Published in final edited form as:

Biochim Biophys Acta. 2010 March ; 1800(3): 305–312. doi:10.1016/j.bbagen.2009.08.010.

Low penetrance of Leber's hereditary optic neuropathy in ten Han Chinese families carrying the ND6 T11484C mutation

Jia Qu^{1,2}, Xiangtian Zhou¹, Fuxin Zhao^{1,2}, Xiaoling Liu¹, Minglian Zhang³, Yan-Hong Sun⁴, Min Liang^{1,2}, Meixia Yuan^{1,2}, Qi Liu², Yi Tong^{1,5}, Qi-Ping Wei⁵, Li Yang⁶, and Min-Xin Guan^{2,6,7,*}

¹School of Ophthalmology and Optometry, Wenzhou Medical College, Wenzhou, Zhejiang 325003, China

²Zhejiang Provincial Key Laboratory of Medical Genetics, School of Life Sciences, Wenzhou Medical College, Wenzhou, Zhejiang 325003, China

³Department of Ophthalmology, Xingta Eye Hospital, Xingta, Hebei 054001, China

⁴Department of Ophthalmology, Beijing University of Chinese Medicine and Pharmacology, Beijing, China

⁵The First Affiliated Hospital, Fujian Medical University, Fuzhou, Fujian 350005, China

⁶Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229, USA

⁷Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229, USA

Abstract

We report there the clinical, genetic and molecular characterization of 10 Han Chinese families with Leber's hereditary optic neuropathy. Clinical evaluation revealed that ten families exhibited extremely low penetrance of visual impairment, with the average of 10%. In particular, ten (8 males/ 2 females) of 114 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 19 years old. Molecular analysis of mitochondrial DNA (mtDNA) identified the homoplasmic ND6 T14484C mutation and distinct sets of variants, belonging to the Asian haplogroups B5b, D4, D4g1b, G3a2, R11, R11a and Z3, respectively. However, there was the absence of secondary LHON-associated mtDNA mutations in these ten Chinese families: The low penetrance of vision loss in these Chinese pedigrees strongly indicated that the T14484C 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 T14484C mutation in those Chinese families with low penetrance of vision loss. However, nuclear modifier genes and environmental factors appear to be modifier factors for the phenotypic manifestation of the T14484C mutation in these Chinese families.

© 2009 Elsevier B.V. All rights reserved.

*Corresponding author: Min-Xin Guan, Ph.D., Division of Human Genetics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, Ohio 45229, USA Telephone: (513) 636-3337; Fax: (513) 636-3486; min-xin.guan@cchmc.org.

Financial Disclosure: The authors have no proprietary or commercial interest in any materials discussed in this article.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Leber's hereditary optic neuropathy (LHON) is a maternally inherited disorder whose typical clinical feature involves a painless, acute or sub-acute, bilateral loss of central vision.^{1,2,3,4} Since the landmark discovery of the ND4 G11778A mutation associated with LHON,⁵ approximately 35 LHON-associated mitochondrial DNA (mtDNA) mutations have been identified in various ethnic populations.^{6,7} Of these, the ND1 G3460A, ND4 G11778A and ND6 T14484C mutations, which involve genes encoding the subunits of respiratory chain complex I, accounts for more than 50% of LHON pedigrees in different ethnic backgrounds.^{8-9,10} Those LHON associated mtDNA mutations, unlike other pathogenic mtDNA mutations such as Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS)-associated tRNA^{Leu(UUR)} A3243G mutation present in heteroplasmy (mixture of mutated and wild-type molecule),¹¹ often occur nearly homoplasmy or homoplasmy. Those mtDNA mutations such as the G11778A or T14484C mutation often exhibit incomplete penetrance since some individuals carrying the mutations(s) have normal vision.^{4,8,10,12} In addition, matrilineal relatives within and among families carrying the 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 event, but the secondary events such as environmental factors, nuclear and mitochondrial genetic modifiers are necessary for the manifestation of the optic neuropathy.^{3,4,13} 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.¹⁴ Furthermore, a group of so-called "secondary" LHON-associated mtDNA mutations including T4216C, A4917G and G13708A¹⁵ were implicated to act in synergy with these primary mtDNA mutations including the G11778A and T14484C mutations.¹⁶ In addition, the mitochondrial haplogroup J was shown to influence the phenotypic manifestation of the primary LHON G11778A and T14484C mutations in a very large cohort of families of European ancestry.^{17,18}

To investigate the role of mitochondrial haplotypes in the development of LHON, 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.^{12,19,21,22,23,24} In the previous investigation, we showed that the ND4 G11696A, tRNA^{Met} A4435G and tRNA^{Thr} A15951G mutations contribute to the high penetrance and expressivity of optic neuropathy in Chinese families carrying the ND4 G11778A mutation.^{19,22,23} However, eight Han Chinese pedigrees carrying the ND4 G11778A mutation displayed the extremely low penetrance of vision loss.²⁴ In addition, four Han Chinese pedigrees carrying the ND6 T14484C mutation exhibited a wide range of penetrance, severity, age-at-onset of LHON.^{20,21} In the present study, we performed the clinical, genetic and molecular characterization of other ten Han Chinese families with LHON. Strikingly, these pedigrees exhibited low penetrance of LHON. Ten (8 males/two female) of 114 matrilineal relatives in these families exhibited the variable severity and age-at-onset in visual dysfunction. Mutational analysis of mtDNA identified the known ND6 T14484C mutation in these Chinese families. To elucidate the role of mitochondrial haplotypes in the phenotypic manifestation of the T14484C mutation, we performed a PCR-amplification of the fragments spanning entire mitochondrial genome and subsequent DNA sequence analysis in the matrilineal relatives of these Chinese families.

