Published in final edited form as:

Ophthalmology. 2009 March ; 116(3): 558–564.e3. doi:10.1016/j.ophtha.2008.10.022.

Extremely Low Penetrance of Leber's Hereditary Optic Neuropathy in 8 Han Chinese Families Carrying the *ND4* G11778A Mutation

Jia Qu, MD^{1,2}, Xiangtian Zhou, MD¹, Juanjuan Zhang, MD^{1,2}, Fuxin Zhao, MS^{1,2}, Yan-Hong Sun, MD³, Yi Tong, MD^{1,4}, Qi-Ping Wei, MD³, Wansi Cai, MS^{1,2}, Li Yang, BA⁵, Constance E. West, MD^{6,7}, and Min-Xin Guan, PhD^{2,5,7}

¹ School of Ophthalmology and Optometry, Wenzhou Medical College, Wenzhou, Zhejiang, China

² Zhejiang Provincial Key Laboratory of Medical Genetics, School of Life Sciences, Wenzhou Medical College, Wenzhou, Zhejiang, China

³ Department of Ophthalmology, Beijing University of Chinese Medicine and Pharmacology, Beijing, China

⁴ The First Affiliated Hospital, Fujian Medical University, Fuzhou, Fujian, China

⁵ Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

⁶ Division of Ophthalmology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

⁷ Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio

Abstract

Purpose—To investigate the role of mitochondrial haplotypes in the development of Leber's hereditary optic neuropathy (LHON) associated with the *ND4* G11778A mutation in Chinese families.

Design—Eight Han Chinese families with maternally transmitted LHON were studied using clinical, genetic, and molecular evaluations.

Participants—One hundred sixty-seven subjects from 8 Chinese families with a wide age range and severity of visual impairment.

Methods—All subjects underwent the clinical and genetic evaluation, as well as molecular analysis of mitochondrial DNA (mtDNA).

Main Outcome Measures—The ophthalmologic examinations included visual acuity, visual field examination, visual evoked potentials, and fundus photography. Mitochondrial DNA analysis included the polymerase chain reaction amplification of the entire mtDNA and subsequent sequence determination.

Results—Eight families exhibited extremely low penetrance of visual impairment, with the average of 13%. In particular, 14 (12 males and 2 females) of 119 matrilineal relatives in these families exhibited the variable severity and age at onset in visual dysfunction. The average age of onset of vision loss was 17 years. Molecular analysis of mtDNA identified the homoplasmic *ND4*

Correspondence: Min-Xin Guan, PhD, Division of Human Genetics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, Ohio 45229. min-xin.guan@cchmc.org.

Financial Disclosure(s): The author(s) have no proprietary or commercial interest in any materials discussed in this article.

G11778A mutation and distinct sets of variants belonging to the Asian haplogroups M8a2, D4g2, B4a1c, B5b, N9a1, D4b2b, C, and M7b1. However, there was an absence of secondary LHON-associated mtDNA mutations in these 8 Chinese families.

Conclusions—The extremely low penetrance of vision loss in these 8 Chinese pedigrees strongly indicates that the G11778A mutation was itself insufficient to produce a clinical phenotype. 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 G11778A mutation in those Chinese families with very low penetrance of vision loss. However, nuclear backgrounds and environmental factors seem to be modifying factors for the phenotypic manifestation of the G11778A mutation in these Chinese families.

Leber's hereditary optic neuropathy (LHON) is a maternally inherited disorder leading to the rapid, painless, and bilateral loss of central vision.^{1–4} Mutations in mitochondrial DNA (mtDNA) are the molecular bases for this disorder.^{5–7} 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 50% of LHON pedigrees worldwide.^{8–10} Those LHON-associated mtDNA mutations, unlike other pathogenic mtDNA mutations such as the Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS)-associated tRNA^{Leu(UUR)} A3243G mutation present in heteroplasmy (mixture of mutated and wild-type molecules),¹¹ often occur nearly homoplasmy or homoplasmy. Such mtDNA mutations as the G11778A mutation often exhibit incomplete penetrance because some individuals carrying the mutations have normal vision.^{4,8,10,12} In addition, matrilineal relatives of intrafamilies or interfamilies, despite carrying the same LHON-associated mtDNA mutations, exhibited a wide range of severity, age at onset, and penetrance in visual impairment. Thus, other modifying factors, including environmental factors and nuclear and mitochondrial genetic modifiers, should modulate the phenotypic manifestation of visual impairment associated with those primary mtDNA mutations.^{3,4,13} In particular, a group of secondary LHON-associated mtDNA mutations such as T4216C, A4917G, G13708A, and haplogroup J were implicated in influencing the phenotypic manifestation of the primary mtDNA mutations, including the G11778A and T14484C mutations.^{14–17}

However, these modifier factors remain poorly defined. With the aim of investigating the role of mitochondrial modifiers in the phenotypic expression of LHON, a systematic and extended mutational screening of mtDNA was been initiated in the large cohort at the Ophthalmology Clinic at the Wenzhou Medical College, Wenzhou, China.^{12,18–21} In a previous investigation, the authors showed that the *ND4* G11696A, tRNA^{Met} A4435G, and tRNA^{Thr} A15951G mutations contribute to the high penetrance and expressivity of vision loss in Chinese families.^{19–21} The present study undertook the clinical, genetic, and molecular characterization of another 8 Chinese families with maternally transmitted LHON. Fourteen (12 males and 2 females) of 119 matrilineal relatives in these families exhibited the variable severity and age at onset in visual dysfunction. Mutational analysis identified the *ND4* G1177A mutation in these Chinese families. To elucidate the role of mitochondrial haplotype in the phenotypic manifestation of the G11778A mutation, polymerase chain reaction (PCR) amplification of the fragments spanning the entire mitochondrial genome and subsequent DNA sequence analysis in the matrilineal relatives of these families was performed.

