Leber's Hereditary Optic Neuropathy Affects Only Female Matrilineal Relatives in Two Chinese Families

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PURPOSE. The purpose of this study was to investigate the role of modifier factors in the expression of Leber's hereditary optic neuropathy (LHON).

METHODS. Thirty-five subjects from two Han Chinese families with maternally transmitted LHON underwent a clinical and genetic evaluation and molecular analysis of mitochondrial (mt)DNA.

RESULTS. Matrilineal relatives in the two Chinese families exhibited a wide range of severity in visual impairment, from blindness to nearly normal vision. Very strikingly, all nine affected individuals of 21 matrilineal relatives (13 females/8 males) were female, which translates to 33% and 57% of penetrance for optic neuropathy in the two families. The average age at onset was 22 and 25 years. These observations were in contrast with typical features in many LHON pedigrees that have a predominance of affected males. Molecular analysis of their mtDNAs identified the homoplasmic *ND4* G11778A mutation and distinct sets of variants belonging to the Asian haplogroups M1 and M10a. Of other variants, the L175F variant in *CO3*; the I58V variant in *ND6*; and the I189V, L292R, and S297A variants

in *CYTb* were located at highly conserved residues of polypeptides.

CONCLUSIONS. Only female matrilineal relatives with a wide range of penetrance, severity, and age at onset of optic neuropathy in these two Chinese pedigrees showed the involvement of X-linked or autosomal recessive modifier genes in the phenotypic manifestation of the G11778A mutation. Furthermore, mitochondrial haplogroup-specific variants, together with epigenetic and environmental factors, may contribute to the phenotypic manifestation of the primary LHON-associated G11778A mutation in these pedigrees. (*Invest Ophthalmol Vis Sci.* 2010;51:4906–4912) DOI:10.1167/iovs.09-5027

Leber's hereditary optic neuropathy (LHON) is a maternally inherited eye disease that generally affects young adults and causes a rapid, painless, bilateral loss of central vision.^{1–4} Mutations in mitochondrial (mt)DNA are the molecular bases for this disorder.^{5–7} Of these, the *ND1* G3460A, *ND4* G11778A, and *ND6* T14484C mutations, which involve genes encoding the subunits of respiratory chain complex I, account for approximately 90% of LHON pedigrees in some countries.^{8–10} Those LHON-associated mtDNA mutations are often nearly homoplasmic or completely homoplasmic occurrences. Typical features in LHON pedigrees carrying the mtDNA mutation(s) are incomplete penetrance and male bias among the affected subjects, reflecting the complex etiology of the disease.^{11,12} In addition, matrilineal relatives within or between families, despite carrying the same LHON-associated mtDNA mutation(s) including G11778A, exhibit a wide range of severity, age at onset, and penetrance in visual impairment. The primary LHON-associated mtDNA mutations such as G11778A are necessary, but by themselves are insufficient to induce the clinical expression of LHON. Thus, other environmental and genetic factors including nuclear modifier genes and mitochondrial haplotypes should modulate the phenotypic manifestation of visual impairment associated with those primary mtDNA mutations.^{3,4,13} In particular, a group of secondary LHON-associated mtDNA mutations such as T4216C, A4917G, and G13708A and haplogroups J, M7b, and M8a, have been implicated in the phenotypic manifestation of the primary mtDNA mutations, including the G11778A and T14484C mutations in Caucasian and Chinese families.^{13–17}

However, these modifier factors remain poorly defined. With the intent of investigating the role of mitochondrial haplotypes in the phenotypic expression of LHON, a systematic and extended mutational screening of mtDNA has been initiated in a large clinical population at the Ophthalmology Clinic at the Wenzhou Medical College, China.^{12,18–21} In the previous investigations, LHON was associated with the *ND4* G11778A mutation in 15 Chinese families with variable penetrance, severity, and age at onset of visual impairment.^{12,18–21} In particular, the *ND4* G11696A, tRNA^{Met} A4435G, and tRNA^{Thr} A15951G mutations contribute to the high penetrance and expressivity of visual loss in Chinese families.^{19–21} In the

