Analysis of Three Primary mtDNA Mutations in 155 Patients with Leber's Hereditary Optic Neuropathy

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Research

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Abstract

Background: Leber's Hereditary Optic Neuropathy (LHON) is a maternal inherited disease caused by mitochondrial DNA (mtDNA) mutations. The aim of the current study is to analysis the frequencies of mitochondrial *ND1* G3460A, *ND4* G11778A and *ND6* T14484C mutations in patients with LHON.

Methods: Our study enrolled 155 patients with LHON and 83 controls, PCR-Sanger sequencing was performed to screen the presence of these primary mutations. Moreover, we performed clinical, genetic and molecular characterizations of five Chinese families carrying LHON-related three primary mutations.

Results: 28 patients with G3460A (18.1%), 86 patients with G11778A (55.5%) and 32 patients carrying T14484C mutation (20.6%) were identified. However, none of these primary mutations were identified in controls. Among them, one patient carrying G3460A, two patients with G11778A and two patients with T14484C mutation had an obvious family history of LHON. Clinical evaluation of these pedigrees showed the variable clinical phenotypes with different age at onset of LHON. Sequence analysis of the complete mtDNA genes from the matrilineal relatives suggested the presence of these primary mutations. However, the lack of any functional variants in mtDNA genes revealed that mitochondrial haplogroups or haplotypes may not play important roles in the clinical phenotypic manifestation of LHON-associated primary mutations.

Conclusions: Our data indicated that screening for the mtDNA primary mutations was necessary for early detection, prevention and diagnosis of LHON.

Background

LHON was a common maternally inherited disease caused by mtDNA mutations, which led to subacute optic atrophy resulting in severe visual impairment in most affected persons [1]. Several documented LHON cases had involved abnormal symptoms, including movement disorders, dystonia or multiple-sclerosis-like symptoms, which remains a big challenge for clinicians [2, 3]. To date, the molecular mechanism underlying LHON was still not fully understood. Since the landmark discovery of the LHON-associated mtDNA *ND4* G11778A mutation [4], more than 30 LHON-related mtDNA mutations had been reported among various ethnic population [5]. Notably, three primary mtDNA mutations: *ND1* G3460A, *ND4* G11778A and *ND6* T14484C, which involved genes encoding the subunits of respiratory chain complex I, accounted for > 90% of LHON pedigrees in some countries [6, 7]. Moreover, LHON mutations were predominantly homoplasmic with incomplete penetrance and marked male bias concerning clinical affection [8], suggesting that additional factors may influence the conversion to symptomatic LHON, like mtDNA genetic background, nuclear genes and environmental factors.

With the aim of exploring the molecular basis for LHON, we recently initiated a mutational screening for the three primary mtDNA mutations in a cohort of 155 LHON patients and 83 healthy subjects. Moreover, we performed clinical, genetic and molecular analysis of five Han Chinese families with LHON-associated mtDNA primary mutations.

Materials And Methods

Subjects and ethics statement

155 patients (99 males and 56 females, aged from 4 to 33 years, with an average of 18 years) received ophthalmological examinations at Fuzhou Second Hospital, affiliated to Xiamen University, were categorized according to the expression of LHON clinical features, such as painless, acute or subacute vision loss. Moreover, a total of 83 control subjects (41 males and 42 females, aged from 8 to 24 years, with an average of 13 years) were obtained from the Physical Examination Center of our hospital. The written informed consents conforming to the tenets of the Declaration of Helsinki were obtained from each participant prior to the study. The institutional review boards of Fuzhou Second Hospital, Xiamen University approved this study.

