Biochemical and Biophysical Research Communications 399 (2010) 179–185

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The *MT-ND1* and *MT-ND5* genes are mutational hotspots for Chinese families with clinical features of LHON but lacking the three primary mutations

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ARTICLE INFO

Article history:
Received 7 July 2010
Available online 17 July 2010

Keywords:
LHON
MT-ND1
MT-ND5
Mutational hotspot
Penetrance
Chinese

ABSTRACT

LHON is one of the most common and primary causes of acute blindness in young male adults. Over 95% of LHON cases are caused by one of the three primary mutations (m.11778G>A, m.14484T>C, and m.3460G>A). In contrast to these genetically diagnosed LHON patients, there are many patients with clinical features of LHON but without the three primary mutations, and these patients have been insufficiently analyzed. We reported 10 suspected Chinese LHON families without the three primary mutations. The overall penetrance (53.4%) in these families is significantly higher than in those families with m.11778G>A (33.3%) or m.3460G>A (25.6%). Complete mtDNA genome sequencing of the 10 families showed that they belonged to different haplogroups and all identified variants (excluding m.12332A>G in mt-tRNA^{Leu}) were previously reported. Eight of 12 private non-synonymous variants in the probands are located in the *MT-ND1* and *MT-ND5* genes, which is substantially higher than that of individuals from general Chinese populations. Comparison of the private variants in the 10 families and in 10 randomly selected mtDNAs from general Chinese populations using resampling simulation strategy further confirmed this pattern. Our results suggest that the *MT-ND1* and *MT-ND5* genes are mutational hotspots for Chinese families with suspected LHON lacking the common primary mutations. Variants m.3736G>A (p.V144I) in family Le1235 and m.10680G>A (p.A71T) in Le1107 can be the pathogenic mutations for LHON.

1. Introduction

Leber hereditary optic neuropathy (LHON, MIM 535000) is one of the most common causes of blindness occurred in young male adults and is characterized by painless, acute or sub-acute bilateral visual loss [1–3]. Three primary mutations, m.11778G>A, m.14484T>C, and m.3460G>A, have been identified to be the essential factors for the onset of LHON [2]. Incomplete penetrance and gender bias of the disease expression are two main unsolved problems in understanding the pathogenesis of LHON. Many factors, including mtDNA haplogroup background, nuclear genes, and environmental factors, have been reported to be associated with the penetrance of LHON [1–7].

Compared to these well-characterized LHON patients whose conditions are mainly caused by one of the three primary mtDNA mutations, there is an abundance of patients with clinical features of LHON who present a family history of the disease but none of the three telltale mtDNA mutations [8]. The exact pathogenic mutations in these families suspected of having LHON have not been well examined. Analysis of the complete mtDNA genome sequences in these patients will help us to genetically diagnose LHON and broaden the available pathogenic mutation spectrum. Indeed, two recent studies of Chinese families with (suspected) LHON have confirmed that m.3635G>A in the *MT-ND1* gene is a rare pathogenic mutation for LHON, thereby enlarging the primary LHON mutation list for Chinese patients [9,10].

We have started a comprehensive study for Chinese patients with LHON in recent years [5,9,11–13]. Currently, we have collected 1626 records of patients with LHON or suspected LHON. Among them are several patients confirmed to lack the three primary mutations but who have a maternally transmitted family history of optic neuropathy or exhibit a mode of disease inheritance that does not exclude the possibility of maternal transmission. In this study, we performed a molecular characterization of 10 such families, with an intention to learn more about the mtDNA mutation spectrum of Chinese families with suspected LHON.

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doi:10.1016/j.bbrc.2010.07.051

2. Materials and methods

2.1. Patient collection and mtDNA complete genome sequencing

We selected 10 unrelated probands from 1626 patients with LHON or suspected LHON for study based on three criteria: (1) patients show typical LHON features, (2) patients have a family history of the disease, (3) genetic diagnosis confirmed the absence of the three primary mutations in each family. All samples were collected at the Eye Hospital, Zhongshan Ophthalmic Center. Informed consent conforming to the tenets of the Declaration of Helsinki was 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.

Total genomic DNA was extracted from whole blood by using the standard phenol/chloroform method. The entire mtDNA sequences of 10 probands were amplified and sequenced by the previously described procedure [11].

2.2. Haplogroup classification and evolutionary conservation analysis

mtDNA sequence variations in each mtDNA were scored relative to the revised Cambridge Reference Sequence (rCRS) [14]. We followed the East Asian mtDNA phylogenetic tree [15], with updated information from the bulk tree at the PhyloTree (http://www.phylotree.org; mtDNA tree Build 8) [16], to classify each lineage. All the sequence variations in the studied families were further presented in a tree profile, as described in our previous studies [5,15,17]. We defined the uniqueness of each private variant that was located in the terminal branch of the tree following the available guideline [18]. Evolutionary conservation analysis for certain mtDNA variant was performed by comparing human mtDNA (GenBank Accession No. J01415) to 43 different vertebrate species (Fig. S1). We followed the reported approach [19] to calculate the conservation index (CI) of certain variant, which is defined by the percentage of species for a list of 44 different vertebrate species (including human) that have the wild type allele of human sequence.

