Leber's hereditary optic neuropathy is potentially associated with a novel m.5587T>C mutation in two pedigrees

YANCHUN JI[1,2], LIHUA QIAO[1,2], XIAOYANG LIANG[1,2], LING ZHU[1,2], YINGLONG GAO[1,2], JUANJUAN ZHANG[3,4], ZIDONG JIA[2], QI-PING WEI[5], XIAOLING LIU[3,4], PINGPING JIANG[1,2] and MIN-XIN GUAN[1,2,6]

[1]Division of Clinical Genetics and Genomics, The Children's Hospital; [2]Institute of Genetics, Zhejiang University and Department of Genetics, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310058; [3]School of Ophthalmology and Optometry, Wenzhou Medical University, Wenzhou, Zhejiang 325025; [4]Attardi Institute of Mitochondrial Biomedicine, Wenzhou Medical University, Wenzhou, Zhejiang 325027; [5]Department of Ophthalmology, Beijing University of Chinese Medicine, Beijing 100029; [6]Joining Institute of Genetics and Genomic Medicine Between Zhejiang University and University of Toronto, Hangzhou, Zhejiang 310058, P.R. China

Received December 30, 2016; Accepted July 21, 2017

DOI: 10.3892/mmr.2017.7734

Abstract. Mitochondrial (mt)DNA mutations have been revealed to be associated with Leber's hereditary optic neuropathy (LHON). The present study conducted clinical, genetic and molecular evaluations of two Han Chinese families. A total of 4 (3 men and 1 female) out of 14 matrilineal relatives in the families exhibited visual impairment with variable severity and age of onset. The average age of onset of visual loss was 20.5 years old. Molecular analysis of the complete mitochondrial genome in these pedigrees demonstrated that the three primary mutations associated with LHON were not detected; however, the homoplasmic m.5587T>C mutation was identified, which was localized at the end of the mitochondrially encoded transfer (t)RNA alanine gene and may alter the tertiary structure of this tRNA. Subsequently, this structural alteration may result in tRNA metabolism failure. In addition, distinct sets of mtDNA polymorphisms belonging to haplogroup F1 were detected in both families tested. The findings of the present study suggested that the m.5587T>C mutation may be involved in the pathogenesis of visual impairment. In addition, the mtDNA variant m.15024G>A(p.C93H) in the mitochondrially encoded cytochrome B gene was detected in both families, which exhibited evolutionary conservation, indicating it may serve a potential modifying role in the development of visual impairment associated with m.5587T>C mutation in these families. Furthermore, other modifying factors, including nuclear modifier genes, and environmental and personal factors may also contribute to the development of LHON in subjects carrying this mutation.

Correspondence to: Dr Yanchun Ji, Division of Clinical Genetics and Genomics, The Children's Hospital, Zhejiang University School of Medicine, 866 Yuhangtang Road, Hangzhou, Zhejiang 310058, P.R. China
E-mail: yanchunji@zju.edu.cn

Key words: Leber's hereditary optic neuropathy, mitochondrial DNA, Chinese, visual loss, mutation

Introduction

Leber's hereditary optic neuropathy (LHON) is a neurodegenerative eye disorder, which is clinically characterized by rapid, painless, bilateral central visual loss, that commonly affects young adults (1-4). The majority of cases of LHON are the result of one of three primary mutations: m.11778G>A/mitochondrially encoded NADH:ubiquinone oxidoreductase core *MT-ND4*, m.3460G>A/*MT-ND1* or m.14484T>C/*MT-ND6* (5-7), whereas the remaining 5% of cases are caused by rare mitochondrial DNA (mtDNA) mutations and/or other factors. At present, ~40 mutations (www.mitomap.org/foswiki/bin/view/MITOMAP/MutationsLHON), which mainly occur in complex I, have been associated with LHON. Therefore, mtDNA mutations are considered the molecular basis for LHON disease (5,8), and they often present near or at homoplasmy. However, male bias and incomplete penetrance are typical clinical characteristics of LHON, thus indicating that LHON has a complex etiology (9,10). Therefore, mtDNA mutations are considered insufficient to result in the phenotypic expression of LHON, and other modifying determinants, including mitochondrial haplotypes and nuclear genetic backgrounds, and environmental factors, are likely to modulate the phenotypic manifestation of LHON-associated common or rare mtDNA mutations (11-14).

LHON is the first disorder that was recognized to be maternally inherited, and is the first to have been attributed to a point mutation in mtDNA (5). In previous investigations, besides the three primary mutations (m.11778G>A, m.3460G>A, m.14484T>C), we identified LHON-associated m.3394T>C/*MT-ND1*,m.3635G>A/*MT-ND1*,m.3866T>C/*MT-ND1*, m.11696G>A/*MT-ND4*, m.12238T>C/*MT-ND5* and m.14502T>C/*MT-ND6* mutations (15-19). In the present

study, the clinical, genetic and molecular features of two Chinese families with maternally transmitted LHON were investigated; the m.5587T>C mutation in the mitochondrially encoded transfer (t)RNA alanine (*MT-TA*) gene was detected in the families, which lacked the three known primary mutations. To elucidate the role of other genetic factors, including mitochondrial haplotype, in the phenotypic expression of the m.5587T>C mutation, 24 overlapping fragments were used to perform polymerase chain reaction (PCR) amplification of fragments spanning the entire mitochondrial genome and a DNA sequence analysis was subsequently conducted.

