Mitochondrial DNA Haplogroups M7b1′2 and M8a Affect Clinical Expression of Leber Hereditary Optic Neuropathy in Chinese Families with the m.11778G→A Mutation

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Leber hereditary optic neuropathy (LHON) is the most extensively studied mitochondrial disease, with the majority of the cases being caused by one of three primary mitochondrial DNA (mtDNA) mutations. Incomplete disease penetrance and gender bias are two features of LHON and indicate involvement of additional genetic or environmental factors in the pathogenesis of the disorder. Haplogroups J, K, and H have been shown to influence the clinical expression of LHON in subjects harboring primary mutations in European families. However, whether mtDNA haplogroups would affect the penetrance of LHON in East Asian families has not been evaluated yet. By studying the penetrance of LHON in 1859 individuals from 182 Chinese families (including one from Cambodia) with the m.11778G→A mutation, we found that haplogroup M7b1′2 significantly increases the risk of visual loss, whereas M8a has a protective effect. Analyses of the complete mtDNA sequences from LHON families with m.11778G→A narrow the association of disease expression to m.12811T→C (Y159H) in the *NADH dehydrogenase 5* gene (*MT-ND5*) in haplogroup M7b1′2 and suggest that the specific combination of amino acid changes (A20T-T53I) in the ATP synthase 6 protein (MT-ATP6) caused by m.8584G→A and m.8684C→T might account for the beneficial background effect of M8a. Protein secondary-structure prediction for the MT-ATP6 with the two M8a-specific amino acid changes further supported our inferences. These findings will assist in further understanding the pathogenesis of LHON and guide future genetic counseling in East Asian patients with m.11778G→A.

Leber hereditary optic neuropathy (LHON, MIM 535000) is a common cause of acute or subacute visual loss in young adults, predominately affecting males.[1–4] The prevalence of LHON in western Europe is about one in 25,000–50,000 individuals.[1,5,6] Genetic defects in the mitochondrial DNA (mtDNA) genome play a key role in the development of LHON, in which the three primary mtDNA mutations (m.11778G→A [R340H] in *NADH dehydrogenase 4* gene [*MT-ND4*, MIM 516003], m.14484T→C [M64V] in *MT-ND6* [MIM 516006], and m.3460G→A [A52T] in *MT-ND1* [MIM 516000]) contribute to about 95% of LHON cases.[1,4,6] However, the phenotypic expression of these primary mutations is very complex. Only about one-third of individuals harboring one of these three mutations eventually develop LHON, and the penetrance varies among different families.[1,7,8] Therefore, identification of other factors affecting LHON penetrance would be of value in elucidating the pathophysiology of retinal neuron loss, as well as in searching for clues that might relieve visual loss or prevent the onset of LHON. Many factors, such as mtDNA background, heteroplasmy of mtDNA mutation, nuclear gene(s), and environmental factors, have been shown to play active roles in the phenotypic expression of LHON.[1,4,9–20]

Most recently, Hudson et al.[7] provided clear evidence that the expression of LHON primary mutations was influenced by the mtDNA haplogroup background in European families. The risk of visual failure is higher when m.11778G→A or m.14484T→C mutations are present in haplogroup J and when m.3460G→A is present in haplogroup K, whereas haplogroup H reduces the disease manifestation in families with m.11778G→A.[7] The cause of the association of mtDNA background effect (subclades J1 and J2b) with LHON expression in families with m.11778G→A or m.14484T→C has been narrowed to two specific combinations of amino acid changes (L236I-F19L and L236I-D171N-V356M) in the *cytochrome b* gene (*MT-CYB*; MIM 516020).[21] Because the distribution patterns of the three primary mutations[7,8] and the matrilineal genetic structures[22,23] differ remarkably in populations from Europe and East Asia, it is indispensable to disclose the potential haplogroup effects on LHON expression in East Asians.

One hundred and seventy-five families with LHON and the m.11778G→A mutation were identified across China from our routine clinical diagnosis of 1369 unrelated subjects suspected of having LHON (including those families that were described in our previous studies[8,24–26]). The presence of the primary mutation m.11778G→A was verified by direct sequencing or allele-specific PCR in all families in the present study. In all cases, we detected no heteroplasmy for the m.11778G→A mutation. The clinical diagnoses were determined at the Zhongshan Ophthalmic Center or by local ophthalmologists. Unaffected individuals were defined as having no vision impairment. Informed

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Table 1. Haplogroup Frequency for the 1859 Subjects, from 182 Pedigrees, with the Primary LHON Mutation m.11778G→A

Haplogroup	No. of Subjects (%)[a]	No. of Families (%)[a]	Pooled Han Chinese (%)[b]	p Value[c]
D4	432 (23.24)	43 (23.63)	56 (13.73)	0.004
D5	161 (8.66)	15 (8.24)	27 (6.62)	0.491
B4	197 (10.60)	19 (10.44)	55 (13.48)	0.347
B5	115 (6.19)	10 (5.49)	17 (4.17)	0.523
M7b1′2	136 (7.32)	15 (8.24)	24 (5.88)	0.370
M7c	122 (6.56)	12 (6.59)	11 (2.70)	0.036
G	139 (7.48)	12 (6.59)	14 (3.43)	0.126
M8a	112 (6.02)	10 (5.49)	18 (4.41)	0.675
M10	113 (6.08)	8 (4.40)	8 (1.96)	0.104
A	75 (4.03)	10 (5.49)	25 (6.13)	0.852
Y	60 (3.23)	6 (3.30)	6 (1.47)	0.203
C	46 (2.47)	7 (3.85)	11 (2.70)	0.605
F	27 (1.45)	4 (2.20)	68 (16.67)	1×10^{-6}
N9a	34 (1.83)	3 (1.65)	15 (3.68)	0.209
M12	35 (1.88)	2 (1.10)	2 (0.49)	0.591
R11	9 (0.48)	2 (1.10)	4 (0.98)	1.000
M9a	8 (0.43)	1 (0.55)	10 (2.45)	0.186
Z	9 (0.48)	1 (0.55)	8 (1.96)	0.287
Other	29 (1.56)	2 (1.10)	29 (7.11)	0.001
Total	1859	182	408	–

[a] The reported families[24–26,28–32] were also included.
[b] Pooled Han Chinese individuals from Yunnan, Hubei, Xinjiang, Liaoning, Shandong, and Guangdong Provinces reported by Yao et al.[23,39] and Kivisild et al.[37]
[c] Fisher's exact test (two-tailed) was performed on the basis of the number of lineages in the pooled Han Chinese and LHON samples.

Table 2. Haplogroup Distribution of Affected and Unaffected Individuals in 182 Families with the Primary Mutation m.11778G→A

Sex and Haplogroup	No. of Individuals with G11778A	
	Affected	Unaffected
Male		
M7b1′2	41	27
M7c	25	42
M8a	15	39
C	20	9
M10	30	33
D4	91	129
D5	37	34
A	24	16
B4	44	57
B5	21	32
G	41	26
Y	14	20
F	7	5
Other	26	35
Total	436	504
Female		
M7b1′2	15	53
M7c	9	46
M8a	7	51
C	3	14
M10	10	40
D4	43	169
D5	23	67
A	7	28
B4	20	76
B5	10	52
G	21	51
Y	1	25
F	3	12
Other	11	52
Total	183	736

The previously reported Chinese families with LHON and m.11778G→A[25,26,28–32] were included. For detailed information, refer to Tables S1 and S2.

consent, 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, was obtained from participants prior to this study, which was approved by the institutional review boards of the Zhongshan Ophthalmic Center and the Kunming Institute of Zoology. We followed the available approach to assign each mtDNA to its respective haplogroup, as previously described.[23,24,27] In brief, each sample was analyzed for a 1.4 kb fragment (region 16024-850) that covers the entire mtDNA control-region sequence and was classified on the basis of the recognition of the haplogroup motif and its matching or near-matching with reported Chinese mtDNAs.[23,27] Coding-region mutation motifs (e.g., m.5178C→A [MT-ND2: L237M; MIM 516001] for haplogroup D, recognized by −5176*Alu*I) were screened to further solidify the inferred haplogroup status for some lineages. In addition, LHON penetrance information of ten reported Chinese families with m.11778G→A[25,26,28–32] was also included for analysis in this study. Note that there were some errors in seven of those reported complete mtDNA sequences[28–32] and that we classified those samples following a well-described strategy.[33,34] The complete mtDNA sequence was determined in probands from seven M7b families, three F families, three M8a families, and two G families via the same strategy and amplification and sequencing conditions as described in our recent study.[25] Sequence variations were scored relative to the revised Cambridge reference sequence (rCRS).[35] The classification tree of the complete mtDNA sequences was drawn via the same procedure as described in our previous studies and others.[33,36–38]

We did not consider the age of subjects as a risk factor for the disease penetrance and included all subjects, irrespective of age, in order to avoid an ascertainment bias in elevating the penetrance value in the pedigree.[7] The following family members were excluded from the analysis: (1) the first generation, (2) spouses of the matrilineal members, and (3) children of the male member in each family. In total, we evaluated the penetrance of LHON in 1859 individuals carrying the m.11778G→A mutation, from 182 Chinese families that were located in South and North China (including one family from Cambodia). Among these subjects, 50.6% were male and 49.4% were female. Binary logistic regression was used for determining the effects of the variables (sex and haplogroup) on the

Table 3. Effect of Gender and mtDNA Haplogroups on Phenotypic Manifestation of the Primary Mutation m.11778G→A

	All 182 Families			140 Families[a]		
Variable	p Value	Odds Ratio	95% CI	p Value	Odds Ratio	95% CI
Sex	2.613×10^{-32}	3.479	2.830–4.277	5.576×10^{-27}	3.324	2.671–4.138
M7b1′2	0.032	1.503	1.035–2.183	0.015	1.631	1.100–2.416
M7c	0.101	0.702	0.460–1.071	0.123	0.706	0.454–1.099
M8a	0.002	0.460	0.282–0.751	0.001	0.391	0.224–0.682
C	0.050	1.845	1.000–3.404	0.154	1.655	0.828–3.308
M10	0.861	1.038	0.685–1.571	0.451	1.173	0.775–1.778
D4	0.213	0.858	0.675–1.092	0.151	0.827	0.638–1.071
D5	0.092	1.353	0.952–1.923	0.076	1.394	0.965–2.013
A	0.157	1.426	0.872–2.331	0.154	1.467	0.867–2.481
B4	0.744	0.947	0.682–1.314	0.911	0.981	0.697–1.381
B5	0.204	0.752	0.484–1.167	0.165	0.714	0.443–1.149
G	0.001	1.827	1.266–2.637	0.003	1.825	1.230–2.709
Y	0.091	0.590	0.320–1.088	0.097	0.580	0.305–1.104
F[b]	0.530	1.302	0.571–2.966	–	–	–
Other	0.429	0.847	0.560–1.280	0.705	0.928	0.629–1.368

Statistical testing was performed on the basis of the original data in Table S2. The visual failure was regarded as the dependent variable in the binary logistic-regression model. The haplogroups (present in at least four families) were separately introduced into the regression equation with the independent variable *sex* and the dependent variable *visual failure*.