PATIENTS AND METHODS

Patients and Subjects

We ascertained ten Han Chinese families (Fig.1) through the School of Ophthalmology and Optometry, Wenzhou Medical College, Zhejiang, Dongfang Hospital, Beijing and Xingta Eye

Hospital, Hebei. 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 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.

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 IIi, SITA Standard; Carl Zeiss Meditec, Oberkochen, Germany), visual evoked potentials (VEP; RETI port gamma, flash VEP; Roland Consult, Brandenburg, 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.

Mutational analysis of the mitochondrial genome—Genomic DNA was isolated from whole blood of participants using the Puregene DNA Isolation Kits (Puregene DNA Isolation Kit; Gentra Systems, Minneapolis, MN). The presence of the G3460A, G11778A and T14484C mutations was examined as detailed elsewhere.⁸ 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,²⁵ respectively. For the detection of the G3460A and G11778A mutations, the amplified PCR segments were digested with restriction enzymes *Bsa*HI and *Tsp*45I, respectively,^{8,12} while the presence of the T14484C mutation was examined by digesting PCR products with a restriction enzyme *Mva*I.8 For the examination of the T14484C mutation, the first PCR segments (938 bp) were amplified using genomic DNA as template and oligodeoxynucleotides corresponding to mtDNA at positions 14000-14998 to rule out the co-amplification of possible nuclear pseudogenes.²⁶ Then, the second PCR product (251 bp) were amplified using the first PCR fragment as template and oligodeoxynucleotides corresponding to mtDNA at positions 14260-14510, and subsequently digested with a restriction enzymes *Mva*I as the T14484C mutation creates the site for this restriction enzyme.⁸ 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 T14484C mutation is in the homoplasmy in these subjects. The entire mitochondrial genomes of ten probands were 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).

Haplogroup analyses

The entire mtDNA sequences of the 10 Chinese probands carrying the T14484C mutation were assigned to the Asian mitochondrial haplogroups by using the nomenclature of mitochondrial haplogroups.^{28,29}

RESULTS

Clinical and genetic evaluation of ten Chinese pedigrees

Ten Han Chinese subjects were diagnosed as LHON by the Eye Clinic at the Wenzhou Medical College. Mutation analysis of the mitochondrial ND6 gene revealed that these ten participants harbored the known ND6 T14484C mutation. A comprehensive history and physical examination as well as ophthalmological examination as detailed in the section of Patients and Method were performed to identify both personal or family medical histories of visual impairments, and other clinical abnormalities in all available members of these Chinese pedigrees. 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 neurological disorders. The restriction enzyme *MvaI* digestion and subsequent electrophoresis analysis of available members in ten pedigrees indicated that the T14484C mutation in the ND6 gene was indeed present in homoplasmy in matrilineal relatives but no other members of these families (data not shown).

In family WZ18, the proband (IV-1) came to the Ophthalmology Clinic of Wenzhou Medical College at the age of 28 year old. He began suffering bilateral visual impairment at the age of 25 years old. His visual impairment occurred within seven months, first in the right eye and then later in the left eye. His visual acuity was 0.05 and 0.04 at the right and left eyes, respectively. Intraocular Pressure (IOP) was 13.7 mmHg and 14.7 mmHg at the right and left eyes, respectively. He saw a dark cloud in the center of vision and had problems appreciating colors that all seemed a dark gray. Visual field testing demonstrated large centrocaecal scotomata in both of his eyes. Fundus examination showed that both his optic disks were abnormal: vascular tortuosity of the central retinal vessels, a circumpapillary telangiectatic microangiopathy, and swelling of the retinal nerve fiber layer. Therefore, he exhibited a typical clinical feature of LHON. However, none of other sixteen matrilineal relatives in this family had visual loss.

In family WZ19, the proband (IV-1) was a 25 years old man. He exhibited bilateral vision loss at the age of 24 years old. He was diagnosed as LHON by Ophthalmology Clinic at the Wenzhou Medical College. His visual acuity was 0.05 and 0.05 at the right and left eyes, respectively. IOP was 18 mmHg in his right eye and 20.3 mmHg in the left eye, respectively. None of other 13 matrilineal relatives in this pedigree had vision failure.

In family WZ20, the proband (III-1) complained of painless, progressive deterioration of bilateral vision impairment at the age of 6 years old and went to Eye Clinic at the Dongfang Hospital at the age of 10 years old. His visual acuity was 0.3 and 0.4 at the right and left eyes, respectively. IOP was 14 mmHg in his right eye and 15 mmHg in the left eye, respectively. Ophthalmological and other clinical evaluations showed that he had a typical clinical feature of LHON. However, other 5 matrilineal relatives in this pedigree had normal vision.

In family WZ21, the proband (III-1) visited Ophthalmology Clinic at the Wenzhou Medical College at the age of 31. He experienced painless, progressive deterioration of bilateral vision loss at the age of 17 years old. His visual acuity was 0.06 and 0.12 at the right and left eyes, respectively. IOP was 11 mmHg in his right eye and 10 mmHg in the left eye, respectively. This subject revealed a typical clinical feature of LHON. None of other 5 matrilineal relatives in this family had vision deficit.