Patients and Methods

Patients

Eight Han Chinese families (Fig 1) were identified through the School of Ophthalmology and Optometry, Wenzhou Medical College, Wenzhou, 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 Institutional 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.

Ophthalmologic Examinations

The ophthalmologic examinations of proband and other members of this family were conducted, including visual acuity, visual field examination (Humphrey Visual Field Analyzer IIi, Swedish Interactive Thresholding Algorithm [SITA] Standard; Humphrey Instruments, San Leandro, CA), visual evoked potentials (VEP) (Roland Consult RETI port gamma, flash VEP; Wiesbaden, Germany), and fundus photography (Canon CR6-45NM fundus camera; Tokyo, Japan). The degree of visual impairment was defined according to the visual acuity as follows: normal, >0.3 ; mild, 0.3 to 0.1 ; moderate, <0.1 to 0.05 ; severe, <0.05 to 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). 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 11295 through 12098²² to rule out the coamplification of possible nuclear pseudogenes.²³ Then, the second PCR product (212 bp) was amplified using the first PCR fragment as template and oligodeoxynucleotides corresponding to mtDNA at positions 11654 through 11865 and subsequently was digested with restriction enzymes *Tsp45I*, because the G11778A mutation creates the site for this restriction enzyme.¹² Equal amounts of various digested samples then were 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 G11778A mutation is in the homoplasmy in these subjects. The entire mitochondrial genome of 8 probands was PCR amplified in 24 overlapping fragments using sets of the light strand and the heavy strand oligonucleotide primers as described previously.²⁴ Each fragment was purified and subsequently was 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_001807).²² DNA and protein sequence alignments were carried out using seqweb program GAP (GCG).

Results

Screening of *ND4* G11778A Mutation in Chinese Subjects with Leber's Hereditary Optic Neuropathy

To elucidate further the molecular basis of vision loss, a mutational screening of the mitochondrial *ND4* gene was performed in a cohort of Han Chinese subjects who were diagnosed with LHON by the Eye Clinic at the Wenzhou Medical College. First, DNA fragments spanning the *ND4* G11778A mutation were PCR amplified from each affected subject. Each fragment was digested by a restriction enzyme *Tsp45I* and subsequent electrophoresis analysis. Of those, 8 subjects harbored the homoplasmic G11778A mutation (data not shown). The presence of the homoplasmic G11778A mutation in those subjects was confirmed by PCR amplification of fragments spanning the G11778A mutation and subsequent DNA sequence analysis (data not shown).

Clinical and Genetic Evaluation of 8 Chinese Pedigrees

A comprehensive history and physical examination as well as ophthalmologic examination were performed to identify both personal and family medical histories of visual impairments and other clinical abnormalities in all available members of 8 Han Chinese pedigrees carrying the G11778A mutation. In fact, comprehensive family medical histories of those probands and other available members of these Chinese families showed no other clinical abnormalities, including diabetes, muscular diseases, hearing dysfunction, and neurologic disorders.

In family WZ41, the proband (III-2), as shown in Table 1, came to the Ophthalmology Clinic of Wenzhou Medical College at the age of 18 years after experiencing painless, progressive deterioration of bilateral visual impairment for 2 weeks. He saw a dark cloud in the center of his vision and had problems appreciating colors, which all seemed a dark gray. His visual acuity was 0.06 and 0.08 in his right and left eyes, respectively. Visual field testing demonstrated large centrocaecal scotomata in both of his eyes. Fundus examination showed that both his optic discs were abnormal: vascular tortuosity of the central retinal vessels, a circumpapillary telangiectatic microangiopathy, and swelling of the retinal nerve fiber layer. Therefore, he exhibited typical clinical features of LHON. However, none of other 6 matrilineal relatives in this family had visual loss.

In family WZ42, the proband (III-11) was diagnosed with LHON by the Ophthalmology Clinic at the Dongfang Hospital at the age of 18 years. He began experiencing bilateral visual impairment at the age of 14 years. His visual acuity was 0.04 and 0.06 in the right and left eyes, respectively. Intraocular pressure (IOP) was 18.3 and 14.7 mmHg in the right and left eyes, respectively. Of other 24 matrilineal relatives in this pedigree, subject III-14 exhibited visual impairment at the age of 15 years. His visual acuity was 0.03 and 1.5 in his right and left eyes, respectively. Other members of this family exhibited normal vision.