[a] 42 small pedigrees were excluded.

[b] When the small pedigrees were not considered, haplogroup F was excluded because of the small number of families.

phenotypic expression of LHON with the use of SPSS 13.0 (SPSS, Chicago, IL). In regard to sample size and statistical power, we only considered the haplogroups shared by at least four families as variables in the statistical analysis. The remaining haplogroups were aggregated together into one variable. In total, 14 variables, including haplogroups M7b1′2, M7c, M8a, C, M10, D4, D5, A, B4, B5, G, Y, F, and others (lumping together Z, M9, M12, N9a, R11, and U), were separately introduced into the regression equation, with the independent variable *sex* and the dependent variable *visual failure*. Other potential variables, such as heteroplasmy of the primary mutation and pedigree generation, were neglected in our study. A p value less than 0.05 was regarded as statistically significant.

Table 1 and Table S1 (available online) list the distribution frequencies of mtDNA haplogroups in our cohort of families. The overall pattern was similar to the general profile that was observed in 41 families in our recent study,[24] with haplogroups D, B, and M7 being the prevalent haplogroups. Only four of the 182 families (2.2%) with m.11778G→A belonged to haplogroup F (including family WZ4 reported by Qian et al.[29]); this frequency was significantly lower than the expected frequency, which ranges from 6%–27% in the regional Chinese populations across China.[23,37,39] However, the overall LHON penetrance in these four families ranged from 25%–75% and was not at a lower rate of penetrance compared to that in those families with other haplogroup status (Table 2 and Table S2). We also failed to observe a significant haplogroup effect of F on the penetrance of LHON (p = 0.530; odds ratio [OR] = 1.302; 95% confidence interval [CI] = 0.571–2.966) in these F families (Table 3). The exact reason for such a low frequency of F in LHON lineages[24] with no effect on penetrance remains unclear. Population stratification might account for this pattern. When we pooled the published regional Han Chinese[23,37,39] as one population, to mimic the heterogeneous nature of the LHON population, haplogroup F was still significantly lower in the LHON population compared to the aggregated sample (Fisher's exact test, two tailed $p = 1 \times 10^{-6}$). Conversely, the frequencies of haplogroups D4 and M7c were significantly higher ($p < 0.05$) in the LHON patients compared to the pooled Han Chinese, but none of them affected the LHON penetrance (Tables 1 and 3). Analysis of the complete mtDNA genomes of the four haplogroup F families with LHON and m.11778G→A failed to provide any useful information (Figure 1 and Table 4), because these mtDNA samples belonged to different subbranches (two F1a, one F1b, and one F2a).

Consistent with a previous report for European LHON patients,[7] sex is also the strongest predictor for visual loss in Chinese families ($p = 2.613 \times 10^{-32}$; OR = 3.479; 95% CI = 2.830–4.277), with a 3.5-fold increased risk of visual failure for males compared with females. Haplogroups M7b1′2 and G increased the risk of visual failure 1.5-fold (p = 0.032; OR = 1.503; 95% CI = 1.035–2.183) and 1.8-fold (p = 0.001, OR = 1.827, 95% CI = 1.266–2.637), respectively. Haplogroup M8a was found to be associated with a reduced risk (p = 0.002; OR = 0.460; 95% CI = 0.282–0.751). Because some of the pedigrees studied here were relatively small, we then excluded 42 pedigrees (each having five maternally related individuals at most) in order to eliminate the potential bias in scoring the affected and unaffected individuals in these small pedigrees. Analysis for the residual 1710 subjects from 140 families then yielded similar results, with an increased risk for haplogroups M7b1′2 (p = 0.015, OR = 1.631, 95% CI = 1.100–2.416) and G (p = 0.003, OR = 1.825, 95% CI = 1.230–2.709) and a reduced risk for

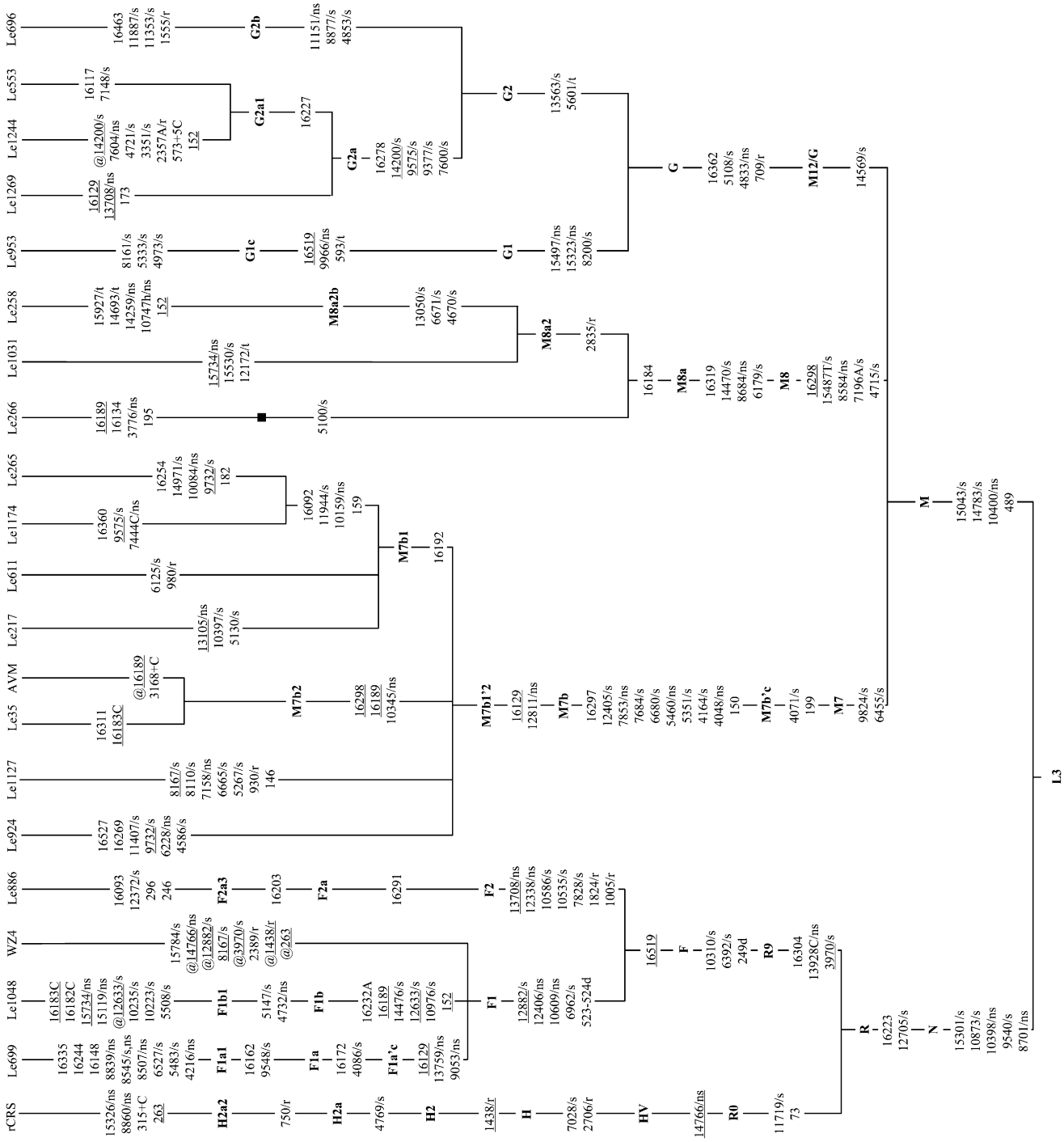

Figure 1. Classification Tree of 20 Complete mtDNAs with m.11778G→A and the Revised Cambridge Reference Sequence

Haplogroup names are inserted along the branches that determine the locations of the corresponding ancestral haplotypes, following the most recent update of the East Asian mtDNA phylogeny.[36] Suffixes "C" and "A" refer to transversions, and "+5C" signifies an insertion of five cytosines. Deletion and heteroplasmy of a mutation are indicated by suffixes "d" and "h," respectively. Back mutations are highlighted by the prefix "@," and recurrent mutations are underlined. The synonymous and nonsynonymous coding-region variants in the samples are further denoted by "/s" and "/ns," respectively. Nucleotide variations that are located in the tRNA genes and the rRNA genes are marked with "/t" and "/r," respectively. Length mutations of the C-tract in region 303–309 and the m.11778G→A mutation in the 20 mtDNAs were omitted from the tree. Sequence WZ4 is taken from Qian et al.[29] but obscured by several errors ([33]). Families Le1244, Le1269, and Le696 are taken from our recent studies.[25,26] The Japanese LHON patient with intracranial arteriovenous malformation (AVM) is taken from Fujitake et al.[40]

Table 4. Private Nonsynonymous mtDNA Sequence Variations in Chinese Families with LHON and m.11778G → A

Family	Haplogroup	Nucleotide Variant (Amino Acid Change)[a]	Gene	Reported (Population Context)[b]	Reported (Disease Context)[b]	Conservation[c]
Le699	F1a1	G8839A (A105T)	*MT-ATP6*	yes	yes	yes
		G8545A (A7T)	*MT-ATP6*	yes	no	no
		A8507G (N48D)	*MT-ATP8*	yes	no	no
		T4216C (Y304H)	*MT-ND1*	yes	yes	no
Le1048	F1b1	G15119A (A125T)	*MT-CYB*	yes	no	yes
		G15734A (A330T)	*MT-CYB*	yes	yes	yes
Le924	M7b1′2	C6228T (L109F)	*MT-CO1*	no	no	no
Le1127	M7b1′2	A7158G (I419V)	*MT-CO1*	yes	yes	no
Le217	M7b1	A13105G (I257V)	*MT-ND5*	yes	yes	no
Le1174	M7b1	G7444C (X514T)	*MT-CO1*	no	no	–
		C10159T (S34F)	*MT-ND3*	no	no	no
Le265	M7b1	T10084C (I9T)	*MT-ND3*	yes	no	no
		C10159T (S34F)	*MT-ND3*	no	no	no
Le266	M8a	G3776A (S157N)	*MT-ND1*	no	no	no
Le1031	M8a2	G15734A (A330T)	*MT-CYB*	yes	yes	yes
Le258	M8a2b	T10747A (L93Q)[d]	*MT-ND4L*	no	no	yes
		G14259A (P139S)	*MT-ND6*	yes	no	no
Le953	G1c	G9966A (V254I)	*MT-CO3*	yes	yes	yes
Le1269	G2a	G13708A (A458T)	*MT-ND5*	yes	yes	no
Le1244	G2a1	G7604A (V7M)	*MT-CO2*	yes	no	no

