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Very low penetrance of Leber's hereditary optic neuropathy in five Han Chinese families carrying the *ND1* G3460A mutation

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Abstract

We report here the clinical, genetic, and molecular characterization of five Han Chinese families with Leber's hereditary optic neuropathy (LHON). Strikingly, there were very low penetrances of visual impairment in these Chinese families, ranging from 4.2% to 22.2%, with an average of 10.2%. In particular, only 7 (4 males/3 females) of 106 matrilineal relatives in these families exhibited the variable severity and age-at-onset in visual dysfunction. The age-at-onset for visual impairment in matrilineal relatives in these families, varied from 20 to 25 years, with an average of 21.8 years old. Molecular analysis of mitochondrial genomes identified the homoplasmic *ND1* G3460A mutation and distinct sets of variants, belonging to the Asian haplogroups B5b, C4a1, D5, F1, and R9, respectively. This suggests that the G3640A mutation occurred sporadically and multiplied through evolution of the mtDNA in China. However, there was the absence of known secondary LHON-associated mtDNA mutations in these Chinese families. Very low penetrance of visual loss in these five Chinese pedigrees strongly indicated that the G3640A mutation was itself insufficient to develop the optic neuropathy. The absence of secondary LHON mtDNA mutations suggest that these mtDNA haplogroup-specific variants may not play an important role in the phenotypic expression of the G3640A mutation in those Chinese families with low penetrance of vision loss. However, nuclear modifier genes, epigenetic and environmental factors appear to be modifier factors for the phenotypic manifestation of the G3640A mutation in these Chinese families.

Keywords

Leber hereditary optic neuropathy; Mitochondrial DNA; Mutation; Visual loss; Penetrance; Haplotype; Modifiers; Chinese; *ND1*

Introduction

Leber's hereditary optic neuropathy (LHON) is a maternally inherited eye disease that generally affects young adults with the rapid, painless, bilateral loss of central vision [1–3]. Mutations in mitochondrial DNA (mtDNA) are the molecular bases for this disorder [2,4–6]. Since the landmark discovery of the LHON-associated *ND4* G11778A mutation [4], more than 30 LHON-associated mtDNA mutations have been identified among various ethnic populations [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 more than 95% of LHON pedigrees in some countries [3,7–10]. Those LHON-associated mtDNA mutations, unlike other pathogenic mtDNA mutations such as MELAS-associated tRNA^{Leu(UUR)} A3243G mutation present in heteroplasmy (mixture of mutated and wild-type molecules) [11], often occur in the nearly homoplasmic or homoplasmic forms. Typical features in LHON pedigrees are incomplete penetrance and male bias among the affected subjects, reflecting the complex etiology of this disease [12–14]. Matrilineal relatives within and among families, despite carrying the same LHON-associated mtDNA mutation(s), exhibited a wide range of severity, age-of-onset, and penetrance of optic neuropathy. Therefore, other modifier factors including environmental factors, nuclear background, and mitochondrial haplotypes should modulate the phenotypic manifestation of visual impairment associated with those primary mtDNA mutations [3,15].

To further elucidate molecular basis of LHON in the Chinese population, a systematic and extended mutational screening of mtDNA has been initiated in the large clinical population of Ophthalmology Clinic at the Wenzhou Medical College, China [15–18]. In the previous investigations, we showed that the LHON was associated with the *ND4* G11778A mutation in 15 Chinese families with variable penetrance and severity and age-at-onset of visual impairment [15–20], the *ND6* T14484C mutation 14 Chinese families [21–23], and the *ND1* G3460A mutation in one Han Chinese family [24]. In addition, we showed that LHON is associated with the *ND4* G11696A and *ND6* T14502C mutations in Chinese families with extremely low penetrances of visual loss [25–27]. In this study, we performed the clinical, genetic, and molecular characterization of another five Chinese families with suggestively maternally transmitted LHON. These pedigrees exhibited very penetrance of optic neuropathy. In particular, only 7 (4 males/3 females) of 106 matrilineal relatives in these families exhibited the variable severity and age-at-onset in visual dysfunction. Molecular analysis has led to identification of the G3460A mutation in *ND1* gene in these Chinese families. With the aim of investigating the role of mitochondrial haplotypes in the phenotypic manifestation of the G3460A mutation, we performed a PCR-amplification of fragments spanning entire mitochondrial genome and subsequent DNA sequence analysis in the matrilineal relatives of those Chinese families.