Screening for three primary mtDNA mutations

For DNA extraction, 5 mL of peripheral blood was collected by cubital vene-puncture from each person (155 LHON patients and 83 controls) enrolled in the study. Following extraction procedure, DNA was quantified and checked for quality by UV spectrophotometry. To screen the LHON-associated primary mtDNA mutations, we carried out the PCR-Sanger sequencing to detect the *ND1* G3460A, *ND4* G11778A and *ND6* T14484C mutations. The affected individuals' DNA fragments spanning these mtDNA mutations were amplified by PCR using oligodeoxynucleotides corresponding to mtDNA at positions 3108-3717 for the *ND1* G3460A mutation, 11654-11865 for the *ND4* G11778A mutation, and 14260-14510 for the *ND6* T14484C mutation [9-12]. The sequences of primers for detecting the *ND1* G3460A mutation were: forward: 5'-ATGAAGAATAGGGCGAAGGG-3', reverse: 5'-TACTTCACAAAGCGCCTTCC-3'; the primers sequences for screening the *ND4* G11778A mutation were: forward: 5'-GGAGAATGGGGGATAGGTGT-3', reverse: 5'-TCACTCTCACTGCCCAAGAA-3', while the primers sequences for screening the *ND6* T14484C mutation were: forward: 5'-AGAATATTGAGGCGCCATTG-3', reverse: 5'-GCATAATTAACTTTACTTC-3'. PCR products were purified and sequenced by Big Dye Terminator chemistry using the Applied Biosystems 3700 automated sequencer (Applied Biosystems). Then the sequence data was compared with the revised Cambridge Reference sequence (rCRS, GenBank accession no. NC_012920.1) [13].

Clinical and molecular features of five LHON pedigrees with three primary mutations

Five Chinese pedigrees, as shown in Figure 2, were ascertained in the Department of Ophthalmology, Fuzhou Second Hospital, Xiamen University. Members of these pedigrees were interviewed at length to identify both personal or family medical histories of visual impairments, and other clinical abnormalities. 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 [14]. The degree of visual loss was classified based on the following criteria: healthy, ≥ 0.3 , mild, 0.1 to 0.299; moderate, 0.05 to 0.099; severe, 0.02 to 0.049; and profound, < 0.02 .

The complete mitochondrial genomes of the affected LHON individuals (FZ010: II-1 and III-8; FZ011: II-6 and III-2; FZ012: III-1 and III-4; FZ013: III-14 and IV-6; FZ014: III-5) were PCR amplified by using 24 primers, as described in a previous study [15]. Each fragment was purified and subsequently was analyzed by direct sequencing in an ABI 3700 automated DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit (Applied Biosystems). These sequence results were compared with the rCRS (GenBank accession number: NC_012920.1) to detect the mutations/variants [13].

Phylogenetic analysis

The mtDNA sequences of 15 different vertebrates were used to conduct an inter-specific analysis as reported previously [16]. The conservation index (CI) of each mtDNA mutation was calculated, we regarded the $CI \geq 75\%$ as having functional potential [17].

Haplogroup analysis

The entire mtDNA sequences of the five Chinese pedigrees carrying the mtDNA primary mutations were assigned to the Asian mitochondrial haplogroups by using the nomenclature of mitochondrial haplogroups [18].

Results

Mutational screening of LHON-associated three primary mutations

The study samples with LHON consisted of 99 males and 56 females. All participants were Han Chinese subjects recruited from Department of Ophthalmology, Fuzhou Second Hospital, Xiamen University in China. After PCR-Sanger sequencing, we identified 28 patients with G3460A mutation (18.1%), 86 patients with G11778A mutation (55.5%) and 32 patients carrying T14484C mutation (20.6%) (Fig. 1). But we failed to detect these primary mutations in 83 healthy controls.

Clinical characterization of five Han Chinese families with mtDNA primary mutations

In our case-control study for genetic screening of LHON-related three primary mtDNA mutations, five Han Chinese families, as shown in Fig. 2, were ascertained in Fuzhou Second Hospital, Xiamen University. In family FZ010, the proband (II-1) was 31-year-old man who lived in Fuzhou City of Fujian Province. The comprehensive history interview indicated that he did not have any other clinical abnormalities such as diabetes, cardiovascular disease, deafness, cancer or neurological disorders. In addition, he began to suffer from painless and progressive bilateral vision loss (right eye: 0.01 and left eye: 0.03) at the age of 18, he observed a dark cloud in the center of his vision and had difficulty discerning colors, which all appeared dark grey. Moreover, the family member (III-8) had vision failure (right eye: 0.1 and left eye: 0.2).

In family FZ011, the proband (III-2) complained of painless, progressive deterioration of bilateral vision impairment at the age of 4 and went to the Eye Center of Fuzhou Second Hospital, Xiamen University. His visual acuity was 0.08 and 0.04 at the right and left. Ophthalmological and other clinical evaluations showed that he had a typical clinical feature of LHON. In addition, the matrilineal relative (II-6) was also LHON carrier.