[a] The nucleotide variants were listed in a format for web-based searches, e.g. mutation G8839A should be presented as 8839G→A and m.8839G→A according to the "traditional" and "approved" formats for mtDNA-mutation nomenclature[56], respectively.
[b] The search was performed on Aug 18, 2008, with the strategy described in Bandelt et al.[41] followed (e.g. both "G8839A mtDNA" and "8839G→A mtDNA" were queried).
[c] The conservation analysis was performed by a comparison of human mtDNA (GenBank accession no. J01415) to eight different vertebrate species, including zebrafish (NC_002333), frog (AB043889), blue whale (NC_001601), mouse (AY466499), cattle (AY526085), horse (EF597513), dog (DQ480502), and gorilla (NC_001645).
[d] This site is heterogeneous for T and A.

haplogroup M8a ($p = 0.001$, OR = 0.391, 95% CI = 0.224–0.682) (Table 3). To minimize the probability of type II errors in the above test, we performed logistic regression by introducing all 14 variables in the regression equation, with the independent variable *sex* and the dependent variable *visual failure*. The increased risk for haplogroups M7b1′2 and G and the decreased risk for haplogroup M8a in phenotypic manifestation of m.11778G→A was further confirmed (considering all 182 families: M7b1′2, $p = 0.012$, OR = 1.630, 95% CI = 1.116–2.382; G, $p = 4.59 \times 10^{-4}$, OR = 1.949, 95% CI = 1.342–2.832; M8a, $p = 0.013$, OR = 0.535, 95% CI = 0.326–0.878; excluding 42 small pedigrees: M7b1′2, $p = 0.008$, OR = 1.714, 95% CI = 1.150–2.554; G, $p = 0.002$, OR = 1.903, 95% CI = 1.275–2.840; M8a, $p = 0.005$, OR = 0.445, 95% CI = 0.254–0.779).

The association between an increased risk of visual loss and haplogroup G is unexpected, because different families belonging to this haplogroup presented strikingly different penetrance patterns.[25,26] In particular, one reported family (Le696)[26] had a very high penetrance (78.6%) and harbored two pathogenic mutations, m.1555A→G (*MT-RNR1*; MIM 561000) and m.11778G→A, which might have enhanced the phenotypic expression and caused a bias in estimation of the haplogroup background effect. Indeed, when we excluded this family, together with four small pedigrees from the analysis, haplogroup G did not significantly increase the penetrance of LHON ($p = 0.051$, OR = 1.526, 95% CI = 0.998–2.335). Therefore, the effect of haplogroup G on the clinical expression of LHON should be treated with caution and further verified in a future study with more pedigrees. Analysis of the five LHON families with G status shows that these mtDNA samples can be grouped into subhaplogroups G1c, G2a, and G2b and thus share only one nonsynonymous haplogroup-specific variant, viz. m.4833A→G (T122A) in the *MT-ND2* gene (Figure 1), which might account for the predisposing effect of haplogroup G in LHON penetrance.

To further define the effect of haplogroup M7b1′2 on LHON penetrance, we narrowed the potential association to specific mtDNA mutations by analyzing the entire mtDNA genomes of eight M7b1′2 families (including one previously reported Japanese LHON proband with intracranial arteriovenous malformation[40]) (Figure 1). All probands shared a string of nonsynonymous mutations (m.4048G→A [D248N] in *MT-ND1*, m.5460G→A [A331T] in *MT-ND2*, and m.7853G→A [V90I] in *MT-CO2* [*cytochrome c oxidase II*; MIM 516040]) and synonymous variants (m.4164A→G in *MT-ND1*, m.5351A→G in *MT-ND2*, m.6680T→C in *MT-CO1* [MIM 516030], m.7684T→C in *MT-CO2*) that are characteristic of haplogroup M7b, as well as m.12811T→C (Y159H) in *MT-ND5* (MIM 516005), which defines haplogroup M7b1′2. At the twig level, we identified five

nonsynonymous mutations in families Le924 (m.6228C→T [MT-CO1: L109F]), Le1127 (m.7158A→G [MT-CO1: I419V]), Le1174 (m.7444G→C [MT-CO1: X514F]; m.10159C→T [MT-ND3: S34F; MIM 516002]), and Le217 (m.13105A→G [MT-ND5: I257V]). With the exception of m.6228C→T, m.7444G→C, and m.10159C→T, all variants can be found in reported mtDNA samples via standard database and web-based searches.[41] Some of the variants are evolutionarily conserved and have been reported in disease context (Table 4). None of these mtDNA variants has been reported to be associated with LHON, except for m.12811T→C, which was considered to be a secondary mutation for LHON expression[42] and was present in two out of 35 Finnish LHON probands.[5] In addition, m.12811T→C could be found in three out of 63 Dutch LHON patients,[43] one case individual with cancer,[44] and one patient with cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (MIM 125310),[45] according to extensive database searches. In the non-European context, m.12811T→C was regarded as a haplogroup-specific polymorphism in East Asians (M7b1′2)[36] and Native Americans (A2h).[46] The potentially synergistic effect of m.11778G→A and m.12811T→C might be the reason for an increased penetrance on the haplogroup M7b1′2 background.

Intriguingly, three of the five M7b1′2 lineages that harbored nonsynonymous mutations at the twig level also had private amino acid changes in the MT-CO1 gene (Figure 1 and Table 4); this suggests that the decreased activity of cytochrome c oxidase and the partial dysfunction of complex IV might be related to the onset of LHON.[9,47–49] For instance, m.7444G→A in the *MT-CO1* gene causes a change of the termination codon of MT-CO1 to lysine and was claimed to be associated with LHON,[47] although this variant was prevalent in haplogroup V and should be categorized as a polymorphism.[33] Note that a recent study showed that pathogenic mutations are also common in the general population.[50] The previously unpublished variant m.7444G→C in Le1174 causes a similar problem as that of m.7444G→A and results in a change of the termination codon to threonine. Whether the change of the mitochondrial respiratory-chain complexes I and IV activities caused by the above mutations in MT-CO1, MT-CO2, MT-ND1, MT-ND2, MT-ND3, and MT-ND5 in M7b1′2 lineages would account for an increased risk for LHON awaits further experimental study. It is worth noting that in a recent study by Kazuno et al.,[51] the four cybrid lines containing mtDNA with haplogroup status G1a1 (two), M7b2, and M7a1a generally had a lower cytosolic calcium response to histamine and a higher-level mitochondrial matrix pH compared to those cybrids containing mtDNA belonging to haplogroups N9a, A, B4, etc. This result suggests that potential alterations in mitochondrial pH and calcium concentration caused by the haplogroup background effects of M7b1′2 and G might be one of the mechanisms for the increased risk of LHON penetrance.

A protective effect of mtDNA haplogroup background has been reported for several diseases; e.g., haplogroup N9a confers resistance to type 2 diabetes in Asians[52] and to metabolic syndrome in Japanese women,[53] and haplogroup H reduces the risk of visual failure in European families with m.11778G→A.[7] In this study, we found that M8a enacted a protective effect on the disease expression in Chinese LHON families, and this protective effect became even more pronounced when we discarded small pedigrees. Analysis of three complete M8a mtDNA sequences from these families showed that each mtDNA had at least one private nonsynonymous nucleotide change at the twig level (Figure 1 and Table 4). Family Le258 had a previously unpublished heterogeneous mutation at site 10747, and this site was conserved in vertebrates. Intriguingly, the haplogroup-specific nonsynonymous-variant pair m.8584G→A and m.8684C→T, causing a combination of amino acid changes A20T and T53I, is located in the *ATP synthase 6* gene (*MT-ATP6*; MIM 516060). We performed protein secondary-structure modeling for the MT-ATP6 protein harboring the two M8a-specific amino acid changes in comparison to mutants containing a well-known pathogenic mutation at site 8993 (m.8993T→G or m.8993T→C), a rare LHON mutation m.9101T→C,[54,55] as well as the wild-type (rCRS) by using the TMpred program. As shown in Figure 2, MT-ATP6 is a largely hydrophobic protein and contains two hydrophilic loops. Both m.8993T→C and m.8993T→G mutants alter the hydrophobicity, but m.8993T→G has a stronger effect, whereas m.9101T→C decreases the hydrophobicity close to the C-terminal end. The amino acid change A20T of M8a decreases the hydrophobicity, but this change is balanced by a reduction of hydrophilicity in the adjacent region, caused by T53I. It thus seems that the two specific amino acid changes are the cause of the protective effect of M8a and that they enhance the activity of the mitochondrial ATP synthase complex. Experimental data will be essential for confirming this speculation.

In summary, by studying 1859 individuals in 182 Chinese families with LHON and m.11778G→A, we found that haplogroup M7b1′2, as well as, possibly, haplogroup G, significantly increased the risk of visual failure in Chinese individuals with m.11778G→A, whereas M8a might have a protective effect on the penetrance of LHON. Sex is the most significant factor for influencing the clinical expression of LHON (3.48-fold) in Chinese families but this influence is lower than that in European LHON families (5.41-fold). Haplogroup F is present at a much lower frequency in these affected families than in the general Han Chinese when we only counted the matrilines, whereas haplogroups D4 and M7c are present at a significantly higher frequency in the affected families. However, none of these haplogroups showed any effect on the penetrance of LHON. Similarly, frequencies of haplogroups M7b1′2, G, and M8a are not significantly increased in LHON pedigrees despite their apparent background effect on the penetrance. The exact reason for this apparent

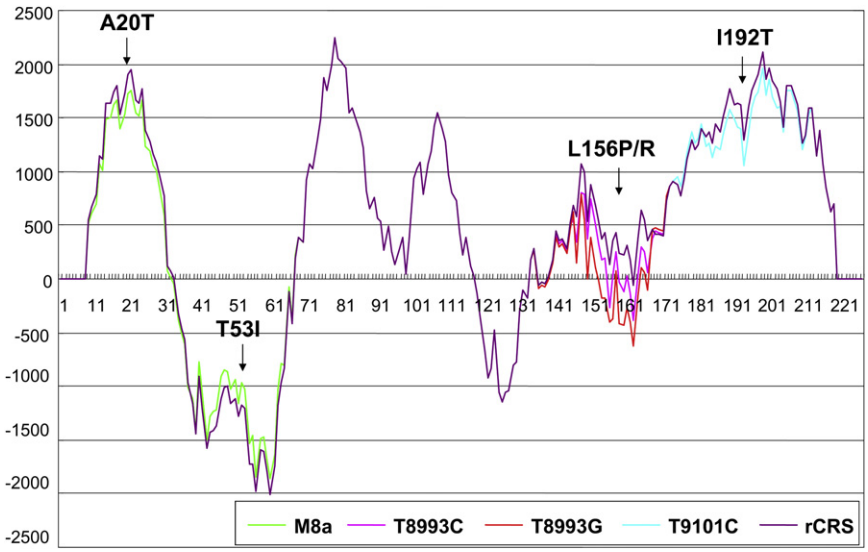

Figure 2. A Hydrophobicity Chart for the MT-ATP6 Protein Predicted by the TMpred Program

