecular characterization of another four Chinese families with suggestively maternally transmitted LHON. Molecular analysis has led to identification of the T3394C mutation in *ND1* gene in these Chinese families. To elucidate the role of mitochondrial haplotype in the phenotypic manifestation of the T3394C mutation, we performed a PCR-amplification of fragments spanning 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, four 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 or family medical histories of visional 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 *BsaH*I [2], while the presence of the T14484C mutation was examined by digesting PCR products with a restriction enzyme *Mva*I [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 four 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 [20]. DNA and protein sequence alignments were carried out using seqweb program GAP (GCG). The allele frequency of T3394C mutation in *ND1* 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 WZ98, the proband (III-5) complained of painless, progressive deterioration of bilateral visual impairment and came to the Ophthalmology Clinic at Wenzhou Medical College at the age of 14. Ophthalmological evaluation showed that her visual acuity was 0.1 in the both eyes. Fundus examination showed that both her temporal optic disks were pale and reflex on fovea centralis was normal. Visual field testing demonstrated large centrocecal scotomata in both her eyes. Therefore, she exhibited a typical clinical feature of LHON. No other abnormality was found on radiological and neurological examination. Furthermore, she had no other significant medical history. The family is originated from Zhejiang Province in Eastern China. However, none of other 16 matrilineal relatives in this family exhibited visual impairment.

In WZ99 pedigree, the proband (III-1) came to Ophthalmology Clinic at Wenzhou Medical College at the age of 21. He suffered from painless, progressive deterioration of bilateral visual impair-

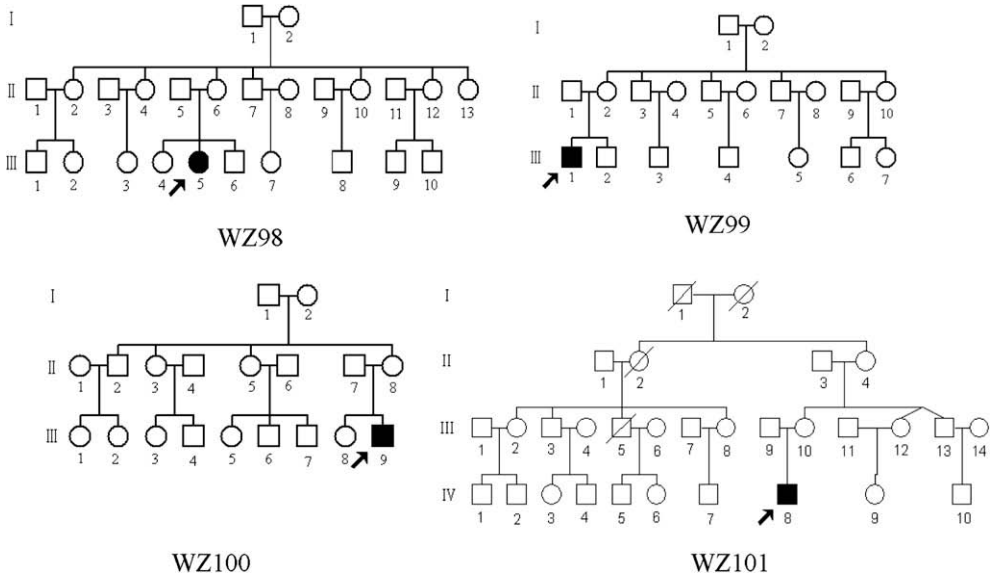

Fig. 1. Four Chinese pedigrees with Leber's hereditary optic neuropathy. Vision impaired individuals are indicated by filled symbols.