2.3. Resampling simulation for private variants

The non-synonymous and synonymous variants that were located in the terminal branches were further compared to those variants in 68 Chinese samples that were taken from Chinese populations [15,20] and one mtDNA generated in this study. We used a resampling strategy to avoid the potential sampling bias in the comparison of the private variants in mtDNAs from general Chinese samples (control) and patients with suspected LHON. We randomly selected 10 mtDNAs from the control mtDNA population and counted the total number of the non-synonymous variants in the terminal branch of the phylogenetic tree for each coding gene. This sampling procedure was repeated 1000 times. The resampling simulation was performed by a program developed using Perl. We employed a box plot to show the difference in the number of private non-synonymous variants of each gene between the compiled simulation result and the counts for the 10 patients. The box plot was produced by using SPSS (version 13; SPSS Inc., Chicago, IL).

2.4. Prediction of secondary structures of the MT-ND1, MT-ND5 proteins and the MT-TL2 gene

We performed a secondary structure modeling for the non-synonymous mutations that are located in the *MT-ND1* and *MT-ND5* genes. The corresponding genes in the rCRS were regarded as wild type. The TMpred program (http://www.ch.embnet.org/software/TMPRED_form.html) was used to predict the secondary structure changes caused by the variants. For comparison, some reported LHON mutations that are located in the *MT-ND1* and *MT-ND5* genes (cf. MITOMAP: http://www.mitomap.org/) were also analyzed. The secondary structure of the $tRNA^{Leu}$ (*MT-TL2*) gene was performed by using the Mobyle method (http://mobyle.pasteur.fr/cgi-bin/portal.py).

3. Results

3.1. Penetrance rate of optic neuropathy

Total of 10 families analyzed in this study evidently had clinical LHON features and either a maternal transmission family history or an otherwise-inherited pattern (Fig. 1). The average disease penetrance rate of the 10 families is 53.4% (31/58), with an almost equal penetrance between male and female [male, 57.1% (16/28); female, 50.0% (15/30)]. The overall penetrance of LHON in the 10 families without the three primary mutations is significantly higher than that of LHON families with m.11778G>A (33.3% [619/1859]; $P = 0.0027$; [5]) or with m.3460G>A mutation (25.6% [10/39]; $P = 0.0072$; [13]) (Fisher's exact test, two-tailed test). The overall ratio of affected male to female patients in the 10 families (1.07:1; 16/15) is significantly lower than the ratio observed in families with m.11778G>A (2.38:1) [5] and m.3460G>A (4:1) [13].

3.2. mtDNA mutation spectrum

Total of 10 complete mtDNA sequences were classified into 10 different haplogroups (Fig. 2). The proband Le370 belonged to the rare haplogroup M74. We defined a subhaplogroup of M74, M74a, according to six shared variants between this mtDNA and the reported M74 lineage (Fig. 2). A total of 68 private variants (these variants that were found in two or more families were counted as one variant) were found in the probands. Most of these variants (53/68) are located in the mtDNA non-coding region or act as synonymous variants in the coding region; 15 variants are non-synonymous or occur in RNA genes and are found in seven families (Table 1). Note that families Le370, Le913, and Le1135 contain no private non-synonymous variants and/or RNA variants. Variant m.12332A>G in family Le256 has not been reported according to our extensive web search [18]. The complete mtDNA sequences of 10 probands have been placed in GenBank under Accession Nos. GU377081–GU377090.

Evolutionary conservation analysis of the 15 private variants showed that the two non-synonymous mutations (m.3395A>G, p.Y30C; m.3736G>A, p.V144I) in the *MT-ND1* gene in family Le1235 were highly conserved in 44 different vertebrate species and had a CI value of 100.0, whereas the CI values for the other variants ranged from 2.3 to 95.5 (Table 1 and Fig. S1). There is no significant difference in the number of terminal non-synonymous variants and synonymous variants between the 10 patients and the 69 control mtDNAs (including one M74a sequence reported in this study) [15,20], although we observed a higher non-synonymous mutation rate in the *MT-ND1* and *MT-ND5* genes in the 10 patients (Table S1 and Fig. S2). Simulation of resampling 10 mtDNAs from the reference population containing 69 individuals showed a relative excess of non-synonymous variants on the terminal branches of the phylogenetic tree for the *MT-CO1* and *MT-ATP6* genes, whereas the *MT-ND1* and *MT-ND5* genes had substantially fewer variants compared to the 10 matrilines from families with suspected LHON (Fig. 3). Note that such a comparison may have a limitation, given the difficulty to match geographic origin and the criterion of having controls from the same haplogroup.