The hydrophobicity of the MT-ATP6 protein harboring the two haplogroup M8a specific amino acid changes (A20T-T53I) is compared to the wild-type MT-ATP6 (rCRS) and the known pathogenic mutants caused by m.8993T→C (L156P) and m.8993T→G (L156R), as well as, a rare LHON mutation m.9101T→C (I192T).

inconsistency remains unclear and this pattern is in contrast to the European study,[7] in which an internal consistency of the haplogroup association was observed, namely, haplogroup J is present at an increased frequency in LHON families with m.11778G→A and m.14484T→C, and subdivisions of this haplogroup have increased penetrance. Analysis of the complete mtDNA sequences of LHON probands with M7b1′2 and G status did not identify the two specific combinations of cytochrome *b* amino acid changes that are responsible for the background effect of haplogroups J1c and J2b in the penetrance of LHON in western European patients,[21] suggesting different mtDNA mutation spectra and mechanisms in the penetrance of LHON in the East and the West. The increased risk of LHON penetrance of haplogroup M7b1′2 may be due to the coexistence of m.11778G→A and m.12811T→C, whereas the effect of haplogroup G as a risk factor in the disease expression may be related to the nonsynonymous mutation m.4833A→G in the *MT-ND2* gene. The haplogroup-specific combination of two amino acid changes A20T and T53I in the MT-ATP6 protein may be the cause for a beneficial background effect of M8a. The identification of haplogroup background in LHON expression in Chinese families will undoubtedly help to understand the pathogenesis of LHON and guide future genetic counseling.

Supplemental Data

Supplemental Data include two tables and can be found with this paper online at http://www.ajhg.org/.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
TMpred, http://www.ch.embnet.org/software/TMPRED_form.html

Accession Numbers

The mtDNA sequences reported herein have been submitted to GenBank under accession numbers FJ198229–FJ198385 and FJ198214–FJ198228.

References

1. Man, P.Y.W., Turnbull, D.M., and Chinnery, P.F. (2002). Leber hereditary optic neuropathy. J. Med. Genet. *39*, 162–169.
2. Carelli, V., Ross-Cisneros, F.N., and Sadun, A.A. (2004). Mitochondrial dysfunction as a cause of optic neuropathies. Prog. Retin. Eye Res. *23*, 53–89.
3. Carelli, V., Rugolo, M., Sgarbi, G., Ghelli, A., Zanna, C., Baracca, A., Lenaz, G., Napoli, E., Martinuzzi, A., and Solaini, G. (2004). Bioenergetics shapes cellular death pathways in Leber's hereditary optic neuropathy: a model of mitochondrial neurodegeneration. Biochim. Biophys. Acta *1658*, 172–179.
4. Yen, M.-Y., Wang, A.-G., and Wei, Y.-H. (2006). Leber's hereditary optic neuropathy: a multifactorial disease. Prog. Retin. Eye Res. *25*, 381–396.
5. Puomila, A., Hamalainen, P., Kivioja, S., Savontaus, M.L., Koivumaki, S., Huoponen, K., and Nikoskelainen, E. (2007).

Epidemiology and penetrance of Leber hereditary optic neuropathy in Finland. Eur. J. Hum. Genet. *15*, 1079–1089.
6. Man, P.Y.W., Griffiths, P.G., Brown, D.T., Howell, N., Turnbull, D.M., and Chinnery, P.F. (2003). The epidemiology of Leber hereditary optic neuropathy in the North East of England. Am. J. Hum. Genet. *72*, 333–339.
7. Hudson, G., Carelli, V., Spruijt, L., Gerards, M., Mowbray, C., Achilli, A., Pyle, A., Elson, J., Howell, N., La Morgia, C., et al. (2007). Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA-haplogroup background. Am. J. Hum. Genet. *81*, 228–233.
8. Jia, X., Li, S., Xiao, X., Guo, X., and Zhang, Q. (2006). Molecular epidemiology of mtDNA mutations in 903 Chinese families suspected with Leber hereditary optic neuropathy. J. Hum. Genet. *51*, 851–856.
9. Smith, K.H., Johns, D.R., Heher, K.L., and Miller, N.R. (1993). Heteroplasmy in Leber's hereditary optic neuropathy. Arch. Ophthalmol. *111*, 1486–1490.
10. Hudson, G., Keers, S., Man, P.Y.W., Griffiths, P., Huoponen, K., Savontaus, M.L., Nikoskelainen, E., Zeviani, M., Carrara, F., Horvath, R., et al. (2005). Identification of an X-chromosomal locus and haplotype modulating the phenotype of a mitochondrial DNA disorder. Am. J. Hum. Genet. *77*, 1086–1091.
11. Man, P.Y., Brown, D.T., Wehnert, M.S., Zeviani, M., Carrara, F., Turnbull, D.M., and Chinnery, P.F. (2002). NDUFA-1 is not a nuclear modifier gene in Leber hereditary optic neuropathy. Neurology *58*, 1861–1862.
12. Ishikawa, K., Funayama, T., Ohde, H., Inagaki, Y., and Mashima, Y. (2005). Genetic variants of TP53 and EPHX1 in Leber's hereditary optic neuropathy and their relationship to age at onset. Jpn. J. Ophthalmol. *49*, 121–126.
13. Danielson, S.R., Carelli, V., Tan, G., Martinuzzi, A., Schapira, A.H., Savontaus, M.L., and Cortopassi, G.A. (2005). Isolation of transcriptomal changes attributable to LHON mutations and the cybridization process. Brain *128*, 1026–1037.
14. Torroni, A., Petrozzi, M., D'Urbano, L., Sellitto, D., Zeviani, M., Carrara, F., Carducci, C., Leuzzi, V., Carelli, V., Barboni, P., et al. (1997). Haplotype and phylogenetic analyses suggest that one European-specific mtDNA background plays a role in the expression of Leber hereditary optic neuropathy by increasing the penetrance of the primary mutations 11778 and 14484. Am. J. Hum. Genet. *60*, 1107–1121.
15. Brown, M.D., Sun, F., and Wallace, D.C. (1997). Clustering of Caucasian Leber hereditary optic neuropathy patients containing the 11778 or 14484 mutations on an mtDNA lineage. Am. J. Hum. Genet. *60*, 381–387.
16. Lamminen, T., Huoponen, K., Sistonen, P., Juvonen, V., Lahermo, P., Aula, P., Nikoskelainen, E., and Savontaus, M.L. (1997). mtDNA haplotype analysis in Finnish families with leber hereditary optic neuroretinopathy. Eur. J. Hum. Genet. *5*, 271–279.
17. Hofmann, S., Jaksch, M., Bezold, R., Mertens, S., Aholt, S., Paprotta, A., and Gerbitz, K.D. (1997). Population genetics and disease susceptibility: characterization of central European haplogroups by mtDNA gene mutations, correlation with D loop variants and association with disease. Hum. Mol. Genet. *6*, 1835–1846.
18. Tsao, K., Aitken, P.A., and Johns, D.R. (1999). Smoking as an aetiological factor in a pedigree with Leber's hereditary optic neuropathy. Br. J. Ophthalmol. *83*, 577–581.
19. Sadun, A.A., Carelli, V., Salomao, S.R., Berezovsky, A., Quiros, P.A., Sadun, F., DeNegri, A.M., Andrade, R., Moraes, M., Passos, A., et al. (2003). Extensive investigation of a large Brazilian pedigree of 11778/haplogroup J Leber hereditary optic neuropathy. Am. J. Ophthalmol. *136*, 231–238.
20. Kerrison, J.B., Miller, N.R., Hsu, F., Beaty, T.H., Maumenee, I.H., Smith, K.H., Savino, P.J., Stone, E.M., and Newman, N.J. (2000). A case-control study of tobacco and alcohol consumption in Leber hereditary optic neuropathy. Am. J. Ophthalmol. *130*, 803–812.
21. Carelli, V., Achilli, A., Valentino, M.L., Rengo, C., Semino, O., Pala, M., Olivieri, A., Mattiazzi, M., Pallotti, F., Carrara, F., et al. (2006). Haplogroup effects and recombination of mitochondrial DNA: novel clues from the analysis of Leber hereditary optic neuropathy pedigrees. Am. J. Hum. Genet. *78*, 564–574.
22. Richards, M., Macaulay, V., Hickey, E., Vega, E., Sykes, B., Guida, V., Rengo, C., Sellitto, D., Cruciani, F., Kivisild, T., et al. (2000). Tracing European founder lineages in the Near Eastern mtDNA pool. Am. J. Hum. Genet. *67*, 1251–1276.
23. Yao, Y.-G., Kong, Q.-P., Bandelt, H.-J., Kivisild, T., and Zhang, Y.-P. (2002). Phylogeographic differentiation of mitochondrial DNA in Han Chinese. Am. J. Hum. Genet. *70*, 635–651.
24. Ji, Y., Jia, X., Zhang, Q., and Yao, Y.-G. (2007). mtDNA haplogroup distribution in Chinese patients with Leber's hereditary optic neuropathy and G11778A mutation. Biochem. Biophys. Res. Commun. *364*, 238–242.
25. Wang, H.-W., Jia, X., Ji, Y., Kong, Q.-P., Zhang, Q., Yao, Y.-G., and Zhang, Y.-P. (2008). 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*, 48–53.
26. Zhang, A.-M., Jia, X., Yao, Y.-G., and Zhang, Q. (2008). Co-occurrence of A1555G and G11778A in a Chinese family with high penetrance of Leber's hereditary optic neuropathy. Biochem. Biophys. Res. Commun. *376*, 221–224.
27. Yao, Y.-G., Kong, Q.-P., Wang, C.-Y., Zhu, C.-L., and Zhang, Y.-P. (2004). Different matrilineal contributions to genetic structure of ethnic groups in the Silk Road region in China. Mol. Biol. Evol. *21*, 2265–2280.
28. Li, R., Qu, J., Zhou, X., Tong, Y., Hu, Y., Qian, Y., Lu, F., Mo, J.-Q., West, C.-E., and Guan, M.-X. (2006). The mitochondrial tRNA(Thr) A15951G mutation may influence the phenotypic expression of the LHON-associated ND4 G11778A mutation in a Chinese family. Gene *376*, 79–86.
29. Qian, Y., Zhou, X., Hu, Y., Tong, Y., Li, R., Lu, F., Yang, H., Mo, J.-Q., Qu, J., and Guan, M.-X. (2005). Clinical evaluation and mitochondrial DNA sequence analysis in three Chinese families with Leber's hereditary optic neuropathy. Biochem. Biophys. Res. Commun. *332*, 614–621.
30. Qu, J., Li, R., Tong, Y., Hu, Y., Zhou, X., Qian, Y., Lu, F., and Guan, M.-X. (2005). Only male matrilineal relatives with Leber's hereditary optic neuropathy in a large Chinese family carrying the mitochondrial DNA G11778A mutation. Biochem. Biophys. Res. Commun. *328*, 1139–1145.
31. Qu, J., Li, R., Zhou, X., Tong, Y., Lu, F., Qian, Y., Hu, Y., Mo, J.Q., West, C.E., and Guan, M.-X. (2006). The novel A4435G mutation in the mitochondrial tRNAMet may modulate the phenotypic expression of the LHON-associated ND4 G11778A mutation. Invest. Ophthalmol. Vis. Sci. *47*, 475–483.
32. Qu, J., Li, R., Zhou, X., Tong, Y., Yang, L., Chen, J., Zhao, F., Lu, C., Qian, Y., Lu, F., et al. (2007). Cosegregation of the ND4 G11696A mutation with the LHON-associated ND4 G11778A mutation in a four generation Chinese family. Mitochondrion *7*, 140–146.