In family WZ43, the proband (III-5) was a 16-year-old boy. He exhibited bilateral vision loss at the age of 15 years. He was diagnosed with LHON by the Ophthalmology Clinic at the Wenzhou Medical College. His visual acuity was 0.02 and 0.02 in the right and left eyes, respectively. His IOP was 16.3 mmHg in the right eye and 15.4 mmHg in the left eye. None of other 8 matrilineal relatives in this pedigree exhibited vision failure.

In family WZ44, the proband (III-1) reported painless, progressive deterioration of bilateral visual impairment at the age of 15 years and came to the Eye Clinic at the Dongfang Hospital at the age of 21 years. His visual acuity was 0.02 and 0.6 in the right and left eyes, respectively. The IOP was 16 mmHg in the right eye and 13 mmHg in the left eye. Ophthalmologic and other clinical evaluations showed that he had a typical clinical feature of LHON. However, the other 16 matrilineal relatives in this pedigree had normal vision.

In family WZ45, the proband (III-1) came to Ophthalmology Clinic at the Wenzhou Medical College at the age of 31 years. He experienced painless, progressive deterioration of bilateral vision loss at the age of 15 years. His visual acuity was <0.01 at both eyes. His sibling (III-2) lost central vision at the age of 17 years. His visual acuity was 0.03 and 0.05 in the right and left eyes, respectively. Both subjects demonstrated typical clinical features of LHON. None of other 10 matrilineal relatives in this family had vision deficit.

In family WZ46, the proband (III-9) came to Ophthalmology Clinic at the Dongfang Hospital at the age of 7 years after experiencing painless, progressive, deterioration of bilateral visual impairment 1 month previously. His visual acuity was 0.4 and 0.25 in the right and left eyes, respectively. Of other matrilineal relatives, subjects II-2 and III-7 exhibited clinical features of LHON at the age of 20 and 16 years, respectively. Visual

acuity of II-2 and III-7 was 0.02 and <0.01 in both eyes, respectively. The other 16 matrilineal relatives in this pedigree had normal vision.

In family WZ47, the proband (III-9) began experiencing painless, progressive deterioration of bilateral visual impairment at the age of 15 years and came to the Ophthalmology Clinic at the Dongfang Hospital at the age of 18 years. Her visual impairment occurred within 3 years, first in the right eye, and then in left eye. She saw a dark cloud in the center of her vision and had problems appreciating colors, which all seemed dark gray. Her visual acuity was 0.1 in both eyes. The IOP was 15 and 18 mmHg in the right and left eyes, respectively. Of other matrilineal relatives, subjects II-1 and II-3 experienced loss of vision at the age of 30 and 36 years, respectively. Visual acuity of II-1 and II-3 was 0.1 and <0.01 in both eyes, respectively. Eight other matrilineal relatives in this pedigree had normal vision.

In family WZ48, the proband (III-10) came to eye clinic at the age of 22 years after experiencing painless, progressive deterioration of bilateral visual impairment 5 years previously. His visual acuity was 0.01 and 0.05 in the right and left eyes, respectively. The IOP was 19 mmHg in the right eye and 17 mmHg in the left eye. Ophthalmologic clinical evaluations showed that he had typical clinical features of LHON. However, none of other 18 matrilineal relatives in this family had vision loss.

Mitochondrial DNA Analysis

To assess the role of mtDNA variants in the phenotypic expression of the G11778A mutation, PCR amplification was performed of fragments spanning entire mitochondrial genome as well as subsequent DNA sequence analysis in 8 probands. In addition to the identical G11778A mutation, as shown in Table 2 (available at <http://aaojournal.org>), these subjects exhibited distinct sets of mtDNA polymorphism. Of other nucleotide changes in these mitochondrial genomes, there are 48 (1 novel and 47 known) variants in the D-loop, 6 known variants in the 12S rRNA gene, 4 known variants in the 16S rRNA gene, the previous identified C02/tRNA^{Lys} intergenic 9-bp deletion corresponding to mtDNA at positions 8271 through 8279,²⁵ the T4386C mutation in the tRNA^{Gln} gene, the G5821A mutation in the tRNA^{Cys} gene, the A12172G mutation in the tRNA^{His} gene, the G15927G mutation in the tRNA^{Thr} gene,²⁶ 53 known and 6 novel silent variants in the protein encoding genes, as well as 25 missense mutations (4 novel and 21 known) in the protein encoding genes.²⁷ These missense mutations are the G4048A (D248N) in the *ND1* gene; the G4959A (A164P), C5178A (L237M), and G5460A (A331T) in the *ND2* gene; the G7853A (V190A) and G8027A (A148T) in the *C02* gene; the C8414T (L17F) in the *A8* gene; the G8584A (A20T), C8684T (T53I), A8701G (T59A), A8860G (T112A), and AG9073G (T183A) in *A6* gene; the T10084C (I8T) and A10398G (T114A) in the *ND3* gene; the A12358G (T8A), A12361G (T9A), T12811C (Y159H), and T13866C (L517P) in the *ND5* gene; the T14318C (N119S) in the *ND6* gene; and the C14766T (T7I), A14978G (I78V), A15326G (T194A), A15662G (I306V), and A15671G (M309V) in the *Cytb* gene. These variants in RNAs and polypeptides were evaluated further by phylogenetic analysis of these variants and sequences from other organisms including mouse,²⁸ bovine,²⁹ and *Xenopus laevis*.³⁰ Of these, the Y159H in ND5 variant located at a highly conserved residue, whereas the G15927A variant located at a highly conserved nucleotide (G28) in the tRNA^{Thr}.²⁶ However, none of other variants showed evolutionary conservation.