Materials and methods

Patients and subjects

As a part of genetic screening program for visual impairment, five Han Chinese families (Fig. 1) were ascertained through the School of Ophthalmology and Optometry, Wenzhou Medical College, Zhejiang and Dongfang Hospital, 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 those pedigrees were interviewed at length to identify both personal or family medical histories of visual impairments and other clinical abnormalities.

Ophthalmological examinations

The ophthalmologic examinations of probands and other members of these families were conducted, including visual acuity, visual field examination (Humphrey Visual Field Analyzer *III*, SITA Standard), visual evoked potentials (VEP) (Roland Consult RETI port gamma, flash VEP), and fundus photography (Canon CR6-45NM fundus camera). The degree of visual impairment was defined according to the visual acuity as follows: normal > 0.3; mild = 0.3–0.1; moderate < 0.1–0.05; severe < 0.05–0.02; and profound < 0.02.

Mutational analysis of the mitochondrial genome

Genomic DNA was isolated from whole blood of participants using the Puregene DNA Isolation Kits (Gentra Systems). The presence of the G3460A, G11778A, and T14484C mutations was examined as detailed elsewhere [2]. Briefly, affected individuals' DNA fragments spanning these mtDNA mutations were amplified by PCR using oligodeoxynucleotides corresponding to mtDNA at positions 3108–3717 for the G3460A mutation, 11654–11865 for the G11778A mutation, and 14260–14510 for the T14484C mutation [28], respectively. For the detection of the G3460A mutation, the amplified PCR segments were digested with a restriction enzyme BsaHI [2], while the presence of the T14484C mutation was examined by digesting PCR products with a restriction enzyme MvaI [2]. For the examination of the G11778A mutation, the amplified PCR segments were digested with the restriction enzyme Tsp45I [16]. The entire mitochondrial genome of four probands was PCR amplified in 24 overlapping fragments using sets of the light (L) strand and the heavy (H) strand oligonucleotide primers as described previously [29]. Each fragment was purified and subsequently analyzed by direct sequencing in an ABI 3700 automated DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit. These sequence results were compared with the updated consensus Cambridge sequence (GenBank Accession No.: NC_001807) [29]. DNA and protein sequence alignments were carried out using seqweb program GAP (GCG).

Haplogroup analyses

The entire mtDNA sequences of five Chinese probands carrying the G3460A mutation were assigned to the Asian mitochondrial haplogroups by using the nomenclature of mitochondrial haplogroups [30,31].

Results

Clinical and genetic evaluation of five Chinese pedigrees

Five Han Chinese subjects, as shown in Table 1, were diagnosed as LHON by the Eye Clinics at the Wenzhou Medical College and Dongfang Hospital. First, we examined three commonly known LHON-associated mtDNA mutations (G3460A, G11778A, and T14484C) by PCR amplification and subsequent restriction enzyme digestion analysis of PCR fragments derived from five probands. Results revealed the presence of *ND1* G3460A mutation, but the absence of G11778A and T14484C mutations in those subjects. A comprehensive history and physical examination as well as ophthalmological examination were performed to identify both personal or family medical histories of visual impairments, and other clinical abnormalities in all available members of these Chinese pedigrees. In fact, comprehensive family medical histories of those probands and other available members of these Chinese families showed no other

clinical abnormalities, including diabetes, muscular diseases, hearing dysfunction, and neurological disorders. Furthermore, there is no evidence that any member of those families had any other known cause to account for visual impairment. The restriction enzyme BsaHI digestion and subsequent electrophoresis analysis of available members in five pedigrees indicated that the G3460A mutation was indeed present in homoplasmy in matrilineal relatives but no other members of these families (data not shown).

In family WZ28, the proband (III-3) came to the Ophthalmology Clinic of Wenzhou Medical College at the age of 16 years old after experiencing painless, progress deterioration of bilateral visual impairment 3 weeks ago. His visual acuity was 0.02 and 0.03 at the right and left eyes, respectively. Intraocular Pressure (IOP) was 13.7 and 14.7 mm Hg at the right and left eyes, respectively. He saw a dark cloud in the center of vision and had problems appreciating colors that all seemed a dark gray. Visual field testing demonstrated large centrocecal scotomata in both of his eyes. Fundus examination showed that both his optic disks were abnormal: vascular tortuosity of the central retinal vessels, a circumpapillary telangiectatic microangiopathy, and swelling of the retinal nerve fiber layer. Therefore, he exhibited a typical clinical feature of LHON. Of other members in this family, only his grandmother (I-2) suffered from moderate visual impairment at the age of 26 years old.