In family FZ012, the proband (III-1) began suffering from painless, progressive deterioration of bilateral vision impairment at the age of 11 years old and came to Ophthalmology Clinic at the Fuzhou Second Hospital, Xiamen University. She saw a dark cloud in the center of vision and had problems appreciating colors that all seemed dark gray. Her visual acuity was 0.01 and 0.03 at the right and left. Among other matrilineal relatives, the subject (III-4) experienced loss of vision at the age of 12, visual acuity of III-4 was 0.1 in both eyes.

In family FZ013, the proband (III-14) was diagnosed with LHON at the age of 25 by the Ophthalmology Clinic at Fuzhou Second Hospital, Xiamen University. His visual acuity was 0.2 in both eyes. Among other members in this pedigree, the subject (IV-6) was LHON carrier, and her visual acuity was 0.03 in both eyes. However, other members in this family were normal vision.

In family FZ014, the proband (III-5) came to the Eye Center of Fuzhou Second Hospital, Xiamen University at the age of 12, he suffered painless, progressive deterioration of visual impairment at the age of 12. He saw a dark cloud in the center of vision and had problems appreciating colors that all seemed a dark gray, and his visual acuity was 0.1 in both eyes. However, none of other members in this family had any vision deficit. The clinical features of several members from these pedigrees were listed in Table 1.

Table 1

Summary of the clinical and molecular data for nine patients carrying LHON-associated three primary mtDNA mutations

Pedigree number	Subject	Sex	Age at test (year)	Age at onset (year)	Primary mtDNA mutation	Visual acuity (Right)	Visual acuity (Left)	Level of visual impairment
FZ010	II-1	Male	31	18	G3460A	0.01	0.03	Severe
FZ010	III-8	Male	5	5	G3460A	0.1	0.2	Mild
FZ011	II-6	Female	21	15	G11778A	0.2	0.1	Mild
FZ011	III-2	Male	4	4	G11778A	0.08	0.04	Severe
FZ012	III-1	Female	15	11	G11778A	0.01	0.03	Profound
FZ012	III-4	Male	12	12	G11778A	0.1	0.1	Mild
FZ013	III-14	Male	25	20	T14484C	0.2	0.2	Mild
FZ013	IV-6	Female	7	6	T14484C	0.03	0.03	Severe
FZ014	III-5	Male	12	12	T14484C	0.1	0.1	Mild

To explore the molecular basis for LHON, we performed the mutational screening for the mtDNA variants in these matrilineal relatives (FZ010: II-1 and III-8; FZ011: II-6 and III-2; FZ012: III-1 and III-4; FZ013: III-14 and IV-6; FZ014: III-5), as well as the healthy subjects. We first amplified the complete mitochondrial genomes of these LHON patients by using 24 primers as previously described [14], the PCR products were purified and subsequently analyzed in an ABI 3700 automated DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit. Sequences were handled by the DNASTAR program (DNAS Inc, Madison, USA). Compared with the rCRS, matrilineal relatives of these families revealed sets of genetic variations belonging to different mitochondrial haplogroup F1a, D5b1, F1, M7b1, M10a, respectively [18] (Table 2). Among them, there were 22 variants in D-loop, 4 variants in 12S rRNA and 3 variants in 16S rRNA, one common 9-bp deletion in the conjunction between tRNA^{Lys} and *CO2* gene, while other variants were found in mitochondrial protein-coding genes. Furthermore, 20 missense mutations were found, including the *ND1* G3316A (Ala to Thr), G3460A (Ala to Thr) and G4048A (Asp to Asn), *ND2* C5178A (Leu to Met), *ATP8* C8414T (Leu to Phe) and A8498G (Lys to Glu), *ATP6* G8584A (Ala to Thr), A8701G (Thr to Ala), A8860G (Thr to Ala) and C9071T (Ser to Leu), *ND3* A10398G (Thr to Ala), *ND4* G11778A (Arg to His), *ND5* A12361G (Thr to Ala), A13681G (Thr to Ala) and G13708A (Ala to Thr), *ND6* T14484C (Met to Val), *CytB* C14766T (Thr to Ile), C14883T (Thr to Ile), A15236G (Ile to Val) and A15326G (Thr to Ala). In addition, evolutionary conservation analysis was performed for these variants between 15 species, including mouse [19], bovine [20] and *Xenopus laevis* [21]. However, besides the *ND4* G11778A mutation, other mutations/variants were well conserved.

