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Very low penetrance of Leber's hereditary optic neuropathy in five Han Chinese families carrying the *ND1* G3460A mutation

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ABSTRACT

We report here the clinical, genetic, and molecular characterization of five Han Chinese families with Leber's hereditary optic neuropathy (LHON). Strikingly, there were very low penetrances of visual impairment in these Chinese families, ranging from 4.2% to 22.2%, with an average of 10.2%. In particular, only 7 (4 males/3 females) of 106 matrilineal relatives in these families exhibited the variable severity and age-at-onset in visual dysfunction. The age-at-onset for visual impairment in matrilineal relatives in these families, varied from 20 to 25 years, with an average of 21.8 years old. Molecular analysis of mitochondrial genomes identified the homoplasmic *ND1* G3460A mutation and distinct sets of variants, belonging to the Asian haplogroups B5b, C4a1, D5, F1, and R9, respectively. This suggests that the G3640A mutation occurred sporadically and multiplied through evolution of the mtDNA in China. However, there was the absence of known secondary LHON-associated mtDNA mutations in these Chinese families. Very low penetrance of visual loss in these five Chinese pedigrees strongly indicated that the G3640A mutation was itself insufficient to develop the optic neuropathy. The absence of secondary LHON mtDNA mutations suggest that these mtDNA haplogroup-specific variants may not play an important role in the phenotypic expression of the G3640A mutation in those Chinese families with low penetrance of vision loss. However, nuclear modifier genes, epigenetic and environmental factors appear to be modifier factors for the phenotypic manifestation of the G3640A 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 [2,4–6]. Since the landmark discovery of the LHON-associated *ND4* G11778A mutation [4], more than 30 LHON-associated mtDNA mutations have been identified among various ethnic populations [7]. Of these, the *ND1* G3460A, *ND4* G11778A, and *ND6* T14484C mutations, which involve genes encoding the subunits of respiratory chain complex I, account for more than 95% of LHON pedigrees in some countries [3,7–10]. Those LHON-associated mtDNA mutations, unlike other pathogenic mtDNA mutations such as MELAS-associated $tRNA^{Leu(UUR)}$ A3243G mutation present in heteroplasmy (mixture of mutated and wild-type molecules) [11], often occur in the nearly homoplasmic or homoplasmic forms. Typical features in LHON pedigrees are incomplete penetrance and male bias among the affected subjects, reflecting the complex etiology of this disease [12–14]. Matrilineal relatives within and among families, despite carrying the same LHON-associated mtDNA mutation(s), exhibited a wide range of severity, age-of-onset, and penetrance of optic neuropathy. Therefore, other modifier factors including environmental factors, nuclear background, and mitochondrial haplotypes should modulate the phenotypic manifestation of visual impairment associated with those primary mtDNA mutations [3,15].

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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 [15–18]. 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 [15–20], the *ND6* T14484C mutation 14 Chinese families [21–23], and the *ND1* G3460A mutation in one Han Chinese family [24]. In addition, we showed that LHON is associated with the *ND4* G11696A and *ND6* T14502C mutations in Chinese families with extremely low penetrances of visual loss [25–27]. In this study, we performed the clinical, genetic, and molecular characterization of another five Chinese families with suggestively maternally transmitted LHON. These pedigrees exhibited very penetrance of optic neuropathy. In particular, only 7 (4 males/3 females) of 106 matrilineal relatives in these families exhibited the variable severity and age-at-onset in visual dysfunction. Molecular analysis has led to identification of the G3460A mutation in *ND1* gene in these Chinese families. With the aim of investigating the role of mitochondrial haplotypes in the phenotypic manifestation of the G3460A 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 and subjects

As a part of genetic screening program for visual impairment, five Han Chinese families (Fig. 1) were ascertained through the School of Ophthalmology and Optometry, Wenzhou Medical College, Zhejiang and Dongfang Hospital, Beijing. 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 visual 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 *IIi*, 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 [28], 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 [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 [29]. Each fragment was purified and subsequently analyzed by direct sequencing in an ABI 3700 automated DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit. These sequence results were compared with the updated con-

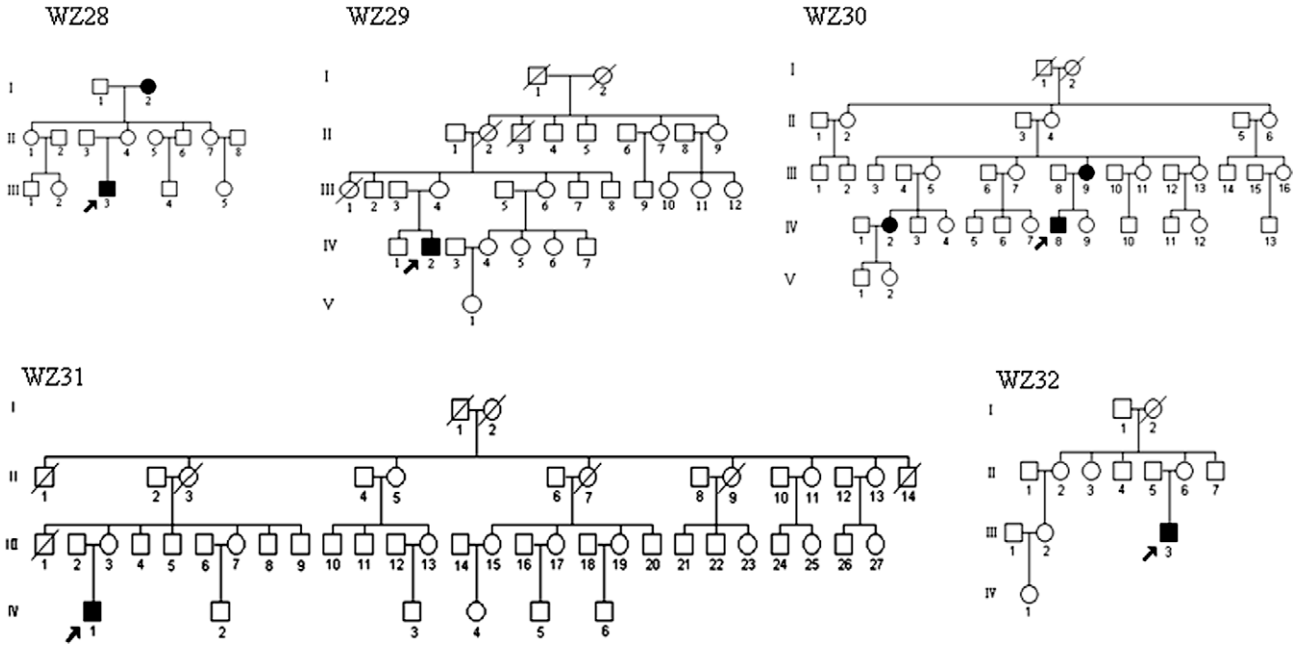

Fig. 1. Five Chinese pedigrees with Leber's hereditary optic neuropathy. Vision impaired individuals are indicated by filled symbols. Arrows denote probands.