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present study, we performed the clinical, genetic, and molecular characterization of another two Han Chinese families with maternally transmitted LHON. Very strikingly, LHON affected only nine females of the 21 matrilineal relatives (13 females/8 males). These nine matrilineal relatives exhibited late-onset/progressive visual impairment with a wide range of severity, from blindness to normal vision. Mutational analysis of mitochondrial genomes identified the known *ND4* G11778A mutation in these families. To elucidate the role of mitochondrial haplotypes in the phenotypic manifestation of the G11778A mutation, we performed a PCR amplification of the fragments, spanning the entire mitochondrial genome and a subsequent DNA sequence analysis in all the matrilineal relatives in the families.

MATERIALS AND METHODS

Patients and Subjects

We ascertained two Han Chinese families (Fig. 1) through the School of Ophthalmology and Optometry, Wenzhou Medical College, Wenzhou and Eye Hospital, Chinese Academy of China Medical Science, Beijing. Informed consent, blood samples, and clinical evaluations were obtained from all participating family members, under protocols approved by the Cincinnati Children's Hospital Medical Center Institute Review Board and the Wenzhou Medical College Ethics Committee. Members of these pedigrees were interviewed at length to identify both personal and family medical histories of visual impairment and other clinical abnormalities.

Ophthalmic Examinations. The ophthalmic examinations of the proband and other family members were conducted, including visual acuity, visual field examination (Humphrey Visual Field Analyzer II, SITA Standard; Carl Zeiss Meditec, Oberkochen, Germany), visual evoked potentials (VEP; RETI-port gamma, flash VEP; Roland Consult, Brandenburg, Germany), and fundus photography (CR6-45NM fundus camera; Canon, Tokyo, Japan). The degree of visual impairment was defined according to visual acuity as follows: normal, >0.3 ; mild, 0.3 to 0.1 ; moderate, <0.1 to 0.05 ; severe, <0.05 to 0.02 ; and profound, <0.02 .

Mutational Analysis of the Mitochondrial Genome. Genomic DNA was isolated from whole blood of the participants (Puregene DNA Isolation Kits; Gentra Systems, Minneapolis, MN). For

the examination of the *ND4* G11778A mutation, the first PCR segments (803 bp) were amplified using genomic DNA as a template and oligodeoxynucleotides corresponding to mtDNA at positions 11295-12098,²² to rule out the co-amplification of possible nuclear pseudogenes.²³ Then, the second PCR product (212 bp) was amplified with the first PCR fragment as a template and oligodeoxynucleotides corresponding to mtDNA at positions 11654-11865 and subsequently was digested with the restriction enzyme *Tsp45I*, as the G11778A mutation creates the site for this restriction enzyme.¹² Equal amounts of various digested samples were then analyzed by electrophoresis through 7% polyacrylamide gel. The proportions of digested and undigested PCR product were determined (Image-Quant; GE Healthcare, Piscataway, NJ) after ethidium bromide staining, to determine whether the G11778A mutation was homoplasmic in these subjects. The entire mitochondrial genome of the two probands was PCR amplified in 24 overlapping fragments by using sets of the light (L)-strand and the heavy (H)-strand oligonucleotide primers, as described elsewhere.²⁴ Each fragment was purified and subsequently analyzed by direct sequencing in an automated DNA sequencer with dye termination chemistry (3700; Applied Biosystems, Inc. [ABI], Foster City, CA, with the Big Dye Terminator Cycle Sequencing Reaction Kit; ABI). These sequence results were compared with the updated consensus Cambridge sequence (GenBank accession number: NC_001807; <http://www.ncbi.nlm.nih.gov/Genbank>; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD).²² DNA and protein sequence alignments were performed (SeqWeb program GAG; GCG Wisconsin Program; Accelerlys, Inc., San Diego, CA).

