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# Mitochondrial haplotypes may modulate the phenotypic manifestation of the LHON-associated m.14484T>C (*MT-ND6*) mutation in Chinese families

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ABSTRACT

Mitochondrial m.14484T>C (*MT-ND6*) mutation has been associated with Leber's hereditary optic neuropathy. Previous investigations revealed that the m.14484T>C mutation is a primary factor underlying the development of optic neuropathy but is not sufficient to produce a clinical phenotype. However, mitochondrial haplogroups have been proposed to modulate the phenotypic manifestation of the m.14484T>C mutation. Here, we performed the clinical, genetic evaluation and complete mitochondrial genome sequence analysis of 41 Han Chinese pedigrees carrying the m.14484T>C mutation. These families exhibited a wide range of penetrances and expressivities of optic neuropathy. The average ratio between affected male/female matrilineal relatives from 41 families was 2:1. The penetrance of optic neuropathy in these Chinese pedigrees ranged from 5.6% to 100%, with the average of 23.8%. Furthermore, the age-of-onset for optic neuropathy varied from 4 to 44 years, with the average of 19.3 years. Sequence analysis of their mitochondrial genomes identified distinct sets of polymorphisms belonging to ten Eastern Asian haplogroups, indicating that the m.14484T>C mutation occurred through recurrent origins and founder events. We showed that mitochondrial haplogroups M9, M10 and N9 increased the penetrance of optic neuropathy in these Chinese families. In particular, these mitochondrial haplogroup specific variants: m.3394T>C (*MT-ND1*), m.14502T>C (*MT-ND4*) and m.14693A>G (*MT-TE*) enhanced the penetrance of visual loss in these Chinese families. These data provided the direct evidence that mitochondrial modifiers modulate the variable penetrance and expressivity of optic neuropathy among Chinese pedigrees carrying the m.14484T>C mutation.



## 1. Introduction

Leber's hereditary optic neuropathy (LHON) is a maternally inherited eye disease that generally affects children to young adults with the rapid, painless, bilateral loss of central vision (Newman, 1993; Nikoskelainen, 1994; Yu-Wai-Man et al., 2009). Mutations in mitochondrial DNA (mtDNA) are one of the molecular bases for this disorder (Howell, 2003; Wallace, 2005; Wallace et al., 1988). Of these, three primary mutations m.3460G>A (*MT-ND1*), m.11778G>A (*MT-ND4*) and m.14484T>C (*MT-ND6*) mutations, which alter genes encoding the subunits of respiratory chain complex I (NADH dehydrogenase), account for approximately 90% of LHON pedigrees in some countries (Brown et al., 1995; Carelli et al., 2009; Mackey et al., 1996; Mashima et al., 1998). The LHON-associated mtDNA mutation(s) often occurred in near homoplasmy or homoplasmy. These data hinted to the mild nature of mutations, evidenced by the fact that relatively mild mitochondrial dysfunction was observed in mutant cells carrying one of these mutations (Brown et al., 2000; Hofhaus et al., 1996; Qian et al., 2011). Typical features in LHON pedigrees carrying the mtDNA mutation(s) are incomplete penetrance and male bias among the affected subjects, reflecting the complex etiology of this disease (Newman et al., 1991; Riordan-Eva et al., 1995; Yu-Wai-Man et al., 2009). In particular, matrilineal relatives within and among families carrying the

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same LHON-associated mtDNA mutation(s) exhibited the wide range of penetrance and expressivity including severity, age-of-onset and progression in visual impairment. These suggest that these LHON-associated mtDNA mutations are the primary causative evidence, but the secondary events such as environmental factors, nuclear and mitochondrial genetic modifiers are necessary for the manifestation of the optic neuropathy (Wallace, 2005; Yen et al., 2006; Yu-Wai-Man et al., 2009). For example, a predominance of male patients presenting with vision loss suggests an X-linked modifier gene for the phenotypic manifestation of LHON-associated mtDNA mutations (Hudson et al., 2005). Furthermore, a group of so-called "secondary" LHON-associated mtDNA mutations including m.4216T>C, m.4917A>G and m.13708G>A was implicated to act in synergy with these primary mtDNA mutations including the m.11778G>A and m.14484T>C mutations (Torroni et al., 1997). In addition, mitochondrial haplogroups influenced the phenotypic manifestation of the primary LHON mtDNA mutations (Brown et al., 2002; Hudson et al., 2007; Kaewsutthi et al., 2011; Pello et al., 2008).