Discussion

In the present study, clinical, genetic, and molecular characterization was performed of 8 Han Chinese families with LHON. Visual impairment as a sole clinical phenotype was present only in the maternal lineage of these pedigrees carrying the homoplasmic *ND4* G11778A mutation. Clinical and genetic evaluations revealed the variable severity and age

at onset in visual impairment in these matrilineal relatives, although these subjects share some common features, specifically, the rapid, painless, bilateral loss of central vision. The average age at onset for visual impairment in matrilineal relatives in these families, as shown in Table 3, varied from 14 years (WZ46) to 27 years (WZ47), with an average of 17 years. As shown in Table 3, in 7 matrilineal relatives in another Chinese family, optic neuropathy developed at the average age of 14, 17, 18, 18, 19, 20, and 22 years, respectively,^{12,18–21} whereas the average ages at onset of visual loss were 24 and 28 years from matrilineal relatives of 66 and 49 white pedigrees carrying the G11778A mutation, respectively.^{31,32} Thus, matrilineal relatives in these families seemed to have an earlier age at onset than those in other families carrying the G11778A mutation. Furthermore, only 2 of 14 affected matrilineal relatives were female among these 8 families. As shown in Table 3, these Chinese families exhibited extremely low penetrance of visual impairment (affected matrilineal relatives/total matrilineal relatives), ranging from 5.3% to 27.3%, with the average of 13%, whereas the penetrance of visual impairment in another 7 Chinese pedigrees carrying the G11778A mutation were 16%, 33%, 33%, 36%, 36%, 57%, and 60%, respectively, with an average of 39%.^{12,18–21} However, in approximately 50% of white males and approximately 10% of white females carrying one of the following LHON-associated mutations, G3460A, G11778A, or T14484C, optic neuropathy indeed developed.^{2,33}

The extremely low penetrance of vision loss in these 8 Chinese pedigrees strongly indicated that the G11778A mutation was itself insufficient to produce a clinical phenotype. Nuclear backgrounds, environmental factors, and mitochondrial haplotypes may contribute to various penetrance and expressivities of visual impairment among families carrying the G11778A mutation. The phenotypic variability of matrilineal relatives from within and among families, including the variable severity of visual impairment, suggests a role of nuclear backgrounds in the phenotypic manifestation of the G11778A mutation as described in other pedigrees.^{3,18,31} Furthermore, it is possible that environmental factors also may contribute to the phenotypic variability of visual loss in matrilineal relatives of these families. In fact, the mitochondrial variants and haplotypes have been shown to influence the penetrance and expressivity of visual loss associated with primary mtDNA mutations. In particular, secondary LHON mutations at positions 4216 and 13708 may increase the penetrance and expressivity of LHON associated with primary LHON mutations, including G11778A and T14484C.^{15,16,34} Furthermore, the G7444A mutation in the *COI* and tRNA^{Ser(UCN)} genes also has been implicated in influencing the penetrance and phenotypic expression of visual loss associated with the primary LHON mutations.⁶ In addition, the *ND4* G11696A, tRNA^{Met} A4435G, and tRNA^{Thr} A15951G mutations may increase the penetrance and expressivity of vision loss in Chinese families.^{19–21}

These Chinese pedigrees showed distinct sets of sequence variations in their mitochondrial genomes. As shown in Table 3, their mtDNAs belong to Eastern Asian haplogroups M8a2, D4g2, B4a1c, B5b, N9a1, D4b2b, C, and M7b1,³⁵ whereas the other 7 Chinese pedigrees carrying the G11778A mutation belong to haplogroups D4, B5, D5, D4, F1, D4b2b, and M10a.^{12,18–21} This suggested that the G11778A mutation, similar to the G11778A mutation in white and Japanese families,^{8,10,15,17} occurred sporadically and multiplied through evolution of the mtDNA in China. Of these mtDNA variants, only the *ND5* Y159H variant located at a highly conserved residue and the G15927A variant located at a highly conserved nucleotide (G28) in the tRNA^{Thr}.²⁶ However, these 8 Chinese families demonstrated an absence of secondary LHON mutations. By contrast, 3 Chinese pedigrees with higher penetrance of vision loss harbored functionally significant mtDNA variants: the *ND4* G11696A, tRNA^{Met} A4435G, and tRNA^{Thr} A15951G.^{19–21} The presences of these mtDNA variants in 3 Chinese pedigrees,^{19–21} but the absences of these mtDNA mutations in these 8 Chinese families, seem to account for different penetrances among these

pedigrees. These data suggest that these mtDNA haplogroup-specific variants may not play an important role in the phenotypic expression of the G11778A mutation in those Chinese families with very low penetrance of vision loss.