Phylogenetic Analysis

Seventeen vertebrate mitochondrial DNA sequences were used in the interspecific analysis: *Bos taurus*, *Cebus albifrons*, *Gorilla gorilla*, *Homo sapiens*, *Hylobates lar*, *Lemur catta*, *Macaca mulatta*, *Macaca sylvanus*, *Mus musculus*, *Nycticebus coucang*, *Pan paniscus*, *Pan troglodytes*, *Pongo pygmaeus*, *Pongo abelii*, *Papio hamadryas*, *Tarsius bancanus*, and *Xenopus laevis* (GenBank). The conservation index (CI) was calculated by comparing the human nucleotide variants with the 16 other vertebrates. The CI was then defined as the percentage of species from the list of 16 vertebrates that have the wild-type nucleotide at that position.

Haplogroup Analyses

The entire mtDNA sequences of two Chinese probands carrying the G11778A mutation were assigned to the Asian mitochondrial haplogroups by using the nomenclature of mitochondrial haplogroups.^{25,26}

RESULTS

Clinical and Genetic Evaluation of Two Chinese Pedigrees Carrying the *ND4* G11778A Mutation

To further elucidate the molecular basis of optic neuropathy, we performed a mutational screening of the mitochondrial *ND4* gene in a large cohort of Han Chinese subjects who had received a diagnosis of LHON at the Eye Clinic at the Wenzhou Medical College. First, DNA fragments spanning the *ND4* G11778A mutation were PCR amplified from each affected subject. Each fragment was digested by the restriction enzyme *Tsp45I* and subsequent electrophoresis analysis. Of those, two subjects harbored the homoplasmic G11778A mutation (data not shown). The presence of the homoplasmic G11778A mutation in those subjects was confirmed by PCR amplification of fragments spanning the G11778A mutation and subsequent DNA sequence analysis (data not shown). A comprehensive history and physical examination as well as an ophthalmic examination were performed to identify both personal and family medical histories of visual impairment and other clinical abnormalities in all available members of the two Han Chinese

FIGURE 1. Two Chinese pedigrees with Leber's hereditary optic neuropathy. Filled symbols: vision-impaired individuals. Arrowheads: the probands.

pedigrees. In fact, the probands and other available members of these Chinese families showed no other clinical abnormalities, including diabetes, muscular diseases, hearing dysfunction, and neurologic disorders.

In family WZ49, the proband (III-6) came to the Ophthalmology Clinic of Eye Hospital, Chinese Academy of Medical Sciences, Beijing, at the age of 19 years after experiencing painless, progressive deterioration of bilateral vision 2 years before. She saw a dark cloud in the center of vision and had problems appreciating colors; all appeared to be dark gray. Visual acuity was 0.01 and 0.04, and intraocular pressure (IOP) was 12.6 and 13 mm Hg in the right and left eyes, respectively. Visual field testing demonstrated large centrocecal scotomata in both of her eyes. A fundus examination showed that both optic discs were abnormal, vascular tortuosity of the central retinal vessels, circumpapillary telangiectatic microangiopathy, and swelling of the retinal nerve fiber layer (Fig. 2). Therefore, she exhibited the typical clinical features of LHON. The family originated from Hebei Province in Northern China, and most of the family members live in the same area. Of eight matrilineal relatives who are the offspring of subject I-2, four female matrilineal relatives exhibited bilateral and symmetric visual impairment as the sole clinical symptom, whereas vision remained normal in all the male matrilineal relatives. The affected matrilineal relatives in this family exhibited early-onset/progressive, but not congenital, visual impairment. Visual acuity testing showed variable acuity in the maternal kindred, ranging from profoundly (II-4) to severely (II-6, III-5) impaired to completely normal (three male matrilineal relatives). In addition, the age at onset of visual impairment in this family varied from 17 (III-5) to 30 (II-4) to 32 (II-6) years. There was no evidence that any family members had another known cause of visual impairment.