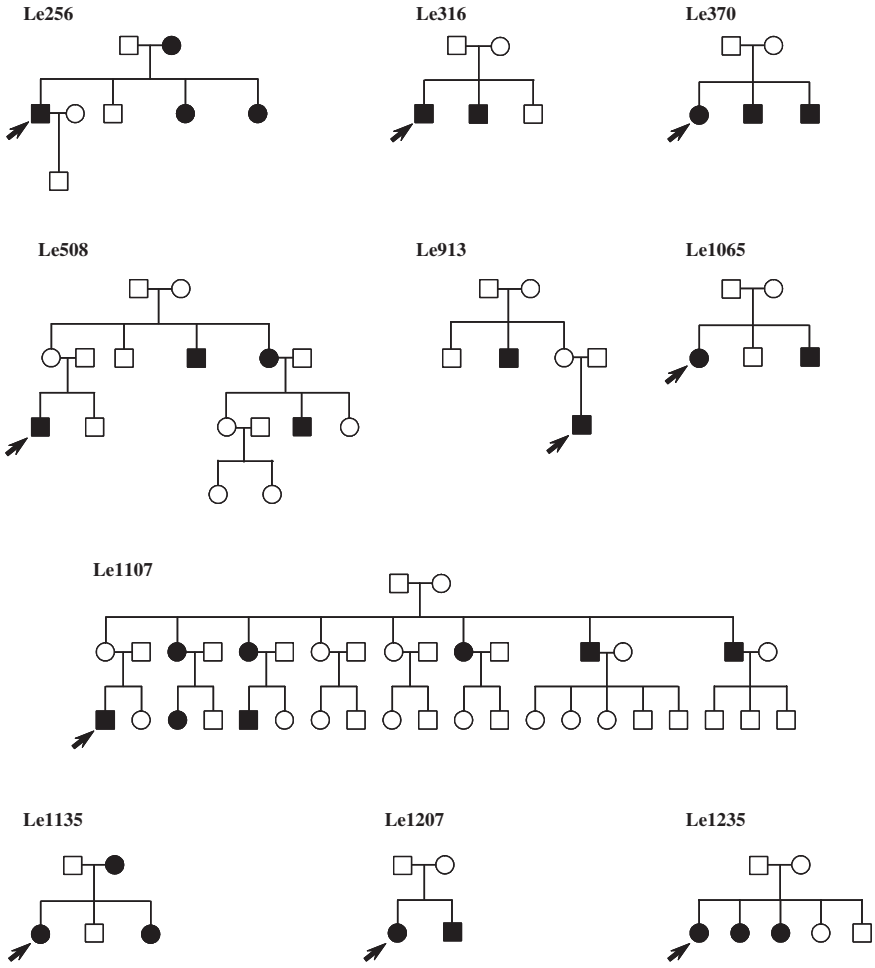

Fig. 1. Pedigree information for 10 Chinese families without the three primary mutations. Affected individuals are marked by a filled symbol. Arrows indicate probands who were analyzed for complete mtDNA sequences.

Most of the non-synonymous MT-ND1 and MT-ND5 variants are located in transmembrane helical structures or along the two outer surfaces of the inner mitochondrial membrane, but they did not significantly change the hydrophobicity of the protein (Fig. 4). The previously unreported mitochondrial $tRNA^{Leu}$ variant m.12332A>G is located in acceptor arm of the *MT-TL2* gene. This variant was not conserved among the examined vertebrate species and caused no change of the predicted secondary structure of the *MT-TL2* gene (Fig. S3).

4. Discussion

Many previous studies have been performed to elucidate the pathogenic mechanism of LHON caused by the three primary mutations, which are not sufficient but essential for the onset of LHON [2]. Among these confirmed LHON patients, over 95% of them harbor one of the three primary mutations [2–4]. Compared with these well-defined LHON patients, mtDNA studies for the families with clinical LHON features but without the three primary mutations are insufficient [8]. Genetic analysis of these patients is of particular importance as these patients account for the majority of patients with blindness caused by unknown reasons [8]. Understanding the role of mtDNA mutation in these patients may identify novel primary mutations and facilitate subsequent genetic diagnosis and consultation.

In this study, we selected 10 Chinese families with typical LHON phenotypes and with a confirmed absence of the three primary mutations. The observed penetrance of bilateral visual loss is significantly higher than that of Chinese LHON families with m.11778G>A [5] or m.3460G>A [13]. Because some of the studied pedigrees were small, we thought this bias would contribute to such an exceptionally high penetrance rate, at least partially. Moreover, the percentage of the affected female subjects in these 10 families is also much higher than that observed for typical LHON families with m.11778G>A or m.3460G>A. All these results suggest that the 10 families with suspected LHON had a different clinical onset pattern compared with those families harboring one of the three primary mutations.

Most of previous studies about LHON patients without the three primary mutations were based on European patients [21,22]. The *MT-ND1* and *MT-ND6* genes have been reported to be mutational hotspots in these LHON patients, and several pathogenic mutations for LHON were characterized [23,24]. For instance, mutation m.3733G>A (p.E143K) changed a conserved glutamic acid at position 143 to lysine in the MT-ND1 protein and could significantly decrease the rotenone sensitivity of complex I [24]. We found that non-synonymous variants in the *MT-ND1* and *MT-ND5* genes, especially on the terminal branches of the phylogenetic tree (Fig. S2), were abundant in the 10 Chinese families. Additionally the variants were located either in the same domain as the reported pathogenic mutations or in some domains without pathogenic mutations (Fig. 4). Variant m.3736G>A (p.V144I) of the *MT-ND1* gene in proband Le1235, which was just one amino acid from m.3733G>A (p.E143K) [24], changed a conserved valine at the 144th position

