·Basic Research·

Complete mitochondrial DNA sequence analysis in two southern Chinese pedigrees with Leber hereditary optic neuropathy revealed secondary mutations along with the primary mutation

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Abstract

• AIM: To investigate mitochondrial factors associated with Leber hereditary optic neuropathy (LHON) through complete sequencing and analysis of the mitochondrial genome of Chinese patients with this disease.

• METHODS: Two unrelated southern Chinese families with LHON and 10 matched healthy controls were recruited, and their entire mitochondrial DNA (mtDNA) was amplified and sequenced with the universal M13 primer. Then DNA sequence analysis and variation identification were perfomed by DNAssist and Chromas 2 software and compared with authoritative databases such as Mitomap.

• RESULTS: Mutational analysis of mtDNA in these two Chinese pedigrees revealed one common LHON-associated mutation, G11778A (Arg→His), in the *MT-ND4* gene. In addition, there were two secondary mutations in Pedigree 1: C3497T (Ala→Val), and C3571T (Leu→Phe) in the *MT-ND1* gene, which have not been reported; and two secondary mutations occurred in Pedigree 2: A10398G (Thr→Ala) in the *MT-ND3* gene, and T14502C (Ile→Val) in the *MT-ND6* gene. Three polymorphisms, A73G, G94A and A263G in the mtDNA control region, were also found.

• CONCLUSION: Our study confirmed that the known *MT-ND4** G11778A mutation is the most significant cause of LHON. The C3497T and C3571T mutations in Pedigree 1 were also both at hot-spots of *MT-ND1*; they may affect the respiratory chain in coordination with the primary mutation G11778A. In Pedigree 2, the two secondary mutations A10398G of *MT-ND3* and T14502C of *MT-ND6* may influence mitochondrial respiratory complex I, leading to the mitochondrial respiratory chain dysfunction which results in optic atrophy together with G11778A. Therefore, not only the common primary LHON mutation is responsible for the visual atrophy, but other secondary mtDNA mutations should also be considered when giving genetic counseling.

• KEYWORDS:Leber hereditary optic neuropathy; mitochondrial DNA; mutation; mitochondrial respiratory complex I

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INTRODUCTION

Leber hereditary optic neuropathy (LHON. OMIM: 535000) is generally regarded as a maternally-transmitted disorder and is the most common cause of sudden bilateral blindness. The mean age at onset has been variously reported from 27 to 34 years. This disease is associated with point mutations in the mitochondrial DNA (mtDNA) that acts autonomously or in association with each other to cause the symptoms [1]. Over 30 mutations have been reported to be responsible for LHON (http://www.mitomap.org/MITOMAP/MutationsLHON). It is known that over 95% of LHON pedigrees are caused by three common

Table 1 Summary of clinical data and mtDNA variants in two Chinese pedigrees with Leber hereditary optic neuropathy (LHON)

Patient	Gender	Onset age (Years)	Visual acuity		Primary mtDNA mutation	Secondary mtDNA mutation	Reported (disease context)*
			left	right			
Pedigree 1							
III-2	Female	24	0.12	0.8	G11778A/*MT-ND4*	C3497T/*MT-ND1* C3571T/*MT-ND1*	Yes No
III-4	Female	43	0.6	0.4	G11778A/*MT-ND4*	C3497T/*MT-ND1* C3571T/*MT-ND1*	Yes No
III-5	Male	15	0.01	0.02	G11778A/*MT-ND4*	C3497T/*MT-ND1* C3571T/*MT-ND1*	Yes No
III-8	Female	30	0.05	0.09	G11778A/*MT-ND4*	C3497T/*MT-ND1* C3571T/*MT-ND1*	Yes No
III-10	Female	19	0.03	0.05	G11778A/*MT-ND4*	C3497T/*MT-ND1* C3571T/*MT-ND1*	Yes No
IV-13 (Proband)	Male	18	0.06	0.01	G11778A/*MT-ND4*	C3497T/*MT-ND1* C3571T/*MT-ND1*	Yes No
Pedigree 2							
III-1 (Proband)	Male	20	0.08	0.2	G11778A/*MT-ND4*	A10398G/*MT-ND3* T14502C/*MT-ND6*	Yes Yes
III-9	Male	16	0.07	0.09	G11778A/*MT-ND4*	A10398G/*MT-ND3* T14502C/*MT-ND6*	Yes Yes
III-13	Male	18	0.1	0.25	G11778A/*MT-ND4*	A10398G/*MT-ND3* T14502C/*MT-ND6*	Yes Yes
III-21	Male	20	0.02	0.06	G11778A/*MT-ND4*	A10398G/*MT-ND3* T14502C/*MT-ND6*	Yes Yes
III-23	Male	48	0.06	0.07	G11778A/*MT-ND4*	A10398G/*MT-ND3* T14502C/*MT-ND6*	Yes
III-26	Female	33	0.07	0.25	G11778A/*MT-ND4*	A10398G/*MT-ND3* T14502C/*MT-ND6*	Yes Yes
IV-12	Male	11	0.6	0.4	G11778A/*MT-ND4*	A10398G/*MT-ND3* T14502C/*MT-ND6*	Yes Yes