In family WZ22, the proband (III-4) came to Ophthalmology Clinic at the Dongfang Hospital at the age of 13 year old after experiencing painless, progressive deterioration of bilateral vision impairment two month ago. His visual acuity was 0.01 at both eyes, respectively. Clinical

evaluation showed that this subject exhibited a typical clinical feature of LHON. Other 6 matrilineal relatives in this pedigree had normal vision.

In family WZ23, the proband (III-1) began suffering from painless, progressive deterioration of bilateral vision impairment at the age of 19 years old and came to Ophthalmology Clinic at the Wenzhou Medical College one month later. He saw a dark cloud in the center of vision and had problems appreciating colors that all seemed dark gray. His visual acuity was 0.04 at the both eyes. All other 12 matrilineal relatives in this pedigree had normal vision.

In family WZ24, the proband (III-2) came to Xingta Eye Hospital at the age of 17 after suffering from painless, progressive deterioration of bilateral vision impairment two month ago. Her visual acuity was 0.03 and 0.04 at the right and left eyes, respectively. IOP was 20.55 mmHg in both eyes, respectively. Ophthalmological clinical evaluations showed that she had a typical clinical feature of LHON. However, none of other 10 matrilineal relatives in this family suffered from vision loss.

In family WZ25, the proband (III-1) complained of painless, progressive deterioration of bilateral vision impairment at the age of 20 years old and came to Ophthalmology Clinic at the Wenzhou Medical College on month later. His visual acuity was 0.1 and 0.4 at the right and left eyes, respectively. Ophthalmological and other clinical evaluations showed that he had a typical clinical feature of LHON. However, none of other 6 matrilineal relatives in this pedigree had vision impairment.

In family WZ26, the proband (III-5) came to Ophthalmology Clinic at the Wenzhou Medical College at the age of 18. He experienced painless, progressive deterioration of bilateral vision loss one month before. His visual acuity was 0.05 at the both eyes. This subject revealed a typical clinical feature of LHON. None of other 11 matrilineal relatives in this family had vision deficit.

In family WZ27, the proband (III-3) came to Ophthalmology Clinic at the Wenzhou Medical College at the age of 29 year old after experiencing painless, progressive deterioration of bilateral vision impairment at the age of 27 year old. His visual acuity was 0.1 and 0.3 at the right and left eyes, respectively. Clinical evaluation showed that this subject exhibited a typical clinical feature of LHON. Other 19 matrilineal relatives in this pedigree exhibited normal vision.

Mitochondrial DNA analysis

To assess the role of mtDNA variants in the phenotypic expression of the T14484C mutation, we performed a PCR-amplification of fragments spanning entire mitochondrial genome and subsequent DNA sequence analysis in ten probands. In addition to the identical T14484C mutation, as shown in Table 2, these subjects exhibited distinct sets of mtDNA polymorphism. Of other nucleotide changes in these mitochondrial genomes, there are 49 known variants in the D-loop, four known variants in 12S rRNA gene, two known variants in the 16S rRNA gene, the previous identified CO2/tRNA^{Lys} intergenic 9 base-pair deletion corresponding to mtDNA at positions 8271-8279, ³⁰ novel CO2/tRNA^{Lys} intergenic 4 base-pair C-insertion at position 8278, the T3290C mutation in the tRNA^{Leu(UUR)} gene, the A4343G mutation in the tRNA^{Glu} gene, the T5774G mutation in the tRNA^{Cys} gene, the T10031C mutation in the tRNA^{Gly} gene, the G15927G mutation in the tRNA^{Thr} gene, 55 (53 known and 2 novel) silent variants in the protein encoding genes as well as 28 missense mutations (1 novel and 27 known) in the protein encoding genes.⁷ These missense mutations are the G3316A (A4T) in the *ND1* gene, the A4833G (T122A) and C5178A (L237M) in the *ND2* gene, the C8414T (L17F) and A8498G (K45D) in the *A8* gene, the G8584A (A20T), A8701G (T59A), A8860G (T112A) and C9071T (S182L) in *A6* gene, the A10398G (T114A) in the *ND3* gene, the C11061T

(S101F), T11087C (F110L), T11204C (F149L) in the *ND4* gene, the A12358G (T8A), A12361G (T9A), A12371G (T13A), A12950G (N205S) and T13681C (T449A) in the *ND5* gene, the T14256C (I140V) in the *ND6*, the C14766T (T7I), 14883T (T46I), A14954T (T70S), T15090C (I115V), T15204C (I153T), A15326G (T194A), A15662G (I306V), A15746G (I334V) and A15851G (I369V) in the *Cytb* gene. These variants in RNAs and polypeptides were further evaluated by phylogenetic analysis of these variants and sequences from other organisms including mouse,³¹ bovine,³² and *Xenopus laevis*.³³ Of tRNA and rRNA variants, the G15927A variant locates at a highly conserved nucleotide (G28) in the tRNA^{Thr}.³⁴ Of variants in the polypeptide encoding genes, the ND4 F110L and F149L and ND5 N205S variants located at highly conserved residues. However, other variants showed no evolutionary conservation.