Materials and methods

Subjects and ophthalmologic examinations. As shown in Fig. 1, the patients from two Han Chinese families (HZL001 and HZL002) were recruited at the School of Ophthalmology and Optometry, Wenzhou Medical University (Wenzhou, China) and at Dongfang Hospital (Beijing, China). A total of 376 control DNA samples were obtained from a panel of unaffected subjects with Han Chinese ancestry from the same region. The ophthalmic examinations of the probands and other matrilineal relatives, including visual field test, visual acuity examination, fundus photography, visual evoked potentials and determination of the degree of visual impairment, as well as other clinical evaluations, were conducted as described previously (11-13). Blood samples were also obtained from the participants. Written informed consent was obtained from the probands and other affected relatives evaluated, and the present study was approved by the Ethic Committees of Wenzhou Medical University and Zhejiang University (Hangzhou, China).

mtDNA mutational analysis. Genomic DNA was isolated from the whole blood samples of participants and controls using QIAamp DNA Blood Mini kit (cat. no. 51104; Qiagen, Inc., Valencia, CA, USA). The presence of the m.11778G>A, m.3460G>A and m.14484T>C mutations was detected as previously described (11-13). Briefly, DNA fragments of probands and affected members spanning these mtDNA mutations were amplified by PCR using oligodeoxynucleotides corresponding to mtDNA at positions 11,654 to 11,865 for the m.11778G>A mutation, 3,108 to 3,717 for the m.3460G>A mutation, and 14,260 to 14,510 for the m.14484T>C mutation (20). Each fragment was purified by PCR clean-up (cat. no. AP-PCR-50; Axygen; Corning Incorporated, Corning, NY, USA) and subsequently analyzed by direct sequencing in an ABI 3700 automated DNA sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) using the Big Dye Terminator Cycle sequencing reaction kit (Thermo Fisher Scientific, Inc.). The three fragment sequence results were compared with the updated consensus Cambridge sequence (GenBank accession no. NC_012920) (13).

For detecting the m.5587T>C mutation in the *MT-TA* gene, PCR amplification using oligodeoxynucleotides corresponding to mtDNA at positions 5,238-6,050 was conducted. The primer pair sequences for PCR were as follows: Forward: 5'-CTAACCGGCTTTTTGCCC-3' and reverse 5'-ACCTAG AAGGTTGCCTGGCT-3', which were designed according to a previously described method (20). Subsequently, this fragment was purified and analyzed as aforementioned.

Haplogroup analyses and phylogenetic analysis. The mtDNA sequences of 17 different vertebrates were used to conduct an interspecific analysis as reported previously (11-13). The conservation index (CI) was calculated by comparing the human nucleotide variants with the other 16 vertebrates. The CI was used to indicate the percentage of species from the list of 16 different vertebrates that have the wild-type nucleotide at the same position.

The Asian mitochondrial haplogroup of these two probands were determined using online software (www.mitotool.org/genomeRSRS.html) or based on the nomenclature of mitochondrial haplogroups previously reported (21,22).

Results

Clinical presentation. Clinical data for the two Chinese probands are summarized in Table I. The four individuals that were affected by LHON in the two pedigrees comprised one woman and three men. Comprehensive medical histories of the probands confirmed that they suffered from no other clinical abnormalities, including hearing dysfunction, muscular diseases, diabetes and neurological diseases. In pedigree HZL001, the proband (III-5) was first examined at the age of 17 at the Ophthalmology Clinic of Wenzhou Medical University. He started to suffer from bilateral visual loss at the age of 15; he observed a dark cloud in the center of his vision and had difficulty discerning colors, which all appeared dark grey. Visual field test detected a large centrocecal scotomata in both eyes. His visual acuity was oculus dexter, 0.02; and oculus sinister, 0.05. Fundus examination demonstrated that both of his optic discs were abnormal: Vascular tortuosity of the central retinal vessels, circumpapillary telangiectatic microangiopathy and swelling of the retinal nerve fiber layer were detected. These findings indicated that the patient exhibited the typical clinical features of LHON. In addition, his uncle (II-1) and grandmother (I-2) exhibited visual loss, as presented in Table I. In family HZL002, the proband (III-1) exhibited bilateral visual loss from the age of 6. He was diagnosed with LHON by the Ophthalmology Clinic at Beijing University of Chinese Medicine and Pharmacology (Beijing, China). The visual field test detected a large centrocecal scotomata in both eyes. His visual acuity was 0.1 in both eyes. In addition, both optic discs were abnormal, as determined by fundus examination: Vascular tortuosity of the central retinal vessels, circumpapillary telangiectatic microangiopathy and swelling of the retinal nerve fiber layer were detected. These findings indicated that the patient exhibited the typical clinical features of LHON. Conversely, none of the other six matrilineal relatives in HZL001 exhibited visual loss.

