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

Yan-Hong Sun [a], Qi-Ping Wei [a], Xiangtian Zhou [b], Yaping Qian [c], Jian Zhou [a], Fan Lu [b], Jia Qu [b,*], Min-Xin Guan [c,d,e,*]

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

* Corresponding authors. Fax: +1 513 636 3486.
E-mail addresses: jqu@wzmc.net (J. Qu), min-xin.guan@cchmc.org (M.-X. Guan).

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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-

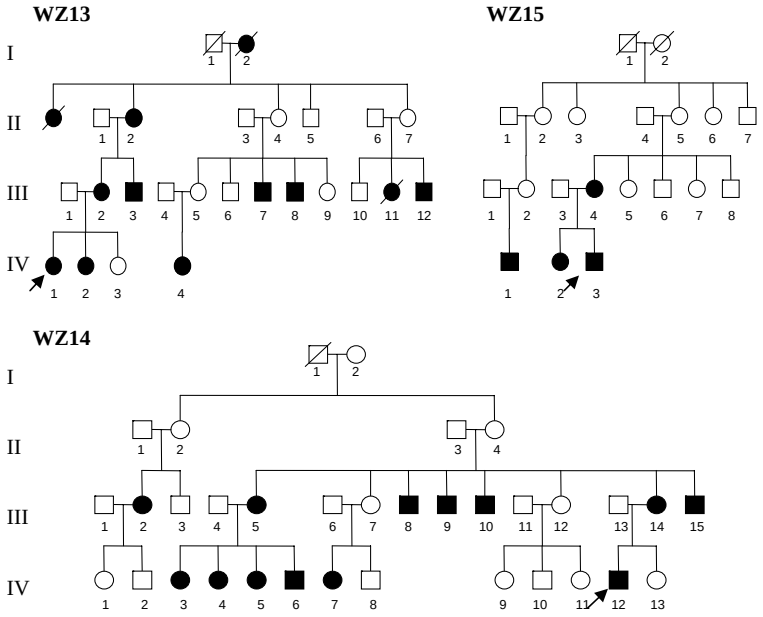

Fig. 1. Three Chinese pedigrees with Leber's hereditary optic neuropathy. Vision impaired individuals are indicated by filled symbols.

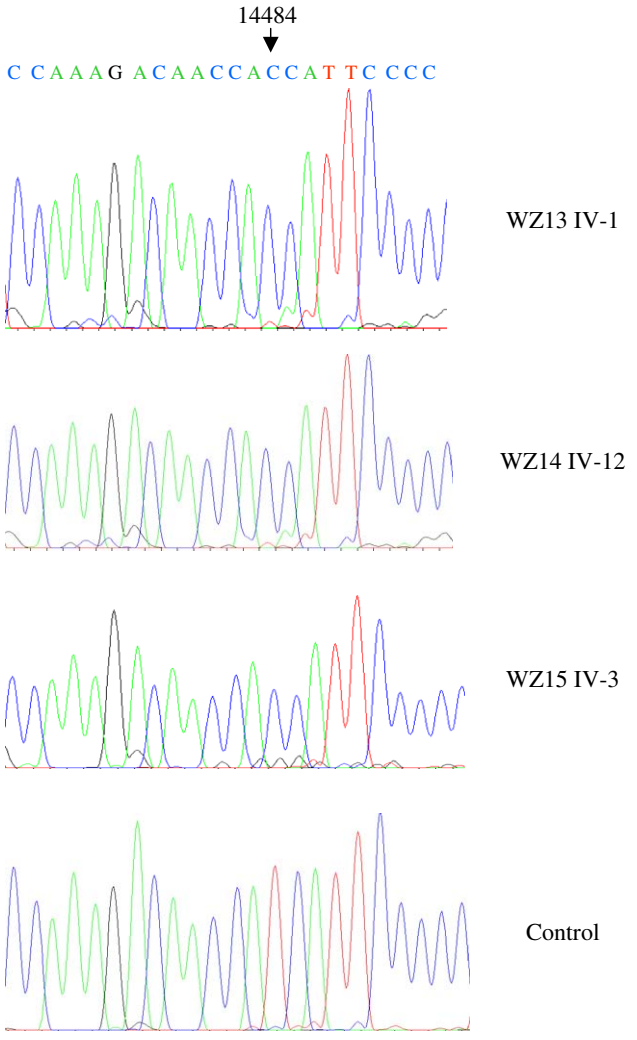

Fig. 2. Identification of the T14484C mutation in the ND6 gene. Partial sequences chromatograms of ND4 gene from three affected individuals and one Chinese control. An arrow indicates the location of the base changes at position 14484.

ing from painless, progressive deterioration of bilateral visual impairment a month ago. Her visual dysfunction occurred within three months, first in the right eye and then 15 days later in the left eye. She saw a dark cloud in the center of vision and had tinnitus. Her visual acuity was 0.01 in the right eye and 0.06 in the left eye. Furthermore, she had normal color vision and movement in both eyes. Fundus examination showed that both her optic disks were abnormal: vascular tortuosity of the central retinal vessels, a circumpapillary telangiectatic microangiopathy, and no reflex on fovea centralis. Intraocular pressure (IOP) appeared normal in both eyes. Visual field testing revealed large centrocecal scotomata in both her eyes. The flash VEP showed that there was the decreased amplitude with delayed latencies at left eye and no wave occurred in right eye. Members of this family are living in the Hebei Province in Northern China. Further familiar history and clinical evaluation revealed that 11 (4 males/7 females) of other 19 matrilineal relatives in this family exhibited a variable severity of visual impairment. Of matrilineal relatives, four suffered from moderate vision loss, two had severe vision loss, two exhibited profound vision loss, and other eight had normal vision. Furthermore, all other family members had normal vision. The age-at-onset of vision loss ranged from 13 years to 40 years, with an average of 24.4 years. Both eyes of six matrilineal relatives exhibited vision loss simultaneously, while vision dysfunction in three other matrilineal relatives occurred within three months, first in one eye and then two weeks to three months in other eye.