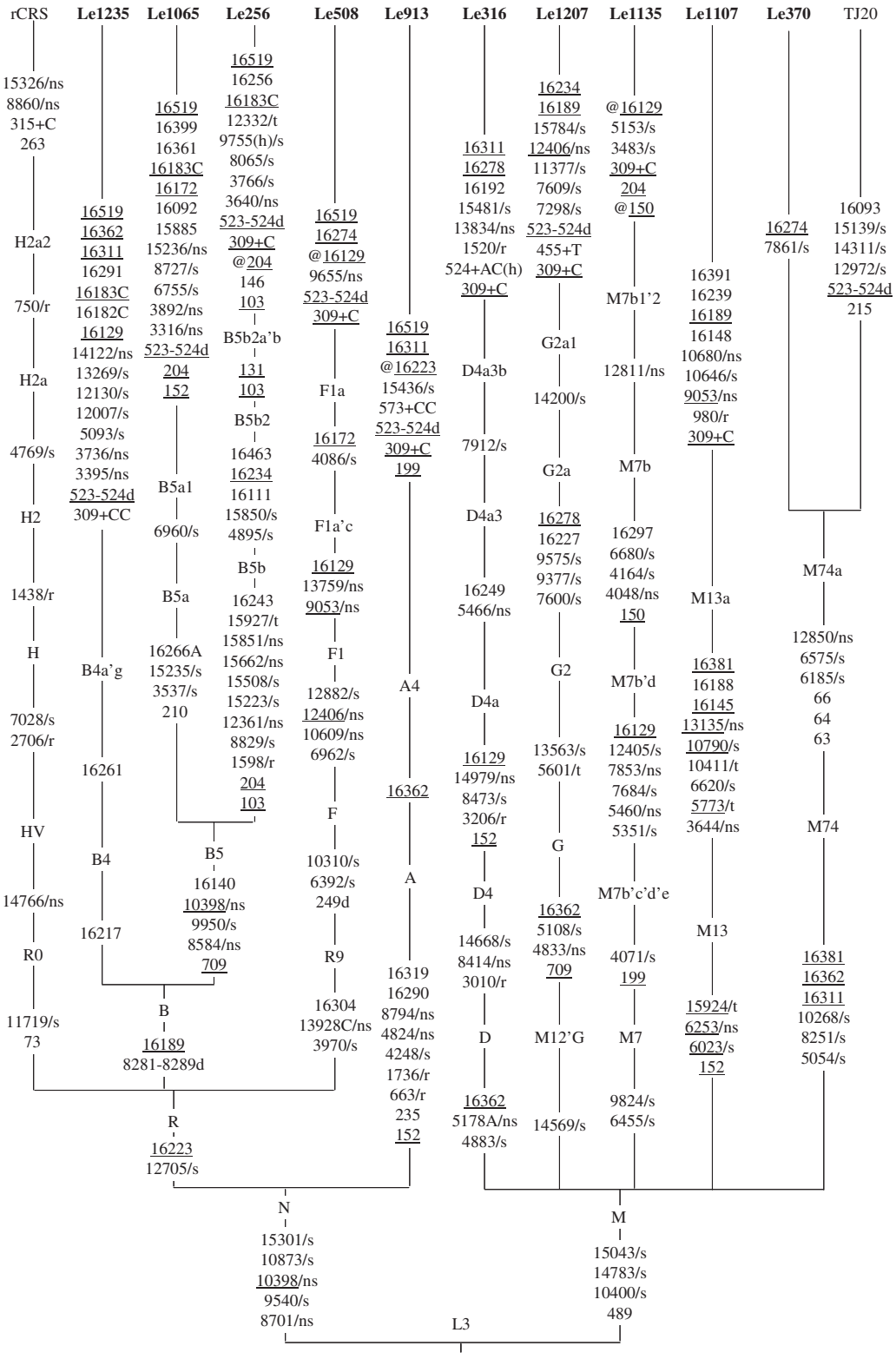

Fig. 2. Classification tree of the complete mtDNA sequences of 11 matrilines sequenced in this study, plus the revised Cambridge reference sequence (rCRS) [14]. Back mutations are highlighted by the prefix "@", and recurrent mutations are underlined. Suffix '+C' indicates an insertion of cytosine. Suffixes A and C refer to transversions, "d" indicates a deletion. Probands Le256 and Le316 had a heteroplasmic status of m.9755G>A and 524+AC, respectively, and were marked by "(*h*)". The synonymous and non-synonymous variants in the mtDNAs are further denoted by "/s" and "/ns", respectively. Variations in the transfer RNA and the ribosomal RNA genes are denoted by "/t" and "/r", respectively.

to isoleucine and most likely account for the onset of LHON in this family. We speculated that both the amino acid changes (p.E143K and p.V144I) shared a similar mechanism of pathogenesis, despite the small physicochemical difference between valine and isoleucine (Grantham value = 29; http://mtsnp.tmig.or.jp/mtsnp/index_e.shtml). Indeed, the predicted structure of the MT-ND1 protein in

Table 1
Private non-synonymous and RNA variants in Chinese families without the three primary mutations.

