Mitochondrial DNA mutation m.3635G>A may be associated with Leber hereditary optic neuropathy in Chinese

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ABSTRACT

Leber hereditary optic neuropathy (LHON) was the first disease to be linked to the presence of a mitochondrial DNA (mtDNA) mutation. Nowadays over 95% of LHON cases are known to be caused by one of three primary mutations (m.11778G>A, m.14484T>C, and m.3460G>A). Reports for other (rare) primary mutations in LHON patients are not infrequent. Among those is the mutation m.3635G>A in the MT-ND1 gene which was reported to be pathogenic in a Russian LHON family. In this study, we report on a Chinese family with clinical features of LHON but without any of the three well-known primary mutations. Analysis of the complete mitochondrial genome in the proband revealed the presence of m.3635G>A and m.6228C>T, along with a full array of other variants that suggest the haplogroup M7b1. Evolutionary analysis indicates that site 3635, but not 6228, is highly conserved in vertebrates. Protein secondary-structure modeling for the MT-ND1 protein harboring amino acid change S110N indicates that mutant m.3635G>A decreases the protein hydrophobicity. Our current observations provide further support for a pathogenic role of m.3635G>A in patients with LHON.

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Materials and methods

Patient. We recently launched a comprehensive survey for mtDNA mutations in Chinese patients with LHON or suspected LHON [7,8,10–12] and have collected samples from over 1500 patients/families. Among them, was a LHON family (Le1143) from Guangdong Province, China, which was seen at the Pediatric and Genetic Clinic of the Eye Hospital, Zhongshan Ophthalmic Center. Informed consents conforming to the tenets of the Declaration of Helsinki and following the guidance of sample collection of Human Genetic Disease (863 program) by the Ministry of Public Health of China were obtained from each participant prior to the study. The institutional review boards of the Zhongshan Ophthalmic Center and the Kunming Institute of Zoology approved this study.

mtDNA genome sequencing. Total genomic DNA was isolated from blood using a standard phenol/chloroform method. The complete mtDNA genome of the proband (III:4) was amplified and sequenced using a modified method as described in our previous study [11]. Sequences were handled by the DNAstar program (DNAS Inc., Madison, WI, USA). Sequence variation was scored relative to the revised Cambridge Reference Sequence (rCRS) [13]. We followed the East Asian mtDNA phylogenetic tree [14] to classify the LHON mtDNA. We defined the uniqueness of the mtDNA variants in this family by an exhaustive database search following the available guidelines [15]. Two other LHON mtDNA sequences, one from family E in Brown et al. [9] showing mutation m.3635G>A and compared the results to the wild-type (rCRS) by using the TMpred program (http://www.ch.embnet.org/software/TMPRED_form.html). This allowed us to analyze the potential change of hydrophobicity between the mutant and wild-type MT-ND1 protein.

Results

Clinical features

The proband is a 18-year-old male. When he first visited the clinic he had been suffering from severely reduced visual acuity of 3–4 months duration without accompanying other symptoms.

![Pedigree](image)

Fig. 1. Pedigree information for a Chinese LHON family (Le1143) with m.3635G>A. Affected individuals are marked by filled symbols. The proband, marked with an arrow, provided the sample for complete mtDNA sequencing.
suffered a similar onset of severely reduced visual acuity (Fig. 1). The penetrance of LHON in family Le1143 is relatively low (16.7%) when compared to most families that we have described before [7,8,11,12].

mtDNA sequence variations and evolutionary analysis

Analysis of the proband’s complete mtDNA genome showed the presence of 46 homoplasmic variations compared to the rCRS and suggest he is a member of haplogroup M7b1 (Fig. 2). This haplogroup is common in Chinese and has a higher frequency in South China than in other regions across China [16]. We recently found that mtDNA background M7b1’2 (M7b1 is a subhaplogroup of this haplogroup) increased the penetrance of LHON in patients with the mutation m.11778G>A [8]. The other private nucleotide substitutions in the proband’s family are m.131T>C in the control region, m.3635G>A in the MT-ND1 gene, m.5899insC in the short non-coding region between tRNA and MT-COI genes, m.6228C>T in the MT-ND1 gene, and m.10232A>G and m.10373G>A in the MT-ND3 gene. None of these variations are novel based on the web-search [15]. Among them, m.3635G>A and m.6228C>T are non-synonymous. Evolutionary analysis based on 10 vertebrate species shows that m.3635G>A changes a highly conserved serine to asparagine at position 110 in MT-ND1. Whereas m.6228C>T changes leucine to phenylalanine at position 109 in MT-COI and this site is not conserved in the human (Fig. 3). The complete mtDNA sequence of the proband has been deposited in GenBank under accession number GQ202273.