In family WZ14, the proband (IV-12) complained of painless, progressive deterioration of bilateral visual impairment and came to the Ophthalmology Clinic at Beijing Dongfang Hospital at the age of 11. His visual dysfunction occurred within three months, first in the left eye and then one week later in the right eye. Ophthalmological evaluation showed that his visual acuity was 0.08 in the right eye and 0.07 in the left eye. Fundus examination showed that both his temporal optic disks were pale and reflex on fovea centralis was normal. IOP in both eyes was at normal ranges. Visual field testing demonstrated large centrocecal scotomata in both his eyes. Therefore, he exhibited a typical clinical feature of LHON. No other abnormality was found on radiological and neurological examination. Furthermore, he had no other significant medical history. The family is originated from Henan Province in Northern China. Twelve matrilineal relatives (5 males/7 females) of other 25 matrilineal relatives (9 males/16 females) in this family exhibited visual impairment, while none of patrilineal relatives had vision deficit. The severity of vision loss in family members ranged from profound vision loss (11 matrilineal relatives), to moderate vision loss (one matrilineal relative) to normal vision (13 matrilineal relatives). The age-at-onset of vision loss in this family varied from 6 years old to 19 years old, with an average of 13.6 years old. Four matrilineal relatives suffered from vision loss in both eyes simultaneously, while the vision dysfunction in other 8 matrilineal relatives occurred within two month ranges, first in one eye and then one week to two months in other eye.

In WZ15 pedigree, the proband (IV-3) came to Ophthalmology clinic at the age of 12. His visual dysfunction occurred within a week, first in the left eye and then three days later in the right eye. He saw a dark cloud in the center of vision. His visual acuity was 0.1 in the right eye and 0.05 in the left eye. Fundus examination showed that both his optic disks were abnormal: vascular tortuosity of the central retinal vessels, a circumpapillary telangiectatic microangiopathy, and no reflex on fovea centralis. IOP was within normal ranges ob both eyes. Encephalon CT showed normal. Visual field testing revealed large centrocecal scotomata in both his eyes. The flash VEP showed bilaterally decreased amplitudes with delayed latencies. Thus, he showed a typical clinical feature of LHON. He had no other significant medical history. The family is originated from Beijing in Northern China. Further familiar history and clinical evaluation revealed that three (two males/one

Table 1
Summary of clinical data for three Chinese families carrying the T14484C mutation

Pedigree	Ratio (affected male/female)	Average age of onset (years)	Number of matrilineal relatives	Penetrance (%)[a]
WZ13	1:2	24	20	60
WZ14	1:1.2	14	26	50
WZ15	1:1	19	15	27

[a] Affected matrilineal relatives/total matrilineal relatives.

female) of other 14 matrilineal relatives in this family exhibited visual impairment, and none of patrilineal relatives had vision problem. Of these, their visual acuity in both eyes varied from moderate (IV-1), to severe (IV-2, III-2) to normal vision, while the age-at-onset ranged from 10 to 25 years, with an average of 18.5 years old. In addition, three matrilineal relatives suffered from vision loss in both eyes simultaneously.

Despite sharing the identical T14484C mutation, the severity and age-of-onset in visual impairment varied in these subjects of intra- or interfamilies, as shown in Table 1. In the affected matrilineal relatives in these pedigrees, similar to other LHON families [4], the loss of central vision is bilateral, the fellow eye becoming affected either simultaneously (45%) or sequentially (55%). However, the age-at-onset for vision loss in those pedigrees seems to be slightly younger than other Caucasian LHON families carrying the C14484T mutation. As shown in Table 1, the average age-at-onset in those Chinese families was 14, 19, and 24 years, respectively, whereas there were 19- and 27-year-olds from 17 and 23 Caucasian pedigrees carrying the T14484C mutation [18,19]. Unlike previous reports that the ratios between affected male and female matrilineal relatives were 2.1:1 and 8:1 from two large cohorts of Caucasian pedigrees carrying the T14484C mutation [18,19], the ratios in these Chinese families were 1:1, 1:1.2, and 1:2, respectively. The penetrance of vision loss in these Chinese pedigrees varied from 27%, 50%, and 60%, respectively. These variable pentrances of vision loss in these Chinese pedigrees are comparable with the 16–60% penetrance of vision loss in six Chinese pedigrees carrying the G11778A mutation [12–15]. In fact, the incomplete penetrance of visual loss and the mild biochemical defect [20] indicted that the T14484C mutation is itself not sufficient to produce the clinical phenotype. Thus, the modifier factors including nuclear backgrounds, other environmental factors, and mitochondrial haplotypes [21,22] contribute to the phenotypic variability and penetrance of vision loss between these Chinese pedigrees and other pedigrees carrying the T14484C mutation.

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