In family WZ50, the proband (III-3) received a diagnosis of LHON at the age of 21 years. She began to notice bilateral visual impairment at the age of 20. Her visual impairment occurred within 3 weeks, first in the right eye and then in left eye. She saw a dark cloud in the center of vision and had problems appreciating colors, with all appearing to be dark gray. Visual field testing demonstrated large centrocecal scotomata in both of her eyes. As showed in Figure 2, a fundus examination showed that both her optic discs were abnormal, with vascular tortuosity of the central retinal vessels, a circum-papillary telangiectatic microangiopathy, and swelling of the

retinal nerve fiber layer. Her visual acuity was 0.03 and 0.15 and IOP was 13.3 and 13 mm Hg in the right and left eyes, respectively. Of the other 12 matrilineal relatives in this pedigree, the female subjects II-2, II-4, and I-2 exhibited visual impairment at the ages of 22, 22, and 24 years, respectively, whereas other members of the family had normal vision. Vision in the maternal kindred varied from profoundly (II-2, II-4) to severely (I-2) impaired to completely normal (four male and four female matrilineal relatives). There is no evidence that any member of this family had any other known cause to account for the optic neuropathy.

Mitochondrial DNA Analysis

To further determine the presence and amount of the G11778A mutation in these matrilineal relatives, nested PCR amplification, as detailed in the Materials and Methods section, was performed to rule out the possible co-amplification of nuclear pseudogenes.²³ The resultant 212-bp PCR segments corresponding to mtDNA at positions 11654-11865 were digested by the restriction enzyme *Tsp45I* and separated by electrophoresis on a 7% polyacrylamide gel. The G11778A mutation appeared to be homoplasmic in the matrilineal relatives of these two families (data not shown). This result strongly indicates that the levels of G11778A mutation in those matrilineal relatives do not correlate with the variability of the severity and age at onset of visual impairment in these families.

To assess the role of mtDNA variants in the phenotypic expression of the G11778A mutation, we performed a PCR amplification of fragments spanning the entire mitochondrial genome and subsequent DNA sequence analysis in two probands. In addition to the identical G11778A mutation shown in Table 1, these subjects exhibited distinct sets of mtDNA polymorphism. The mtDNAs of WZ49 and WZ50 pedigrees belong to the East Asian haplogroups M1 and M10a,^{25,26} respectively. In fact, these probands shared 27 known variants. Of interest, the WZ50 pedigree carried more variants than did the WZ49 family. Of other nucleotide changes in these mitochondrial genomes, there are 13 variants in the D-loop, 4 variants in the 12S rRNA gene, 2 known variants in the 16S rRNA gene, 28 (26 known and 2 novel) silent variants in the protein-encoding genes, and 13 missense mutations (11 known and 2 novel) in the protein encoding genes.²⁷ These missense mutations are A8701G (T59A) and A8860G (T112A) in the *A6* gene; C9729T (L175F) in the *CO3* gene²⁶; A10398G (T114A) in the *ND3* gene; A12358G (T8A) in the *ND5* gene; T14502C (I58V) in the *ND6* gene; and C14766T (T7D), T15071C (Y109H), A15218G (T158A), A15311G (I189V), A15326G (T194A), T15621G (L292R) and T15635G (S297A) in the *CYTb* gene.

These variants in RNAs and polypeptides were further evaluated by phylogenetic analysis of these variants and sequences from the 16 other vertebrates, including mouse,²⁸ bovine,²⁹ and *Xenopus laevis*.³⁰ The conservation index (CI) was calculated by comparing the human nucleotide variants with other 16 vertebrates. Of these variants, the CIs of the *CO3* L175F, *ND6* I58V, and *CYTb* I189V, L292R, and S297A variants were 94.1%, 76.5%, 88.2%, 88.2%, and 100%, respectively. These CIs were above the threshold level to be functionally significant in terms of mitochondrial physiology, as proposed by Wallace.³¹ On the other hand, CIs of other variants were <70%, indicating that these variants may not be functionally significant. Here, the *ND6* I58V variant, which is a haplogroup M10-specific variant,^{25,26} was involved in LHON in Chinese families,³² the *CO3* L175F variant was associated with LHON in Thai families,³³ and the *CYTb* I189V variant was found in hypertensive individuals.³⁴ Furthermore, the *CYTb* I189V, L292R, and S297A variants appear to be novel.