In family WZ29, the proband (IV-2) was a 23-year-old man. He exhibited bilateral vision loss at the age of 22 years old. He was diagnosed as LHON by Ophthalmology Clinic at the Dongfang Hospital. His visual acuity was 0.1 at the right and left eyes, respectively. IOP was 15 mm Hg in his right eye and 17 mm Hg in the left eye, respectively. However, all other 23 matrilineal relatives in this pedigree had normal vision.

In family WZ30, the proband (IV-8) complained of painless, progressive deterioration of bilateral vision impairment at the age of 18 years old and went to Ophthalmology Clinic at the Dongfang Hospital 3 months later. His visual acuity was 0.02 and 0.03 at the right and left eyes, respectively. IOP was 14 mm Hg in his right eye and 15 mm Hg in the left eye, respectively. Ophthalmological and other clinical evaluations showed that he had a typical clinical feature of LHON. Family history and clinical evaluation showed that only two (WZ30-III-9 and WZ30-IV-2) of other 27 matrilineal relatives suffered from moderate and mild visual loss at the age of 24 and 20 years old, respectively.

In family WZ31, the proband (IV-1) came to the Ophthalmology Clinic at the Dongfang Hospital at the age of 27 years. He exhibited bilateral visual impairment at the age of 25 years old. His visual acuity was 0.1 in both his eyes. His visual dysfunction occurred in both eyes simultaneously. The flash VEP showed bilaterally decreased amplitudes with delayed latencies. IOP was 11 mm Hg in both eyes, Encephalon CT showed normal. Clinical evaluation revealed that he had a typical clinical feature of LHON. The family originated from Shanxi Province in Northwestern China. Further familial history and clinical evaluation revealed that all other matrilineal relatives in this family exhibited normal vision.

In family WZ32, the proband (III-3) was diagnosed as LHON by Ophthalmology clinic at the Dongfang Hospital, Beijing at the age of 20 years old. He began suffering bilateral visual impairment 2 months ago. He saw a dark cloud in the center of vision and had problems appreciating colors that all seemed a dark gray. Ophthalmological examination showed that his visual acuity was 0.08 in the right eye and 0.03 in the left eye. Fundus examination showed that both his temporal optic disks were pale and reflex on fovea centralis was normal. Visual field testing demonstrated large centrocecal scotomata in both eyes. Encephalon CT showed normal. However, none of other 8 matrilineal relatives in this family had visual deficit.

Mitochondrial DNA analysis

To assess the role of mitochondrial haplotypes in the phenotypic manifestation of the G3460A mutation, the DNA fragments spanning entire mitochondrial genome from five probands were PCR amplified, purified, and subsequently analyzed by direct sequence analysis. In addition to the G3460A mutation, as shown in Table 2, these subjects exhibited distinct sets of mtDNA polymorphisms. Of these other nucleotide changes in these mitochondrial genomes, there were 38 known variants in the D-loop, 8 known variants in 12S rRNA gene, 5 known variants in the 16S rRNA gene, the previous identified CO2/tRNA^{Lys} intergenic 9 base-pair deletion corresponding to mtDNA at positions 8271–8279 [17], the novel A4316G variant in the tRNA^{Ile} gene, the T4336C variant in the tRNA^{Gln} gene, the A15924G and G15927A variants in the tRNA^{Thr} gene, the T15968C variant in the tRNA^{Pro} gene, 55 (2 novel and 53 known) silent variants in the protein encoding genes as well as 28 known missense mutations in the protein encoding genes [7]. These missense mutations are the G4048A (D248N) in the *ND1* gene, the G4924A (S152N) and C5178A (L237M) in the *ND2* gene, the T6253C (M117T) in *COI* gene, the G8584A (A20T), A8701G (T59A), G8723A (R66Q), and A8860G (T112A) in *A6* gene, the A10398G (T114A) in the *ND3* gene, the T10609C (M47T) in the *ND4L* gene, the T11204C (F149L) and G11969A (A404T) in the *ND4* gene, the A12361G (T9A), G12406A (V24I), G12940A (A202T), G13135A (A267T), A13637G (N434R), and G13928C (S531T) in the *ND5* gene, the T14318C (N119S) and T14502C (I58V) in the *ND6*, the C14766T (T7I), G14831A (A29T), T15071C (Y109H), T15204C (I153T), A15218G (T158A), A15326G (T194A), A15662G (I306V), and A15851G (I369V) in the *Cytb* gene. These variants in RNAs and polypeptides were further evaluated by phylogenetic analysis of these variants and sequences from other organisms including mouse [32], bovine [33], and *Xenopus laevis* [34]. Of tRNA and rRNA variants, the G15927A variant locates at a highly conserved nucleotide (G42) in the tRNA^{Thr} [35], while the novel A4316G variant resides at the conserved adenine (A58) in the tRNA^{Ile} [36,37]. Of variants in the polypeptide encoding genes, the ND4 F110L [23] and ND6 I58V variants [27] located at highly conserved residues of corresponding polypeptides. However, other variants showed no evolutionary conservation.