The entire mtDNA sequences of the 10 Chinese probands carrying the T14484C mutation were assigned to the Asian mitochondrial haplogroups B5b, D4, D4g1b, G3a2, R11, R11a and Z3, respectively, by using the nomenclature of mitochondrial haplogroups.^{28,29}

DISCUSSION

In the present study, we have performed the clinical, genetic, and molecular characterization of ten Han Chinese families with Leber's hereditary optic neuropathy. Visual impairment as a sole clinical phenotype was only present in the maternal lineage of these pedigrees carrying the homoplasmic ND6 T14484C mutation. Clinical and genetic evaluations revealed the variable severity and age-of-onset in visual impairment in these matrilineal relatives, although these subjects shared some common features: being the rapid, painless, bilateral loss of central vision. The age-at-onset for vision impairment in matrilineal relatives in these families, as shown in Table 3, varied from 6 years (WZ20) to 27 years (WZ27), with the average of 18.6 years old. As shown in Table 3, the average age-at-onset was 12.5, 14, 19, and 24 years, in other four Chinese families carrying the T14484C mutation,^{20,21} and 19- and 27-year-old from 17 and 23 Caucasian pedigrees carrying the T14484C mutation.^{35,36} Unlike previous reports that the ratios between affected male and female matrilineal relatives were 2.1:1 and 7.7:1 from two large cohorts of Caucasian pedigrees carrying the T14484C mutation,^{35,36} 8 males/2 female of 114 matrilineal relatives in these ten families exhibited vision impairment. As shown in Table 3, these Chinese families exhibited extremely low penetrance of vision impairment (affected matrilineal relatives/total matrilineal relatives), ranging from 4.8% to 16.7%, with the average of 10.2%, while the penetrances of vision impairment in other four Chinese pedigrees carrying the T14484C mutation were 27%, 36%, 48% and 60%, respectively.^{20,21} On the other hand, there were extremely low penetrance of vision loss observed in 10 Han Chinese families carrying the G11778A mutation. 24 Furthermore, ~50% males and ~10% females in Caucasians carrying one of LHON associated G3460A, G11778A and T14484C mutations indeed developed the optic neuropathy.^{2,37,38}

The low penetrance of vision loss in these ten Chinese pedigrees strongly indicated that the ND6 T14484C mutation, similar to the G11778A mutation,²⁴ was itself insufficient to produce a clinical phenotype. Other environmental and genetic factors including nuclear modifier genes and mitochondrial haplotypes may contribute to the variability of penetrances and expressivities of LHON among families carrying the T14484C mutation. The phenotypic variability of matrilineal relatives within and among families including the severity of vision impairment suggests a role of nuclear backgrounds in the phenotypic manifestation of the T14484C mutation as described other pedigrees.^{3,14,18,35,36} Furthermore, it is possible that environmental factors may also contribute to the phenotypic variability of visual loss in matrilineal relatives of these families. In fact, the mitochondrial variants/haplotypes have been shown to influence the penetrance and expressivity of vision 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 the primary LHON mutations including G11778A and T14484C,^{15,16,17,18} or deafness associated with the primary tRNA^{Ser(UCN)} A7445A mutation.³⁹ Furthermore, the G7444A mutation in the CO1 and tRNA^{Ser(UCN)} genes has also been implicated to influence the penetrance and phenotypic expression of visual loss associated with the primary G11778A and T14484C mutations,⁸ or deafness associated with the primary 12S rRNA A1555G mutation.⁴⁰ In addition, our previous investigations showed that the ND4 G11696A, tRNA^{Met} A4435G and tRNA^{Thr} A15951G mutations may increase the penetrance and expressivity of vision loss in Chinese families.^{19, 22,23}

Here, there were the distinct sets of sequence variations in mitochondrial genomes of these Chinese pedigrees. As shown in Table 3, their mtDNAs belong to East Asian haplogroups D4, D4g1b, B5b, N9a, G3a2, R11, R11a and Z3, respectively.^{28,29} This suggested that the T14484C mutation, similar to these Caucasian and Japanese families carrying the T14484C mutation,^{8,10,15,18} occurred sporadically and multiplied through evolution of the mtDNA in China. Of these mtDNA variants, the ND4 F110L and F149L and ND5 N205S variants located at highly conserved residues, while and the G15927A variant locates at a highly conserved nucleotide (G28) in the tRNA^{Thr}.³⁴ However, there was the absence of secondary LHON mutations in these ten Chinese families. These data suggest that these mtDNA haplogroup-specific variants may not play an important role in the phenotypic expression of the ND6 T14484C mutation in those Chinese families with very low penetrance of vision loss.

Acknowledgments

This work was supported by National Institutes of Health (NIH) grants RO1DC05230 and RO1DC07696 from the National Institute on Deafness and Other Communication Disorders to M.X.G., a Chinese Young Scholar Award (30628013) from National Science Foundation of China and a key research grant Z204492 from Zhejiang Provincial Natural Science Foundation of China to M.X.G., a project grant ZB0202 from Zhejiang Provincial Natural Science Foundation of China and a Key Research and Development Program project grant 2004C14005 from Zhejiang Province, China to J.Q.

Funding support This work was supported by National Institutes of Health (NIH) grants RO1DC05230 and RO1DC07696 from the National Institute on Deafness and Other Communication Disorders to M.X.G., a Chinese Young Scholar Award (30628013) from National Science Foundation of China and a key research grant Z204492 from Zhejiang Provincial Natural Science Foundation of China to M.X.G., a project grant ZB0202 from Zhejiang Provincial Natural Science Foundation of China and a Key Research and Development Program project grant 2004C14005 from Zhejiang Province, China to J.Q.