FIGURE 2. Fundus photograph of two affected subjects (WZ49-III-6, WZ50-III-3) and a married-in control subject (WZ50-II-1).

TABLE 1. mtDNA Variants in Two Chinese Pedigrees with Leber's Hereditary Optic Neuropathy

Gene	Position	Replacement	Conservation (H/B/M/X)*	CRS†	WZ49	WZ50	Previously Reported‡
<i>D to loop</i>	73	A to G		A	G	G	Yes
	263	A to G		A	G	G	Yes
	310	T to CTC		T	TC	CTC	Yes
	489	T to C		T	C	C	Yes
	568	C to CCCC		C	CCCC		Yes
	16066	A to G		A		G	Yes
	16163	A to G		A		G	Yes
	16178	T to C		T		C	Yes
	16223	C to T		C	T	T	Yes
	16256	C to T		C	T		Yes
	16299	A to G		A	G		Yes
	16311	T to C		T	C	C	Yes
	16519	T to C		T		C	Yes
	709	G to A	G/A/A/	G	A	A	Yes
	750	A to G	A/G/G/	A	G	G	Yes
<i>12S rRNA</i>	953	T to C	T/G/C/T	T	C		Yes
	1438	A to G	A/A/A/G	A	G	G	Yes
	2706	A to G	A/G/A/A	A	G	G	Yes
	3172	C to CC	C/G/A/T	C	CC	CC	Yes
<i>16S rRNA</i>	3774	A to G		A		G	No
	4140	C to T		C	T	T	Yes
<i>ND1</i>	4769	A to G		A	G	G	Yes
	4823	T to C		T	C		Yes
	5426	T to C		T		C	Yes
<i>CO1</i>	5964	T to C		T		C	Yes
	7028	C to T		C	T	T	Yes
	7250	A to G		A		G	Yes
<i>CO2</i>	7888	C to T		C		T	Yes
<i>ATP6</i>	8701	A to G	Thr to Ala	A	G	G	Yes
	8793	T to C		T		C	Yes
	8856	G to A		G		A	Yes
<i>CO3</i>	8860	A to G	Thr to Ala	A	G	G	Yes
	9540	T to C		T	C		Yes
	9644	A to G		A	G		Yes
<i>ND3</i>	9729	C to T	Leu to Phe	C	T		Yes
	10398	A to G	Thr to Ala	A	G	G	Yes
	10400	C to T		C	T	T	Yes
<i>ND4L</i>	10646		G to A	G		A	Yes
	10724	T to C		T		C	Yes
	10873	T to C		T		C	Yes
<i>ND4</i>	11647	C to T		C		T	Yes
	11719	G to A		G	A	A	Yes
	11778	G to A	Arg to His	G	A	A	Yes
	12358	A to G	Thr to Ala	A		G	Yes
	12549	C to T		C	T	T	Yes
	12705	C to T		C	T	T	Yes
	12976	C to T		C	T		No
	13152	A to G		A	G	G	Yes
<i>ND6</i>	14502	T to C	Ile to Val	T	C	C	Yes
<i>Cytb</i>	14766	C to T	Thr to Ile	C	T	T	Yes
	14783	T to C		T	C	C	Yes
	15040	C to T		C	T	T	Yes
	15043	G to A		G	A	A	Yes
	15071	T to C	Tyr to His	T	C	C	Yes
	15218	A to G	Thr to Ala	A		G	Yes
	15301	G to A		G		A	Yes
	15311	A to G	Ile to Val	G	A		Yes
	15326	A to G	Thr to Ala	A	G	G	Yes
	15621	T to G	Leu to Arg	T	G		No
	15635	T to G	Ser to Ala	T	G		No
	15784	T to C		T		C	Yes

* Conservation of amino acid for polypeptides or nucleotide for RNAs in human (H), bovine (B), mouse (M), and *Xenopus laevis* (X).† CRS, Cambridge reference sequence²²‡ See online mitochondrial genome databases: <http://www.mitomap.org> and <http://www.genpat.uu.se/mtDB>