Based on the nomenclature of mitochondrial haplogroups [30,31], we used the mtDNA sequence variations among five Chinese probands to establish the haplogroup affiliation of each mtDNA. These included the haplogroup D5 specific variants T1107C, C4883T, C5178A and A5301G in the mtDNA of pedigree WZ28, the haplogroup B5b specific variants 9 base-pair deletion corresponding to mtDNA at positions 8271–8279, T9950C and G1598A of pedigree WZ29, the haplogroup R9 specific variants C3970T, C12705T, G13928C, and C16223T of pedigree WZ30, the haplogroup F1 specific variants G6962A, T10609C, G12406A, and C12882T of pedigree WZ31 as well as the haplogroup C4a1 specific variants T195C, 2232insA, G11969A, A12672G, T15204C, and T15968C of pedigree WZ32.

Discussion

In this study, we have performed the clinical, genetic, and molecular characterization of five Han Chinese families with Leber's hereditary optic neuropathy. Visual impairment as a sole clinical phenotype was only present in the maternal lineage of these pedigrees carrying the known G3460A mutation. In fact, the G3460A mutation has been associated with LHON in many families worldwide [2,5,8,9,14,24,38–41]. Here, clinical and genetic evaluations of these five Chinese families revealed the variable severity and age-of-onset in visual impairment in these matrilineal relatives, although these subjects shared some common features: being the rapid, painless, bilateral loss of central vision. The age-at-onset for visual impairment in matrilineal relatives in these families, as shown in Table 3, varied from 20 years (WZ32) to 25 years (WZ31), with the average of 21.8 years old. However, as shown in Table 3, the average age-at-onset was 14 years old in other Chinese family (WZ17) carrying the G3460A mutation

[24]. Furthermore, the average age-of-onset for visual loss was 20 and 29 years old from matrilineal relatives of 8 and 9 Caucasian pedigrees carrying the G3460A mutation, respectively [42,43]. Thus, matrilineal relatives in these five Chinese families appeared to be comparable with those in these Caucasian families carrying the G3460A mutation. The ratios between affected male and female matrilineal relatives were 2.3:1 and 4:3 in 8 and 9 Caucasian pedigrees carrying the G3460A mutation, respectively [42,43], while the ratio (affected male/female) is 1:0, 1:1, and 1:2 in these Chinese families. As shown in Table 3, these five Chinese families exhibited low penetrance of optic neuropathy (affected matrilineal relatives/total matrilineal relatives), ranging from 4.2% to 22.2%, with the average of 10.2%. Similarly, there were extremely low penetrances of vision loss observed in 10 Han Chinese families carrying the T14484C mutation [23] and 9 Han Chinese families carrying the G11778A mutation [16, 17]. However, ~43% of matrilineal relatives of a Chinese family carrying both *ND1* G3460A and tRNA^{Glu} A14693G mutations exhibited optic neuropathy [24], and ~27% of matrilineal relatives of three Finnish families carrying the G3460A mutation developed optic neuropathy [38].