REFERENCE

1. Newman NJ. Leber's hereditary optic neuropathy. *Ophthalmol Clin NA* 1993;4:431–447.
2. Brown MD, Wallace DC. Spectrum of mitochondrial DNA mutations in Leber's hereditary optic neuropathy. *Clin Neurosci* 1994;2:134–145.
3. Man PY, Turnbull DM, Chinnery PF. Leber hereditary optic neuropathy. *J Med Genet* 2002;39:162–169. [PubMed: 11897814]
4. Howell N. Leber hereditary optic neuropathy: mitochondrial mutations and degeneration of the optic nerve. *Vision Res* 1987;37:3495–3507. [PubMed: 9425526]
5. Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Elsas LJ, Nikoskelainen EK. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* 1988;242:1427–1430. [PubMed: 3201231]
6. Howell N. LHON and other optic nerve atrophies: the mitochondrial connection. *Dev Ophthalmol* 2003;37:94–108. [PubMed: 12876832]
7. Brandon MC, Lott MT, Nguyen KC, Spolim S, Navathe SB, Baldi P, Wallace DC. MITOMAP: a human mitochondrial genome database--2004 update. *Nucleic Acids Res* 2005;33:D611–613. [PubMed: 15608272]

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;6:311–325. [PubMed: 8680405]
9. Mackey DA, Oostra RJ, Rosenberg T, Nikoskelainen E, Bronte-Stewart J, Poulton J, Harding AE, Govan G, Bolhuis PA, Norby S, Bleeker-Wagemakers EM, Savontaus M-L, Cahn C, Howell N. Primary pathogenic mtDNA mutations in multigeneration pedigrees with Leber hereditary optic neuropathy. *Am J Hum Genet* 1996;59:481–485. [PubMed: 8755941]
10. Mashima Y, Yamada K, Wakakura M, Kigasawa K, Kudoh J, Shimizu N, Oguchi Y. Spectrum of pathogenic mitochondrial DNA mutations and clinical features in Japanese families with Leber's hereditary optic neuropathy. *Curr Eye Res* 1998;17:403–408. [PubMed: 9561832]
11. Goto Y, Noaka L, Horai S. A mutation in the tRNA^{Leu(UUR)} gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 1990;148:651–653. [PubMed: 2102678]
12. Qu J, Li R, Tong T, Hu Y, Zhou X, Qian Y, Lu F, Guan MX. 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;328:1139–1145. [PubMed: 15707996]
13. Wallace DC. Mitochondrial diseases in man and mouse. *Science* 1999;283:1482–1488. [PubMed: 10066162]
14. Shankar SP, Fingert JH, Carelli V, Valentino ML, King TM, Daiger SP, Salomao SR, Berezovsky A, Belfort R Jr, Braun TA, Sheffield VC, Sadun AA, Stone EM. Evidence for a novel x-linked modifier locus for Leber hereditary optic neuropathy. *Ophthalmic Genet* 2008;29:17–24. [PubMed: 18363168]
15. Johns DR, Berman J. Alternative, simultaneous Complex I mitochondrial DNA mutations in Leber's hereditary optic neuropathy. *Biochem Biophys Res Commun* 1991;174:1324–1330. [PubMed: 1900003]
16. Torroni A, Petrozzi M, D'Urbano L, Sellitto D, Zeviani M, Carrara F, Carducci C, Leuzzi V, Carelli V, Barboni P, De Negri A, Scozzari R. 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;60:1107–1121. [PubMed: 9150158]
17. Brown MD, Starikovskaya E, Derbeneva O, Hosseini S, Allen JC, Mikhailovskaya IE, Sukernik RI, Wallace DC. The role of mtDNA background in disease expression: a new primary LHON mutation associated with Western Eurasian haplogroup J. *Hum Genet* 2002;110:130–138. [PubMed: 11935318]
18. Howell N, Oostra RJ, Bolhuis PA, Spruijt L, Clarke LA, Mackey DA, Preston G, Herrnstadt C. Sequence analysis of the mitochondrial genomes from Dutch pedigrees with Leber hereditary optic neuropathy. *Am J Hum Genet* 2003;72:1460–1469. [PubMed: 12736867]
19. Qu J, Li R, Tong Y, Zhou X, Lu F, Qian Y, Hu Y, Mo JQ, West CE, Guan MX. 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 Ophth Vis Sci* 2006;47:475–83.
20. Sun YH, Wei QP, Zhou X, Qian Y, Zhou J, Lu F, Qu J, Guan MX. Leber's hereditary optic neuropathy is associated with the mitochondrial ND6 T14484C mutation in three Chinese families. *Biochem Biophys Res Commun* 2006;347:221–225. [PubMed: 16806060]
21. Wei QP, Zhou X, Yang L, Sun YH, Zhou J, Li G, Jiang R, Lu F, Qu J, Guan MX. The coexistence of mitochondrial ND6 T14484C and 12S rRNA A1555G mutations in a Chinese family with Leber's hereditary optic neuropathy and hearing loss. *Biochem Biophys Res Commun* 2007;357:910–916. [PubMed: 17452034]
22. Qu J, Li R, Zhou X, Tong Y, Yang L, Chen J, Zhao F, Lu C, Qian Y, Lu F, Guan MX. Cosegregation of the ND4 G11696A mutation with the LHON-associated ND4 G11778A mutation in a four generation Chinese family. *Mitochondrion* 2007;7:140–146. [PubMed: 17300996]
23. Li R, Qu J, Zhou X, Tong Y, Lu F, Qian Y, Hu Y, Mo JQ, West CE, Guan MX. 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]