Table 2
mtDNA sequence variants in five LHON families

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	rCRS ^b	FZ010	FZ011	FZ012	FZ013	FZ014	Previously reported ^c
D-loop	73	A to G		A	G	G	G	G	G	Yes
	143	G to A		G		A		A	A	Yes
	146	T to C		T	C				C	Yes
	150	C to T		C	T	T	T	T	T	Yes
	152	T to C		T		C		C		Yes
	185	G to A		G		A	A			Yes
	195	T to C		T	C			C	C	Yes
	207	G to A		G	A					Yes
	263	A to G		A	G	G			G	Yes
	310	T to TC/CTC		T	TC	CTC	CTC	TC	CTC	Yes
	489	T to C		T	C	C	C	C	C	Yes
	515	Del A		A		Del A				Yes
	568	C to CCCC		C				CCCC		Yes
	16066	A to G		A	G		G		G	Yes
	16163	A to G		A		G		G		Yes
	16172	T to C		T		C			C	Yes
	16189	T to C		T		C	C		C	Yes
	16223	C to T		C	T		T			Yes
	16266	C to T		C		T			T	Yes
	16319	G to A		G	A		A	A		Yes
	16360	C to T		C		T		T		Yes
	16519	T to C		T			C		C	Yes
12S rRNA	709	G to A	G/A/A/A	G	A		A		A	Yes
	750	A to G	A/A/A/G	A	G	G	G	G	G	Yes
	1438	A to G	A/A/A/G	A	G	G	G	G	G	Yes
	1598	G to A	G/A/T/A	G	A			A		Yes
16S rRNA	2120	G to A	G/A/T/T	G		A			A	Yes
^a Conservation of amino acids in polypeptides or nucleotides in RNA in humans (H), bovine (B), mouse (M) and <i>Xenopus laevis</i> (X)										
^b rCRS: reversed Cambridge Reference Sequence										
^c As presented in online mitochondrial genome databases: www.mitomap.org and www.genpat.uu.se/mtDB .										

<i>Gene</i>	Position	Replacement	Conservation (H/B/M/X) ^a	rCRS ^b	FZ010	FZ011	FZ012	FZ013	FZ014	Previously reported ^c
	2706	A to G	A/G/A/A	A	G	G	G	G		Yes
	3010	G to A	G/G/A/A	G		A	A		A	Yes
ND1	3316	G to A (Ala to Thr)	A/I/I/I	G	A				A	Yes
	3460	G to A (Ala to Thr)	A/A/A/G	G	A					Yes
	3606	A to G			G		G		G	Yes
	3970	C to T		C	T	T		T	T	Yes
	4048	G to A (Asp to Asn)	D/N/Y/F	G		A	A			Yes
	4086	C to T		C		T	T			Yes
ND2	4715	A to G		A		G		G		Yes
	4769	A to G		A	G	G	G	G	G	Yes
	4883	C to T		C	T	T	T	T	T	Yes
	5178	C to A (Leu to Met)	L/T/T/T	C		A			A	Yes
	5301	A to G		A		G		G		Yes
CO1	5978	A to G		A		G	G			Yes
	6026	G to A		G					A	Yes
	7028	C to T		C	T	T	T	T	T	Yes
	7196	C to A		C		A			A	Yes
NC7	8271– 8279	Del 9-bp		9-bp			Del 9- bp	Del 9- bp		Yes
ATP8	8414	C to T (Leu to Phe)	F/M/L/W	C	T	T	T	T	T	Yes
	8498	A to G (Lys to Glu)	K/L/M/S	A				G		Yes
ATP6	8584	G to A (Ala to Thr)	A/V/V/T	G	A		A		A	Yes
	8701	A to G (Thr to Ala)	T/S/L/Q	A	G	G	G	G	G	Yes
	8860	A to G (Thr to Ala)	T/A/A/T	A	G	G	G	G	G	Yes
	9071	C to T (Ser to Leu)	S/M/M/L	C		T			T	Yes

^aConservation of amino acids in polypeptides or nucleotides in RNA in humans (H), bovine (B), mouse (M) and *Xenopus laevis* (X)

^brCRS: reversed Cambridge Reference Sequence

^cAs presented in online mitochondrial genome databases: www.mitomap.org and www.genpat.uu.se/mtDB.