The very low penetrance of optic neuropathy in these Chinese pedigrees strongly indicated that the *ND1* G3460A mutation, similar to the homoplasmic G11778A and T14484C mutations [17,23] and deafness-associated 12S rRNA C1494T and A1555G mutations [44,45], was necessary but itself insufficient to induce the clinical expression of LHON. Other environmental and genetic factors including nuclear modifier genes and mitochondrial haplotypes may contribute to the variability of penetrances and expressivities of LHON among families carrying the G3460A mutation. The phenotypic variability of matrilineal relatives within and among families including the severity of vision impairment suggests a role of nuclear backgrounds in the phenotypic manifestation of the G3460A mutation. Furthermore, it is possible that environmental factors may also contribute to the phenotypic variability of visual loss in matrilineal relatives of these families. In fact, the mitochondrial variants/haplogroups have been shown to influence the penetrance and expressivity of visual loss associated with the primary mtDNA mutations. In particular, the secondary LHON mutations at positions 4216 and 13708 can increase the penetrance and expressivities of the primary LHON (G11778A and T14484C) mutations [9,46–49] or the deafness-associated tRNA^{Ser(UCN)} A7445G mutation [50]. Furthermore, the G7444A mutation in the CO1 and tRNA^{Ser(UCN)} genes has also been implicated to influence the penetrance and phenotypic expression of visual loss associated with the primary LHON mutations [9] and hearing loss associated with the A1555G mutation [51]. Most recently, we showed that the *ND4* G11696A, tRNA^{Met} A4435G, and tRNA^{Thr} A15951G mutations have a potential modifier role in increasing the penetrance and expressivity of the G11778A mutation [15,18,20]. Furthermore, the tRNA^{Glu} A14693G mutation was implicated to increase the penetrance and expressivity of optic neuropathy in a Chinese family carrying the G3460A mutation [24] and those of hearing loss in another Chinese family carrying the A1555G mutation [52].

Here, there were the distinct sets of sequence variations in mitochondrial genomes of these Chinese pedigrees. As shown in Table 3, mtDNAs of these five families and WZ17 [24] pedigree belong to Eastern Asian haplogroups B5b, C4a1, D5, F1, R9, and M7b2, respectively [30,31]. This suggested that the G3460A mutation, similar to the T14484C and G11778A mutations [17,23], occurred sporadically and multiplied through evolution of the mtDNA in China. Here, the novel A4316G variant resides at the conserved adenine (A58) in the tRNA^{Ile} [36,37] and the G15927A variant locates at a highly conserved nucleotide (G42) in the tRNA^{Thr} [35], while the known ND4 F110L [23] and ND6 I58V variants [27] located at highly conserved residues of corresponding polypeptides, respectively. However, the very low penetrance of optic neuropathy among these five Chinese families, despite carrying one of these four functionally significant mtDNA variants, indicated that these mtDNA haplogroup-specific variants may not play an important role in the phenotypic expression of the G3460A

mutation. In fact, the significant reductions in complex I and II-dependent ATP production were observed in both LHON patients and carriers carrying the *ND1* G3460A, *ND4* G11778A, or *ND6* T14484C mutation [53]. However, an increase in mitochondrial density per cell compensated for the dysfunctional mitochondria in LHON carriers, whereas early-onset LHON patients failed to compensate impaired oxidative phosphorylation [53]. Thus, nuclear modifier genes or epigenetic factors as well as environmental factors including food intake may contribute to the phenotypic manifestation of the G3460A mutation in these Chinese families. The understanding of these modifier factors will be important for the elucidation of the pathogenic mechanism of LHON and the open revenue for therapeutic interventions.

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Fig. 1. Five Chinese pedigrees with Leber's hereditary optic neuropathy. Vision impaired individuals are indicated by filled symbols. Arrows denote probands.

Table 1
Summary of clinical data for affected matrilineal relatives in five Chinese pedigrees.

Subject	Gender	Age of test (years)	Age-of-onset (years)	Visual acuity		Level of visual impairment	
				Right eye	Left eye		
WZ28-III-3	M	16	16	0.02	0.03		Severe
WZ28-I-2	F	32	26	0.03	0.05		Moderate
WZ29-IV-2	M	23	22	0.1	0.1		Mild
WZ30-IV-8	M	18	18	0.02	0.03		Severe
WZ30-III-9	F	25	24	0.03	0.04		Moderate
WZ30-IV-2	F	21	20	0.2	0.1		Mild
WZ31-IV-1	M	27	25	0.1	0.1		Mild
WZ32-III-3	M	20	20	0.08	0.03		Moderate

Table 2

mtDNA variants in five Chinese families with Leber's hereditary optic neuropathy.