33. Yao, Y.-G., Salas, A., Bravi, C.M., and Bandelt, H.-J. (2006). A reappraisal of complete mtDNA variation in East Asian families with hearing impairment. Hum. Genet. *119*, 505–515.
34. Bandelt, H.-J., Achilli, A., Kong, Q.-P., Salas, A., Lutz-Bonengel, S., Sun, C., Zhang, Y.-P., Torroni, A., and Yao, Y.-G. (2005). Low "penetrance" of phylogenetic knowledge in mitochondrial disease studies. Biochem. Biophys. Res. Commun. *333*, 122–130.
35. Andrews, R.M., Kubacka, I., Chinnery, P.F., Lightowlers, R.N., Turnbull, D.M., and Howell, N. (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat. Genet. *23*, 147.
36. Kong, Q.-P., Bandelt, H.-J., Sun, C., Yao, Y.-G., Salas, A., Achilli, A., Wang, C.-Y., Zhong, L., Zhu, C.-L., Wu, S.-F., et al. (2006). Updating the East Asian mtDNA phylogeny: a prerequisite for the identification of pathogenic mutations. Hum. Mol. Genet. *15*, 2076–2086.
37. Kivisild, T., Tolk, H.-V., Parik, J., Wang, Y., Papiha, S.S., Bandelt, H.-J., and Villems, R. (2002). The emerging limbs and twigs of the East Asian mtDNA tree. Mol. Biol. Evol. *19*, 1737–1751.
38. Derenko, M., Malyarchuk, B., Grzybowski, T., Denisova, G., Dambueva, I., Perkova, M., Dorzhu, C., Luzina, F., Lee, H.K., Vanecek, T., et al. (2007). Phylogeographic analysis of mitochondrial DNA in northern Asian populations. Am. J. Hum. Genet. *81*, 1025–1041.
39. Yao, Y.-G., Kong, Q.-P., Man, X.-Y., Bandelt, H.-J., and Zhang, Y.-P. (2003). Reconstructing the evolutionary history of China: a caveat about inferences drawn from ancient DNA. Mol. Biol. Evol. *20*, 214–219.
40. Fujitake, J., Mizuta, H., Fujii, H., Ishikawa, Y., Sasamoto, K., Goto, Y., Nonaka, I., and Tatsuoka, Y. (2002). Leber's hereditary optic neuropathy with intracranial arteriovenous malformation: a case report. Acta Neurol. Belg. *102*, 82–86.
41. Bandelt, H.-J., Salas, A., Taylor, R.W., and Yao, Y.-G. (2008). The exaggerated status of "novel" and "pathogenic" mtDNA sequence variants due to inadequate database searches. Hum. Mutat., in press. 10.1002/humu.20846.
42. Huoponen, K., Lamminen, T., Juvonen, V., Aula, P., Nikoskelainen, E., and Savontaus, M.L. (1993). The spectrum of mitochondrial DNA mutations in families with Leber hereditary optic neuroretinopathy. Hum. Genet. *92*, 379–384.
43. Howell, N., Oostra, R.-J., Bolhuis, P.A., Spruijt, L., Clarke, L.A., Mackey, D.A., Preston, G., and Herrnstadt, C. (2003). Sequence analysis of the mitochondrial genomes from Dutch pedigrees with Leber hereditary optic neuropathy. Am. J. Hum. Genet. *72*, 1460–1469.
44. Gasparre, G., Porcelli, A.M., Bonora, E., Pennisi, L.F., Toller, M., Iommarini, L., Ghelli, A., Moretti, M., Betts, C.M., Martinelli, G.N., et al. (2007). Disruptive mitochondrial DNA mutations in complex I subunits are markers of oncocytic phenotype in thyroid tumors. Proc. Natl. Acad. Sci. USA *104*, 9001–9006.
45. Annunen-Rasila, J., Finnilä, S., Mykkänen, K., Moilanen, J.S., Veijola, J., Pöyhönen, M., Viitanen, M., Kalimo, H., and Majamaa, K. (2006). Mitochondrial DNA sequence variation and mutation rate in patients with CADASIL. Neurogenetics *7*, 185–194.
46. Achilli, A., Perego, U.A., Bravi, C.M., Coble, M.D., Kong, Q.-P., Woodward, S.R., Salas, A., Torroni, A., and Bandelt, H.-J. (2008). The phylogeny of the four pan-American mtDNA haplogroups: implications for evolutionary and disease studies. PLoS ONE *3*, e1764.
47. Brown, M.D., Yang, C.C., Trounce, I., Torroni, A., Lott, M.T., and Wallace, D.C. (1992). A mitochondrial DNA variant, identified in Leber hereditary optic neuropathy patients, which extends the amino acid sequence of cytochrome c oxidase subunit I. Am. J. Hum. Genet. *51*, 378–385.
48. Mather, M.W., and Rottenberg, H. (1998). Intrinsic uncoupling of cytochrome c oxidase may cause the maternally inherited mitochondrial diseases MELAS and LHON. FEBS Lett. *433*, 93–97.
49. Brown, M.D., Torroni, A., Huoponen, K., Chen, Y.S., Lott, M.T., and Wallace, D.C. (1994). Pathological significance of the mtDNA COX III mutation at nucleotide pair 9438 in Leber hereditary optic neuropathy. Am. J. Hum. Genet. *55*, 410–412.
50. Elliott, H.R., Samuels, D.C., Eden, J.A., Relton, C.L., and Chinnery, P.F. (2008). Pathogenic mitochondrial DNA mutations are common in the general population. Am. J. Hum. Genet. *83*, 254–260.
51. Kazuno, A.A., Munakata, K., Nagai, T., Shimozono, S., Tanaka, M., Yoneda, M., Kato, N., Miyawaki, A., and Kato, T. (2006). Identification of mitochondrial DNA polymorphisms that alter mitochondrial matrix pH and intracellular calcium dynamics. PLoS Genet. *2*, e128.
52. Fuku, N., Park, K.S., Yamada, Y., Nishigaki, Y., Cho, Y.M., Matsuo, H., Segawa, T., Watanabe, S., Kato, K., Yokoi, K., et al. (2007). Mitochondrial haplogroup N9a confers resistance against type 2 diabetes in Asians. Am. J. Hum. Genet. *80*, 407–415.
53. Tanaka, M., Fuku, N., Nishigaki, Y., Matsuo, H., Segawa, T., Watanabe, S., Kato, K., Yokoi, K., Ito, M., Nozawa, Y., et al. (2007). Women with mitochondrial haplogroup N9a are protected against metabolic syndrome. Diabetes *56*, 518–521.
54. Lamminen, T., Majander, A., Juvonen, V., Wikström, M., Aula, P., Nikoskelainen, E., and Savontous, M.L. (1995). A mitochondrial mutation at nt 9101 in the ATP synthase 6 gene associated with deficient oxidative phosphorylation in a family with Leber hereditary optic neuroretinopathy. Am. J. Hum. Genet. *56*, 1238–1240.
55. Majander, A., Lamminen, T., Juvonen, V., Aula, P., Nikoskelainen, E., Savontaus, M.L., and Wikström, M. (1997). Mutations in subunit 6 of the F1F0-ATP synthase cause two entirely different diseases. FEBS Lett. *412*, 351–354.
56. den Dunnen, J.T., and Antonarakis, S.E. (2001). Nomenclature for the description of human sequence variations. Hum. Genet. *109*, 121–124.

Supplemental Data

Mitochondrial DNA Haplogroups M7b1'2 and M8a Affect Clinical Expression of Leber Hereditary Optic Neuropathy in Chinese Families with the m.11778G→A Mutation

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Table S1. mtDNA Sequence Variation and Haplogroup Classification of 175 Families with m.11778G→A and LHON

Sample	Haplogroup	Region 16024-16569 (16000+)	Region 1-850 (all with 73 and 315+C)	Coding Region Mutation [a]
Le1053	A	223 290 293C 319 519	152 263 309+C 523-524d 663 750	
Le1096	A4	223 290 319 362	152 200 235 263 309+C 523-524d 663 750	+663*Hae*III; +5176*Alu*I; –9820*Hinf*I
Le1351	A4	209 223 274 290 319 362	152 207 235 309+C 523-524d 663 750	
Le216	A4	223 290 319 362	152 207 235 309+C 329 523-524d 663 750	+663*Hae*III
Le243	A4	223 290 319 362 519	195 200 235 263 309+C 523-524d 663 750	
Le296	A4	124 223 290 319 362	152 235 263 309+C 523-524d 663 750	+663*Hae*III
Le615	A4	223 290 319 325 362	41 152 235 263 523-524d 663 750	+663*Hae*III
Le67	A4	223 290 319 362	152 200 235 263 309+C 523-524d 663 750	+663*Hae*III
Le917	A4	124 223 290 319 362	151 152 200 235 263 309+C 523-524d 663 750	+663*Hae*III
Le977	A4	129 223 290 319 362	152 179 235 263 663 750	
Le458	B4	182C 183C 189 217 261	263 309+CC 523-524d 750	9bpd

