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Leber's hereditary optic neuropathy is associated with mitochondrial ND6 T14502C mutation

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Abstract

We report here the clinical, genetic and molecular characterization of three Chinese families with Leber's hereditary optic neuropathy (LHON). There were variable severity and age-of-onset in visual impairment among these families. Strikingly, there were extremely low penetrances of visual impairment in these Chinese families. Sequence analysis of complete mitochondrial genomes in these pedigrees showed the homoplasmic T14502C (I58V) mutation, which localized at a highly conserved isoleucine at position 58 of ND6, and distinct sets of mtDNA polymorphisms belonging to haplogroups M10a, F1a1 and H2. The occurrence of T14502C mutation in these several genetically unrelated subjects affected by visual impairment strongly indicates that this mutation is involved in the pathogenesis of visual impairment. Here, mtDNA variants I187T in the ND1, A122V in CO1, S99A in the in the A6 and V254L in CO3 exhibited an evolutionary conservation, indicating a potential modifying role in the development of visual impairment associated with T14502C mutation in those families. Furthermore, nuclear modifier gene(s) or environmental factor(s) may play a role in the phenotypic manifestation of the LHON-associated T14502C mutation in these Chinese.

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Keywords

Mitochondrial DNA; ND6; Mutation; Leber's hereditary optic neuropathy; Visual loss; Penetrance; Haplotype; Maternally; Chinese

INTRODUCTION

Leber's hereditary optic neuropathy (LHON) is a maternally inherited eye disease that generally affects young adults with the rapid, painless, bilateral loss of central vision [1-3]. Mutations in mitochondrial DNA (mtDNA) are the molecular bases for this disorder [5-7]. Up to date, more than 30 mtDNA mutations have been associated LHON worldwide [7]. Of these, the *ND1* G3460A, *ND4* G11778A and *ND6* T14484C mutations, in the genes encoding the subunits of respiratory chain complex I, are the most commonly LHON-associated mtDNA mutations, accounting for more than 50% of LHON pedigrees in different ethnic origins worldwide [2,7-10]. Typical features in LHON pedigrees are incomplete penetrance and male bias among the affected subjects, reflecting the complex etiology of this disease [11-12]. These primary LHON-associated mtDNA mutations such as *ND4* G11778A mutation by themselves are insufficient to produce a clinical phenotype. Therefore, other modifier factors including the nuclear modifier genes, mitochondrial haplotypes and environmental factors modify the risk of optic neuropathy [12-13].

With the aim of investigating the molecular basis of LHON in the Chinese population, 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 [11-20]. In the previous investigations, we showed that the LHON was associated with the *ND4* G11778A mutation in 15 Chinese families with variable penetrance and severity and age-at-onset of visual impairment [11-16]. Furthermore, *ND6* T14484C mutation and *ND1* G3460A mutation were identified in three Han Chinese families and one Chinese family, respectively [17,18]. In addition, we showed that LHON is associated with the *ND4* G11696A and *ND1* T3394C mutations in Chinese families with extremely low penetrances of visual loss [19,20]. In the present study, we performed the clinical, genetic and molecular characterization of other three Chinese families with suggestively maternally transmitted LHON. Molecular analysis has led to identification of the known T14502C mutation in *ND6* gene in these Chinese families. To elucidate the role of mitochondrial haplotype in the phenotypic manifestation of the T14502C mutation, we performed a PCR-amplification of fragments spanning entire mitochondrial genome and subsequent DNA sequence analysis in the matrilineal relatives of those Chinese families.

MATERIALS AND METHODS

Patients

As a part of genetic screening program for visual impairment, three Chinese families (Figure 1) were ascertained through the School of Ophthalmology and Optometry, Wenzhou Medical College, and Ophthalmology Clinic, Beijing Dongfang Hospital, respectively. 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 ethics committee. Members of those pedigrees were interviewed at length to identify both personal or family medical histories of visual impairments, and other clinical abnormalities. The 167 control DNA samples used for screening for the presence of mtDNA mutations were obtained from a panel of unaffected individuals from Chinese ancestry.

Ophthalmological examinations—The ophthalmologic examinations of probands and other members of these families were conducted, including visual acuity, visual field examination (Humphrey Visual Field Analyzer Iii, SITA Standard), visual evoked potentials (VEP) (Roland Consult RETI port gamma, flash VEP), and fundus photography (Canon CR6-45NM fundus camera). 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 (Gentra Systems). The presence of the G3460A, G11778A and T14484C mutations was examined as detailed elsewhere [2]. 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 [20], respectively. For the detection of the G3460A mutation, the amplified PCR segments were digested with a restriction enzyme *BsaHI* [2], while the presence of the T14484C mutation was examined by digesting PCR products with a restriction enzyme *MvaI* [2]. For the examination of the G11778A mutation, the amplified PCR segments were digested with the restriction enzyme *Tsp45I* [11-16].

