

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

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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.

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

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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'-CTAACCGGCTTTTTGCC-3' and reverse 5'-ACCTAG AAGTTGCCTGGCT-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

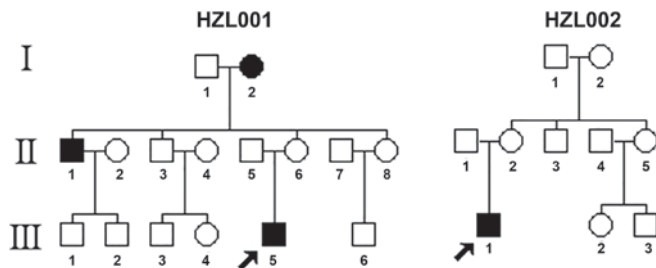


Figure 1. Two Chinese pedigrees with Leber's hereditary optic neuropathy. Vision-impaired individuals are indicated by filled symbols; females are indicated by circles; males are indicated by squares; arrows indicate the probands.

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

^aAccording to the national standard visual acuity chart (41). F, female; M, male; CF, counting fingers.

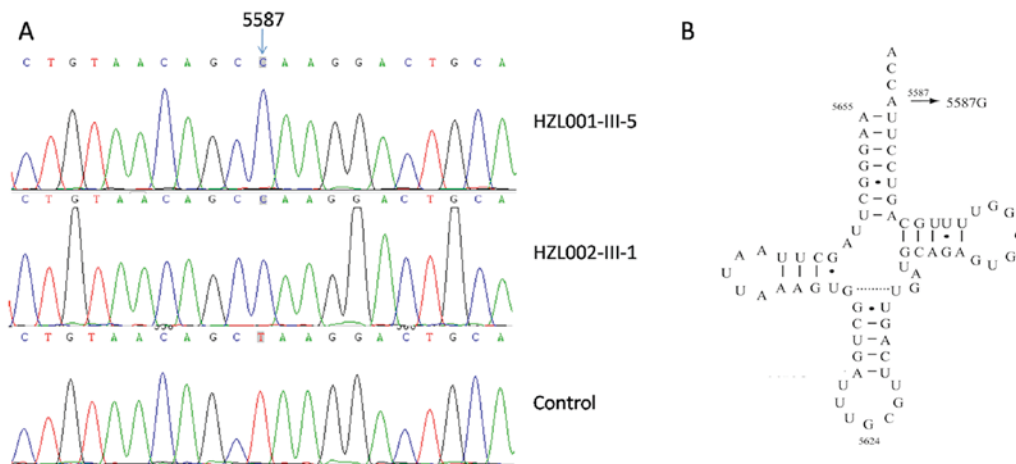


Figure 2. (A) Identification of the m.5587T>C mutation in the *MT-TA* gene. Partial sequence chromatograms of the *MT-TA* gene from two affected individuals and one Chinese control. The arrow indicates the location of the base change at position 5,587. (B) Location of the m.5587T>C mutation in the *MT-TA* 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-7A* gene from 17 different species. Position 73 is the location of the m.5587T>C mutation.