Acknowledgments

Supported by the National Institutes of Health, Bethesda, Maryland (grant nos.: R01DC05230 and R01DC07696 [M.X.G.]); the National Science Foundation of China, Beijing, China (Chinese Young Scholar Award no.: 30628013); the Zhejiang Provincial Natural Science Foundation, Hangzhou, Zhejiang, China (grant nos.: Z204492 [M.X.G.] and ZB0202); and Zhejiang Province, Hangzhou, Zhejiang, China (Key Research and Development Program project grant no. 2004C14005 [J.Q.]).

References

1. Newman NJ, Wallace DC. Mitochondria and Leber's hereditary optic neuropathy. *Am J Ophthalmol* 1990;109:726–30. [PubMed: 2346203]
2. Brown MD, Wallace DC. Spectrum of mitochondrial DNA mutations in Leber's hereditary optic neuropathy. *Clin Neurosci* 1994;2:134–45.
3. Man PY, Turnbull DM, Chinnery PF. Leber hereditary optic neuropathy. *J Med Genet* 2002;39:162–9. [PubMed: 11897814]
4. Howell N. Leber hereditary optic neuropathy: mitochondrial mutations and degeneration of the optic nerve. *Vision Res* 1997;37:3495–507. [PubMed: 9425526]
5. Wallace DC, Singh G, Lott MT, et al. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* 1988;242:1427–30. [PubMed: 3201231]
6. Howell N. LHON and other optic nerve atrophies: the mitochondrial connection. *Dev Ophthalmol* 2003;37:94–108. [PubMed: 12876832]
7. Servidei S. Mitochondrial encephalomyopathies: gene mutation. *Neuromuscul Disord* 2004;14:107–16. [PubMed: 14702949]
8. Brown MD, Torroni A, Reckord CL, Wallace DC. Phylogenetic analysis of Leber's hereditary optic neuropathy mitochondrial DNA's indicates multiple independent occurrences of the common mutations. *Hum Mutat* 1995;6:311–25. [PubMed: 8680405]
9. Mackey DA, Oostra RJ, Rosenberg T, et al. Primary pathogenic mtDNA mutations in multigeneration pedigrees with Leber hereditary optic neuropathy. *Am J Hum Genet* 1996;59:481–5. [PubMed: 8755941]
10. Mashima Y, Yamada K, Wakakura M, et al. Spectrum of pathogenic mitochondrial DNA mutations and clinical features in Japanese families with Leber's hereditary optic neuropathy. *Curr Eye Res* 1998;17:403–8. [PubMed: 9561832]
11. Goto Y, Nonaka L, Horai S. A mutation in the tRNA^{Leu(UUR)} gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 1990;148:651–3. [PubMed: 2102678]
12. Qu J, Li R, Tong T, et al. Only male matrilineal relatives with Leber's hereditary optic neuropathy in a large Chinese family carrying the mitochondrial DNA G11778A mutation. *Biochem Biophys Res Commun* 2005;328:1139–45. [PubMed: 15707996]
13. Hudson G, Carelli V, Spruijt L, et al. Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA-haplogroup background. *Am J Hum Genet* 2007;81:228–33. [PubMed: 17668373]
14. Johns DR, Berman J. Alternative, simultaneous complex I mitochondrial DNA mutations in Leber's hereditary optic neuropathy. *Biochem Biophys Res Commun* 1991;174:1324–30. [PubMed: 1900003]
15. Torroni A, Petrozzi M, D'Urbano L, et al. Haplotype and phylogenetic analyses suggest that one European-specific mtDNA background plays a role in the expression of Leber hereditary optic neuropathy by increasing the penetrance of the primary mutations 11778 and 14484. *Am J Hum Genet* 1997;60:1107–21. [PubMed: 9150158]
16. Brown MD, Starikovskaya E, Derbeneva O, et al. The role of mtDNA background in disease expression: a new primary LHON mutation associated with Western Eurasian haplogroup. *J Hum Genet* 2002;110:130–8.