Analysis of MT-ND1 protein secondary-structure

As shown in Fig. 4, the amino acid change S110N caused by m.3635G>A decreased the hydrophobicity of the MT-ND1 protein, despite the fact that both amino acids are hydrophilic. In contrast, the known primary mutation m.3460G>A affected the hydrophilicity of a proximal domain. This result suggested that the stability of the oxidative phosphorylation (OXPHOS) supercomplexes may alter in the presence of m.3635G>A, thus leading to a potential pathogenicity [17]. The L109F change caused by variant m.6228C>T in MT-COI did not change the hydrophobicity of the MT-COI protein (data not shown).

Discussion

Although, nearly two decades have elapsed since the first identification of the effect of a mtDNA mutation in LHON [18], the exact pathogenesis of this disease has not been well elucidated [1–4]. The etiology of the disease in many patients with the clinical features of LHON remains unclear. In an initial survey looking for mtDNA mutations in LHON patients, we found that the overall distribution patterns of the three primary mutations (m.3460G>A, m.11778G>A, and m.14484T>C) in the Chinese population were significantly different from those found in western European populations [5,7]. We also identified a co-existence of the LHON primary mutation m.11778G>A and a deafness-associated mutation m.1555A>G in a family with a high penetrance of LHON [12]. Moreover, we demonstrated that mtDNA haplogroups M7b1’2 and M8a had quite different roles on the clinical expression of LHON in Chinese [8]. All these studies undoubtedly broadened our knowledge about LHON expression in Chinese. Because the majority of patients with similar LHON features do not have the three known primary mutations, identifying new mtDNA mutation(s) associated with LHON will have important implications for clinical diagnosis and genetic counseling.

In this study, we report the identification of a Chinese LHON family in which the proband has the mtDNA mutation m.3635G>A. This mutation, first reported by Brown et al. [9] in a Russian LHON family, could substantially decrease the ADP-stimulated oxygen consumption and succinate-normalized state III ratios in lymphoblast and transmitochondrial cybrid mitochondria from the proband as compared to controls. Mutation m.3635G>A received a pathogenicity score of nine (maximum score of 40) in the complex I pathogenicity scoring system proposed by Mitchell et al. [19]. We searched for mutation m.3635G>A in the PhyloTree.org website (http://www.phyloTree.org/), where 5794 complete, or near-complete,
mtDNA sequences were listed (version dated on May 9, 2009), but didn’t find any new sequence harboring m.3635G>A. However, a Google search with “G3635A” as described in our recent study [15] identified three further LHON cases with this mutation in Chinese people: two mtDNA sequences are available from GenBank (Access Nos. FJ969382 and FJ969383) and belong to haplogroup R11a and D4g, respectively. By using a phylogenetic approach [20–22], we proofread the two complete mtDNA sequences and identified several sequencing errors. The third case, said to be from haplogroup F1, was only mentioned in an abstract indexed at website (http://www.medcon.org.cn/2009/cmao/cn/news.asp?abid=3238.html). Taken together, it is obvious that m.3635G>A occurs independently in Chinese LHON patients from different mtDNA backgrounds. Although, we failed to get more blood samples from family Le1143 to perform further functional assays for m.3635G>A, we believe this mutation is associated with LHON in Chinese families based on the following lines of evidence. First, m.3635G>A has not been found in the available complete mtDNA sequences across the world (most of them are from general populations) but exists in five LHON families without the three primary mutations (p < 0.01). Second, protein secondary-structure modeling for the MT-ND1 protein with S110N change showed a decrease of hydrophobicity. Third, this mutation was found to cause a respiration defect with complex-I–linked substrates [9].

Haplogroups M7b1’2 and J have been found to increase penetrance in East Asian and western European LHON families with m.11778G>A, respectively [5,8]. Coincidentally, the proband in this study and the family reported by Brown et al. [9] belonged to these two high-risk haplogroups. Whether the two mtDNA haplogroups affect the penetrance of LHON in patients with m.3635G>A remains unresolved.

Intriguingly, m.6228C>T in family Le1143 was also present in LHON family (Le924) with m.11778G>A from our previous studies [8]. Both families, Le924 and Le1143, belong to haplogroup M7b1’2 but have different penetrance rates (33.3% versus 16.7%). Because the amino acid change L109F caused by m.6228C>T in MT- COI is not conserved in the human and does not affect the hydrophobicity of this protein, we are inclined to believe that m.6228C>T might not be pathogenic. It is possible that different primary mutations in the two families may contribute to the variance of LHON penetrance. Additional evidence will be needed to test this possibility.

In summary, we identified mutation m.3635G>A in a Chinese LHON family without the three primary mutations. This mutation has been identified in three other Chinese LHON families based on information from web-searches and in one Russian LHON family [9]. Further study will be essential to verify the pathogenic role of mutation m.3635G>A in LHON.

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