mtDNA analysis. To explore the molecular basis of visual loss in the two pedigrees, a mutational analysis of the mitochondrial genome in the two Chinese families was conducted. Initially, three well known LHON-associated mtDNA mutations (m.11778G>A, m.3460G>A and m.14484T>C) were detected by PCR amplification and restriction enzyme digestion analysis

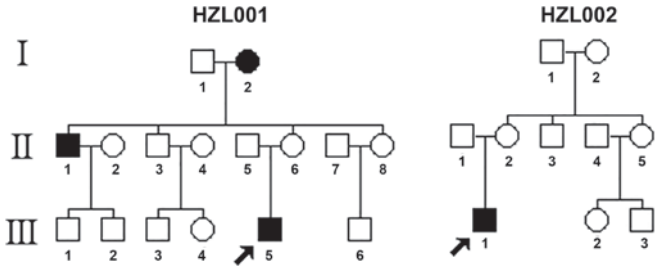

Figure 1. Two Chinese pedigrees with Leber's hereditary optic neuropathy. Vision-impaired individuals are indicated by filled symbols; females are indicated by circles; males are indicated by squares; arrows indicate the probands.

of PCR fragments derived from each proband (data not shown). The m.11778G>A, m.3460G>A and m.14484T>C mutations were not detected. Subsequently, PCR amplification of fragments spanning the entire mitochondrial genome and a DNA sequence analysis was conducted using DNA obtained from the two probands and the affected matrilineal relatives. The homoplasmic m.5587T>C mutation was detected (Fig. 2A). The m.5587T>C mutation refers to a T to C transition at position 5,587 in the *MT-TA* gene, which is localized in the end of the *MT-TA* gene (position 73; Fig. 2B), and was detected in the two pedigrees evaluated in the present study. As shown in Table II, this point at position 73 in the *MT-TA* gene is highly conserved among all 17 organisms analyzed, with the exception of *Hylobates lar*. Notably, the m.5587T>C mutation has been reported to be associated with progressive unstable gait, dysarthria, hearing loss, muscle cramps and myalgia (23,24). Further analysis of this gene fragment sequence, demonstrated that the homoplasmic m.5587T>C mutation was detected in matrilineal relatives of the two families but not in non-matrilineal relatives (data not shown). Furthermore, none of the 376 unrelated Chinese control subjects carried the m.5587T>C mutation.

As presented in Tables II and III, in addition to the m.5587T>C mutation, which exhibited evolutionary conservation among 17 organisms, there are many mtDNA polymorphic loci in the two families investigated in the present study. Of the other identified nucleotide alterations in these two mitochondrial genomes, there were 15 known variants in the D-loop, two known variants in the 12S ribosomal (r)RNA gene, one known variant in the 16S rRNA gene, 23 silent variants (one novel) in protein-encoding genes, and seven missense mutations in protein-encoding genes. The missense mutations were as follows: m.8860A>G(p.T112A) in the mitochondrially encoded ATP synthase 6 gene, m.10609T>C(p.M47T) in the *MT-ND4 L* gene, m.12406G>A(p.V24I) and m.13928G>C(p.S531T) in the *MT-ND5* gene, m.14766C>T(p.T7I), m.15024G>A (p.C93H) and m.15326A>G(p.T194A) in the mitochondrially encoded cytochrome B (*MT-CYB*) gene. Subsequently, these RNA and polypeptide variants were further assessed by phylogenetic analysis of these variants and sequences from other organisms, including mice (25), cows (26) and *Xenopus laevis* (27). The mtDNA variant m.15024G>A(p.C93H) in the *MT-CYB* gene exhibited evolutionary conservation among the four organisms. However, none of the other variants exhibited evolutionary conservation, suggesting that these variants may not be functionally significant. Based on the nomenclature of mitochondrial haplogroups, the mtDNA sequence variations among two Chinese probands were used to establish the haplogroup affiliation of each mtDNA (23,24). In the present study, the mtDNAs of the two pedigrees belonged to haplogroup F1.

Discussion

In the present study, the genetic, clinical and molecular features of two Chinese pedigrees with LHON were reported. The primary characteristic of LHON is bilateral visual loss, which was only present in the maternal lineage of the pedigrees evaluated, thus suggesting that mtDNA mutations are the molecular basis for this disorder. Sequence analysis of the complete mitochondrial genomes of these pedigrees demonstrated that the three primary mutations associated with LHON were not present; however, distinct mtDNA polymorphisms and the m.5587T>C mutation in the *MT-TA* gene were detected in both pedigrees. Notably, this homoplasmic mutation was only present in the maternal lineage of the pedigrees, but not in the other members of these families. Position 73 is evolutionarily conserved in the *MT-TA* gene. This mutation may influence the 3' end sequences of the amino acid arm of tRNA; amino acids linked affect the tRNA structure and infer structural changes. Therefore, this mutation may affect the efficiency of amino acid translation, hinder protein synthesis and induce mitochondrial dysfunction; in particular it may affect encoding of the compound enzyme that initiates mitochondrial oxidative phosphorylation required for respiratory chain and enzyme activity. In response to abnormal oxidative phosphorylation, a series of pathological alterations may be induced, including production of oxygen free radicals and a reduction in the use of nitric oxide. In addition, this mutation may have a potential modifying role in deafness by worsening m.7505T>C mutation-induced mitochondrial dysfunction (23). Furthermore, in a previous study, a 28-year-old woman that carried this mutation presented with a 16-year history of progressive unstable gait, dysarthria, hearing loss, muscle cramps and myalgia (24). Notably, in the present study, the presence of the m.5587T>C mutation in the two genetically different pedigrees, both influenced by visual loss, indicated that this mutation may be associated with the pathogenesis of visual loss.