Family[a]	Haplogroup	Nucleotide variant (amino acid change)	Gene	Conservation index (CI)[b]	Reported (population context)[c]	Reported (disorder context)[c]	Haplogroup specific variant[d]	Variant frequency[e]
Le256	B5b2	m.3640G>A (p.A112T)	*MT-ND1*	65.9	Yes	No[f]	No	1/2196
		m.12332A>G	*MT-TL2*	86.4	No	No	No	0/2196
Le316	D4a3	m.1520T>C	*MT-RNR1*	19.5	Yes	Yes	No	2/2196
		m.13834A>G (p.T500A)	*MT-ND5*	6.8	Yes	Yes	No	2/2196
Le508	F1a	m.9655G>A (p.S150N)	*MT-CO3*	95.5	No	Yes	No	0/2196
Le1065	B5a2	m.3316G>A (p.A4T)	*MT-ND1*	6.85	Yes	Yes	Yes (A2f, D1e, D4e1, etc.)	10/2196
		m.3892A>G (p.T196A)	*MT-ND1*	13.65	Yes	Yes	No	0/2196
		m.15236A>G (p.I164V)	*MT-CYB*	77.3	Yes	Yes	Yes (B4b1a1, M7c3, M12b, etc.)	6/2196
Le1107	M13a	m.980T>C	*MT-RNR1*	40.93	Yes	Yes	Yes (M27a, M61, U7)	6/2196
		m.9053G>A (p.S176N)	*MT-ATP6*	25.0	Yes	Yes	Yes (F1a'c, K2c, Z5)	8/2196
		m.10680G>A (p.A71T)	*MT-ND4L*	95.5	No	Yes	No	0/2196
Le1207	G2a1	m.12406G>A (p.V24I)	*MT-ND5*	2.3	Yes	Yes	Yes (F1, P5, R7a1, etc.)	9/2196
Le1235	B4a'g	m.3395A>G (p.Y30C)	*MT-ND1*	100.0	Yes	Yes	Yes (B5b1c)	3/2196
		m.3736G>A (p. V144I)	*MT-ND1*	100.0	Yes	Yes	No	3/2196
		m.14122A>G (p.I596V)	*MT-ND5*	20.9	Yes	Yes	No	0/2196

[a] The complete mtDNAs of Le370, Le913, and Le1135 contain no private non-synonymous and RNA variants and are not included in Table 1.
[b] The conservation index is the percentage of species for a list of 44 different vertebrate species that have the wild type human sequence (GenBank Accession No. J01415) [19]. The entire list of the species was shown in Fig. S1. Only 43 and 41 species were considered when we calculated the CI of variants m.14122A>G and m.1520T>C, respectively, because the gaps existed in this amino acid position of a few species.
[c] The search was performed on 27 April 2010 following the described strategy [18] (e.g. both 'G3640A mtDNA' and '3640G>A mtDNA' were queried).
[d] The column "Haplogroup specific variant" refers to the presence or absence of the corresponding variants in the world mtDNA phylogeny displayed at http://www.phylotree.org/tree/main.htm (mtDNA tree Build 8; 21 March 2010) [16]. In round brackets we indicate the haplogroup status as is defined in that tree.
[e] The variant frequency was adopted from Soares et al. [28], which was based on the number of occurrences for each variant in 2196 complete mtDNA sequences.
[f] There were many hits when we searched "G3640A" using Google, but all of these hits actually referred to spelling error for "G3460A".

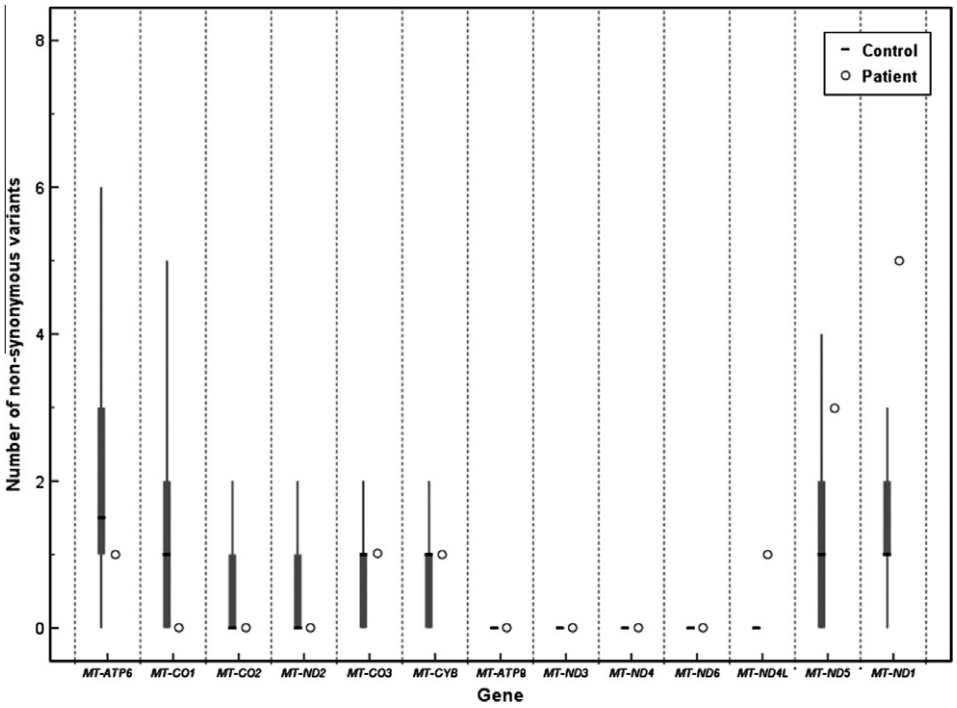

Fig. 3. Box plot of the number of mtDNA non-synonymous variants on the terminal branches of the phylogenetic tree. The simulation resampling of 10 mtDNAs from the reference populations which was composed of 69 complete mtDNAs ([15,20] and this study) were performed by a program developed using Perl.