Le856	B4	182C 183C 189 217 223 519	263 309+C 573+6C 709 750 827	
Le1007	B4a	129 182C 183C 189 217 261 311	263 309+CC 523-524d 750	+5176*Alu*I; 9bpd
Le546	B4a	150 182C 183C 189 217 240 261	143 263 309d 523-524d 750	
Le942	B4a	182C 183C 189 217 261 519	263 309+C 523-524d 573+5C 750	9bpd
Le991	B4a	129 182C 183C 189 217 261 519	263 309+C 523-524d 750	
Le1064	B4a'g	129 182C 183C 189 217 261 356	93 263 309+CC 513 523-524d 750	
Le1321	B4a'g	168 182C 183C 189 217 261 311 519	182 185 263 309+C 523-524d 750	
Le1003	B4b1	136 183C 187A 189 217 519	263 309+C 499 750 827	
Le353	B4b1	136 154 183C 189 217 218 519	150 249d 263 309+CC 499 709 750 827	
Le269	B4b1a	136 183C 189 217 519	152 207 263 499 750 827	
Le780	B4b1a	93 136 183C 189 217 519	207 263 499 750 827	
Le931	B4b1a	136 183C 189 217 519	207 263 309+CC 499 523-524d 750 827	9bpd
Le804	B4b1b'c	86 136 183C 189 217 218 519	263 309+C 499 750 827	
Le406	B4b'd	183C 189 217 519	263 309+CC 316 750 827	
Le1188	B4c1	168 182C 183C 189 217 311 519	263 309+CC 523-524d 750 794	–663*Hae*III; +5176*Alu*I; –9820*Hinf*I; 9bpd
Le782	B4c1b	129 140 166 179 182C 183C 189 217 274 335 465 519	150 195 263 309d 709 750	9bpd
Le1114	B4g	181C 182C 183C 189 213 217 261 278 292 519	263 302C 309+CC 455+T 523-524d 750	–663*Hae*III; +5176*Alu*I; –9820*Hinf*I; 9bpd
Le1344	B4g	90 181C 182C 183C 189 213 217 261 292 519	61A 62 263 309+CC 523-524d 750	
Le1121	B5a	93 140 183C 189 260 266G 311 519	210 263 294 309+C 709 750	–663*Hae*III; +5176*Alu*I; –9820*Hinf*I; 9bpd
Le1365	B5a	140 183C 189 266A 519	210 263 309+CC 523-524d 709 750	
Le1366	B5a	140 183C 189 218 266A 519	210 263 523-524d 709 750	
Le18	B5a	93 140 183C 189 260 266G 519	210 263 294 309+C 709 750	–663*Hae*III; 9bpd
Le416	B5a	93 140 182C 183C 189 266A 519	210 263 309+CC 523-524d 709 750	9bpd
Le74	B5a	140 183C 189 266A 362 519	150 210 263 309+CC 455+T 523-524d 709 750	+5176*Alu*I; –9820*Hinf*I; 9bpd
Le555	B5a2	140 187 189 256 266G 519	93 210 263 523-524d 709 750	
Le246	B5b	140 145 182C 183C 189 243 257 362 519	103 146 204 263 709 750	–9820*Hinf*I; 9bpd
Le487	B5b	140 182C 183C 189 243 519	103 195 198 204 207 263 309+CC 523-524d 709 750	

Le204	C	223 298 327 519	249d 263 489 750	
Le51	C	183C 189 223 298 327 519	249d 263 309+CC 489 593 750	+5176*Alu*I
Le534	C	223 298 327 519	146 249d 263 309+C 489 750	
Le767	C	213 223 298 327 519	249d 263 309+C 489 750	
Le867	C	223 298 327 519	146 249d 263 489 750	
Le889	C	129 150 189 223 298 327 519	195 249d 263 309+CC 489 750	
Le912	C	93 184 223 298 327 519	152 249d 263 309+C 489 750	
Le1278	D4	174 362	263 309+C 489 750	
Le1120	D4	223 271 362 519	263 298 489 593 750	–663*Hae*III; 3010; –5176*Alu*I; –9820*Hinf*I
Le1168	D4	192 223 362	263 489 750 869	–663*Hae*III; 3010; –5176*Alu*I; –9820*Hinf*I
Le1191	D4	223 362	263 309+C 489 750	–5176*Alu*I
Le1211	D4	192 223 362	263 489 750 869	–5176*Alu*I
Le1367	D4	223 362	263 309+C 489 750	–5176*Alu*I
Le385	D4	174 223 352 362	263 309+C 489 523-524d 750	3010; –5176*Alu*I; 10400
Le394	D4	192 223 362 519	263 309+C 489 593 709 750	–5176*Alu*I
Le442	D4	129 223 274h 311h 362	263 298h 309+CC 489 750	–5176*Alu*I
Le519	D4	174 223 343 362	263 489 750	–5176*Alu*I
Le565	D4	86 188 223 362	263 489 750	–5176*Alu*I
Le604	D4	223 249 250 309 362	263 309+C 489 750	–5176*Alu*I
Le631	D4	172 362 519	194 263 489 523-524d 722 750	–5176*Alu*I
Le638	D4	174 362	263 309+C 489 750	–5176*Alu*I
Le698	D4	223 362 519	152 195 263 309+C 489 750	–5176*Alu*I
Le954	D4	42 214 223 362	263 309+C 489 709 750	–663*Hae*III; 3010; –5176*Alu*I
Le812	D4	174 177 223 362	263 309+C 489 573+5C 750	3010; –5176*Alu*I
Le854	D4	92 223 270 362	263 309+C 489 750	–5176*Alu*I
Le894	D4	184 311 362 519	194 263 489 523-524d 750	–5176*Alu*I
Le930	D4	223 362	263 489 750	–5176*Alu*I

Le952	D4	223 362	263 309+C 489 523-524d 750 789	–5176*Alu*I
Le976	D4	93 188 214 223 362	146 263 309+C 489 750	–5176*Alu*I
Le343	D4a	129 223 362 519	152 263 309+CC 489 750	–5176*Alu*I
Le392	D4a	129 223 256 299 362 519	152 263 489 750	–5176*Alu*I
Le457	D4a	129 223 263 362	151 152 263 309+C 489 750	
Le655	D4a	129 223 362 519	152 199 263 309+CC 489 750	
Le811	D4a3	93 129 223 249 362	143 146 152 196 263 309+C 489 750	
Le998	D4b1	185 189 193d 223 232A 319 362	263 309+CC 489 523-524d 750	
Le539	D4b1b	93 223 287 319 362 380	263 309+C 431 489 523-524d 750	–5176*Alu*I; 9bpd
Le78	D4b1c	158 223 284 287 319	152 204 207 263 309+C 489 523-524d 750	–663*Hae*III; –9820*Hinf*I; 10398, 10400
Le285	D4b2b	223 362 519	150 194 263 489 523-524d 750	
Le422	D4b2b	223 283T 362 519	152 194 263 489 523-524d 573+3C 750	
Le1017	D4e1a1	93 176 223 362	94 194 263 309+C 489 750	3010; –5176*Alu*I
Le1013	D4g2a	93 223 274 362	263 298 309+C 489 499 750	
Le1139	D4h	174 177 223 362	263 309+C 489 573+5C 750	–663*Hae*III; 3010; –5176*Alu*I; –9820*Hinf*I
Le1140	D4h	174 223 352 362	263 309+C 489 523-524d 750	–663*Hae*III; 3010; –5176*Alu*I; –9820*Hinf*I
Le1215	D4k	192 223	195 263 309+C 489 750	
Le423	D4k	192 223	195 263 309+C 489 750	
Le884	D4k	131 192 222 223 316 362	184 195 263 309+C 489 750	
Le877 [b]	D5	93 129 189 362 519	63 152 263 309+C 489 750	
Le1012	D5a	164 172 182C 183C 189 209 223 266 362	150 263 489 523-524d 750 752 870	
Le1057	D5a2	164 182C 183C 189 223 266 362 519	150 263 489 523-524d 750 752	
Le1108	D5a2a	92 164 167 172 182C 183C 189 223 266 362	150 235 263 309+C 489 523-524d 750 752	–663*Hae*III; –5176*Alu*I; –9820*Hinf*I
Le563	D5a2a	92 167 172 182C 183C 189 223 266 274 293 362	150 263 489 523-524d 750 752	–5176*Alu*I
Le1104	D5b	91 167 189 223 362	150 189 263 456 489 681 750	
Le1231	D5b	189 223 362 519	150 263 309+C 456 489 681 750	–663*Hae*III; –5176*Alu*I;

				–9820*Hinf*I; 10343, 10397, 10398, 10400
Le141	D5b	129 148 183C 189 362 519	150 152 185 263 309+C 456 489 523-524d 681 750	
Le465	D5b	148 183C 189 223 362 519	150 152 185 188 263 309+CC 456 489 681 750	–5176*Alu*I
Le779	D5b	183C 189 223 249 357 362 519	150 263 309+CC 456 489 681 750	
Le835	D5b	148 183C 189 223 362 519	150 152 185 263 309+CC 456 489 523-524d 681 750	–5176*Alu*I
Le853	D5b	148 179 183C 189 223 362 519	150 152 185h 195 263 309+C 456 489 681 750	–5176*Alu*I
Le873	D5b	91 167 189 223 362	150 189 263 456 489 681 750	
Le90	D5b	183C 189 223 362 519	150 263 456 489 511 681 750	–5176*Alu*I; –9820*Hinf*I
Le1357	D5b2b	223 362 519	41 194 263 489 523-524d 750	
Le699	F1a1	129 148 162 172 244 304 335 519	249d 263 309+C 523-524d 750	
Le1048	F1b	182C 183C 189 232A 304 519	152 249d 263 309d 523-524d 750	–663*Hae*III; +5176*Alu*I; –9820*Hinf*I; 10223, 10235, 10310, 10609
Le886	F2a3	93 203 291 304 519	246 249d 263 296 309+C 750	
Le554	G	93 223 362 519	263 489 593 709 750	+5176*Alu*I
Le953	G1c	223 362 519	263 309+C 489 593 709 750	
Le1355	G1a1	189 221 223 325 362 519	150 263 489 709 750	
Le1269	G2a	129 223 278 362	173 263 489 709 750	–663*Hae*III; +5176*Alu*I; –9820*Hinf*I; 10398, 10400
Le1244	G2a1	223 227 278 362	152 263 489 573+5C 709 750	
Le553	G2a1	117 223 227 278 362	263 489 709 750	
Le1336	G2a2	51 86 150 223 278 362	263 489 709 750	
Le696	G2b	223 362 463	263 309+C 489 709 750	+5176*Alu*I
Le145	G3	93 223 274 362	263 309+C 489 709 750	
Le237	G3	223 274 362	143 152 263 489 709 750	
Le647	G3	223 274 362 390 519	263 489 574 709 750	+5176*Alu*I
Le1081	G3a	223 274 362	143 146 152 263 489 709 750	
Le1295	M10	223 248 271 311 519	263 309+CC 489 573+5C 709 750	
Le245	M10	129 223 311	263 309+C 489 573+5C 709 750	–663*Hae*III; –9820*Hinf*I;