However, the role of these genetic modifiers, especially mitochondrial haplogroups/variants, in the phenotypic expression of these primary LHON-associated mtDNA mutations remains poorly defined. In the previous investigation, we showed that the m.3394T>C (*MT-ND1*), m.11696G>A (*MT-ND4*), m.14502T>C (*MT-ND6*), m.4435A>G (*MT-TM*) and m.15951A>G (*MT-TT*) mutations contributed to the high penetrance and expressivity of optic neuropathy in Chinese families carrying the m.11778G>A (*MT-ND4*) mutation (Li et al., 2006; Qu et al., 2006, 2007; J. Zhang et al., 2010; M. Zhang et al., 2010). In the present investigation, we carried out a systematic and extended mutational screening of m.14484T>C (*MT-ND6*) mutation in a large cohort of 844 Han Chinese subjects with LHON. This analysis identified 41 individuals harboring the m.14484T>C mutation. We then performed the clinical, genetic and molecular characterization of these vision-impaired subjects carrying the m.14484T>C mutation. A wide range of penetrance, severity and age-at-onset of visual loss was observed in the matrilineal relatives within and among these Chinese families. To assess the contribution that mtDNA variants or haplogroups make toward the variable penetrance and expressivity of visual loss in these pedigrees, we performed PCR-amplification of fragments spanning the entire mtDNA and subsequent DNA sequence analysis in the matrilineal relatives of those families. These analyses showed that there were distinct sets of mtDNA variants belonging to ten Eastern Asian haplogroups in these Chinese pedigrees carrying the m.14484T>C mutation. Furthermore, we evaluated the potential role of these mtDNA haplogroups and variants in the phenotypic manifestation of the m.14484T>C mutation in these Chinese families.

## 2. Patients and methods

### 2.1. Patients and subjects

We ascertained 27 Han Chinese families (Fig. 1) through the Eye clinics of Wenzhou Medical College, Zhejiang, Dongfang Hospital, Beijing and Xingtai Eye Hospital, Hebei. Informed consent, blood samples, and clinical evaluations were obtained from all participating family members, under the protocols approved by the Zhejiang University Institute Review Board and the Wenzhou Medical College Ethic Committee. Members of these pedigrees were interviewed at length to identify both personal or family medical histories of visual impairments, and other clinical abnormalities.

#### 2.1.1. Ophthalmological examinations

The ophthalmic examinations of the proband and other members of these families were conducted, including visual acuity, visual field examination (Humphrey Visual Field Analyzer II*i*, SITA Standard; Carl Zeiss Meditec, Oberkochen, Germany), visual evoked potentials (VEP; RETI port gamma, flash VEP; Roland Consult, Brandenberg, Germany), and fundus photography (CR6-45NM fundus camera; Canon, Lake Success, NY). 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.

#### 2.1.2. Mutational analysis of the mitochondrial genome

Genomic DNA was isolated from the whole blood of participants using the Puregene DNA Isolation Kits (Puregene DNA Isolation Kit; Gentra Systems, Minneapolis, MN). The presence of the m.14484T>C mutations was examined as detailed elsewhere (Brown et al., 1995). Briefly, the first PCR segments (938 bp) were amplified using genomic DNA from affected subjects as template and oligodeoxynucleotides corresponding to mtDNA at positions 14000–14998 to rule out the co-amplification of possible nuclear pseudogenes (Woischnik and Moraes, 2002). Then, the second PCR product (251 bp) was amplified using the first PCR fragment as template and oligodeoxynucleotides corresponding to mtDNA at positions 14260–14510, and subsequently digested with a restriction enzyme *Mva*I as the m.14484T>C mutation creates the site for this restriction enzyme (Brown et al., 1995). Equal amounts of various digested samples were then analyzed by electrophoresis through 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 m.14484T>C mutation is in the homoplasmy in these subjects.

The entire mitochondrial genomes of 27 probands and 102 control subjects were PCR amplified in 24 overlapping fragments using sets of the light (L) strand and the heavy (H) strand oligonucleotide primers as described previously (Rieder et al., 1998). Each fragment was purified and subsequently analyzed by direct sequencing in an ABI 3700 automated DNA sequencer using the BigDye Terminator Cycle sequencing reaction kit. These sequence results were compared with the updated consensus Cambridge sequence (GenBank accession number: NC_012920) (Andrews et al., 1999). DNA and protein sequence alignments were carried out using seqweb program GAP (GCG).

### 2.2. Phylogenetic analysis

A total of 17 vertebrates 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) (Supplemental Table 1). The conservation index (CI) was calculated by comparing the human mtDNA variants with other 16 vertebrates.

### 2.3. Haplogroup analyses

The entire mtDNA sequences of the 38 Chinese probands carrying the m.14484T>C mutation were assigned to the Asian mitochondrial haplogroups by using the nomenclature of mitochondrial haplogroups (Kong et al., 2006; Tanaka et al., 2004). The phylogenetic tree of 38 Chinese probands carrying the m.14484T>C mutation was generated by Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0., as detailed elsewhere (Tamura et al., 2007).

### 2.4. Statistical analysis

Statistical analysis was carried out using the Student's unpaired, two-tailed *t*-test contained in the Microsoft-Excel program. Unless indicated otherwise, a $p$ value <0.05 was considered statistically significant.