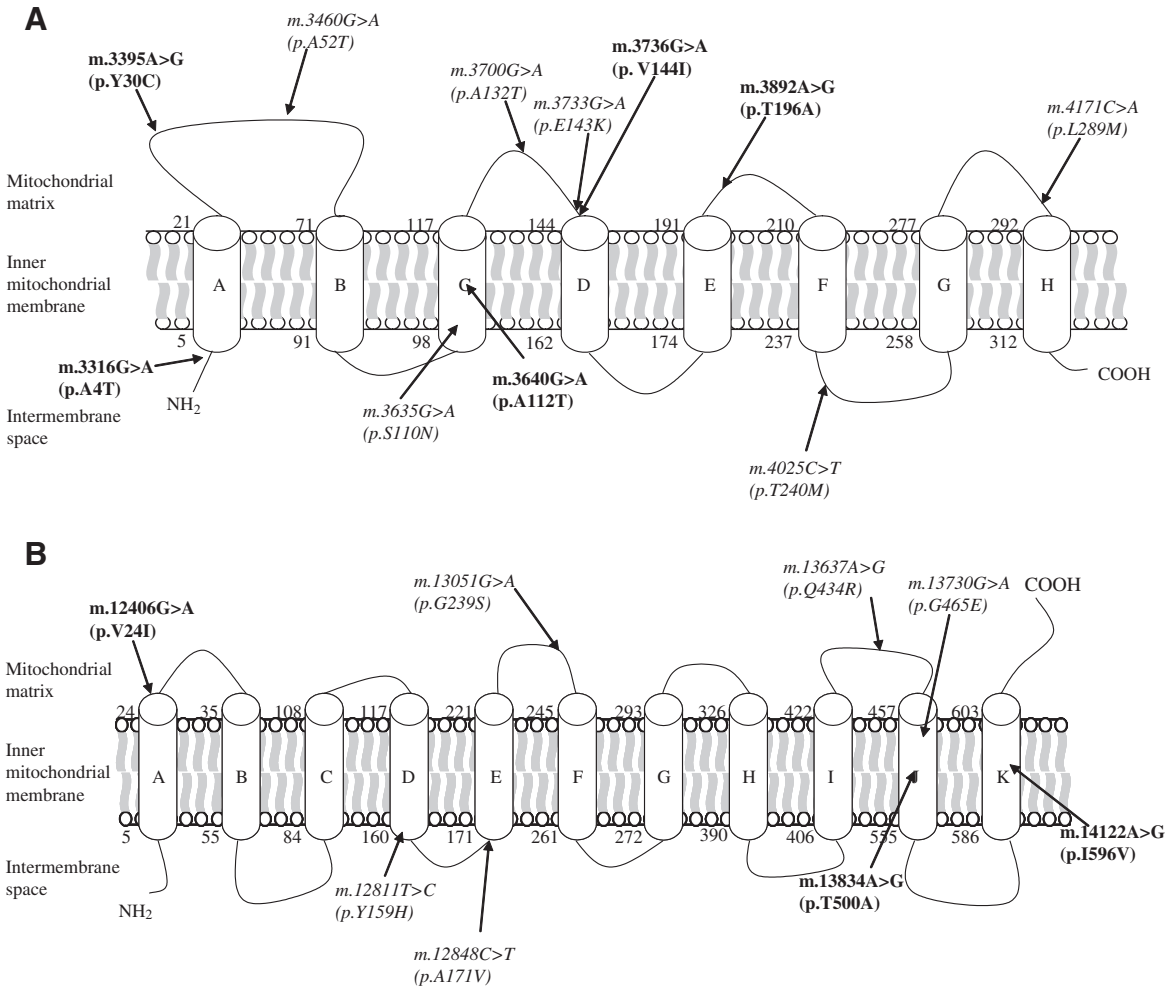

Fig. 4. Predicted transmembrane structures of human MT-ND1 (A) and MT-ND5 (B) proteins by the TMpred program. Cylinders represent transmembrane helices. The *MT-ND1* gene (A) and the *MT-ND5* gene (B) variants identified in this study are in bold and the reported variants that were associated with LHON (cf. MITOMAP) are in italics.

the presence of m.3736G>A (p.V144I) was similar to that of m.3733G>A (p.E143K) (Fig. S4). Intriguingly, proband Le1235 contained an additional private variant m.3395A>G (p.Y30C) in the *MT-ND1* gene. The Y30C replacement caused a reduction of hydrophilicity (Fig. S4) and might render MT-ND1 protein more susceptible to oxidative stress. This variant was reported in two young obese adults [25], but there was no functional assay to characterize its potentially pathogenic role in obesity. The strongly high conservation of m.3736G>A and m.3395A>G would also suggest importance of both positions in MT-ND1 protein. No private non-synonymous variant was found in the *MT-ND*6 gene in this study, despite the fact that mutations in this gene were reported in LHON patients outside of East Asia [23,26,27]. This result may suggest a racial difference but need further study due to limited number of families studied.