				10245, 10398, 10400, 10646
Le1136	M10a1	129 193 223 311 357 497	146 152 263 309+C 489 523-524d 573+4C 709 750	–663*Hae*III; +5176*Alu*I; –9820*Hinf*I; 10398, 10400
Le255	M10a1	129 193 223 311 357 497	146 263 489 523-524d 573+5C 709 750	
Le515	M10a1	193 223 311 357 497	146 263 489 523-524d 573+5C 709 750	
Le580	M10a1	129 193 223 311 357 497	146 152 263 309+C 489 523-524d 573+4C 709 750	
Le549	M10b	66 220 223 311 519	263 489 573+4C 593 709 750	
Le869	M12	223 234 287 290 362	125 127 128 263 309+C 318 489 513 523	
Le918	M12	223 234 287 290 362	125 127 128 263 309+C 318 489 513 523	
Le1074	M7b1	93 129 192 213 223 297 309 519	150 199 263 309+C 489 709 750	
Le1142	M7b1	129 192 223 297	150 199 263 309+C 489 750	
Le1174	M7b1	92 129 192 223 297 360	150 159 199 263 309+CC 489 750	-663*Hae*III; +5176*Alu*I; +9820*Hinf*I
Le217	M7b1	129 192 223 297	150 199 263 309+C 489 750	+9820*Hinf*I
Le265	M7b1	92 129 192 223 254 297	150 159 182 199 263 489 750	+9820*Hinf*I
Le359	M7b1	129 192 223 297	150 199 263 489 750	
Le611	M7b1	129 192 223 297	150 199 263 309+CC 489 750	+9820*Hinf*I
Le666	M7b1	129 192 223 297	150 199 263 309+C 489 750	
Le777	M7b1	129 192 223 297	150 199 263 309+C 489 750	+9820*Hinf*I
Le807	M7b1	129 192 223 297	150 195 199 263 309+C 489 750	+9820*Hinf*I
Le1127	M7b1'2	129 223 297	146 150 199 263 309+C 489 750	–663*Hae*III; +5176*Alu*I; +9820*Hinf*I
Le924	M7b1'2	129 223 269 297 527	150 199 263 309+C 489 750	–663*Hae*III; +5176*Alu*I; +9820*Hinf*I
Le978	M7b1'2	129 189 223 297	150 199 204 263 309+CC 456 489 750	+5176*Alu*I; +9820*Hinf*I
Le35	M7b2	129 183C 189 223 297 298 311	150 199 263 309+CC 489 750	+5176*Alu*I; +9820*Hinf*I; 10345, 10398, 10400
Le485	M7b2	129 189 223 297 298	150 199 263 489 750	
Le1349	M7c	172 223 291 295 311 519	146 153 263 309+C 489 523-524d 750	
Le497	M7c1	183C 189 223 274 295 519	146 152 199 263 309+CC 489 523-524d 750	

Le529	M7c1	223 295 519 527	146 152 199 263 309+C 489 523-524d 750	
Le731	M7c1	172 223 295 519	146 199 263 309+CC 489 523-524d 750	
Le980	M7c1	223 293 295 519	146 199 263 309+C 489 523-524d 750	
Le262	M7c1'2	223 249 295 519	146 152 199 263 309+C 489 523-524d 750	+9820*Hinf*I
Le97	M7c1'2	172 223 295 519	146 152 199 263 489 523-524d 750	+9820*Hinf*I
Le124	M7c3	519	146A 199 263 309+CC 489 523-524d 750	+9820*Hinf*I
Le1346	M7c3	223 293T 295 362 519	72 146 199 263 309+C 489 523-524d 750	
Le238 [b]	M7c3	183C 189 274 295 519	146 152 199 263 309+C 489 523-524d 750	
Le47	M7c3	519	146A 183 199 204 263 489 523-524d 750	+9820*Hinf*I
Le586	M7c3	519	146A 199 204 263 489 523-524d 750	
Le1329	M8a	134 184 189 223 298 319	195 263 309+CC 489 750	
Le266	M8a	134 184 189 223 298 319	195 263 309+CC 489 750	
Le719	M8a	42 223 298 319	185 203 263 489 750	+5176*Alu*I; –9820*Hinf*I; 10398, 10400
Le251	M8a1	185 223 260 298 311 519	152 249d 263 309+CC 489 593 709 750	
Le1031	M8a2	184 223 298 319	263 309+C 489 750	
Le288	M8a2	93 134 184 223 298 319 519	263 309+C 489 750	
Le381	M8a2	134 184 223 298 319	263 309+C 489 750	
Le548	M8a2	184 223 271 298 319	152 195 263 489 750	
Le84	M8a2	134 184 189 223 298 319	195 263 309+CC 489 750	+5176*Alu*I
Le258	M8a2b	184 223 298 319	152 263 309+C 489 750	
Le393	M9a	223 234 294 316	153 263 309+C 489 750	
Le1014	N9a	51 223 257A 261	150 185 263 309+C 750	
Le54	N9a	183C 189 223 257A 261	150 263 309+CC 750	
Le874	N9a	189 223 257A	150 263 309+C 750	
Le675	R11	92 145 182C 183C 189 311 390 519	185 189 195 263 709 750 789	
Le771	R11	182C 183C 189 290 311 390	151 152 185 189 234 263 309+C 709 750	+5176*Alu*I
Le537	U2b	51 93 209 239 263 311 352 353	146 152 234 263 292 309+CC 750	
Le399	U4a	92 134 172 356 519	152 195 263 309+C 499 750	

Le1200	Y	86 126 176 231 399 519	146 152 263 750	
Le1261	Y1b	172 231 357 519	146 263 309+C 750	–663*Hae*III; +5176*Alu*I; –9820*Hinf*I; 10238, 10398
Le1328	Y1b	126 231 249 266 319 399 519	146 263 309+CC 709 750	
Le1361	Y1b	126 231 266 519	146 263 309+C 750	
Le310	Y1b	126 231 384 519	146 207 263 309+C 750	
Le1159	Y2	126 231 311	151 263 309+C 482 523-524d 750	
Le584	Z	185 209 223 260 298 437	143 152 249d 263 309+CC 489 750	

Note – Sequence variation was scored relative to the revised Cambridge reference sequence (rCRS) [1]. Suffixes A, G, C, and T indicate transversions; suffixes “h”, “d”, and “+” indicate heteroplasmy, deletions, and insertions, respectively. Indels (insertion and deletion) are recorded at the last possible site. “+” and “–” denote the presence and absence of the restriction site, respectively. The deletion of the 9-bp (CCCCCTCTA) repeat in the COII/tRNA^{lys} intergenic region is abbreviated as “9bpd”. When sequence information was not available, slots have been left blank. The samples that were analyzed for the entire mtDNA sequences are marked with grey shade, with the sample name in italic and bold font. Samples Le696, Le1269, and Le1244 were from our previous studies [2,3]. The mtDNA sequences reported in Ji et al. [4] were also included, with the exception of sample Le508, for we could not confirm the presence of m.11778G→A in this patient in this study.

[a] The coding region mutations and the 9-bp deletion were screened using the same method as described in Yao et al.[5].

[b] Samples Le238 and Le877 contained missing information in regions 16200-16253 and 16194-16291, respectively.

Table S2. Penetrance of LHON in 182 Families with m.11778G→A

Sample	Haplo-group	Location	No. of subjects	No. of affected subjects		No. of unaffected subjects	
				Males	Females	Males	Females
Le1053	A	Shanxi	9	4	0	2	3
Le1096	A4	Hunan	8	2	1	2	3
Le296	A4	Hubei	7	2	1	3	1
Le67	A4	Hunan	11	2	1	3	5
Le917	A4	Hubei	19	4	2	3	10
Le977	A4	Hebei	6	3	1	1	1
Le216	A4	Fujian	4	2	0	1	1
Le615	A4	Hunan	6	3	0	1	2
Le1351	A4	Jiangxi	2	1	0	0	1
Le243	A4	Tianjin	3	1	1	0	1
Le458	B4	Fujian	13	3	1	7	2
Le856	B4	Jiangsu	7	3	0	1	3
Le546	B4a	Hebei	9	1	1	2	5
Le942	B4a	Guangdong	14	2	2	4	6
Le991	B4a	Hebei	15	1	1	3	10
Le1007	B4a	Hubei	6	1	1	3	1
Le1064	B4a'g	Guangdong	22	5	0	11	6
Le1321	B4a'g	Hunan	10	3	2	2	3
Le1003	B4b1	Unknown	7	2	1	1	3
Le353	B4b1	Inner Mongolia	4	3	0	0	1
Le780	B4b1a	Shanxi	2	1	1	0	0
Le269	B4b1a	Shandong	16	2	0	4	10
Le931	B4b1a	Jiangxi	8	2	0	3	3
Le804	B4b1b'c	Hebei	11	2	1	2	6
Le406	B4b'd	Hebei	9	2	2	1	4
Le1188	B4c1	Guangdong	19	4	3	4	8
Le782	B4c1b	Guangdong	13	3	3	3	4
Le1114	B4g	Guangdong	4	2	0	2	0
Le1344	B4g	Guangxi	8	2	1	4	1
WZ1	B5a	Fujian	38	6	0	16	16
Le74	B5a	Hunan	9	2	3	1	3
Le1121	B5a	Guangdong	8	1	1	3	3
Le18	B5a	Guangdong	9	3	1	2	3
Le416	B5a	Hunan	5	3	0	0	2
Le1365	B5a	Sichuan	10	1	1	2	6
Le1366	B5a	Zhejiang	6	0	2	2	2
Le555	B5a2	Jiangsu	2	1	1	0	0
Le487	B5b	Shanxi	25	3	1	5	16
Le246	B5b	Guangdong	3	1	0	1	1
Le51	C	Guangdong	8	3	2	2	1