In these two families, the age of onset for visual impairment ranged between 6 and 32 years old (average, 20.5 years old). Previous studies confirmed that the age of onset for visual impairment in the two pedigrees harboring the m.5587T>C mutation was similar to that in other Chinese families with LHON carrying the m.11778G>A mutation (28-32). In contrast, a number of Chinese subjects carrying the m.11778G>A mutation, which exhibited profound visual loss, the affected subjects carrying the m.5587T>C mutation suffered from mild to profound visual impairment, similar to that exhibited in patients carrying m.3394T>C, m.3635G>A, m.3866T>C, m.11696G>A, m.12238T>C and m.14502T>C mutations (15-19,33). Furthermore, there is a high penetrance of visual loss in some Chinese subjects harboring the m.11778G>A mutation (28,29,31-34); however, the penetrance of visual impairment was very low in the two Chinese

Table I. Summary of clinical data for four patients with Leber's hereditary optic neuropathy from two pedigrees carrying the m.5587T>C mutation.

Subject	Sex	Age of test (years)	Age of onset (years)	Visual acuity, right[a]	Visual acuity, left[a]	Level of visual impairment
HZL001-III-5	F	17	15	0.02	0.05	Severe
HZL001-II-1	M	42	29	0.01	CF/30 cm	Profound
HZL001-I-2	M	65	32	0.2	0.1	Mild
HZL002-III-1	M	11	6	0.1	0.1	Moderate

[a]According to the national standard visual acuity chart (41). F, female; M, male; CF, counting fingers.

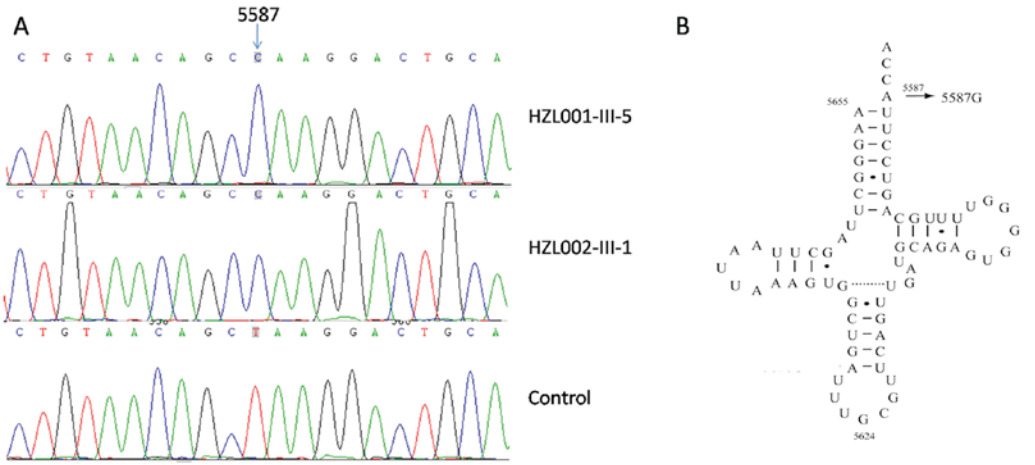

Figure 2. (A) Identification of the m.5587T>C mutation in the *MT-TA* gene. Partial sequence chromatograms of the *MT-TA* gene from two affected individuals and one Chinese control. The arrow indicates the location of the base change at position 5,587. (B) Location of the m.5587T>C mutation in the *MT-TA* gene. Arrow indicates the position of the m.5587T>C mutation. *MT-TA*, mitochondrially encoded transfer tRNA alanine.

pedigrees carrying the m.5587T>C mutation. In addition, the m.5587T>C mutation was not detected in the 376 Chinese control subjects. Similar to the three common mutations, and m.3394T>C, m.33635G>A, m.3866T>C, m.11696G>A, m.12238T>C and m.14502T>C mutations (15-19), the incomplete penetrance of visual loss associated with the m.5587T>C mutation, and the lack of the mutation in any of the 376 control subjects, indicated that the m.5587T>C mutation is itself insufficient to result in the clinical phenotype. These findings suggested that other modifying factors, including mitochondrial haplotype, environmental factors and nuclear background are required for the phenotypic manifestation of the m.5587T>C mutation. In particular, mitochondrial haplotypes have been reported to influence the penetrance and expressivity of visual loss associated with primary mtDNA mutations. Therefore, secondary LHON mutations, such as m.4216T>C and m.13708G>A, have been implicated to increase the penetrance of LHON-associated primary m.11778G>A or m.14484T>C mutations (35). In addition, in a large cohort of subjects with European ancestry, the mitochondrial haplogroup J was able to affect the phenotypic manifestation of LHON-associated m.11778G>A and m.14484T>C mutations (36,37). Furthermore, the m.11696G>A, m.14502T>C, m.15951A>G and m.4435A>G mutations have been reported to increase LHON penetrance in Chinese subjects (28,31,38). In the two pedigrees evaluated in the present study, the mtDNA variants p.C93H in the *MT-CYB* gene exhibited evolutionary conservation; however, other known mutations were not detected in the LHON pedigrees. This mtDNA variant may have a potential modifying role in the development of visual impairment associated with the m.5587T>C mutation in these two pedigrees.