24. Qu J, Zhou X, Zhang J, Zhao F, Sun YH, Yong Y, Wei QP, Cai W, Weat CE, Guan MX. Extremely low penetrance of Leber's hereditary optic neuropathy (LHON) in eight Han Chinese families carrying the ND4 G11778A mutation. *Ophthalmology* 2009;116:558–564. [PubMed: 19167085]
25. Anderson S, Bankier AT, Barrell BG, deBruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Rose BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young I. Sequence and organization of the human mitochondrial genome. *Nature* 1981;290:457–465. [PubMed: 7219534]
26. Woischnik M, Moraes CT. Pattern of organization of human mitochondrial pseudogenes in the nuclear genome. *Genome Res* 2002;12:885–893. [PubMed: 12045142]
27. 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;26:967–973. [PubMed: 9461455]
28. Tanaka M, Cabrera VM, Gonzales AM, Larruga JM, Takeyasu T, Fuku N, Guo L-J, Hirose R, Fujita Y, Kurata M, Shinoda K-i, Umetsu K, Yamada Y, Oshida Y, Sato Y, Hattori N, Mizuno Y, Arai Y, Hirose N, Ohta S, Ogawa O, Tanaka Y, Kawamori R, Shamoto-Nagai M, Maruyama W, Shimokata H, Suzuki R, Shimodaira H. Mitochondrial genome variation in eastern Asia and the peopling of Japan. *Genom Res* 2004;14:1832–50.
29. Kong QP, Bandelt HJ, Sun C, Yao YG, Salas A, Achilli A, Wang CY, Zhong L, Zhu CL, Wu SF, Torroni A, Zhang YP. Updating the East Asian mtDNA phylogeny: a prerequisite for the identification of pathogenic mutations. *Hum. Mol. Genet* 2006;15:2076–2086. [PubMed: 16714301]
30. Young WY, Zhao L, Qian Y, Wang Q, Li N, Greinwald JH, Guan MX. Extremely low penetrance of hearing loss in four Chinese families with the mitochondrial 12S rRNA A1555G mutation. *Biochem Biophys Res Commun* 2005;328:1244–1251. [PubMed: 15708009]
31. Bibb MJ, Van Etten RA, Wright CT, Walberg MW, Clayton DA. Sequence and gene organization of mouse mitochondrial DNA. *Cell* 1981;26:167–180. [PubMed: 7332926]
32. Gadaleta G, Pepe G, De Candia G, Quagliariello C, Sbisà E, Saccone C. 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]
33. Roe A, Ma DP, Wilson RK, Wong JF. The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. *J Biol Chem* 1985;260:9759–9774. [PubMed: 4019494]
34. Chen B, Sun D, Yang L, Zhang C, Yang A, Zhu Y, Zhao J, Chen Y, Guan M, Wang X, Li R, Tang X, Wang J, Tao Z, Lu J, Guan MX. 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]
35. Johns DR, Heher KL, Miller NR, Smith KH. Leber's hereditary optic neuropathy. Clinical manifestations of the 14484 mutation. *Arch Ophthalmol* 1993;111:495–8. [PubMed: 8470982]
36. Macmillan C, Kirkham T, Fu K, Allison V, Andermann E, Chitayat D, Fortier D, Gans M, Hare H, Quercia N, Zackon D, Shoubridge EA. Pedigree analysis of French Canadian families with T14484C Leber's hereditary optic neuropathy. *Neurology* 1998;50:417–22. [PubMed: 9484365]
37. Nikoskelainen EK. Clinical pictures of LHON. *Clin Neurosci* 1994;2:115–120.
38. Seedorff T. The inheritance of Leber's disease. A genealogical follow-up study. *Acta Ophthalmol* 1985;63:135–45.
39. Guan MX, Enriquez JA, Fischel-Ghodsian N, Puranam R, Lin CP, Marion MA, Attardi G. The Deafness-associated mtDNA 7445 mutation, which affects tRNA^{Ser(UCN)} precursor processing, has long-range effects on NADH dehydrogenase ND6 subunit gene expression. *Mol. Cell. Biol* 1998;18:5868–5879. [PubMed: 9742104]
40. Yuan H, Qian Y, Xu Y, Cao J, Bai L, Shen W, Ji F, Zhang X, Kang D, Mo JQ, Greinwald JH, Han D, Zhai S, Young WY, Guan MX. Cosegregation of the G7444A mutation in the mitochondrial COI/tRNA^{Ser(UCN)} genes with the 12S rRNA A1555G mutation in a Chinese family with aminoglycoside-induced and nonsyndromic hearing loss. *Am J Med Genet* 2005;138A:133–140. [PubMed: 16152638]

Figure 1. Ten Chinese pedigrees with Leber's hereditary optic neuropathy
Vision impaired individuals are indicated by filled symbols. Arrow denotes the probands.