The entire mitochondrial genome of three probands was PCR amplified in 24 overlapping fragments using sets of the light (L) strand and the heavy (H) strand oligonucleotide primers as described previously [21]. Each fragment was purified and subsequently analyzed by direct sequencing in an ABI 3100 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) [22]. DNA and protein sequence alignments were carried out using seqweb program GAP (GCG). The allele frequency of T14502C mutation in *ND6* gene was determined by PCR amplification of fragments spanning the corresponding regions, using the genomic DNA derived from Chinese controls as templates and performing subsequent sequence analysis of PCR products, as described above.

RESULTS

Clinical presentation

In family WZ401, the proband (III-1), as shown in Table 1, came to the Ophthalmology Clinic of Wenzhou Medical College at the age of 17 years. She began experiencing bilateral visual impairment at the age of 11 years. She saw a dark cloud in the center of her vision and had problems appreciating colors, which all seemed a dark gray. Her visual acuity was 0.1 in both eyes. Visual field testing demonstrated large centrocaecal scotomata in both eyes. Fundus examination showed that both of her optic discs were abnormal: vascular tortuosity of the central retinal vessels, a circumpapillary telangiectatic microangiopathy, and swelling of the retinal nerve fiber layer. The flash VEP showed that there was the decreased amplitude with delayed latencies in right eyes. Therefore, she exhibited typical clinical features of LHON. However, other 7 matrilineal relatives in this family had normal vision.

In family WZ402, the proband (III-2) was diagnosed with LHON by the Ophthalmology Clinic at the Dongfang Hospital at the age of 4 years. He began experiencing bilateral visual impairment at the age of 2 years. His visual acuity was 0.1 in both eyes. Intraocular pressure (IOP) was 11 and 11.3 mmHg in the right and left eyes, respectively. The flash VEP showed that there was the decreased amplitude with delayed latencies in right eye. Ophthalmological and other clinical evaluation showed that he had a typical clinical feature of LHON. However, none of other 5 matrilineal relatives in this pedigree had visual deficit.

In family WZ403, the proband (II-12) exhibited bilateral vision loss at the age of 30 years. She was diagnosed with LHON by the Ophthalmology Clinic at the Wenzhou Medical College. Her visual acuity was 0.3 and 0.2 in the right and left eyes, respectively. Visual field testing demonstrated large centrocaecal scotomata in both of her eyes. Fundus examination showed that both her optic disks were abnormal: vascular tortuosity of the central retinal vessels, a circumpapillary telangiectatic microangiopathy, and swelling of the retinal nerve fiber layer. Therefore, she exhibited a typical clinical feature of LHON. By contrast, none of other 15 matrilineal relatives in this pedigree exhibited a visual failure.

Furthermore, there is no evidence that any member of those families had any other known cause to account for visual impairment. Comprehensive family medical histories of these individuals showed no other clinical abnormalities, including diabetes, muscular diseases, hearing impairment and neurological disorders.

Mitochondrial DNA analysis

To elucidate the molecular basis of visual impairment, we have performed a mutational analysis of the mitochondrial genome in these families. First, we examined three commonly known LHON-associated mtDNA mutations (G3460A, G11778A and T14484C) by PCR amplification and subsequent restriction enzyme digestion analysis of PCR fragments derived from each proband of those families. We failed to detect the presence of the G3460A, G11778A and T14484C mutations. We then performed a PCR-amplification of fragments spanning entire mitochondrial genome and subsequent DNA sequence analysis in these probands. As shown in Figure 2, the T-to-C transition at position 14502 (T14502C) in *ND6* gene, resulted in the substitution of an isoleucine for valine (I58V) at amino acid position 58, has been found in those subjects. As shown in Figure 3, the isoleucine at position 58 in *ND6* is highly conserved among 20 organisms [23]. Indeed, this mutation has been associated with LHON in Chinese families [14,24] and idiopathic cardiomyopathy in Japanese families [25]. Further sequence analysis, as shown in Figure 2, confirmed the presence of the homoplasmic T14502C mutation in matrilineal relatives of these families but not other members of these families. The allele frequency analysis of the T14502C mutation showed that one (18 year old female) of 167 unrelated Chinese control subjects carried the T14502C mutation.