17. Howell N, Oostra RJ, Bolhuis PA, et al. Sequence analysis of the mitochondrial genomes from Dutch pedigrees with Leber hereditary optic neuropathy. *Am J Hum Genet* 2003;72:1460–9. [PubMed: 12736867]
18. Qian Y, Zhou X, Hu Y, et al. Clinical evaluation and mitochondrial DNA sequence analysis in three Chinese families with Leber's hereditary optic neuropathy. *Biochem Biophys Res Commun* 2005;332:614–21. [PubMed: 15896721]
19. Qu J, Li R, Zhou X, et al. The novel A4435G mutation in the mitochondrial tRNA^{Met} may modulate the phenotypic expression of the LHON-associated ND4 G11778A mutation in a Chinese family. *Invest Ophthalmol Vis Sci* 2006;47:475–83. [PubMed: 16431939]
20. Li R, Qu J, Zhou X, et al. The mitochondrial tRNA^{Thr} A15951G mutation may influence the phenotypic expression of the LHON-associated ND4 G11778A mutation in a Chinese family. *Gene* 2006;376:79–86. [PubMed: 16624503]
21. Qu J, Li R, Zhou X, et al. Cosegregation of the ND4 G11696A mutation with the LHON-associated ND4 G11778A mutation in a four generation Chinese family. *Mitochondrion* 2007;7:140–6. [PubMed: 17300996]
22. Anderson S, Bankier AT, Barrell BG, et al. Sequence and organization of the human mitochondrial genome. *Nature* 1981;290:457–65. [PubMed: 7219534]
23. Woischnik M, Moraes CT. Pattern of organization of human mitochondrial pseudogenes in the nuclear genome. *Genome Res* 2002;12:885–93. [PubMed: 12045142]
24. Rieder MJ, Taylor SL, Tobe VO, Nickerson DA. Automating the identification of DNA variations using quality-based fluorescence re-sequencing: analysis of the human mitochondrial genome. *Nucleic Acids Res* 1998;26:967–73. [PubMed: 9461455]
25. Young WY, Zhao L, Qian Y, et al. Extremely low penetrance of hearing loss in four Chinese families with the mitochondrial 12S rRNA A1555G mutation. *Biochem Biophys Res Commun* 2005;328:1244–51. [PubMed: 15708009]
26. Chen B, Sun D, Yang L, et al. Mitochondrial ND5 T12338C, tRNA^{Cys} T5802C, and tRNA^{Thr} G15927A variants may have a modifying role in the phenotypic manifestation of deafness-associated 12S rRNA A1555G mutation in three Han Chinese pedigrees. *Am J Med Genet* 2008;146A:1248–58. [PubMed: 18386806]
27. Brandon MC, Lott MT, Nguyen KC, et al. MITOMAP: a human mitochondrial genome database—2004 update. *Nucleic Acids Res* 2005;33(database issue):D611–3. [PubMed: 15608272]
28. Bibb MJ, Van Etten RA, Wright CT, et al. Sequence and gene organization of mouse mitochondrial DNA. *Cell* 1981;26:167–80. [PubMed: 7332926]
29. Gadaleta G, Pepe G, De Candia G, et al. The complete nucleotide sequence of the *Rattus norvegicus* mitochondrial genome: cryptic signals revealed by comparative analysis between vertebrates. *J Mol Evol* 1989;28:497–516. [PubMed: 2504926]
30. Roe A, Ma DP, Wilson RK, Wong JF. The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. *J Biol Chem* 1985;260:9759–74. [PubMed: 4019494]
31. Harding AE, Sweeney MG, Govan GG, Riordan-Eva P. Pedigree analysis in Leber hereditary optic neuropathy families with a pathogenic mtDNA mutation. *Am J Hum Genet* 1995;57:77–86. [PubMed: 7611298]
32. Newman NJ, Lott MT, Wallace DC. The clinical characteristics of pedigrees of Leber's hereditary optic neuropathy with the 11778 mutation. *Am J Ophthalmol* 1991;111:750–62. [PubMed: 2039048]
33. Nikoskelainen EK. Clinical picture of LHON. *Clin Neurosci* 1994;2:115–20.
34. Howell N, Kubacka I, Xu M, McCullough DA. Leber hereditary optic neuropathy: involvement of the mitochondrial ND1 gene and evidence for an intragenic suppression mutation. *Am J Hum Genet* 1991;48:935–42. [PubMed: 2018041]
35. Tanaka M, Cabrera VM, González AM, et al. Mitochondrial genome variation in eastern Asia and the peopling of Japan. *Genome Res* 2004;14:1832–50. [PubMed: 15466285]

Figure 1. Eight Chinese pedigrees with Leber's hereditary optic neuropathy. Visually impaired individuals are indicated by filled symbols. Arrows denote the probands.

Table 1
Summary of Clinical Data for 14 Subjects of 8 Chinese Pedigrees Carrying the G11778A Mutation

Subject	Gender	Age at Test (yrs)	Age at Onset (yrs)	Visual Acuity		Level of Visual Impairment
				Right Eye	Left Eye	
WZ41-III-2	M	18	18	0.06	0.08	Moderate
WZ42-III-11	M	18	14	0.044	0.06	Severe
WZ42-III-14	M	17	15	0.03	1.5	Mild
WZ43-III-5	M	16	15	0.02	0.02	Profound
WZ44-III-1	M	20	15	0.02	0.06	Severe
WZ45-III-1	M	31	15	<0.01	<0.01	Profound
WZ45-III-2	M	28	17	0.03	0.5	Severe
WZ46-II-2	F	40	20	0.02	0.02	Severe
WZ46-III-7	M	17	16	<0.01	<0.01	Profound
WZ46-III-9	M	7	7	0.4	0.25	Mild
WZ47-II-1	M	58	30	0.1	0.1	Mild
WZ47-II-3	M	52	36	<0.01	<0.01	Profound
WZ47-III-9	F	18	15	0.1	0.1	Mild
WZ48-III-10	M	22	16	0.01	0.05	Severe

F = female; M = male.