<i>Gene</i>	<i>Position</i>	<i>Replacement</i>	<i>Conservation (H/B/M/X)^a</i>	<i>rCRS^b</i>	<i>FZ010</i>	<i>FZ011</i>	<i>FZ012</i>	<i>FZ013</i>	<i>FZ014</i>	<i>Previously reported^c</i>
CO3	9540	T to C		T	C	C	C	C		Yes
	9950	T to C		T	C				C	Yes
ND3	10398	A to G (Thr to Ala)	T/T/T/A	A		G		G	G	Yes
	10400	C to T		C	T			T	T	Yes
ND4	10873	T to C		T	C	C	C	C	C	Yes
	11719	G to A		G		A			A	Yes
	11778	G to A (Arg to His)	R/R/R/R	G		A	A			Yes
ND5	12705	C to T		C	T	T	T	T	T	Yes
	12361	A to G (Thr to Ala)	T/L/L/L	A				G	G	Yes
	13104	A to G		A		G	G	G		Yes
	13681	A to G (Thr to Ala)	T/L/D/T	A	G				G	Yes
	13708	G to A (Ala to Thr)	A/T/T/I	G		A	A	A		Yes
ND6	14484	T to C (Met to Val)	M/M/L/L	T				C	C	Yes
	14569	G to A		G	A	A	A	A	A	Yes
	14668	C to T		C	T	T	T	T	T	Yes
CytB	14766	C to T (Thr to Ile)	T/S/T/S	C	T	T		T	T	Yes
	14783	T to C		T	C	C	C	C	C	Yes
	14883	C to T (Thr to Ile)	T/L/I/I	C		T		T		Yes
	15043	G to A		G	A	A	A	A	A	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	Yes
	15326	A to G (Thr to Ala)	T/M/I/I	A	G	G	G	G	G	Yes
	15784	T to C		T			C		C	Yes
^a Conservation of amino acids in polypeptides or nucleotides in RNA in humans (H), bovine (B), mouse (M) and <i>Xenopus laevis</i> (X)										
^b rCRS: reversed Cambridge Reference Sequence										
^c As presented in online mitochondrial genome databases: www.mitomap.org and www.genpat.uu.se/mtDB .										

Table 3

Summary of clinical and molecular data for 19 Chinese pedigrees carrying LHON-related three primary mutations

Pedigree	Ratio (affected male/female)	Average age of onset (years)	Number of matrilineal relative	Penetrance (%)	mtDNA primary mutations	mtDNA haplogroup	Secondary Mutations	References
FZ010	2:0	11.5	12	16.7	G3460A	F1a1	None	This study
FZ011	1:1	9.5	8	25.0	G11778A	D5b1	None	This study
FZ012	1:1	11.5	4	50.0	G11778A	F1	None	This study
FZ013	1:1	13	9	22.2	T14484C	M7b1	None	This study
FZ014	1:0	12	6	16.7	T14484C	M10a	None	This study
WZ33	1:5	28.7	21	28.6	G3460A	A	None	12
WZ34	0:1	19	14	7.1	G3460A	M7b1	None	12
WZ35	2:0	23	6	33.3	G3460A	M12	None	12
WZ36	1:0	26	7	14.3	G3460A	M7c1	None	12
WZ41	1:0	18	7	14.3	G11778A	M8a2	None	27
WZ42	2:0	14.5	25	8	G11778A	D4g2	None	27
WZ43	1:0	15	9	11.1	G11778A	B4a1c	None	27
WZ2	3:1	14	14	57.1	G11778A	D5	tRNA ^{Met} A4435G	28
WZ3	2:1	19	10	60	G11778A	D4	tRNA ^{Thr} A15951G	29
WZ18	0:1	25	17	5.9	T14484C	D4	None	9
WZ19	1:0	24	13	7.7	T14484C	D4g1b	None	9
WZ20	1:0	6	9	11.1	T14484C	R11	None	9
WZ21	1:0	17	6	16.7	T14484C	R11a	None	9
WZ22	1:0	13	7	14.3	T14484C	D4	None	9

Discussion

In the present study, we analyzed the frequencies of three primary mtDNA mutations in a cohort of 155 patients with LHON and 83 control subjects. In fact, the *ND1* G3460A, *ND4* G11778A and *ND6* T14484C mutations accounted for more than 90% of cases of LHON worldwide. The prevalence of each mutation varied among different populations, but the average was 69 ~ 92% for *ND4* G11778A, and 3 ~ 19% for *ND6* T14484C and *ND1* G3460A [22–26]. However, these mtDNA mutations were only responsible for 38.3% cases in a cohort of 903 Chinese Han families with LHON [2], indicating the variation in the incidence and spectrum of mtDNA mutations among the different ethnic backgrounds. Here, we showed that the incidence of the *ND1* G3460A, *ND4* G11778A and *ND6* T14484C mutation was 18.1%, 55.5% and 20.6%, respectively.