* See online mitochondrial genome database: http://www.mitomap.org/MITOMAP/MutationsLHON.

primary mutations: G11778A in the *MT-ND4* gene, G3460A in the *MT-ND1* gene, and T14484C in the *MT-ND6* gene. The proportions of the three point mutations in Caucasian LHON families are reported to be 56.6% for G11778A/*MT-ND4*, 22.6% for G3460A/*MT-ND1*, and 20.8% for T14484C/*MT-ND6* [2]. However, these proportions were reported as 90.2% , 8.7% and 1.1% respectively on a Chinese genetic background [3]. Although the primary etiological factor in LHON is the mtDNA mutation, the presence of such a mutation does not necessarily lead to visual loss. Families carrying the same LHON-associated mutation include patients who present a wide range of phenotypes. The pathogenesis of LHON remains obscure. The marked incomplete penetrance and gender bias indicate that additional genetic (nuclear or mitochondrial) and epigenetic factors (e.g., smoking and drinking) may also be involved [4, 5].

To further explain the mitochondrial basis of LHON, we investigated the factors associated with LHON by sequencing and analyzing the complete mitochondrial genome of two southern Chinese LHON pedigrees.

MATERIALS AND METHODS

Patients Two unrelated southern Han Chinese families with LHON and 10 ethnically-matched healthy controls were recruited from the Department of Ophthalmology, Xiangshan First People's Hospital, Xiangshan County, Ningbo, Zhejiang Province, China. Diagnosis was made according to Kanski's *Clinical Ophthalmology: A Systematic Approach* (7th edition, 2011, W.B. Saunders Co.). The eye examinations of family members of both pedigrees and controls were performed by two independent ophthalmologists. The tests included visual acuity, color vision, and a funduscopic examination (data not shown). Pedigree 1 (P1) was a four-generation family with 8 affected members (Figure 1A). The penetrance of visual impairment (affected matrilineal relatives/total matrilineal relatives) in this pedigree was 34.8%. The ratio of affected males to females was 3/5. Age at onset ranged from 18 to 43 years (Table 1). Pedigree 2 (P2) was a four-generation family with 7 affected individuals (Figure 1B). Penetrance: 28%; ratio: 6/1; age at onset: 11 to 48 years (Table 1).

This study was conducted in conformity with the Declaration of Helsinki and approved by the Zhejiang University Review Board. Written informed consent was given by all participants.