It has been suggested that mitochondrial haplotypes may affect the penetrance and expressivity of LHON associated with the three primary mutations (m.11778G>A, m.3460G>A and m.14484T>C). In European pedigrees, mitochondrial haplogroup J may raise the risk of LHON-associated m.11778G>A or m.14484T>C mutations (14,39), whereas haplogroup K may aggrandize the penetrance of primary LHON-associated m.3460G>A mutation (14). Conversely, haplogroup H may reduce the risk of LHON-associated m.11778G>A mutation (14). In addition, the haplogroup M7b1'2 significantly increases the occurrence of LHON, whereas the haplogroup M8a has a protective effect in Chinese pedigrees (40). In the two families analyzed in the present study, the mitochondrial genomes of the HZL001 and HZL002 pedigrees belonged to the Eastern Asian haplogroup F1. However, since data from only two families were analyzed, and due to the lack of a large data analysis, the role the mitochondrial haplotype serves in the two families cannot be fully

Table II. Alignment of the *MT-TA* gene from 17 different species. Position 73 is the location of the m.5587T>C mutation.

Species	Acc-stem, pos 1	Pos 8	D-stem, pos 10	D-loop, pos 14	D-stem, pos 22	Pos 26	Ac-stem, pos 27	Anticd-loop, pos 32	Ac-stem, pos 39	V-region, pos 44	T-stem, pos 49	T-loop, pos 54	T-stem, pos 61	Acc-stem, pos 66	CCA tail, pos 73
Cebus albifrons	GAGGGCT	TA	GCTT	AATTA	AAGT	A	GTTGA	TTTGCGT	TCAAT	TGAT	GCAAG	GTATAG	TTTGC	AGTCCTT	A
Cercopithecus aethiops	AGGGGCT	TA	GCTT	AATTA	AAGT	G	GTTGA	TTTGCGT	TCAAT	TGAT	GCAGA	GTAGGTT	TTTGC	AGTCCTT	A
Colobus guereza	AAGGGCT	TA	GCTT	AATGA	AAGT	G	ATTGA	TTTGCGT	TCAGT	TGAT	GCAGA	GTAGAGT	TTTGC	AGTCCTT	A
Gorilla gorilla	AAGGGCT	TA	GCTT	AATTA	AAGT	G	GCTGA	TTTGCGT	TCAGT	TGAT	GCAGA	GTAGGGT	TTTGC	AGTCCTT	A
Homo sapiens	AAGGGCT	TA	GCTT	AATTA	AAGT	G	GCTGA	TTTGCGT	TCAGT	TGAT	GCAGA	GTGGGGT	TTTGC	AGTCCTT	A
Hylobates lar	AAGGGCT	TA	GCTT	AATTA	AAGT	G	ACTGA	TTTGCGT	TCGGT	TGAT	GCAAA	GTGGGC	TTTGC	AGTCCTT	G
Lemur catta	GAGGATT	TA	GCTT	AATTA	AAGT	G	ATTGA	TTTGCGT	TCAGT	TGAT	GTAAG	ATATAAT	CTTGC	AGTCCTT	A
Macaca mulatta	AAGGGCT	TA	GCTT	AATTA	AAGT	G	GTTGA	TTTGCGT	TCAAT	TGAT	GCAGA	GTAGGTG	TTTGC	AGTCCTT	A
Macaca sylvanus	AAGGGCT	TA	GCTT	AATTA	AAGT	A	GTTGA	TTTGCGT	TCAAT	TGAT	GCAGA	GCAGGTG	TTTGC	AGTCCTT	A
Nycticebus coucang	GAGGACT	TA	GCTT	AATTA	AAGT	A	ATTGA	TTTGCGT	TCAGT	TGAT	GTAGG	AGAAGT	CTTGC	AGTCCTT	A
Pan paniscus	AAGGGCT	TA	GCTT	AATTA	AAGT	G	GCTGA	TTTGCGT	TCAGT	TGAT	GCAGA	GTGGGGT	TTTGC	AGTCCTT	A
Pan troglodytes	AAGGGCT	TA	GCTT	AATTA	AAGT	G	GCTGA	TTTGCGT	TCAGT	TGAT	GCAGA	GTGGGGT	TTTGC	AGTCCTT	A
Papio hamadryas	AAGGGCT	TA	GTTT	AATTA	AAGC	G	ATTGA	TTTGCGT	TCAGT	TGAT	GCGGA	GTAGGTG	TCTGC	AGTCCTT	A
Pongo pygmaeus	GAGGGCT	TA	GCTT	AATTA	AAGT	G	GCTGA	TTTGCGC	TCAGT	TGAT	GCAAA	GTGGGGT	TTTGC	AGTCCTT	A
Pongo pygmaeus abelii	GAGGGCT	TA	GCTT	AATTA	AAGT	G	GCTGG	TTTGCGT	TCAGT	TGAT	GCAGA	GCGGGGC	TTTGC	AGTCCTT	A
Tarsius bancanus	GAGGACT	TA	GCTT	AAGTTA	AAGT	A	GCTAA	TTTGCAG	TTAGT	TGAT	GTAGA	GTGAGTC	TTTGC	AGTCCTT	A
Trachypithecus bscurus	AAGGGCT	TA	GCTT	AATTA	AAGT	A	ACTGG	TTTGCGT	TCAGT	TGAT	GCAGA	ATGAGAT	TCTGT	AGTCCTT	A

Pos, position.