Le534	C	Henan	12	5	1	4	2
Le767	C	Henan	7	2	0	1	4
Le867	C	Heilongjiang	3	2	0	0	1
Le912	C	Cambodia	3	2	0	0	1
Le204	C	Hebei	4	3	0	0	1
Le889	C	Hebei	9	3	0	2	4
Le1278	D4	Ningxia	11	2	1	3	5
Le854	D4	Guangdong	10	0	2	5	3
Le394	D4	Hebei	14	2	2	3	7
Le519	D4	Guangxi	8	1	1	3	3
Le565	D4	Hebei	24	1	1	8	14
Le638	D4	Hebei	2	1	1	0	0
Le954	D4	Hubei	2	1	1	0	0
Le1168	D4	Guangdong	10	2	1	2	5
Le894	D4	Hebei	7	2	1	2	2
Le930	D4	Hebei	18	2	1	6	9
Le976	D4	Hebei	33	4	2	10	17
Le1120	D4	Jiangxi	5	2	0	1	2
Le1191	D4	Hunan	1	1	0	0	0
Le1211	D4	Guangdong	3	1	0	2	0
Le385	D4	Hunan	7	1	0	3	3
Le442	D4	Hebei	3	1	1	0	1
Le604	D4	Hebei	20	4	0	5	11
Le698	D4	Hebei	1	1	0	0	0
Le812	D4	Hunan	5	2	0	2	1
Le952	D4	Beijing	10	2	0	4	4
Le1357	D4	Shandong	11	2	1	6	2
Le1367	D4	Hubei	13	3	2	5	3
Le631	D4	Henan	13	2	2	6	3
Le655	D4a	Hebei	5	0	2	2	1
Le457	D4a	Hebei	6	2	1	2	1
Le343	D4a	Jiangxi	12	3	0	3	6
Le392	D4a	Unknown	4	2	0	1	1
Le811	D4a3	Shandong	7	1	1	2	3
Le998	D4b1	Shandong	11	2	0	3	6
Le539	D4b1b	Hunan	13	3	0	4	6
WZ3	D4b1b2	Jiangsu	9	4	2	0	3
Le78	D4b1c	Guangdong	4	2	1	0	1
WZ5	D4b2b	Unknown	13	4	1	4	4
Le285	D4b2b	Shanxi	12	2	1	6	3
Le422	D4b2b	Zhejiang	8	2	0	3	3
Le1017	D4e1a1	Anhui	4	1	1	1	1
Le1013	D4g2a	Hebei	8	1	2	3	2
Le1140	D4h	Hunan	14	3	1	7	3

Le1139	D4h	Hunan	9	3	0	3	3
WZ12	D4j	Zhejiang	28	7	3	7	11
Le884	D4k	Hebei	5	1	2	0	2
Le1215	D4k	Hebei	13	4	3	0	6
Le423	D4k	Hebei	16	4	2	2	8
Le877	D5	Shandong	10	2	1	4	3
WZ2	D5a	Anhui	13	6	2	1	4
Le1012	D5a2	Hebei	20	3	10	4	3
Le1057	D5a2	Hebei	4	2	0	0	2
Le563	D5a2a	Guangdong	7	2	1	0	4
Le1108	D5a2a	Sichuan	21	4	0	5	12
Le1231	D5b	Hubei	10	1	3	0	6
Le873	D5b	Henan	4	2	1	0	1
Le1104	D5b	Unknown	16	3	1	5	7
Le141	D5b	Hebei	3	2	0	0	1
Le465	D5b	Guangdong	8	2	0	2	4
Le835	D5b	Guangxi	13	1	0	5	7
Le853	D5b	Hubei	6	2	1	2	1
Le90	D5b	Guangdong	10	2	0	1	7
Le779	D5b	Unknown	16	3	3	5	5
WZ4	F1	Zhejiang	8	3	0	2	3
Le699	F1a1	Hebei	4	2	1	0	1
Le1048	F1b	Hunan	7	2	0	0	5
Le886	F2a3	Hebei	8	0	2	3	3
Le554	G	Henan	9	0	2	0	7
Le953	G1	Hebei	5	2	1	0	2
Le1355	G1a1	Jiangxi	4	1	1	1	1
Le1269	G2a	Henan	30	9	7	5	9
Le553	G2a1	Hebei	7	2	1	3	1
Le1244	G2a1	Jiangxi	13	3	0	4	6
Le1336	G2a2	Hebei	10	7	0	0	3
Le696	G2b	Hebei	14	8	3	2	1
Le647	G3	Hebei	21	2	3	8	8
Le145	G3	Unknown	5	2	1	0	2
Le237	G3	Jilin	5	2	1	0	2
Le1081	G3a	Hebei	16	3	1	3	9
Le245	M10	Guangdong	7	3	1	2	1
Le1295	M10	Hebei	33	10	3	9	11
WZ6	M10a	Unknown	30	6	4	12	8
Le255	M10a1	Henan	13	2	1	3	7
Le515	M10a1	Hebei	6	2	0	1	3
Le580	M10a1	Jiangsu	7	2	0	1	4
Le1136	M10a1	Hubei	7	2	0	2	3
Le549	M10b	Anhui	10	3	1	3	3

Le918	M12	Hubei	17	4	3	4	6
Le869	M12	Hubei	18	4	2	5	7
Le978	M7b	Guangdong	6	5	0	0	1
Le1142	M7b1	Guangxi	7	1	1	2	3
Le265	M7b1	Hunan	9	3	3	0	3
Le611	M7b1	Guangxi	3	1	1	0	1
Le777	M7b1	Guangdong	5	1	1	3	0
Le217	M7b1	Guangdong	11	5	4	0	2
Le359	M7b1	Hebei	16	2	1	5	8
Le1074	M7b1	Jilin	7	4	0	1	2
Le1174	M7b1	Guangdong	7	3	0	1	3
Le666	M7b1	Guangdong	6	2	0	1	3
Le807	M7b1	Guangdong	8	3	0	0	5
Le1127	M7b1'2	Guangdong	16	2	0	5	9
Le924	M7b1'2	Guangdong	9	3	0	4	2
Le485	M7b2	Hebei	21	3	4	4	10
Le35	M7b2	Guangdong	5	3	0	1	1
Le1349	M7c	Hunan	9	3	0	3	3
Le529	M7c1	Anhui	12	1	3	3	5
Le731	M7c1	Hebei	9	1	2	3	3
Le497	M7c1	Hebei	17	2	1	7	7
Le980	M7c1	Guangdong	13	2	0	3	8
Le97	M7c1'2	Guangdong	9	3	1	3	2
Le262	M7c1'2	Guangdong	1	1	0	0	0
Le124	M7c3	Guangxi	19	2	0	7	10
Le47	M7c3	Guangdong	9	3	0	3	3
Le586	M7c3	Hebei	12	3	1	4	4
Le1346	M7c3	Hunan	8	1	1	6	0
Le238	M7c3	Beijing	4	3	0	0	1
Le719	M8a	Unknown	8	2	0	2	4
Le1329	M8a	Guangdong	14	2	0	6	6
Le266	M8a	Guangdong	17	2	0	7	8
Le251	M8a1	Henan	5	2	3	0	0
Le381	M8a2	Hebei	14	0	2	5	7
Le548	M8a2	Hebei	8	0	1	4	3
Le288	M8a2	Henan	9	2	1	2	4
Le1031	M8a2	Henan	18	2	0	4	12
Le84	M8a2	Guangdong	14	2	0	6	6
Le258	M8a2b	Shandong	5	1	0	3	1
Le393	M9a	Hebei	8	1	3	2	2
Le1014	N9a	Hebei	10	3	0	3	4
Le874	N9a	Henan	18	2	2	7	7
Le54	N9a	Guangdong	6	2	0	2	2
Le675	R11	Hebei	7	4	0	1	2

Le771	R11	Shanxi	2	2	0	0	0
Le537	U2b	Xinjiang	15	2	0	4	9
Le399	U4a	Unknown	14	1	0	4	9
Le1200	Y	Guangdong	13	2	0	6	5
Le1261	Y1b	Sichuan	10	2	0	1	7
Le310	Y1b	Tianjin	16	4	0	5	7
Le1328	Y1b	Beijing	2	2	0	0	0
Le1361	Y1b	Guangdong	11	2	1	5	3
Le1159	Y2	Shandong	8	2	0	3	3
Le584	Z	Henan	9	1	1	3	4
Total			1859	436	183	504	736

Note – Samples WZ1-WZ6 and WZ12 were taken from the published resources [6-10]. Samples Le696, Le1269, and Le1244 were from our previous studies [2,3].

Supplemental References

1. Andrews, R.M., Kubacka, I., Chinnery, P.F., Lightowlers, R.N., Turnbull, D.M., and Howell, N. (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat. Genet. *23*, 147.
2. Wang, H.-W., Jia, X., Ji, Y., Kong, Q.-P., Zhang, Q., Yao, Y.-G., and Zhang, Y.-P. (2008). 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*, 48-53.
3. Zhang, A.-M., Jia, X., Yao, Y.-G., and Zhang, Q. (2008). Co-occurrence of A1555G and G11778A in a Chinese family with high penetrance of Leber's hereditary optic neuropathy. Biochem. Biophys. Res. Commun. *376*, 221-224.
4. Ji, Y., Jia, X., Zhang, Q., and Yao, Y.-G. (2007). mtDNA haplogroup distribution in Chinese patients with Leber's hereditary optic neuropathy and G11778A mutation. Biochem. Biophys. Res. Commun. *364*, 238-242.
5. Yao, Y.-G., Kong, Q.-P., Bandelt, H.-J., Kivisild, T., and Zhang, Y.-P. (2002). Phylogeographic differentiation of mitochondrial DNA in Han Chinese. Am. J. Hum. Genet. *70*, 635-651.
6. Li, R., Qu, J., Zhou, X., Tong, Y., Hu, Y., Qian, Y., Lu, F., Mo, J.-Q., West, C.-E., and Guan, M.-X. (2006). The mitochondrial tRNA(Thr) A15951G mutation may influence the phenotypic expression of the LHON-associated ND4 G11778A mutation in a Chinese family. Gene *376*, 79-86.
7. Qian, Y., Zhou, X., Hu, Y., Tong, Y., Li, R., Lu, F., Yang, H., Mo, J.-Q., Qu, J., and Guan, M.-X. (2005). Clinical evaluation and mitochondrial DNA sequence analysis in three Chinese families with Leber's hereditary optic neuropathy. Biochem. Biophys. Res. Commun. *332*, 614-621.
8. Qu, J., Li, R., Tong, Y., Hu, Y., Zhou, X., Qian, Y., Lu, F., and Guan, M.-X. (2005). Only male matrilineal relatives with Leber's hereditary optic neuropathy in a large Chinese family carrying the mitochondrial DNA G11778A mutation. Biochem. Biophys. Res. Commun. *328*, 1139-1145.
9. Qu, J., Li, R., Zhou, X., Tong, Y., Lu, F., Qian, Y., Hu, Y., Mo, J.-Q., West, C.-E., and Guan, M.-X. (2006). The novel A4435G mutation in the mitochondrial tRNAMet may modulate the phenotypic expression of the LHON-associated ND4 G11778A mutation. Invest. Ophthalmol. Vis. Sci. *47*, 475-483.
10. Qu, J., Li, R., Zhou, X., Tong, Y., Yang, L., Chen, J., Zhao, F., Lu, C., Qian, Y., Lu, F., and Guan, M.-X. (2007). Cosegregation of the ND4 G11696A mutation with the LHON-associated ND4 G11778A mutation in a four generation Chinese family. Mitochondrion *7*, 140-146.