Haplogroup classification of the 10 families shows that these lineages belonged to a diverse pool and suggests no role of haplogroup background effect. Therefore, the potentially pathogenic mutations in the 10 families are located in the terminal branch of the mtDNA tree, instead of being presented as shared variants on the internal branches. An extensive analysis of the previously published mtDNA complete sequence data by Soares et al. [28] found that non-synonymous mutations are more frequently detected in the new branches of phylogenetic tree of mtDNA. We evaluated the non-synonymous/synonymous ratio between our patients and 69 Chinese samples ([15,20] and this study). Although we found no statistical difference between the two groups, the non-synonymous mutation rate in the *MT-ND1* and *MT-ND5* genes are substantially higher in our patients than in controls (Table S1). This pattern was further confirmed by our simulation assay (Fig. 3). Evidently, our findings confirmed a notion that mutations in the respiratory chain complex I and the decreased functional activity of this complex may be crucial for the onset of optic neuropathy [29,30].

A rare mtDNA variant m.10680G>A in the *MT-ND4L* gene was recently reported to have a synergistic role with the primary mutation m.14484T>C because of the complete penetrance of LHON in that family [31]. This variant was found in family Le1107 but showed no co-segregation with a primary mutation. This may explain why only 40% of the members in this family show a penetrance of the disease. Based on the available data, m.10680G>A could well be a primary pathogenic LHON mutation in the pedigree Le1107 that shows a quite clear maternal inheritance. Further functional assays are essential to determine the pathogenicity of this mutation.

The novel tRNA variant m.12332A>G in family Le256 is located in the acceptor arm of the *MT-TL2* gene. This variant did not change the predicted secondary structure of this gene (Fig. S3). Two other variants near this location, m.12331A>G and m.12334G>A, express completely contrary functions: m.12331A>G is a polymorphism in world populations, whereas m.12334G>A is claimed to be the pathogenic mutation for muscle disease [32]. Whether

m.12332A>G is a polymorphism or a pathogenic mutation is still an open question.

5. Conclusion

To our knowledge, this is the first study for the penetrance and complete mtDNA genome sequence variation in Chinese patients with LHON features but without the three primary mutations. We found that the *MT-ND1* and *MT-ND5* genes are mutational hotspots for these patients. In particular, we identified several (presumably) pathogenic mutations, e.g. variants m.3736G>A (p.V144I) and m.10680G>A (p.A71T), in our patients. All these results suggest that the mutation pattern in Chinese patients with the typical LHON phenotype but lacking the three primary mutations is quite different from the pattern observed in western European patients.

Acknowledgments

We thank the patients who participated in this study and Miss Diana Chen for language editing. This study was supported by the National Natural Science Foundation of China (30925021), Yunnan Province (2009CI119), Guangdong Province (2009B091300150), and the Chinese Academy of Sciences.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.07.051.