Table III. mtDNA variants in two Chinese families with Leber's hereditary optic neuropathy.

Gene	Position	Replacement	Conservation (H/B/M/X)	CRS	HZL001	HZL002	Previously reported[a]
D-loop	73	A-G		A	G	G	Yes
	150	C-T		C		T	Yes
	195	T-C		T		C	Yes
	204	T-C		T	C		Yes
	207	G-A		G	A		Yes
	215	A-G		A	G		Yes
	249	delA		A	del	del	Yes
	263	A-G		A	G	G	Yes
	310	T-CTC		T	CTC	CTC	Yes
	522	Del C		C	Del		Yes
	523	Del A		A	Del		Yes
	16,183	A-C		A	C		Yes
	16,189	T-C		T	C		Yes
	16,304	T-C		T	C	C	Yes
	16,519	T-C		T	C	C	Yes
MT-RNR1	750	A-G	A/A/A/-	A	G	G	Yes
	1,438	A-G	A/A/A/G	A	G	G	Yes
MT-RNR2	2,706	A-G	A/G/A/A	A	G	G	Yes
MT-ND1	3,970	C-T		C	T	T	Yes
MT-ND2	4,769	A-G		A	G	G	Yes
	5,201	T-C		T	C		Yes
MT-TA	5,587	T-C	T/T/-/T	T	C	C	Yes
MT-CO1	6,182	G-A		G		A	Yes
	6,392	T-C		T	C	C	Yes
	6,962	G-A		G	A	A	Yes
	7,028	C-T		C	T	T	Yes
MT-CO2	8,149	A-G		A		G	Yes
	8,152	G-A		G	A		Yes
MT-ATP6	8,860	A-G(Thr-Ala)	T/A/A/T	A	G	G	Yes
	9,165	T-C		T		C	Yes
MT-CO3	10,310	G-A		G	A	A	Yes
MT-ND4L	10,490	T-C		T	C		Yes
	10,609	T-C(Met-Thr)	M/T/T/T	T	C	C	Yes
MT-ND4	11,471	C-T		C		T	Yes
	11,719	G-A		G	A	A	Yes
MT-ND5	12,406	G-A(Val-Ile)	V/F/S/F	G	A	A	Yes
	12,882	C-T		C	T	T	Yes
	13,707	G-A		G	A		Yes
	13,928	G-C(Ser-Thr)	S/T/S/T	G	C	C	Yes
MT-CYB	14,766	C-T(Thr-Ile)	T/S/T/S	C	T	T	Yes
	15,024	G-A(Cys-His)	C/C/C/C	G	A	A	No
	15,326	A-G(Thr-Ala)	T/M/I/I	A	G	G	Yes

Conservation of amino acids in polypeptides or nucleotides in RNA in humans (H), cows (B), mice (M) and *Xenopus laevis* (X). CRS, Cambridge reference sequence. [a]As presented in online mitochondrial genome databases: www.mitomap.org and www.genpat.uu.se/mtDB.

explained. In addition, nuclear modifier genes, including the tyrosyl-tRNA synthetase 2 gene in Chinese families (11), or environmental factors, may have an important role in the phenotypic expression of the LHON-associated m.5587T>C mutation in the two Chinese pedigrees. Thus, the results of the present study may provide novel insights into the understanding of clinical diagnosis and valuable information on the management of LHON.

Acknowledgements

The present study was supported by grants from the Natural Science Foundation of China (grant nos. 31471191, 81400434 and 31601025); the College Students' Science and Technology Innovation (Xinmiao Talents Program) in Zhejiang province (grant no. 2015R401217); and a general financial grant from China Postdoctoral Science Foundation (grant no. 2016M591986).