Table 1
Summary of clinical data for ten probands carrying the ND6 T14484C mutation

Subject	Gender	Age at test (yrs)	Age at onset (yrs)	Visual acuity right	Visual acuity left	Level of visual impairment
WZ18-□-1	F	27	25	0.05	0.04	Severe
WZ19-□-1	M	25	24	0.05	0.05	Moderate
WZ20-□-1	M	10	6	0.3	0.4	Mild
WZ21-□-1	M	17	17	0.12	0.02	Severe
WZ22-□-4	M	13	13	0.1	0.1	Mild
WZ23-□-1	M	19	19	0.04	0.04	Moderate
WZ24-□-2	F	17	17	0.03	0.04	Moderate
WZ25-□-1	M	20	20	0.1	0.4	Mild
WZ26-□-5	M	18	18	0.05	0.05	Moderate
WZ27-□-3	M	29	27	0.1	0.3	Mild

F= Female; M=Male

Table 2

mDNA Variants in Ten Chinese Pedigrees with Leber's hereditary optic neuropathy

Gene	Position	Replacement	Conservation (H/B/M/Y) ^a	CRS ^b	WZ18	WZ19	WZ20	WZ21	WZ22	WZ23	WZ24	WZ25	WZ26	WZ27	Previously reported ^c
D-loop	73	A to G		A	G	G	G	G	G	G	G	G	G	G	Yes
	143	G to A		G										A	Yes
	146	T to C		T	C										Yes
	150	C to T		C								T			Yes
	152	T to C		T	C			C		C	C			C	Yes
	185	G to A		G				A	A						Yes
	189	A to G		A				G							Yes
	195	T to C		T				C							Yes
	199	T to C		T										C	Yes
	200	A to G		A					G						Yes
	204	T to C		T				C				C			Yes
	207	G to A		G										A	Yes
	214	A to G		A							G				Yes
	234	A to G		A											Yes
	249	Del A		A				G							Yes
	263	A to G		A	G	G	G	G	G	G	Del A				Yes
	309	C to CC		C				CC						G	Yes
	310	T to CTC		T	CTC	CTC	CTC	CTC	CTC	CTC		CTC			Yes
	310	T to TC		T	TC				TC		TC			TC	Yes
	374	A to G		A											Yes
	489	T to C		T	C	C			C	C	C			C	Yes
	522	Del C		C									Del C		Yes
	523	Del A		A									Del A		Yes
	573	InsC(6)		C										InsC(6)	Yes
	16093	T to C		T								C			Yes
	16114	C to A		C						A					Yes
	16140	T to C		T									C		Yes
	16182	A to C		A											Yes

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS ^b	WZ18	WZ19	WZ20	WZ21	WZ22	WZ23	WZ24	WZ25	WZ26	WZ27	Previously reported ^c
12S rRNA	16183	A to C		A			C	C					C		Yes
	16185	C to T		C						T					Yes
	16189	T to C		T			C	C					C	C	Yes
	16194	A to C		A									C		Yes
	16223	C to T		C	T	T			T	T	T			T	Yes
	16256	C to T		C					T						Yes
	16257	C to A		C								A			Yes
	16260	C to T		C	T					T					Yes
	16261	C to T		C							T				Yes
	16265	A to C		A										C	Yes
16S rRNA	16274	G to A		G										A	Yes
	16278	C to T		C	T										Yes
	16292	C to T		C	T										Yes
	16298	T to C		T						C					Yes
	16304	T to C		T				C							Yes
	16311	T to C		T			C	C	C						Yes
	16319	G to A		G				A							Yes
	16320	C to T		C		T									Yes
	16362	T to C		T	C	C			C	C				C	Yes
	16390	G to A		G			A								Yes
16S rRNA	16519	T to C		T			C	C							Yes
	709	G to A		G											Yes
	750	A to G		A			A	A			A		A	A	Yes
	1438	A to G		A	G	G	G	G	G	G	G	G	G	G	Yes
16S rRNA	1598	G to A		G		G	G	G					A		Yes
	2706	A to G		A	G	G	G	G	G	G	G	G	G	G	Yes
	3010	G to A		G	A	A			A						Yes
	3290	T to C		T				C							Yes
tRNA ^{Leu(UUR)}															
ND1	3316	G to A (Ala to Thr)		G	A	A			A	A					Yes
	3606	A to G		A	G										Yes

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS ^b	WZ18	WZ19	WZ20	WZ21	WZ22	WZ23	WZ24	WZ25	WZ26	WZ27	Previously reported ^c
rRNA ^{Glu} ND2	4343	A to G	A/A/C/A	A	G										Yes
	4706	A to G		A			G								Yes
	4715	A to G		A			G			G					Yes
	4769	A to G		A	G	G	G	G	G	G	G	G	G		Yes
	4833	A to G (Thr to Ala)	T/I/I/L	A										G	Yes
rRNA ^{Cys} COI	4853	G to A		G							A				Yes
	4883	C to T		C	T	T		T	T						Yes
	4985	G to A		G	A		A								Yes
	5108	T to C		T										C	Yes
	5178	C to A (Leu to Met)	L/T/T/T	C	A	A		A	A						Yes
	5231	G to A		G							A				Yes
	5417	G to A		G							A				Yes
	5471	G to A		G				A							Yes
	5774	T to G	T/A/C/A	T								G			Yes
	6086	T to C		T										C	Yes
COII NC7	6221	T to C		T										C	Yes
	6752	A to G		A											Yes
	6968	C to T		C							G				Yes
	7028	C to T		C	T	T	T	T	T	T	T	T	T		No
	7196	C to A		C											Yes
	7621	T to C		T						A					Yes
	8277	T to C		T			C	C						C	Yes
	8278	C to CCCC		C			CCCC								Yes
	8271-79	9bpDel		9 bp									9bpDel		No
	8414	C to T (Leu to Phe)	L/F/M/W	C	T	T			T	T					Yes
ATP8	8498	A to G (Lys to Glu)	K/L/M/S	A									G		No
	8584	G to A (Ala to Thr)	A/V/V/T	G							A		A		Yes
	8701	A to G (Thr to Ala)	T/S/L/Q	A	G				G	G	G			G	Yes