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS	WZ28	WZ29	WZ30	WZ31	WZ32	Previously reported ^b
D-loop	73	A to G		A	G	G	G	G	G	Yes
	103	G to A		G	A					Yes
	146	T to C		T		C				Yes
	150	C to T		C	T					Yes
	152	T to C		T		C	C	C		Yes
	195	T to C		T		C		C		Yes
	204	T to C		T		C				Yes
	249	A to Del		A				A-Del	A-Del	Yes
	263	A to G		A	G	G	G	G	G	Yes
	309	C to Del		C					C-Del	Yes
	310	T to CTC		T	CTC	CTC	CTC			Yes
	310	T to TC		T			TC			Yes
	456	C to T		C	T					Yes
	489	T to C		T	C			C		Yes
	522	C to Del		C			C-Del			Yes
	523	A to Del		A		A-Del	A-Del			Yes
	524	C to Del		C		C-Del				Yes
	573	Ins6C		C		Ins6C				Yes
	16086	T to C		T	C					Yes
	16093	T to C		T			C			Yes
	16129	G to A		G				A		Yes
	16140	T to C		T		C				Yes
	16150	C to T		C				T		Yes
	16167	C to T		C	T					Yes
	16182	A to C		A		C	C	C		Yes
	16183	A to C		A	C	C		C		Yes
	16189	T to C		T		C		C		Yes
	16223	C to T		C	T		T		T	Yes
	16243	T to C		T		C				Yes

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS	WZ28	WZ29	WZ30	WZ31	WZ32	Previously reported ^b
12S rRNA	16291	C to T		C				T		Yes
	16298	T to C		T					C	Yes
	16304	T to C		T				C		Yes
	16311	T to C		T						Yes
	16327	C to T		C			C			Yes
	16357	T to C		T			C		T	Yes
	16362	T to C		T	C					Yes
	16497	A to G		A			G			Yes
	16519	T to C		T		C		C		Yes
	681	T to C	T/T/A/-	T	C					Yes
16S rRNA	709	G to A	G/A/A/A	G		A				Yes
	750	A to G	A/A/A/G	A	G	G	G	G	G	Yes
	961	C to CC	T/A/T/A	C		CC				Yes
	1048	C to T	C/C/T/C	C	T					Yes
	1107	T to C	T/C/T/T	T	C					Yes
	1438	A to G	A/A/A/G	A	G		G	G	G	Yes
	1598	G to A	G/A/T/T	G		A				Yes
	1715	C to T	C/A/A/T	C					T	Yes
	2232	A to AA	A/A/A/A	A					AA	Yes
	2389	C to T	C/T/T/C	C				T		Yes
ND1	2706	A to G	A/G/A/A	A	G	G	G	G	G	Yes
	3172	C to CC	C/C/T/T	C			CC			Yes
	3460	G to A (Ala to Thr)	A/A/A/G	G	A	A	A	A	A	Yes
	3552	T to A		T					A	Yes
	3834	G to A		G		A				Yes
	3970	C to T		C				T		Yes
	4048	G to A (Asp to Asn)	D/N/Y/F	G				A		Yes
	4140	C to T		C						Yes
	4316	A to G	A/A/A/A	A			G			No
	4336	T to C	T/T/A/G	T	C					Yes
ND2	4715	A to G		A					G	Yes

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS	WZ28	WZ29	WZ30	WZ31	WZ32	Previously reported ^b
CO1	4769	A to G		A	G	G	G	G	G	Yes
	4883	C to T		C	T					Yes
	4924	G to A (Ser to Asn)	S/N/T/P	G		A				Yes
	4985	G to A		G	A					Yes
	5153	A to G		A	G					Yes
	5178	C to A (Leu-Met)	L/T/T/T	C	A					Yes
	5301	A to G		A	G					Yes
	6026	G to A		G				A		Yes
	6253	T to C (Met-Thr)	M/M/M/G	T	C					Yes
	6392	T to C		T				C		Yes
CO2	6962	G to A						A		Yes
	7028	C to T			T	T	T	T	T	Yes
	7196	C to A						A		Yes
	7250	A to G				G				Yes
	7933	A to G						G		Yes
	7999	T to C							C	Yes
	8281_9	9bpDel				9bpDel				Yes
	8584	G to A (Ala to Thr)	A/V/V/I			A		A		Yes
	8701	A to G (Thr to Ala)	T/S/L/Q	G			G		G	Yes
	8723	G to A (Arg to Gln)	R/Q/R/H							Yes
CO3	8793	T to C					C			Yes
	8794	C to T (His to Tyr)	H/H/H/Y							Yes
	8829	C to T				T				Yes
	8856	G to A					A			Yes
	8860	A to G (Thr to Ala)	T/A/A/T		G	G	G	G	G	Yes
	8973	A to G						G		Yes
	9180	A to G								Yes
	9540	T to C								Yes
	9545	A to G					C		C	Yes
	9950	T to C							G	Yes
ND3	10310	G to A				C				Yes
	10397	A to G			G			A		Yes