References

1. Rasool N, Lessell S and Cestari DM: Leber hereditary optic neuropathy: Bringing the lab to the clinic. Semin Ophthalmol 31: 107-116, 2016.
2. Kirches E: LHON: Mitochondrial mutations and more. Curr Genomics 12: 44-54, 2011.
3. Yu-Wai-Man P, Griffiths PG, Hudson G and Chinnery PF: Inherited mitochondrial optic neuropathies. J Med Genet 46: 145-158, 2009.
4. Carelli V, La Morgia C, Valentino ML, Barboni P, Ross-Cisneros FN and Sadun AA: Retinal ganglion cell neurodegeneration in mitochondrial inherited disorders. Biochim Biophys Acta 1787: 518-528, 2009.
5. Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Elsas LJ II and Nikoskelainen EK: Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242: 1427-1430, 1988.
6. Huoponen K, Vilkki J, Aula P, Nikoskelainen EK and Savontaus ML: A new mtDNA mutation associated with Leber hereditary optic neuroretinopathy. Am J Hum Genet 48: 1147-1153, 1991.
7. Johns DR, Neufeld MJ and Park RD: An ND-6 mitochondrial DNA mutation associated with Leber hereditary optic neuropathy. Biochem Biophys Res Commun 187: 1551-1557, 1992.
8. Howell N: LHON and other optic nerve atrophies: The mitochondrial connection. Dev Ophthalmol 37: 94-108, 2003.
9. Yu-Wai-Man P, Griffiths PG and Chinnery PF: Mitochondrial optic neuropathies-disease mechanisms and therapeutic strategies. Prog Retin Eye Res 30: 81-114, 2011.
10. Qu J, Li R, Tong Y, Hu Y, Zhou X, Qian Y, Lu F and 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 328: 1139-1145, 2005.
11. Jiang P, Jin X, Peng Y, Wang M, Liu H, Liu X, Zhang Z, Ji Y, Zhang J, Liang M, *et al*: The exome sequencing identified the mutation in YARS2 encoding the mitochondrial tyrosyl-tRNA synthetase as a nuclear modifier for the phenotypic manifestation of Leber's hereditary optic neuropathy-associated mitochondrial DNA mutation. Hum Mol Genet 25: 584-596, 2016.
12. Jiang P, Liang M, Zhang J, Gao Y, He Z, Yu H, Zhao F, Ji Y, Liu X, Zhang M, *et al*: Prevalence of mitochondrial ND4 mutations in 1281 Han Chinese subjects with leber's hereditary optic neuropathy. Invest Ophthalmol Vis Sci 56: 4778-4788, 2015.
13. Liang M, Jiang P, Li F, Zhang J, Ji Y, He Y, Xu M, Zhu J, Meng X, Zhao F, *et al*: Frequency and spectrum of mitochondrial ND6 mutations in 1218 Han Chinese subjects with leber's hereditary optic neuropathy. Invest Ophthalmol Vis Sci 55: 1321-1331, 2014.
14. Hudson G, Carelli V, Spruijt L, Gerards M, Mowbray C, Achilli A, Pyle A, Elson J, Howell N, La Morgia C, *et al*: Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA-haplogroup background. Am J Hum Genet 81: 228-233, 2007.
15. Liang M, Guan M, Zhao F, Zhou X, Yuan M, Tong Y, Yang L, Wei QP, Sun YH, Lu F, *et al*: Leber's hereditary optic neuropathy is associated with mitochondrial ND1 T3394C mutation. Biochem Biophys Res Commun 383: 286-292, 2009.
16. Zhang J, Jiang P, Jin X, Liu X, Zhang M, Xie S, Gao M, Zhang S, Sun YH, Zhu J, *et al*: Leber's hereditary optic neuropathy caused by the homoplasmic ND1 m.3635G>A mutation in nine Han Chinese families. Mitochondrion 18: 18-26, 2014.
17. Zhou X, Qian Y, Zhang J, Tong Y, Jiang P, Liang M, Dai X, Zhou H, Zhao F, Ji Y, *et al*: Leber's hereditary optic neuropathy is associated with the T3866C mutation in mitochondrial ND1 gene in three Han Chinese families. Invest Ophthalmol Vis Sci 53: 4586-4594, 2012.
18. Zhou X, Wei Q, Yang L, Tong Y, Zhao F, Lu C, Qian Y, Sun Y, Lu F, Qu J and Guan MX: Leber's hereditary optic neuropathy is associated with the mitochondrial ND4 G11696A mutation in five Chinese families. Biochem Biophys Res Commun 340: 69-75, 2006.
19. Zhao F, Guan M, Zhou X, Yuan M, Liang M, Liu Q, Liu Y, Zhang Y, Yang L, Tong Y, *et al*: Leber's hereditary optic neuropathy is associated with mitochondrial ND6 T14502C mutation. Biochem Biophys Res Commun 389: 466-472, 2009.
20. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM and Howell N: Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23: 147, 1999.
21. Kong QP, Bandelt HJ, Sun C, Yao YG, Salas A, Achilli A, Wang CY, Zhong L, Zhu CL, Wu SF, *et al*: Updating the East Asian mtDNA phylogeny: A prerequisite for the identification of pathogenic mutations. Hum Mol Genet 15: 2076-2086, 2006.
22. Tanaka M, Cabrera VM, González AM, Larruga JM, Takeyasu T, Fuku N, Guo LJ, Hirose R, Fujita Y, Kurata M, *et al*: Mitochondrial genome variation in eastern Asia and the peopling of Japan. Genome Res 14: 1832-1850, 2004.
23. Tang X, Li R, Zheng J, Cai Q, Zhang T, Gong S, Zheng W, He X, Zhu Y, Xue L, *et al*: Maternally inherited hearing loss is associated with the novel mitochondrial tRNA Ser (UCN) 7505T>C mutation in a Han Chinese family. Mol Genet Metab 100: 57-64, 2010.
24. Crimi M, Sciacco M, Galbiati S, Bordoni A, Malferrari G, Del Bo R, Biunno I, Bresolin N and Comi GP: A collection of 33 novel human mtDNA homoplasmic variants. Hum Mutat 20: 409, 2002.
25. Bibb MJ, Van Etten RA, Wright CT, Walberg MW and Clayton DA: Sequence and gene organization of mouse mitochondrial DNA. Cell 26: 167-180, 1981.
26. Gadaleta G, Pepe G, De Candia G, Quagliariello C, Sbisà E and Saccone C: The complete nucleotide sequence of the *Rattus norvegicus* mitochondrial genome: Cryptic signals revealed by comparative analysis between vertebrates. J Mol Evol 28: 497-516, 1989.
27. Roe BA, Ma DP, Wilson RK and Wong JF: The complete nucleotide sequence of the Xenopus laevis mitochondrial genome. J Biol Chem 260: 9759-9774, 1985.
28. Qu J, Li R, Zhou X, Tong Y, Lu F, Qian Y, Hu Y, Mo JQ, West CE and Guan MX: The novel A4435 G mutation in the mitochondrial tRNAMet may modulate the phenotypic expression of the LHON-associated ND4 G11778A mutation. Invest Ophthalmol Vis Sci 47: 475-483, 2006.
29. Qu J, Li R, Zhou X, Tong Y, Yang L, Chen J, Zhao F, Lu C, Qian Y, Lu F and Guan MX: Cosegregation of the ND4 G11696A mutation with the LHON-associated ND4 G11778A mutation in a four generation Chinese family. Mitochondrion 7: 140-146, 2007.
30. Qu J, Zhou X, Zhang J, Zhao F, Sun YH, Tong Y, Wei QP, Cai W, Yang L, West CE and Guan MX: Extremely low penetrance of Leber's hereditary optic neuropathy in 8 Han Chinese families carrying the ND4 G11778A mutation. Ophthalmology 116: 558-564. e3, 2009.
31. Li R, Qu J, Zhou X, Tong Y, Hu Y, Qian Y, Lu F, Mo JQ, West CE and 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 376: 79-86, 2006.
32. Xie S, Zhang J, Sun J, Zhang M, Zhao F, Wei QP, Tong Y, Liu X, Zhou X, Jiang P, *et al*: Mitochondrial haplogroup D4j specific variant m.11696G>A(MT-ND4) may increase the penetrance and expressivity of the LHON-associated m.11778G>A mutation in Chinese pedigrees. Mitochondrial DNA A DNA Mapp Seq Anal 28: 434-441, 2017.
33. Liu XL, Zhou X, Zhou J, Zhao F, Zhang J, Li C, Ji Y, Zhang Y, Wei QP, Sun YH, *et al*: Leber's hereditary optic neuropathy is associated with the T12338C mutation in mitochondrial ND5 gene in six Han Chinese families. Ophthalmology 118: 978-985, 2011.
34. Zhou X, Zhang H, Zhao F, Ji Y, Tong Y, Zhang J, Zhang Y, Yang L, Qian Y, Lu F, *et al*: Very high penetrance and occurrence of Leber's hereditary optic neuropathy in a large Han Chinese pedigree carrying the ND4 G11778A mutation. Mol Genet Metab 100: 379-384, 2010.
35. Torroni A, Petrozzi M, D'Urbano L, Sellitto D, Zeviani M, Carrara F, Carducci C, Leuzzi V, Carelli V, Barboni P, *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 60: 1107-1121, 1997.