References

[1] V. Carelli, F.N. Ross-Cisneros, A.A. Sadun, Mitochondrial dysfunction as a cause of optic neuropathies, Prog. Retin. Eye Res. 23 (2004) 53–89.
[2] P. Yu-Wai-Man, P.G. Griffiths, G. Hudson, et al., Inherited mitochondrial optic neuropathies, J. Med. Genet. 46 (2009) 145–158.
[3] M.-Y. Yen, A.-G. Wang, Y.-H. Wei, Leber's hereditary optic neuropathy: a multifactorial disease, Prog. Retin. Eye Res. 25 (2006) 381–396.
[4] G. Hudson, V. Carelli, L. Spruijt, et al., Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA-haplogroup background, Am. J. Hum. Genet. 81 (2007) 228–233.
[5] Y. Ji, A.-M. Zhang, X. Jia, et al., Mitochondrial DNA haplogroups M7b1'2 and M8a affect clinical expression of leber hereditary optic neuropathy in Chinese families with the m.11778G>A mutation, Am. J. Hum. Genet. 83 (2008) 760–768.
[6] M.A. Kirkman, P. Yu-Wai-Man, A. Korsten, et al., Gene-environment interactions in Leber hereditary optic neuropathy, Brain 132 (2009) 2317–2326.
[7] N. Phasukkijwatana, B. Kunhapan, J. Stankovich, et al., Genome-wide linkage scan and association study of PARL to the expression of LHON families in Thailand, Hum. Genet. 128 (2010) 39–49.
[8] N.J. Newman, Hereditary optic neuropathies: from the mitochondria to the optic nerve, Am. J. Ophthalmol. 140 (2005) 517–523.
[9] A.-M. Zhang, Y. Zou, X. Guo, et al., Mitochondrial DNA mutation m.3635G>A may be associated with Leber hereditary optic neuropathy in Chinese, Biochem. Biophys. Res. Commun. 386 (2009) 392–395.
[10] J. Yang, Y. Zhu, Y. Tong, et al., Confirmation of the mitochondrial ND1 gene mutation G3635A as a primary LHON mutation, Biochem. Biophys. Res. Commun. 386 (2009) 50–54.
[11] H.-W. Wang, X. Jia, Y. Ji, et al., Strikingly different penetrance of LHON in two Chinese families with primary mutation G11778A is independent of mtDNA haplogroup background and secondary mutation G13708A, Mutat. Res. 643 (2008) 48–53.
[12] X. Jia, S. Li, X. Xiao, et al., Molecular epidemiology of mtDNA mutations in 903 Chinese families suspected with Leber hereditary optic neuropathy, J. Hum. Genet. 51 (2006) 851–856.
[13] D. Yu, X. Jia, A.-M. Zhang, et al., Molecular characterization of six Chinese families with m.3460G>A and Leber hereditary optic neuropathy, Neurogenetics 11 (2010) 349–356.
[14] R.M. Andrews, I. Kubacka, P.F. Chinnery, et al., Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA, Nat. Genet. 23 (1999) 147.
[15] Q.-P. Kong, H.-J. Bandelt, C. Sun, et al., Updating the East Asian mtDNA phylogeny: a prerequisite for the identification of pathogenic mutations, Hum. Mol. Genet. 15 (2006) 2076–2086.
[16] M. van Oven, M. Kayser, Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation, Hum. Mutat. 30 (2009) E386–E394.
[17] Y.-G. Yao, A. Salas, C.M. Bravi, et al., A reappraisal of complete mtDNA variation in East Asian families with hearing impairment, Hum. Genet. 119 (2006) 505–515.
[18] H.-J. Bandelt, A. Salas, R.W. Taylor, et al., Exaggerated status of "novel" and "pathogenic" mtDNA sequence variants due to inadequate database searches, Hum. Mutat. 30 (2009) 191–196.
[19] E. Ruiz-Pesini, D. Mishmar, M. Brandon, et al., Effects of purifying and adaptive selection on regional variation in human mtDNA, Science 303 (2004) 223–226.
[20] Q.-P. Kong, Y.-G. Yao, C. Sun, et al., Phylogeny of east Asian mitochondrial DNA lineages inferred from complete sequences, Am. J. Hum. Genet. 73 (2003) 671–676.
[21] S. Fauser, J. Luberichs, D. Besch, et al., Sequence analysis of the complete mitochondrial genome in patients with Leber's hereditary optic neuropathy lacking the three most common pathogenic DNA mutations, Biochem. Biophys. Res. Commun. 295 (2002) 342–347.
[22] N. Howell, R.-J. Oostra, P.A. Bolhuis, et al., Sequence analysis of the mitochondrial genomes from Dutch pedigrees with Leber hereditary optic neuropathy, Am. J. Hum. Genet. 72 (2003) 1460–1469.
[23] P.F. Chinnery, D.T. Brown, R.M. Andrews, et al., The mitochondrial ND6 gene is a hot spot for mutations that cause Leber's hereditary optic neuropathy, Brain 124 (2001) 209–218.
[24] M.L. Valentino, P. Barboni, A. Ghelli, et al., The ND1 gene of complex I is a mutational hot spot for Leber's hereditary optic neuropathy, Ann. Neurol. 56 (2004) 631–641.
[25] L.-J. Guo, Y. Oshida, N. Fuku, et al., Mitochondrial genome polymorphisms associated with type-2 diabetes or obesity, Mitochondrion 5 (2005) 15–33.
[26] J. Luberichs, B. Leo-Kottler, D. Besch, et al., A mutational hot spot in the mitochondrial ND6 gene in patients with Leber's hereditary optic neuropathy, Graefes Arch. Clin. Exp. Ophthalmol. 240 (2002) 96–100.
[27] S.I. Zhadanov, V.V. Atamanov, N.I. Zhadanov, et al., A novel mtDNA ND6 gene mutation associated with LHON in a Caucasian family, Biochem. Biophys. Res. Commun. 332 (2005) 1115–1121.
[28] P. Soares, L. Ermini, N. Thomson, et al., Correcting for purifying selection: an improved human mitochondrial molecular clock, Am. J. Hum. Genet. 84 (2009) 740–759.
[29] C. Bonnet, S. Augustin, S. Ellouze, et al., The optimized allotopic expression of ND1 or ND4 genes restores respiratory chain complex I activity in fibroblasts harboring mutations in these genes, Biochim. Biophys. Acta 1783 (2008) 1707–1717.
[30] M.D. Brown, I.A. Trounce, A.S. Jun, et al., Functional analysis of lymphoblast and cybrid mitochondria containing the 3460, 11778, or 14484 Leber's hereditary optic neuropathy mitochondrial DNA mutation, J. Biol. Chem. 275 (2000) 39831–39836.
[31] J. Yang, Y. Zhu, Y. Tong, et al., The novel G10680A mutation is associated with complete penetrance of the LHON/T14484C family, Mitochondrion 9 (2009) 273–278.
[32] C. Vives-Bauza, J. Gamez, M. Roig, et al., Exercise intolerance resulting from a muscle-restricted mutation in the mitochondrial tRNA(Leu (CUN)) gene, Ann. Med. 33 (2001) 493–496.