In addition to the identical T14502C mutation, as shown in Table 2, these subjects exhibited distinct sets of mtDNA polymorphism. Of other nucleotide changes in these mitochondrial genomes, there are 21 known variants in the D-loop, four known variants in 12S rRNA gene, two known variants in the 16S rRNA gene, the known G5824A mutation variant in the tRNA^{Cys} gene, the A8296G variant in the NC7, the T15900C variant in the tRNA^{Thr} gene, 37 silent variants (one novel) in the protein encoding genes as well as 19 missense mutations in the protein encoding genes [7]. These missense mutations are the T3866C (I187T) in the *ND1* gene, the C6268T (A122V) in *CO1* gene, the T8618C (I31T1), the A8701G (T59A), T8821G (S99A), A8860G (T112A) and G9053A (S176E) in *A6* gene, the G9966A (V254L) in *CO3* gene, the A10398G (T114A) in the *ND3* gene, the T10609C (M47T) in *ND4L* gene, the T10908C (F50S) in *ND4* gene, the G12406A (V20L), G13135A (A267T), G13759A (A475T) and G13928C (S531T) in the *ND5* gene, the C14766T (T7I), T15071C (Y109H), A15218G (T158A) and A15326G (T194A) 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 [26], bovine [27], and *Xenopus laevis* [28]. Here, I187T in the *ND1*, A122V in *CO1*, S99A in the in the *A6* and V254L in *CO3* exhibited evolutionary conservation among these four organisms. However, none of other variants showed evolutionary conservation. Based on the nomenclature of mitochondrial haplogroups [29,30], we used the mtDNA sequence variations among 3 Chinese probands to establish the haplogroup

affiliation of each mtDNA. Here, mtDNAs of pedigree WZ401, WZ402 and WZ403 belong to the Eastern Asian haplogroups M10a, F1a1 and H2, respectively.

DISCUSSION

In the present study, we have performed the clinical, genetic, and molecular characterization of three Chinese families with Leber's hereditary optic neuropathy. The bilateral visual impairment as a sole clinical phenotype was only present in the maternal lineage of those pedigrees, suggesting that the mtDNA mutation is the molecular basis for this disorder. Sequence analysis of the complete mitochondrial genomes in these pedigrees showed the distinct sets of mtDNA polymorphism, in addition to the identical T14502C (I58V) mutation in *ND6* gene. Indeed, this mutation is present in homoplasmy only in the maternal lineage of those pedigrees but not other members of these families. The isoleucine at amino acid position 58 is extremely conserved in ND6 polypeptide among different organisms [23]. This mutation has been associated with other clinical abnormalities including LHON [14,24] and diopathic cardiomyopathy [25]. In fact, the occurrence of the T14502C mutation in these several genetically unrelated subjects affected by visual impairment strongly indicates that this mutation is involved in the pathogenesis of visual impairment.

In contrast to our previous data that there is a high penetrance of visual loss in some Chinese pedigrees carrying the G11778A mutation [13,15,16,31], the penetrances of visual impairment in these three Chinese families carrying the T14502C mutation were extremely low. In particular, only one matrilineal relative of each Chinese pedigree exhibited optic neuropathy. In addition, one of 167 Chinese control subjects carried this mutation. By contrast, the coexistence of the T14502C mutation with the G11778A mutation and T14484C mutation in Chinese families exhibited higher penetrance of optic neuropathy than these three families carrying only T14502C mutation [14,24]. Furthermore, as shown in Table 1, the age-at-onset for vision loss in those subjects varied from 2 to 30 years old, with an average of 14.3 year old. This data suggested that the age-at-onset for visual loss in three Chinese families carrying the T14502C mutation was younger than those in other Chinese families with LHON [11-19]. Unlike the fact that some Chinese subjects carrying the G11778A mutations exhibited profound visual loss [11,13,15,16], those affected subjects carrying the T14502C mutation suffered from mild visual impairment.