Species	Acc-stem, pos 1	D-stem, pos 8	D-loop, pos 10	D-stem, 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
<i>Cebus albifrons</i>	GAGGGCT	TA	GCTT	AATTA	AAGT	A	GTTGA	TTTGGCT	TCAAT	TGAT	GCAAG	GTATAG	TTTGC	AGTCCTT	A
<i>Cercopithecus aethiops</i>	AGGGGCT	TA	GCTT	AATTA	AAGT	G	GTTGA	TTTGGCT	TCAAT	TGAT	GCAGA	GTAGGTT	TTTGC	AGTCCTT	A
<i>Colobus guereza</i>	AAGGGCT	TA	GCTT	AATGA	AAGT	G	ATTGA	TTTGGCT	TCAGT	TGAT	GCAGA	GTAGAGT	TTTGC	AGTCCTT	A
<i>Gorilla gorilla</i>	AAGGGCT	TA	GCTT	AATTA	AAGT	G	GCTGA	TTTGGCT	TCAGT	TGAT	GCAGA	GTAGGGT	TTTGC	AGTCCTT	A
<i>Homo sapiens</i>	AAGGGCT	TA	GCTT	AATTA	AAGT	G	GCTGA	TTTGGCT	TCAGT	TGAT	GCAGA	GTGGGGT	TTTGC	AGTCCTT	A
<i>Hylobates lar</i>	AAGGGCT	TA	GCTT	AATTA	AAGT	G	ACTGA	TTTGGCT	TCGGT	TGAT	GCAAA	GTGGGC	TTTGC	AGTCCTT	G
<i>Lemur catta</i>	GAGGATT	TA	GCTT	AATTA	AAGT	G	ATTGA	TTTGGCT	TCAGT	TGAT	GTAAAG	ATATAAT	CTTGC	AGTCCTT	A
<i>Macaca mulatta</i>	AAGGGCT	TA	GCTT	AATTA	AAGT	G	GTTGA	TTTGGCT	TCAAT	TGAT	GCAGA	GTAGGTG	TTTGC	AGTCCTT	A
<i>Macaca sylvanus</i>	AAGGGCT	TA	GCTT	AATTA	AAGT	A	GTTGA	TTTGGCT	TCAAT	TGAT	GCAGA	GCAGGTG	TTTGC	AGTCCTT	A
<i>Nycticebus coucang</i>	GAGGACT	TA	GCTT	AATTA	AAGT	A	ATTGA	TTTGGCT	TCAGT	TGAT	GTAGG	AGAAAGT	CTTGC	AGTCCTT	A
<i>Pan paniscus</i>	AAGGGCT	TA	GCTT	AATTA	AAGT	G	GCTGA	TTTGGCT	TCAGT	TGAT	GCAGA	GTGGGGT	TTTGC	AGTCCTT	A
<i>Pan troglodytes</i>	AAGGGCT	TA	GCTT	AATTA	AAGT	G	GCTGA	TTTGGCT	TCAGT	TGAT	GCAGA	GTGGGGT	TTTGC	AGTCCTT	A
<i>Papio hamadryas</i>	AAGGGCT	TA	GTTT	AATTA	AAGC	G	ATTGA	TTTGGCT	TCAGT	TGAT	GCGGA	GTAGGTG	TCTGC	AGTCCTT	A
<i>Pongo pygmaeus</i>	GAGGGCT	TA	GCTT	AATTA	AAGT	G	GCTGA	TTTGGC	TCAGT	TGAT	GCAAA	GTGGGGT	TTTGC	AGTCCTT	A
<i>Pongo pygmaeus abelii</i>	GAGGGCT	TA	GCTT	AATTA	AAGT	G	GCTGG	TTTGGCT	TCAGT	TGAT	GCAGA	GCGGGGC	TTTGC	AGTCCTT	A
<i>Tarsius bancanus</i>	GAGGACT	TA	GCTT	AAGTTA	AAGT	A	GCTAA	TTTGCAG	TTAGT	TGAT	GTAGA	GTGAGTC	TTTGC	AGTCCTT	A
<i>Trachypithecus bescurus</i>	AAGGGCT	TA	GCTT	AATTA	AAGT	A	ACTGG	TTTGGCT	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
<i>MT-RNR1</i>	750	A-G	A/A/A/-	A	G	G	Yes
	1,438	A-G	A/A/A/G	A	G	G	Yes
<i>MT-RNR2</i>	2,706	A-G	A/G/A/A	A	G	G	Yes
<i>MT-ND1</i>	3,970	C-T		C	T	T	Yes
<i>MT-ND2</i>	4,769	A-G		A	G	G	Yes
	5,201	T-C		T	C		Yes
<i>MT-TA</i>	5,587	T-C	T/T/-/T	T	C	C	Yes
<i>MT-CO1</i>	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
<i>MT-CO2</i>	8,149	A-G		A		G	Yes
	8,152	G-A		G	A		Yes
<i>MT-ATP6</i>	8,860	A-G(Thr-Ala)	T/A/A/T	A	G	G	Yes
	9,165	T-C		T		C	Yes
<i>MT-CO3</i>	10,310	G-A		G	A	A	Yes
<i>MT-ND4L</i>	10,490	T-C		T	C		Yes
	10,609	T-C(Met-Thr)	M/T/T/T	T	C	C	Yes
<i>MT-ND4</i>	11,471	C-T		C		T	Yes
	11,719	G-A		G	A	A	Yes
<i>MT-ND5</i>	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
<i>MT-CYB</i>	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. ^aAs 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.

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