DISCUSSION

In the present study, we performed the clinical, genetic, and molecular characterization of two Han Chinese families with

Leber's hereditary optic neuropathy. Optic neuropathy as a sole clinical phenotype was present only in the maternal lineage of the pedigrees carrying the homoplasmic *ND4* G11778A mutation. Very strikingly, all 9 affected subjects of 21 matriline-

eal relatives (13 females/8 males) were females in these Chinese families. These results were in contrast with the previous observations that the ratios between the affected males and affected females were 4.5:1 in 49 Caucasian pedigrees,³⁵ 3.7:1 in 66 Caucasian families,³⁶ 5.3:1 in 10 Caucasian pedigrees,³⁷ and 3.4:1 in 15 Chinese families carrying the G11778A mutation.¹¹ There was a wide range of severity and age at onset in optic neuropathy in the matrilineal relatives of these Chinese families, although these subjects share the common feature of a rapid, painless, bilateral loss of central vision. The average age at onset of visual impairment in matrilineal relatives in these families, as shown in Table 2, was 25 (WZ49) and 22 (WZ50) years, respectively. However, matrilineal relatives in the other 15 Chinese families carrying the G11778A mutation developed optic neuropathy at ages ranging from 7 to 27 years,^{11,12,18–21} whereas the average age at onset of visual impairment was 24, 28, and 29 years from matrilineal relatives of 66, 49, and 10 Caucasian pedigrees carrying the G11778A mutation, respectively.^{35–37} As shown in Table 2, the penetrance of visual impairment (affected matrilineal relatives/total matrilineal relatives) in these 2 Chinese pedigrees was 33.3% and 56.6%, respectively, whereas the penetrance of optic neuropathy in the other 15 Chinese pedigrees carrying the G11778A mutation ranged from 5.3% to 60%, with an average of 25%.^{11,12,18–21} However, ~50% of male and ~10% of female Caucasians carrying one of the LHON associated mutations—G3460A, G11778A, and T14484C—indeed developed optic neuropathy.^{2,38}

The wide range of phenotypic variability of matrilineal relatives within and among families including the severity, age at onset, and penetrance of visual impairment indicated the involvement of other modifier factors including nuclear modifier genes, mitochondrial haplotypes, and epigenetic and environmental factors in the phenotypic manifestation of LHON-associated G11778A mutation. In fact, the predominance of affected males in many LHON families suggests that the existence of a recessive X-linked susceptibility gene acting in synergy with the primary LHON-associated mtDNA mutation to precipitate the optic neuropathy.^{39,40} An X-linked susceptibility locus closely linked to the DXS7 region was the first putative nuclear modifier for the phenotypic manifestation of the G11778A mutation in Finnish families.⁴¹ Several other susceptibility loci were also identified on the X chromosome from LHON families from different ethnic populations. In particular, two overlapping disease loci with highly significant LOD

scores at Xp21-Xq21^{42,43} and Xq25–27.2⁴⁴ were identified using a larger number of European and Brazilian pedigrees. Most recently, an autosomal nuclear modifier *PARL* was implicated in the LHON in Thai families.⁴⁵ Despite the statistical support for the linkages of those putative nuclear modifier loci, none of the mutations in these modifier genes has been identified. In this investigation, the fact that optic neuropathy affected only female matrilineal relatives in two Chinese pedigrees indicates the involvement of X-linked modifier genes in the phenotypic manifestation of the G11778A mutation. Indeed, a missense mutation (R15W) of the *GJB1* gene on the X chromosome was shown to be responsible for only female family members with Charcot-Marie-Tooth neuropathy in a European pedigree.⁴⁶ Alternatively, an autosomal nuclear modifier, similar to that in the Thai families,⁴⁵ may be involved in the clinical expression of LHON. However, the genetic etiology of LHON may be more complex, with epistatic interaction of these multiple nuclear susceptibility loci and genetic heterogeneity.