The extremely low penetrance of visual loss and the presence of one/167 controls indicated that the T14502C mutation, similar to *ND1* T3394C, *ND4* G11778A and G11696A mutations [12,17,20], is itself insufficient to produce the clinical phenotype. Thus, the modifier factors including nuclear backgrounds, other environmental factors and mitochondrial haplotypes are necessary for the phenotypic manifestation of the T14502C mutation. In particular, the mitochondrial haplotypes have been shown to influence the penetrance and expressivity of vision loss associated with primary mtDNA mutations. mtDNA mutations at positions 4216 and 13708 labeled as second LHON mutations were implicated to increase the penetrance of the LHON-associated G11778A and T14484C mutations [32]. Furthermore, the mitochondrial haplogroup J can influence the phenotypic manifestation of the primary LHON G11778A and T14484C mutations in a very large cohort of families of European ancestry [33-34]. Most recently, the *ND4* G11696A, tRNA^{Thr} A15951G and the tRNA^{Met} A4435G mutations have been implicated to increase the penetrance of LHON in Chinese families [13,15,16,33]. Here, these three Chinese pedigrees shared the identical eleven mtDNA variants. These mitochondrial genomes of W401, WZ402, and WZ403 pedigrees belong to the Eastern Asian haplogroups M10a, F1a1 and H2, respectively, while mtDNA of one Chinese pedigree carrying the T14502C belonged to the haplogroup M10 [14]. This implied that the T14502C mutation, similar to G11778A mutation (2,12), occurred sporadically and multiplied through evolution of the mtDNA (3,11-16). Here, Here, the variants I187T in the ND1, A122V in CO1, S99A in

the in the A6 and V254L in CO3 exhibited an evolutionary conservation, despite the absence of known secondary LHON mutations in Chinese pedigrees. These mtDNA variants may have a potential modifying role in the development of visual impairment associated with T14502C mutation in those families. However, nuclear modifier gene(s) or environmental factor(s) appear to play a role in the phenotypic expression of the LHON-associated T14502C mutation in these Chinese.

Acknowledgments

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Figure 1. Three Chinese pedigrees with Leber's hereditary optic neuropathy
Vision impaired individuals are indicated by filled symbols.

Figure 2. Identification of the T14502C mutation in the *ND6* gene

Partial sequences chromatograms of *ND6* gene from three affected individuals and one Chinese control. An arrow indicates the location of the base changes at position 14502.

Species	50	↓60	70
Homo sapiens	ILNFGGGGYMGLMVFLIYLGGMVVFGYT		
Bovine	VLNFGGGSFLGLMVFLIYLGGMVVFGYT		
Xenopus laevis	IVSFGGSSFLSIIVFLIYLGGMVVFGYT		
Balaenoptera musculus	VLSFGGGSFLGLMVFLIYLGGMVVFGYT		
Bos taurus	VLNFGGGSFLGLMVFLIYLGGMVVFGYT		
Ceratotherium simum	VMSFGGGSFLGLMVFLIYLGGMVVFGYT		
Equus asinus	IMNFGGGSFLGLMVFLIYLGGMVVFGYT		
Felis catus	VLNFGGGSFLGLMVFLIYLGGMVVFGYT		
Gorilla gorilla	ILNCGGGGYMGLMVFLIYLGGMVVFGYT		
Hylobates lar	ILNCGGGGYLGLMVFLIYLGGMVVFGYT		
Macropus robustus	VVSLEDVFLGLIVFLIYLGGMVVFGYT		
Mus musculus	VLGFGGGSFLGLMVFLIYLGGMVVFGYT		
Myoxus glis	VLYFGGGSFLGLMVFLIYLGGMVVFGYT		
Oryctolagus cuniculus	VLSFGGGSFLGLMMFLIYLGGMVVFGYT		
Ovis aries	VLNFGGGSFLGLMVFLIYLGGMVVFGYT		
Pan paniscus	ILNYGGGYMGLMVFLIYLGGMVVFGYT		
Papio hamadryas	ILNYGGAYLGLVMFLIYLGGMVVFGYT		
Phoca vitulina	VLSFGGGSFLGLMVFLIYLGGMVVFGYT		
Rhinoceros unicornis	VMSFGGGSFLGLMVFLIYLGGMVVFGYT		
Sus scrofa	VLNFGGGSFLGLMVFLIYLGGMVVFGYT		

Figure 3. Alignment of partial ND6 polypeptides from different species

Arrow indicates the isoleucine at position 58 (I58) at the partial ND6 polypeptide, corresponding to the T14502C mutation.

Table 1
Summary of clinical and molecular data for affected matrilineal relatives of three Chinese families with LHON

Subjects	Gender	Age of test (yrs)	Age of onset (yrs)	Visual acuity		Level of visual impairment	Number of matrilineal relatives	mtDNA haplogroup
				Right	Left			
WZ401-III-1	F	17	11	0.1	0.1	Mild	8	M10a
WZ402-III-2	M	4	2	0.1	0.1	Mild	6	F1a1
WZ403-II-12	F	40	30	0.3	0.2	Mild	16	H2

Table 2

mtDNA variants in three Chinese families with Leber's hereditary optic neuropathy.