Table 2

mtDNA Variants in Eight Chinese Pedigrees with Leber's hereditary Optic Neuropathy

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS ^b	WZ41	WZ42	WZ43	WZ44	WZ45	WZ46	WZ47	WZ48	Previously Reported ^c
D-loop	73	A to G		A	G	G	G	G	G	G	G	G	Yes
	94	G to A		G				A					Yes
	103	G to A		G				A					Yes
	131	T to C		T				C					Yes
	146	T to C		T			C			C			Yes
	150	C to T		C					T			T	Yes
	194	C to T		C						T			Yes
	199	T to C		T								C	Yes
	228	G to T		G							T		Yes
	249	Del A		A							Del A		Yes
	263	A to G		A	G	G	G	G	G	G	G	G	Yes
	298	C to T		C		T							Yes
	309	C to CC		C			CC					CC	Yes
	310	T to CTC		T	TC	TC	CTC	CTC	CTC	TC	CTC	CTC	Yes
	481	C to T		C				T					No
	489	T to C		T	C	C				C	C	C	Yes
	514	Del C		C			Del C	Del C		Del C			Yes
	515	Del A		A			Del A	Del A		Del A			Yes
	573	C to CCC		C			CCC						Yes
	16093	T to C		T			C	C					Yes
	16111	G to A		G					A				Yes
	16129	G to A		G					A			A	Yes
	16140	T to C		T				C					Yes
	16167	C to T		C			T						Yes
	16172	T to C		T						C			Yes
	16182	A to C		A			C						Yes
	16183	A to C		A			C	C					Yes
	16184	C to T		C	T								Yes

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS ^b	WZ41	WZ42	WZ43	WZ44	WZ45	WZ46	WZ47	WZ48	Previously Reported ^c
12S rRNA	16189	T to C		T			C	C		C			Yes
	16192	C to T		C							T		Yes
	16217	T to C		T			C						Yes
	16223	C to T		C	T	T		T		T	T		Yes
	16234	C to T		C				T					Yes
	16243	T to C		T				C					Yes
	16257	C to A		C					A				Yes
	16261	T to C		T					C				Yes
	16261	C to T		C			T						Yes
	16271	T to C		T		C							Yes
	16289	T to C		T						C			Yes
	16293	A to C		A			C						Yes
	16297	T to C		T							C		Yes
	16298	T to C		T	C					C			Yes
	16319	G to A		G	A								Yes
	16327	C to T		C							T		Yes
	16335	A to G		A				G					Yes
	16362	T to C		T		C				C			Yes
	16463	A to G		A				G					Yes
16S rRNA	16519	T to C		T			C	C		C			Yes
	709	G to A	G/A/A/-	G			A	A					Yes
	722	C to T	C/T/A-	C									Yes
	750	A to G	A/A/A/-	A	G	G	G	G		G	G		Yes
	1382	A to C	A/A/A/G	A						C			Yes
	1438	A to G	A/A/A/G	A	G	G	G	G	G	G	G		Yes
	1598	G to A	G/A/T/T	G				A					Yes
16S rRNA	1719	G to A	G/G/T/C	G				A					Yes
	2706	A to G	A/G/A/A	A	G	G	G	G	G	G	G		Yes
	2835	C to T	C/A/A/A	C	T								Yes
	3010	G to A	G/G/A/A	G					A				Yes
ND1	3552	T to A		T		A					A		Yes

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS ^b	WZ41	WZ42	WZ43	WZ44	WZ45	WZ46	WZ47	WZ48	Previously Reported ^c
tRNA ^{Gln} <i>ND2</i>	4048	G to A (Asp to Asn)	D/N/Y/F	G							A	Yes	
	4071	C to T		C							T	Yes	
	4161	C to T		C				T				No	
	4164	A to G		A							G	Yes	
	4386	T to C	T/T/A/C	T				C				Yes	
	4715	A to G		A	G					G		Yes	
	4769	A to G		A	G	G	G	G	G	G	G	Yes	
	4883	C to T		C		T						Yes	
	4895	A to G		A			G					Yes	
	4959	G to A (Ala to Pro)	A/I/M/I	G		A						Yes	
tRNA ^{Cys} <i>COI</i>	5178	C to A (Leu to Met)	L/T/T/T	C	A			A				Yes	
	5231	G to A		G	A			A				Yes	
	5351	A to G		A							G	Yes	
	5417	G to A		G				A				Yes	
	5460	G to A (Ala to Thr)	A/V/I/I	G							A	Yes	
	5465	T to C		T			C					Yes	
	5821	G to A	G/A/A/A	G						A		Yes	
	6053	C to T		C				T				No	
	6179	G to A		G	A							Yes	
	6338	A to G		A						G		Yes	
CO2	6357	C to T		C					T			Yes	
	6455	C to T		C							T	Yes	
	6680	T to C		T							C	Yes	
	7028	C to T		C	T	T	T	T	T	T	T	Yes	
	7043	C to T		C	T							Yes	
	7052	A to G		A			G					Yes	
	7196	C to A		C	A					A		Yes	
	7271	A to G		A			G					Yes	
	7684	T to C		T							C	Yes	
	7853	G to A (Val to Ala)	V/I/I/V	G							A	Yes	
	8020	G to A		G				A				Yes	