Mitochondrial haplotypes have been thought to influence the clinical expression of LHON-associated primary mtDNA mutations. In European families, the risk of optic neuropathy increases when the G11778A and T14484C mutations are present in haplogroup J^{13,15,47,48} and when the G3460A mutation occurs in haplogroup K.¹³ On the other hand, the risk of visual failure reduces when the G11778A mutation is present in haplogroup H.¹³ In the Chinese families carrying the G11778A mutation, haplogroup M7b1'2 significantly increases the risk of optic neuropathy, whereas haplogroup M8a has a protective effect.¹⁶ Furthermore, secondary LHON mutations such as *ND1* T4216C and *ND5* G13708A may increase the penetrance and expressivity of LHON associated with the primary LHON G11778A and T14484C mutations.^{8,14,15,49} In addition, the G7444A mutation in the *CO1* and *tRNA^{Ser(UCN)}* genes has also been thought to influence the penetrance and phenotypic occurrence of visual loss associated with the primary LHON mutations.⁸ Most recently, we have shown that the *ND4* G11696A, *tRNA^{Met}* A4435G, and *tRNA^{Thr}* A15951G mutations increase the penetrance and expressivity of visual loss in Chinese families carrying the G11778A mutation.^{19–21} In these two Chinese families with the mutation, there were distinct sets of sequence variations in their mitochondrial genomes belonging to haplogroup M1 and M10a, respectively. As shown in Table 2, mtDNAs of the other 15 Chinese pedigrees carrying the G11778A mutation belong to haplogroups B4a, B5, C, D4, D5, D4, F1, M7b, M8a, M10a, and N9a.^{12,18–21} This

TABLE 2. Summary of Clinical and Molecular Data for 17 Chinese Families Carrying the ND4 G11778A Mutation

Pedigree	Ratio (Affected Male/Female)	Average Age at Onset (y)	Number of Matrilineal Relatives	Penetrance (%)*	Secondary Mutations	mtDNA Haplogroup
WZ49	0:5	12	25	56.5		M1
WZ50	0:4	17	22	33.3		M10a
WZ41 ¹¹	1:0	18	7	14.3		M8a2
WZ42	2:0	14.5	25	8.0		D4g2
WZ43	1:0	15	9	11.1		B4a1c
WZ44	1:0	15	17	5.9		B5b
WZ45	2:0	16	12	16.7		N9a1
WZ46	2:1	14	19	15.8		D4b2b
WZ47	2:1	27	11	27.3		C
WZ48	1:0	16	19	5.3		M7b1
WZ1 ¹²	6:0	22	38	15.8		B5
WZ2 ¹⁹	3:1	14	14	57.1	A4435G	D5
WZ3 ²⁰	2:1	19	10	60.0	A15951G	D4
WZ4 ¹⁸	3:0	17	9	33.3		F1
WZ5	4:1	20	14	35.7		D4b2b
WZ6	1.2:1	18	31	35.5		M10a
WZ12 ²¹	3.5:1	18	30	33.0	G11696A	D4

* Penetrance: affected matrilineal relatives/total matrilineal relatives.

finding suggests that the G11778A mutation, similar to the mutation in Caucasian and Japanese families,^{8,10,13,15,17} occurs sporadically and that it multiplies through the evolution of the mtDNA in the Chinese population. Of these mtDNA variants in two Chinese families, the L175F variant in *CO3*; the I58V variant in the *ND6*; and the I189V, L292R, and S297A variants in *CYTb* are located at highly conserved residues of polypeptides. The highly evolutionary conservation of these variants makes them likely to be functionally significant in terms of mitochondrial physiology.³¹ These notions further supported recent observations that the ND6 I58V variant is involved in LHON in Chinese families,³² the *CO3* L175F variant is associated with LHON in Thai families,³³ and the *CYTb* I189V variant is found in hypertensive individuals.³⁴ Thus, these mitochondrial haplogroup-specific variants, together with nuclear modifier genes and epigenetic and environmental factors, may contribute to the phenotypic manifestation of LHON-associated G11778A mutation in these two Chinese pedigrees. The understanding of these modifier factors is important for the elucidation of the pathophysiology of LHON and to open avenues for therapeutic interventions for this disorder.

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