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS ^b	WZ18	WZ19	WZ20	WZ21	WZ22	WZ23	WZ24	WZ25	WZ26	WZ27	Previously reported ^c
	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	G	G	G		Yes
	9004	C to T	C	C	T										Yes
	9071	C to T (Ser to Leu)	S/M/M/L	C											Yes
	9078	T to C	T	T					C						Yes
	9090	T to C	T	T						C					Yes
COIII	9540	T to C	T	T	C	C		C	C	C	C		C		Yes
	9896	A to G	A	A	G										Yes
	9950	T to C	T	T								C			Yes
tRNA ^{Gly}	10031	T to C	T/T/A/A	T			C	C							Yes
ND3	10208	T to C	T	T						C					Yes
	10398	A to G (Thr to Ala)	T/T/T/A	A	G	G	G	G	G	G	G	G	G		Yes
	10400	C to T	C	C	T	T			T	T	T			T	Yes
ND4L	10694	A to G	A	A								G			Yes
ND4	10873	T to C	T	T	C	C			C	C	C		C		Yes
	10978	A to G	A	A				G							Yes
	11061	C to T (Ser to Phe)	S/S/S/F	C			T	T							Yes
	11087	T to C (Phe to Leu)	F/F/F/F	T	C	C									Yes
	11368	T to C	T	T								C			Yes
	11204	T to C (Phe to Leu)	F/F/F/F	T				C							Yes
	11719	G to A	G	A	A	A	A	A	A	A	A	A	A	A	Yes
	11899	T to C	T	T				C							Yes
ND5	12358	A to G (Thr to Ala)	T/S/I/M	A				G				G			Yes
	12361	A to G (Thr to Ala)	T/L/L/L	A									G		Yes
	12372	G to A	G	G								A			Yes
	12373	A to G (Thr to Ala)	T/L/I/S	A	G										Yes
	12612	A to G	A	A				G							Yes
	12705	C to T	C	C	T	T		T	T	T	T	T		T	Yes

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS ^b	WZ18	WZ19	WZ20	WZ21	WZ22	WZ23	WZ24	WZ25	WZ26	WZ27	Previously reported ^c
ND6	12950	A to G (Asn to Ser)	N/N/N/N	A		G	G								Yes
	13104	A to G		A	G										Yes
	13269	A to G		A		G									Yes
	13512	A to G		A	G										Yes
	13681	A to G (Thr to Ala)	T/L/D/T	A		G	G								Yes
ND6	14484	T to C (Met to Val)	M/M/L/L	T	C	C	C	C	C	C	C	C	C		Yes
	14569	G to A		G									A		Yes
	14668	C to T		C	T	T		T	T	T					Yes
	14766	C to T (Thr to Ile)	T/S/T/S	C	T	T	T	T	T	T	T	T	T		Yes
Cytb	14783	T to C		T	C	C		C	C	C	C			C	Yes
	14883	C to T (Thr to Ile)	T/L/I/I	C							T				No
	14905	G to A		G	A										Yes
	14954	A to T (Thr to Ser)	T/C/C/C	A								T			Yes
	14968	T to C		T						C					Yes
	15043	G to A		G	A	A		A	A	A	A			A	Yes
	15090	T to C (Ile to Val)	I/V/V/V	T							C				Yes
	15130	C to T		C	T										No
	15172	G to A		G									A		Yes
	15204	T to C (Ile to Thr)	I/I/I/R	T					C						Yes
	15223	C to T		C								T			Yes
	15236	A to G (Ile to Val)	I/I/I/S	A				G				G			Yes
	15301	G to A		G	A	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		G	Yes
	15487	A to T		A					T		T				Yes
	15508	C to T		C											Yes
	15518	C to T		C		T							T		Yes
	15662	A to G (Ile to Val)	I/L/F/L	A								G			Yes
	15746	A to G (Ile to Val)	I/T/I/I	A										G	Yes
	15784	T to C		T							C				Yes
	15851	A to G (Ile to Val)	I/A/S/M	A									G		Yes

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS ^b	WZ18	WZ19	WZ20	WZ21	WZ22	WZ23	WZ24	WZ25	WZ26	WZ27	Previously reported ^c
------	----------	-------------	--	------------------	------	------	------	------	------	------	------	------	------	------	-------------------------------------

tRNA^{Thr}

15927 A to G

A/A/A/A

A

G

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 databases <http://www.mitomap.org> and <http://www.genpat.uu.se/mitDB/>

Table 3

Summary of clinical and molecular data for fourteen Chinese families carrying the T14484C mutation

Pedigree	Ratio (affected male/female)	Average Age of onset (y)	Number of matrilineal relatives	Penetrance (%) ^a	mtDNA haplogroup
WZ18	0:1	25	17	5.9	D4
WZ19	1:0	24	13	7.7	D4g1b
WZ20	1:0	6	9	11.1	R11
WZ21	1:0	17	6	16.7	R11a
WZ22	1:0	13	7	14.3	D4
WZ23	1:0	19	13	7.7	D4
WZ24	0:1	17	11	9.1	Z3
WZ25	1:0	20	6	16.7	N9a
WZ26	1:0	18	12	8.3	B5b
WZ27	1:0	27	21	4.8	G3a2
WZ13 ^b	1:2	24	20	60	–
WZ14	1:1.2	14	27	48	–
WZ15	1:1	19	15	27	–
WZ16 ^c	1:1	12.5	11	36	H2

^aPenetrance: affected matrilineal relatives/total matrilineal relatives

^bSun et al.(2006)

^cWei et al.(2007)