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Mitochondrial haplogroup M9a specific variant *ND1* T3394C may have a modifying role in the phenotypic expression of the LHON-associated *ND4* G11778A mutation

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ABSTRACT

We report here the clinical, genetic and molecular characterization of four Han Chinese families with Leber's hereditary optic neuropathy (LHON). The penetrances of optic neuropathy in these Chinese pedigrees were 38%, 38%, 44% and 56%. This observation is in contrast with the previously identified 14 Chinese families with very low penetrance of LHON. The age-at-onset for visual impairment in matrilineal relatives in these Chinese families varied from 18 to 30 years. Furthermore, the ratios between affected male and female matrilineal relatives in these families were 3:0, 3:0, 3:1 and 2:3, respectively. Molecular analysis of mitochondrial genomes identified the known *ND4* G11778A mutation and distinct sets of variants belonging to the Asian haplogroups M9a. Of these, the *ND1* T3394C mutation caused the substitution of a highly conserved histidine for tyrosine (Y30H) at amino acid position 30. This mutation was associated with LHON in other families with low penetrance of optic neuropathy and other clinical abnormalities. The presence of both G11778A and T3394C mutations appears to contribute to higher penetrance of optic neuropathy in these four Chinese families than other Chinese families carrying only the G11778A mutation. Therefore, the mitochondrial haplogroup M9a specific variant T3394C may modulate the phenotypic manifestation of LHON-associated G11778A mutation in these Chinese pedigrees.

1. 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 [3,7–11]. Those LHON-associated mtDNA mutations often occur in the nearly homoplasmy or homoplasmy. Typical features in those LHON pedigrees are incomplete penetrance and male bias among the affected subjects [12–14]. Matrilineal relatives within and among families, despite carrying the identical LHON-associated mtDNA mutation(s), exhibited a wide range of severity, age-of-onset and penetrance of optic neuropathy. Therefore, other modifier factors including nuclear modifier genes, mitochondrial haplotypes, epigenetic factors and environmental factors should modulate the phenotypic manifestation of optic neuropathy associated with those primary mtDNA mutations [3,10,15,16].

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 the Ophthalmology Clinic at the Wenzhou Medical College, China [17–24]. In the previous investigation, we showed that the *ND4* G11696A, $tRNA^{Met}$ A4435G and $tRNA^{Thr}$ A15951G as well as G6480A/T12811C/

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A15935G mutations contribute to the high penetrance and expressivity of visual loss in four Chinese families [17–20]. On other hand, other nine Chinese families carrying the only G11778A mutation exhibited extremely low penetrances of LHON [21,22]. In the present study, we performed the clinical, genetic and molecular characterization of another four Han Chinese families with maternally transmitted LHON. In contrast with nine Chinese pedigrees with low penetrance of LHON [21,22], 38%, 38%, 44% and 56% of matrilineal relatives in these four families displayed a wide range of severity and age-of-onset in visual impairment, respectively. Mutational analysis of their mitochondrial genomes identified the known *ND4* G11778A mutation in these Chinese families. To elucidate the role of mitochondrial haplotypes in the phenotypic manifestation of the G11778A mutation, we performed a PCR-amplification of the fragments spanning an entire mitochondrial genome and subsequent DNA sequence analysis in the matrilineal relatives of these families. Interestingly, the known *ND1* T3394C mutation [24] was implicated to influence the phenotypic manifestation of the G11778A mutation in these families.

2. Materials and methods

2.1. Patients and subjects

We ascertained four Han Chinese families (Fig. 1) through the School of Ophthalmology and Optometry, Wenzhou Medical College, Wenzhou. 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 these pedigrees were interviewed at length to identify both personal or family medical histories of visual impairments, and other clinical abnormalities.

2.2. 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.