Clinical and genetic evaluations revealed the variable severity and age at onset in visual impairment in these matrilineal relatives, although these subjects shared some common features, specifically, the rapid, painless, bilateral loss of central vision. The average age at onset for visual impairment in matrilineal relatives in these families, as shown in Table 3, varied from 9.5 years (FZ011) to 13 years (FZ013). Moreover, a wide range of penetrance and expressivities of LHON was observed in these Chinese families, penetrances of optic neuropathy (affected matrilineal relatives/total matrilineal relatives) in these five

Chinese families ranged from 16.7 to 50%, with the average of 26.12%. Furthermore, the average age-of-onset for optic neuropathy ranged from 6 to 28.7 years, with the average of 18.7 years in other 14 Chinese families [9, 12, 27–28] (Table 3).

The relative low penetrances of LHON in these five families strongly indicated that the *ND1* G3460A, *ND4* G11778A and *ND6* T14484C mutations were not sufficient to produce the clinical phenotypes, hence, other modified factors such as environmental factors, nuclear genes, mtDNA haplogroups and epigenetic modification may contribute to the clinical manifestation of LHON-associated mtDNA primary mutations [29, 30].

Recently, many studies reported that mtDNA background affected the expression of LHON. For example, in the European families, the risk of optic neuropathy increased when the *ND4* G11778A and *ND6* T14484C mutations were present in the haplogroup J [31, 32], and when the *ND1* G3460A mutation occurred in the haplogroup K [32]. Furthermore, secondary LHON mutations such as *ND1* T4216C and *ND5* G13708A may increase the penetrance and expressivity of LHON associated with the primary *ND4* G11778A and *ND6* T14484C mutations [32]. In the Chinese families carrying the G11778A mutation, the haplogroup M7b1'2 significantly increased the risk of optic neuropathy, whereas the haplogroup M8a had a protective effect [33]. Moreover, the mitochondrial haplogroup M9a specific variant *ND1* T3394C may modify the penetrance and expressivity of *ND4* G11778A mutation [34]. In the present study, sequence analysis of the complete mitochondrial genomes of the matrilineal relatives of these five pedigrees revealed the presence of *ND1* G3460A, *ND4* T11778A and *ND6* T14484C, as well as set of genetic polymorphisms belonging to human mitochondrial haplogroup F1a1, D5b1, F1, M7b1 and M10a [18] (Tables 2 and 3). Whereas the other 14 Chinese pedigrees carrying the *ND1* G3460A or *ND4* G11778A or *ND6* T14484C mutation belong to haplogroup A, M7b1, M12, M7c1, M8a2, D4g2, B4a1c, D5, D4, D4g1b, R11 and R11a, respectively [18]. This suggested that the G3460A, G11778A and T14484C mutations occurred sporadically and multiplied through evolution of the mtDNA in China. However, there was the absence of secondary LHON mutations in these five Chinese families. By contrast, two Chinese families with higher penetrance of vision loss harbored functionally significant mtDNA variants: the tRNA^{Met} A4435G, and tRNA^{Thr} A15951G [28, 29]. These data suggested that these mtDNA haplogroup-specific variants may not play an important role in the phenotypic expression of the LHON-related mtDNA primary mutations in these pedigrees.

Conclusions

In summary, our study indicated that mitochondrial *ND1* G3460A, *ND4* G11778A and *ND6* T14484C mutations were the molecular basis for LHON, screening for these primary mtDNA mutations were recommended for early detection and diagnosis of inherited optic neuropathy. The main limitation of this study was the relatively small sample size, further studies including more LHON patients were needed to be performed.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

YJY and XMZ participated in the design of this study, FZ collected the samples and controls, YJY performed the genetic, molecular analysis and drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

This work was approved by the Ethics Committee of Fuzhou Second Hospital, Xiamen University.

Consent for publication

Not applicable.

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Figures

Figure 1

Identification of mitochondrial ND1 G3460A, ND4 G11778A and ND6 T14484C mutations by direct Sanger sequence. MT: mutant; WT: wild type.

Figure 2

Five Han Chinese pedigrees with LHON, arrows indicate the proband, the affected subjects are indicated by filled symbols.