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS ^b	WZ41	WZ42	WZ43	WZ44	WZ45	WZ46	WZ47	WZ48	Previously Reported ^c
NC7	8027	G to A (Ala148Thr)	A/M/L/S	G			9bpDel	9bpDel	A				Yes
	8271_9	9bpDel		9bp									Yes
	8414	C to T (Leu to Phe)	L/F/M/W	C		T							Yes
ATP8	8584	G to A (Ala to Thr)	A/V/V/I	G	A			A					Yes
ATP6	8684	C to T (Thr to Ile)	T/V/I/F	C	T								No
	8701	A to G (Thr to Ala)	T/S/L/Q	A	G	G			G	G			Yes
	8829	C to T		C				T					Yes
	8860	A to G (Thr to Ala)	T/A/A/T	A	G	G	G	G	G	G			Yes
	8964	C to T		C					T				Yes
	9073	A to G (Thr to Ala)	T/S/N/S	A							G		No
CO3	9123	G to A		G			A						Yes
	9296	C to T		C									Yes
	9540	T to C		T	C	C							Yes
	9545	A to G		A						G			Yes
	9824	T to C		T							C		Yes
	9950	T to C		T									Yes
ND3	10084	T to C (Ile to Thr)	I/T/I/I	T				C			C		Yes
	10238	T to C		T									Yes
	10398	A to G (Thr to Ala)	T/T/T/A	A	G	G		G		G	G		Yes
	10400	C to T		C	T	T					T		Yes
	10873	T to C		T	C	C				C	C		Yes
ND4	11101	A to G		A				G					No
	11296	C to T		C									Yes
	11719	G to A		G	A	A	A	A	A	A	A		Yes
	11778	G to A (Arg to His)	R/R/R/R	G	A	A	A	A	A	A	A		Yes
	11914	G to A		G							A		Yes
	11935	T to C		T		C							Yes
tRNA ^{His}	12007	G to A		G									Yes
	12172	A to G	A/A/A/T	A					A				Yes
	12358	A to G (Thr to Ala)	T/S/I/M	A					G				Yes
ND5	12361	A to G (Thr to Ala)	T/L/L/L	A					G	G			Yes
								G					Yes

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS ^b	WZ41	WZ42	WZ43	WZ44	WZ45	WZ46	WZ47	WZ48	Previously Reported ^c
ND6	12372	G to A		G				A					Yes
	12405	C to T		C							T		Yes
	12705	C to T		C	T	T		T	T	T	T		Yes
	12811	T to C (Tyr to His)	Y/Y/Y/Y	T								C	Yes
	12957	T to C		T						C			Yes
	12972	A to G		A		G							No
	13104	A to G		A	G								Yes
	13263	A to G		A			G			G			Yes
	13886	T to C (Leu to Pro)	L/S/N/T	T			C						No
	14318	T to C (Asn to Ser)	N/N/D/S	T						C			Yes
Cytb	14383	C to T		C					T				No
	14470	T to C		C	T								Yes
	14668	C to T		C		T				T			Yes
	14766	C to T (Thr to Ile)	T/S/T/S	C	T	T	T	T	T	T	T		Yes
	14783	T to C		T	C	C			C	C	C		Yes
	14978	A to G (Ile to Val)	I/I/L/L	A						G			Yes
	15043	G to A		G	A	A			A	A	A		Yes
	15223	C to T		C			T						No
	15301	G to A		G	A	A			A	A	A		Yes
	15326	A to G (Thr to Ala)	T/M/I/I	A	G	G	G	G	G	G	G		Yes
tRNA ^{Thr}	15487	A to T		A	T						T		Yes
	15508	C to T		C			T						Yes
	15661	C to T		C			T						Yes
	15662	A to G (Ile to Val)	I/L/F/L	A			G						Yes
	15671	A to G (Met to Val)	M/T/T/T	A							G		No
	15927	G to A	G/G/G/G	G			A						Yes

^aConservation of amino acid for polypeptides or nucleotide for RNAs in human (H), bovine (B), mouse (M), and *Xenopus laevis* (X).

^bCRS, Cambridge reference sequence (Andrews et al., 1999).

^cSee online mitochondrial genome database <http://www.mitomap.org>.

Table 3

Summary of Clinical and Molecular Data for 15 Chinese Families Carrying the ND4 G11778A Mutation

Pedigree	Ratio of Affected Males to Affected Females	Average Age at Onset (yrs)	No. of Matrilineal Relatives	Penetrance (%) [*]	Secondary Mutations	Mitochondrial DNA Haplogroup
WZ41	1:0	18	7	14.3		M8a2
WZ42	2:0	14.5	25	8		D4g2
WZ43	1:0	15	9	11.1		B4a1c
WZ44	1:0	15	17	5.9		B5b
WZ45	2:0	16	12	16.7		N9a1
WZ46	2:1	14	19	15.8		D4b2b
WZ47	2:1	27	11	27.3		C
WZ48	1:0	16	19	5.3		M7b1
WZ112	6:0	22	38	15.8		B5
WZ219	3:1	14	14	57.1	A4435G	D5
WZ320	2:1	19	10	60	A15951G	D4
WZ418	3:0	17	9	33.3		F1
WZ5	4:1	20	14	35.7		D4b2b
WZ6	1.2:1	18	31	35.5		M10a
WZ1221	3.5:1	18	30	33	G11696A	D4

^{*} Number of affected matrilineal relatives per total number of matrilineal relatives. Qu et al. (2005)¹², Qu et al. (2006)¹⁹, Li et al. (2006)²⁰, Qian et al. (2005)¹⁸, Qu et al. (2007)²¹