36. Brown MD, Starikovskaya E, Derbeneva O, Hosseini S, Allen JC, Mikhailovskaya IE, Sukernik RI and Wallace DC: The role of mtDNA background in disease expression: A new primary LHON mutation associated with Western Eurasian haplogroup J. Hum Genet 110: 130-138, 2002.
37. Howell N, Oostra RJ, Bolhuis PA, Spruijt L, Clarke LA, Mackey DA, Preston G and Herrnstadt C: Sequence analysis of the mitochondrial genomes from Dutch pedigrees with Leber hereditary optic neuropathy. Am J Hum Genet 72: 1460-1469, 2003.
38. Jiang P, Liang M, Zhang C, Zhao X, He Q, Cui L, Liu X, Sun YH, Fu Q, Ji Y, *et al*: Biochemical evidence for a mitochondrial genetic modifier in the phenotypic manifestation of Leber's hereditary optic neuropathy-associated mitochondrial DNA mutation. Hum Mol Genet 25: 3613-3625, 2016.
39. Brown MD, Sun F and Wallace DC: Clustering of Caucasian Leber hereditary optic neuropathy patients containing the 11778 or 14484 mutations on an mtDNA lineage. Am J Hum Genet 60: 381-387, 1997.
40. Ji Y, Zhang AM, Jia X, Zhang YP, Xiao X, Li S, Guo X, Bandelt HJ, Zhang Q and Yao YG: Mitochondrial DNA haplogroups M7b1'2 and M8a affect clinical expression of leber hereditary optic neuropathy in Chinese families with the m.11778G>a mutation. Am J Hum Genet 83: 760-768, 2008.
41. Bailey IL and Lovie JE: New design principles for visual acuity letter charts. Am J Optom Physiol Opt 53: 740-745, 1976.