Gene	PositionReplacement	Conservation (H/B/M/X) ^a	CRS	WZ401	WZ402	WZ403	Previously reported ^b
D-loop	73A to G		A	G	G	G	Yes
	195T to C		T			C	Yes
	249Del A		A		Del A		Yes
	263A to G		A	G	G	G	Yes
	310T to TC or CTC		T	CTC	TC	TC	Yes
	489T to C		T	C			Yes
	522Del C		C		Del C		Yes
	523Del A		A		Del A		Yes
	573C-G(6)		C	C6			Yes
	16093T to C		T	C			Yes
	16129G to A		G	A	A		Yes
	16162A to G		A		G		Yes
	16172T to C		T		C		Yes
	16176C to T		C			T	Yes
	16189T to C		T		C		Yes
	16223C to T		C	T		T	Yes
	16304T to C		T		C	T	Yes
	16311T to C		C	C			Yes
	16355C to T		T	C		T	Yes
12S rRNA	16497A to G		A				Yes
	16519T to C		T		G	C	Yes
	709G to A	G/A/A/-	G	A			Yes
	750A to G	A/A/A/-	A	G	G	G	Yes
16S rRNA	813A to G	A/A/CT	A				Yes
	1438A to G	A/A/A/G	A	G	G	G	Yes
	2706A to G	A/G/A/A	A	G	G	G	Yes
	3172C-CC	C/C/T/T	C	CC			Yes
ND1	3866T to C (Ile to Thr)	I/I/I	T	C			Yes
	3970C to T		C		T		Yes
	4086C to T		C		T		Yes
	4140C to T		C				Yes
ND2	4769A to G		A	T	G		Yes
	5824G to A		G			G	Yes
	6268C to T (Ala to Val)	G/G/A/C	C		T	A	No
	6392T to C	A/A/A/A	T		C		Yes
Cys	6674T to C		T				Yes
	6962G to A		G		A		Yes
	6357C to T		C				Yes
	7028C to T		C				Yes
CO1	7250A to G		A	T	T	T	Yes
	7975A to G		A	G			Yes
	8296A to G		A				Yes
	8618T to C (Ile to Thr)	I/F/F/I	T				Yes
CO2	8701A to G (Thr to Ala)	T/S/L/Q	A	G			Yes
	8793T to C		T	C			Yes
	8821T to G (Ser to Ala)	S/S/S/S	T	G			Yes
	8856G to A		G				Yes
NC7	8860A to G (Thr to Ala)	T/A/A/T	A	G			Yes
	8928T to C		T				Yes
	9053G to A (Ser to Asp)	S/G/G/T	G		A		Yes
	9180A to G		A			G	Yes

Gene	PositionReplacement	Conservation (H/B/M/X) ^a	CRS	WZ401	WZ402	WZ403	Previously reported ^b
CO3	9540T to C		T	C			Yes
	9548G to A		G		A		Yes
	9966G to A (Val to Ile)	V/V/V/V	G	A			Yes
	10289A to G		A			G	Yes
ND3	10310G to A		G		A		Yes
	10398A to G (Thr to Ala)	T/T/T/A	A	G			Yes
	10400C to T		C	T			Yes
	10609T to C (Met to Thr)	M/T/T/T	T		C		Yes
ND4L	10646G to A		G	A			Yes
	10873T to C		T	C			Yes
	10908T to C (Phe to Ser)	F/L/K/H	T	T		C	Yes
	11257C to T		C				Yes
ND4	11581C to A		C			A	No
	11719G to A		G	A	A		Yes
	12406G to A (Val to Ile)	V/F/S/F	G	A			Yes
	12549C to T		C	T			Yes
ND5	12705C to T		C	T	T	T	Yes
	C to T		C				Yes
	G to A (Ala to Thr)		G	A			Yes
	A to G		A	G			Yes
ND6	13759		G				Yes
	13928	A/T/T/I S/T/S/T	G		A		Yes
	14311T to C		A	C		G	Yes
	T to C		T			C	Yes
ND6	14502		T			C	Yes
	14766		T	C	C	C	Yes
	14783T to C	T/S/T/S	C	T	T	T	Yes
	15040C to T		C	T			Yes
ND6	15043G to A		G	A			Yes
	T to C (Tyr to His)	Y/F/F/Y	T	C			Yes
	G to A		A	G			Yes
	A to G (Thr to Ala)		G	A	G	G	Yes
ND6	15514T to C		T			C	Yes
	15900T to C		T			C	Yes

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

^bSee the online mitochondrial genome database <http://www.mitomap.org> and <http://www.genpat.uu.se/mtDB>