Methods

Mutation analysis of the mitochondrial genome Genomic DNA was isolated from the peripheral blood of 22 family members by the standard protocol. Nine members (II-4, III-2, III-4, III-5, III-8, III-10, IV-6, IV-8, and IV-13)

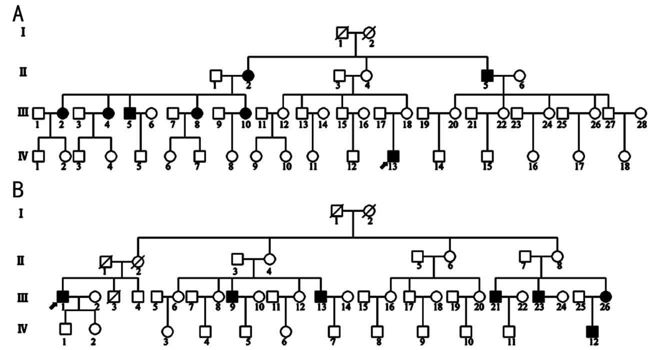

Figure 1 Pedigrees 1 and 2 of the southern Chinese LHON families investigated. Black filled symbols represent the affected Arrow shows the proband.

from P1 and 13 members (III-1, III-6, III-9, III-10, III-13, III-17, III-20, III-21, III-22, III-23, III-26, IV-11 and IV-12) from P2 were available. The entire mitochondrial genome was amplified by polymerase chain reaction in 24 overlapping fragments using sets of heavy-strand and light-strand oligonucleotide primers as described previously [6]. Amplified fragments were bidirectionally sequenced with the universal M13 primer. Then DNA sequence analysis and variation identification were performed by DNAssist and Chromas 2 software, and compared with the authoritative databases Mitomap (www.mitomap.org) and secondary Cambridge sequence(GenBank accession number:NC_012920) [7].

RESULTS

One common primary LHON-associated point mutation, G11778A (Arg→His) in the *MT-ND4* gene, was revealed in both Chinese LHON pedigrees (Figure 2A). In addition, there were two secondary mutations in patients from P1, C3497T (Ala→Val) (Figure 2B), and C3571T (Leu→Phe) in the *MT-ND1* gene, which have not been reported (Figure 2C), while in patients from P2, A10398G (Thr→Ala) in the *MT-ND3* gene (Figure 2D) and T14502C (Ile→Val) in the *MT-ND6* gene were found (Figure 2E) (Table 1). All the pathogenic mtDNA mutations were homoplastic. Three polymorphisms, A73G, G94A and A263G in the mtDNA control region were also found in P2.

None of the above mutations were present in 10 normal controls.

DISCUSSION

LHON most often occurs in young men in their second or third decade of life. Usually both eyes are involved. But the onset in each eye is not synchronous, with an interval of either some months or even longer than a decade [8]. LHON patients present acute or sub-acute, painless, central vision loss leading to a central scotoma with little probability of partial visual recovery. Neuro-ophthalmologic examination commonly reveals peripapillary telangiectasia, microangiopathy, disc pseudoedema, and vascular tortuosity. The disease finally leads to optic disc atrophy [1]. In a few families, mtDNA complex I mutations cause optic atrophy

