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Leber's hereditary optic neuropathy is associated with the mitochondrial ND6 T14484C mutation in three Chinese families

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Abstract

We report here the clinical, genetic, and molecular characterization of three Chinese families with maternally transmitted Leber's hereditary optic neuropathy (LHON). Clinical and genetic evaluations revealed the variable severity and age-of-onset in visual impairment in these families. In the affected matrilineal relatives, the loss of central vision is bilateral, the fellow eye becoming affected either simultaneously (45%) or sequentially (55%). The penetrances of vision loss in these pedigrees were 27%, 50%, and 60%, respectively. The age-at-onset of vision loss in these families was 14, 19, and 24 years, respectively. Furthermore, the ratios between affected male and female matrilineal relatives were 1:1, 1:1.2, and 1:2, respectively. Mutational analysis of mitochondrial DNA revealed the presence of homoplasmic ND6 T14484C mutation, which has been associated with LHON. The incomplete penetrance and phenotypic variability implicate the involvement of nuclear modifier gene(s), environmental factor(s) or mitochondrial haplotype(s) in the phenotypic expression of the LHON-associated T14484C mutation in these Chinese pedigrees.

Keywords: Leber's hereditary optic neuropathy; ND6 T14484C mutation; Penetrance; Vision loss; Mitochondrial DNA; Chinese

Leber's hereditary optic neuropathy (LHON) is a maternally inherited disorder leading to the rapid, painless, bilateral loss of central vision [1–4]. The maternal transmission of visual dysfunction in many families with LHON indicates that mutations in mitochondrial DNA (mtDNA) are the molecular basis for this disorder. The sequence analysis of the mitochondrial genome of families with LHON led to the landmark discovery of the ND4 G11778A mutation associated with LHON [5]. Up to date, approximately 35 LHON-associated mtDNA mutations have been identified in various ethnic populations [6–8]. 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 ~80% of LHON pedigrees in different ethnic backgrounds [2,9–11].

To further elucidate molecular basis of LHON in the Chinese population, a systematic and extended mutational screening of mitochondrial genome has been initiated in the large clinical population of Ophthalmology Clinics at the Wenzhou Medical College and Beijing Dongfang Hospital, China [12–16]. In the previous investigations, we showed that the LHON was associated with the ND4 G11778A mutation in six Chinese families and with the ND4 G11696A mutation in five Chinese pedigrees with variable penetrance and severity and age-at-onset of visual impairment [12–16]. In the present investigation, we performed

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the clinical, genetic, and molecular characterization of another three Chinese families with maternally transmitted LHON associated with the ND6 T14484C mutation.

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 or family medical histories of visional impairments, and other clinical abnormalities.

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 [17], respectively. For the detection of the G3460A mutation, the PCR amplified segments were digested with a restriction enzyme *Bsa*HI [2], while the PCR amplified segments were digested with restriction enzymes *Mae*III for the examination of the G11778A mutation [12–14]. Furthermore, the presence of the T14484C mutation was examined by digesting PCR products with a restriction enzyme *Mva*I [2].

Results and discussion

To further elucidate the molecular basis of visual impairment, we have performed a mutational analysis of the mitochondrial genome in a cohort of Chinese subjects, who were diagnosed as LHON by Ophthalmology Clinics at the Wenzhou Medical College and Beijing Dongfang Hospital, China. First, we examined three commonly known LHON-associated mtDNA mutations (G3460A, G11778A, and T14484C) by PCR amplification and subsequent restriction enzyme digestion analysis of PCR fragments derived from each proband of those families. Of these subjects, three subjects with LHON carried the homoplasmic T14484C mutation (data not shown). To confirm the presence of the T14484C mutation, these PCR-amplified segments were then purified and subsequently analyzed by DNA sequencing. Indeed, the sequence analysis, as shown in Fig. 2, confirmed the presence of T14484C mutation. A comprehensive history and physical examination as well as ophthalmologic examination were performed to identify any syndromic findings, and genetic factors related to the vision impairment in all available members of three Chinese families carrying the T14484C mutation. In fact, comprehensive family medical histories of those probands and other members of these Chinese families showed no other clinical abnormalities, including diabetes, muscular diseases, hearing loss, and neurological disorders. Subsequent restriction enzyme digestion and electrophoresis analysis indicated that the T14484C mutation was indeed present in nearly homoplasmy in other matrilineal relatives of these families (data not shown).

Of these families, the proband (IV-1) in WZ13 pedigree came to ophthalmology clinic at the age of 16 after suffer-

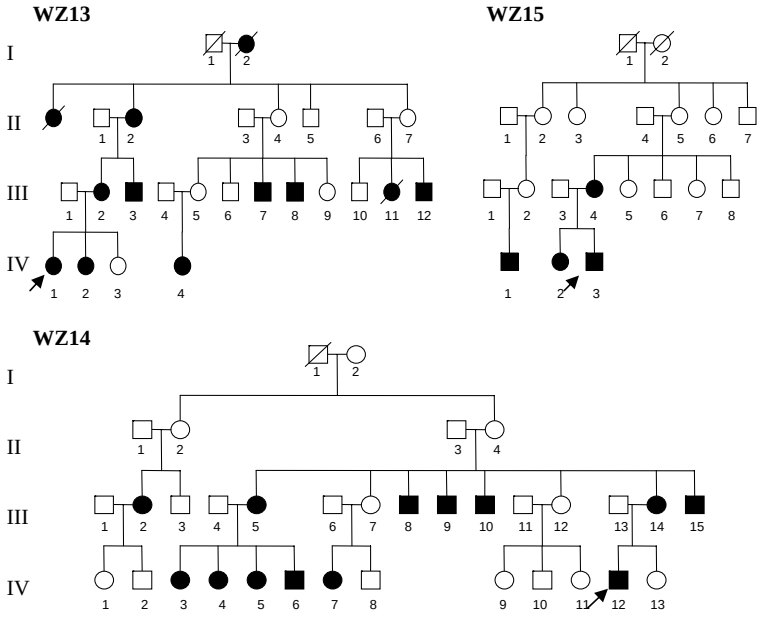

Fig. 1. Three Chinese pedigrees with Leber's hereditary optic neuropathy. Vision impaired individuals are indicated by filled symbols.