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS	WZ28	WZ29	WZ30	WZ31	WZ32	Previously reported ^b
ND4L	10398	A to G (Thr to Ala)	T/T/T/A	G	G	G	G	G	G	Yes
	10400	C to T		T		T	T	T	T	Yes
	10529	A to G				G	G			Yes
	10609	T to C (Met to Thr)	M/T/T/T					C		Yes
ND4	10646	G to A				A	A			Yes
	10873	T to C		C		C	C		C	Yes
	11204	T to C (Phe to Leu)	F/F/F/F					C		Yes
	11335	T to C		C						Yes
ND5	11719	G to A		A	A	A	A	A	A	Yes
	11914	G to A								Yes
	11969	G to A (Ala to Thr)	A/A/G/A							Yes
	12007	G to A		A						Yes
ND6	12361	A to G (Thr to Ala)	T/L/L/L		G					Yes
	12372	G to A							A	Yes
	12406	G to A (Val to Ile)	V/F/S/F					A		Yes
	12549	C to T				T	T			Yes
Cytb	12630	G to A				A	A			Yes
	12672	A to G							G	Yes
	12705	C to T				T	T		T	Yes
	12882	C to T						T		Yes
ND6	12940	G to A (Ala to Thr)	A/F/M/F						A	Yes
	13135	G to A (Ala to Thr)	A/T/T/M				A			Yes
	13152	A to G				G	G			Yes
	13263	A to G							G	Yes
ND6	13637	A to G	Q/Q/K/H				G			Yes
	13928	G to C (Ser to Thr)	S/T/S/T					C		Yes
	14028	A to G			G					No
	14318	T to C (Asn to Ser)	N/N/D/S						C	Yes
Cytb	14410	G to A			A					No
	14502	T to C (Ile to Val)	I/I/I				C			Yes
	14766	C to T (Thr to Ile)	T/S/T/S		T		T	T	T	Yes
	14783	T to C		C			C		C	Yes

Gene	Position	Replacement	Conservation (H/B/M/X) ^a	CRS	WZ28	WZ29	WZ30	WZ31	WZ32	Previously reported ^b
tRNA ^{Thr}	14831	G to A (Ala to Thr)	A/S/S/S		A					Yes
	15040	C to T				T				Yes
	15043	G to A			A		A	A		Yes
	15071	T to C (Tyr to His)	Y/F/F/Y				C			Yes
	15204	T to C (Ile to Thr)	I/I/K						C	Yes
	15218	A to G (Thr to Ala)	T/T/T/N			G				Yes
	15223	C to T				T				Yes
	15299	T to C				C				Yes
	15301	G to A			A		A	A		Yes
	15326	A to G (Thr to Ala)	T/M/I/I		G		G	G		Yes
	15487	A to T						T		Yes
	15508	C to T				T				Yes
	15662	A to G (Ile to Val)	I/L/F/L			G				Yes
	15851	A to G (Ile to Val)	I/A/S/M			G				Yes
	15924	A to G	A/A/AG		G					Yes
	15927	G to A	G/A/T/G			A				Yes
tRNA ^{Pro}	15968	T to C	T/A/A/A						C	Yes

CRS, consensus Cambridge sequence.

^aConservation of amino acid for polypeptides or nucleotide for RNAs in human (H), bovine (B), mouse (M), and *Xenopus laevis* (X).

^bSee the online mitochondrial genome database <http://www.mitomap.org> and <http://www.genpat.uu.se/mtDB>.

Table 3
Summary of clinical and molecular data for six Chinese families carrying the G3460A mutation.

Pedigree	Ratio of affected males to affected females	Average age-at-onset (years)	Number of matrilineal relatives	Penetrance (%) ^a	mtDNA haplogroup
WZ28	1/1	21	9	22.2	D5
WZ29	1/0	22	24	4.2	B5b
WZ30	1/2	21	28	10.7	R9
WZ31	1/0	25	36	2.8	F1
WZ32	1/0	20	9	11.1	C4aI
WZ17 ^b	1/2	14	7	42.9	M7b2

^a Affected matrilineal relatives/total matrilineal relatives.

^b Tong et al. [24].