2.3. Mutational analysis of the mitochondrial genome

Genomic DNA was isolated from whole blood of participants using the Puregene DNA Isolation Kits (Gentra Systems). For the examination of the *ND4* G11778A mutation, the first PCR segments (803 bp) were amplified using genomic DNA as template and oligodeoxynucleotides corresponding to mtDNA at positions 11,295–12,098 [25] to rule out the co-amplification of possible nuclear pseudogenes [26]. Then, the second PCR product (212 bp) was amplified using the first PCR fragment as template and oligodeoxynucleotides corresponding to mtDNA at positions 11,654–11,865, and subsequently digested with the restriction enzyme *Tsp45*I as the G11778A mutation creates the site for this restriction enzyme [22]. Equal amounts of various digested samples were then analyzed by electrophoresis through a 7% polyacrylamide gel. The proportions of digested and undigested PCR product were determined by the Image-Quant program after ethidium bromide staining to determine if the G11778A mutation is in the homoplasmy in these subjects. 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 [27]. 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 consensus Cambridge sequence (GenBank accession number: NC_012920) [25]. DNA and protein sequence alignments were carried out using seqweb program GAP (GCG).

2.4. Phylogenetic analysis

A total of 17 vertebrate mitochondrial DNA sequences were used in the interspecific analysis. These include: *Bos Taurus*, *Cebus albifrons*, *Gorilla gorilla*, *Homo sapiens*, *Hylobates lar*, *Lemur catta*, *Macaca mulatta*, *Macaca sylvanus*, *Mus musculus*, *Nycticebus coucang*, *Pan paniscus*, *Pan troglodytes*, *Pongo pygmaeus*, *Pongo abelii*, *Papio hamadryas*, *Tarsius bancanus*, and *Xenopus laevis* (Genbank). The conservation index (CI) was calculated by comparing the human nucleotide variants with the other 16 vertebrates. The CI was then

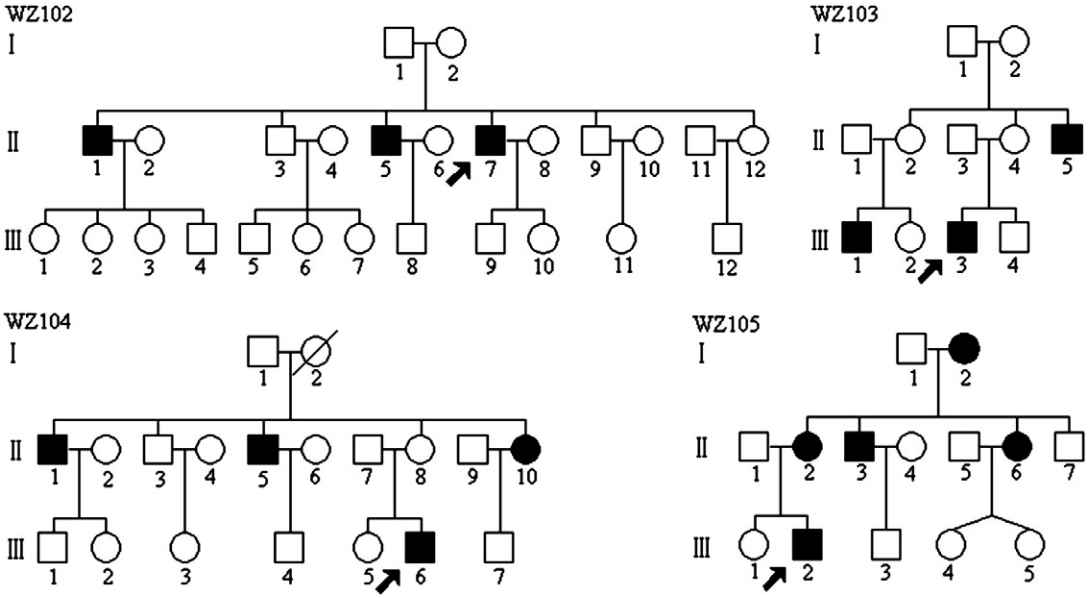

Fig. 1. Four Chinese pedigrees with Leber's hereditary optic neuropathy. Vision impaired individuals are indicated by filled symbols. Arrows denote the probands.