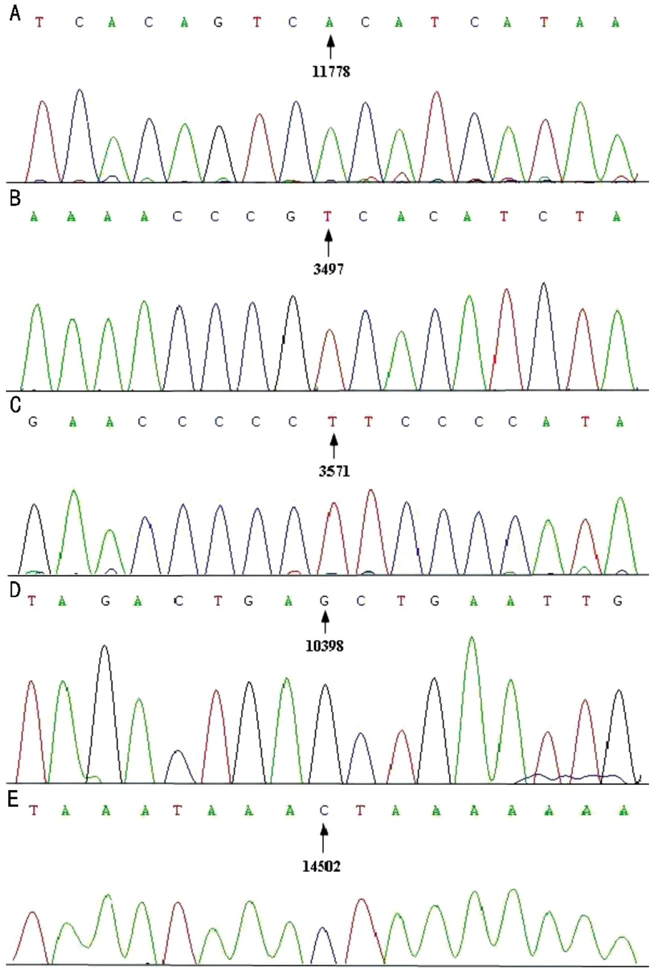

Figure 2 Partial sequence chromatograms of the mtDNA in LHON patients Arrows indicate the positions of the base changes.A:G11778A/*MTND4* mutation in P1-III-1;B: C3497T/*MTND4* mutation in P1-III-1; C: C3571T/*MTND4* mutation in P1-III-1; D:A10398G/*MTND3* mutation in P2-III-1;E: T14502C/*MTND4*mutation in P2-III-1.

in association with severe neurologic deficits such as ataxia, dystonia, and encephalopathy [9]. Some patients, usually women, may develop a progressive multiple sclerosis-like illness. These individuals manifest not only severe bilateral optic neuropathy, but also disseminated central nervous system demyelination, with characteristic periventricular white matter lesions and unmatched cerebrospinal fluid oligoclonal bands [10]. The variable phenotypes of LHON suggest that other modifying factors play an important role in this disease.

In the present study, we clinically and genetically characterized two southern Chinese families with LHON. Visual impairment was only present in the maternal lineage of these pedigrees carrying the G11778A/*MT-ND4* mutation. They all exhibited a rapid, painless, bilateral loss of central vision, but with different ages at onset and variable severity. LHON exhibits incomplete penetrance with a male predominance. Here, the ratios between the affected males and females in the two families were 3/5

(P1) and 6/1 (P2). Qu *et al.* reported an average ratio of 3.4/1 after studying 15 Chinese families carrying the G11778A mutation [11]. The ratio in our P1 was much lower than the average. This unusual male/female ratio suggests other modifying factors at work. The penetrance in the two pedigrees was 34.8% (P1) and 28% (P2). The penetrance of optic neuropathy in other Chinese pedigrees carrying the primary G11778A/*MT-ND4* mutation range from 5% to 83%, with an average of 34% [11-13]. There was no significant discrepancy of penetrance between the LHON pedigrees.

mtDNA variants may influence the penetrance and expressivity of visual impairment associated with the primary mtDNA mutation. In P1, there were two secondary mutations, C3497T and C3571T, in the *MT-ND1* gene. Phasukkijwatana *et al* [14] studied 30 Thai LHON pedigrees with G11778A/*MT-ND4* and found that the secondary mutation C3497T/*MT-ND1* had a synergistic deleterious effect with G11778A/*MT-ND4*, accelerating the onset of the disease in their patients. Yusnita *et al* [15] proposed that this point mutation, which lies in a highly-conserved region, is likely to alter the structure and function of the ND1 protein. This mutation combined with other mtDNA variants, might cause slight changes that generate higher levels of toxic reactive oxygen species. The mutation C3571T/*MT-ND1* is first reported here, and we speculate that it may modulate the phenotypic expression of the major G11778A/*MT-ND4* mutation with C3497T/*MT-ND1*.

There were also two secondary mutations in P2, A10398G/*MT-ND3* and T14502C/*MT-ND6*. Sudoyo *et al* [16] found a significant association between A10398G/*MT-ND3* and the primary G11778A/*MT-ND4* mutation in LHON. This might act synergistically to increase the penetrance of the LHON mutation. The T14502C/*MT-ND6* mutation causes the substitution of a highly-conserved isoleucine for valine at position 58 in the ND6 molecule, which has been associated with LOHN in some Chinese pedigrees [17].

In summary, LHON is a complicated disease. Not only are the common primary LHON mutations responsible for the visual atrophy, but other secondary mtDNA mutations such as those reported here should also be considered when giving genetic counselling [18].

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