Mitochondrial DNA analysis in the Turkish Leber's hereditary optic neuropathy population

C.F. DOGULU, T. KANSU, V. SEYRANTEPE, M. OZGUC, H. TOPALOGLU, D.R. JOHNS

CLINICAL STUDY

Abstract

Purpose **To define the prevalence of a panel of mitochondrial DNA (mtDNA) mutations associated with Leber's hereditary optic neuropathy (LHON) in the Turkish LHON population. LHON-associated mtDNA mutations have been found in LHON patients from around the world, but the Turkish LHON population has not been studied.**
Methods **Thirty-two Turkish patients were defined clinically as having LHON on the basis of painless, subacute, bilateral optic neuropathy and the exclusion of other causes of subacute optic neuropathy. mtDNA was extracted from blood of the 32 probands and assayed for a panel of primary and secondary LHON-associated mtDNA mutations by polymerase chain reaction (PCR)-based methods. We studied three well-known LHON-associated primary mutations (at nucleotide positions 11778, 3460 and 14484) and one common secondary mutation (at nucleotide 15257) in all 32 probands. In addition to these mutations, 18 of the 32 probands were tested for the Complex IV, COX III gene, LHON associated 9804 and 9438 mutations and secondary LHON mutations at nucleotide positions 3394, 4160, 4216, 4917, 5244, 7444, 7706, 13708, 13730 and 15812.**
Results **Among the 32 probands tested for four common LHON mutations, 3 carried the 14484 mutation, 1 carried the 11778 mutation, 1 carried the 3460 mutation and 1 carried the 15257 mutation. Among the 18 LHON patients who tested for additional mutations, 1 proband harboured the 9804 mutation and 4 carried the secondary mutations at nucleotide positions 4216, 4917 and 13708.**
Conclusion **The results of mtDNA analysis of the Turkish LHON patients appear to be different from those of previous reports.**

Key words LHON, mtDNA, mtDNA mutations

Leber's hereditary optic neuropathy (LHON) is a maternally inherited disease of young adults, predominantly males, characterised by acute or subacute, simultaneous or sequential, bilateral central visual loss and, ultimately, optic atrophy. LHON is the first human disease associated with a mtDNA point mutation.[1] Analysis of the mtDNA molecules of LHON patients of different ethnic groups has indicated that three point mutations at nucleotide positions (np) 11778 (ND4 gene), 3460 (ND1 gene) and 14484 (ND6 gene), all of which occur in subunits of Complex I of the mitochondrial respiratory chain, have a primary pathogenetic significance in LHON. All three of these mutations alter evolutionarily conserved amino acids, and are not found in control individuals. They seem to be sufficient in themselves to cause the disease. The relative frequency of the three primary LHON mtDNA mutations varies considerably between populations, although the 11778 is the most prevalent mutation. The ND4/11778 mutation accounts for 50–76% of all LHON families, the ND1/3460 mutation is detected in 7–30% of cases and 10–31% of LHON families have the ND6/14484 mutation.[1–5]

Recently, two missense mutations at np 9438 and 9804, in the cytochrome *c* oxidase subunit III gene (Complex IV), have been identified in 8 independent LHON probands who lack the three primary LHON mutations, and it has been proposed that these mutations are of primary pathogenetic importance in LHON.[6] The Complex IV, COX III/9804 mutation has been found in 3 probands, and the COX III/9438 mutation in 5 probands; neither has been found in controls.

In addition to these primary mutations, several other mutations of uncertain significance – so-called secondary mutations at np 3394 (Complex I/ND1 gene), 4160 (Complex I/ND1 gene), 4216 (Complex I/ND1 gene), 4917 (Complex I/ND2 gene), 5244 (Complex I/ND2 gene), 7444 (Complex IV/COXI gene), 7706 (Complex IV/COXII gene), 13708 (Complex I/ND5 gene), 13730 (Complex I/ND5 gene) and 15812 (Complex III/apocyt b gene) – have been reported in LHON pedigrees.[7–10] These mutations are also detected at low frequency in control individuals, and they change evolutionarily less conserved amino acids. It has been suggested that secondary LHON

C.F. Dogulu
T. Kansu
Institute of Neurological Sciences and Psychiatry
Department of Neurology
Neuro-Ophthalmology Unit
Hacettepe University
Ankara, Turkey

V. Seyrantepe
M. Ozguc
Department of Medical Biology
Hacettepe University
Faculty of Medicine
Ankara, Turkey

H. Topaloglu
Department of Neurology
Pediatric Neurology Division
Hacettepe University
Hospitals
Ankara, Turkey

D.R. Johns
Department of Neurology
Neuromuscular Disease Division
Beth Israel Hospital
Harvard Medical School
Boston, MA, USA

Cigdem F. Dogulu, MD ✉
Hacettepe University
Hospitals
Department of Neurology
Ankara 06100, Turkey
Tel: +90 312 305 1806
Fax: +90 312 309 3451
e-mail: cd05-k@tr-net.net.tr

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Table 1. *Ophthalmological findings of the Turkish LHON patients*

Patient no.	Sex	Age of onset (years)	Onset interval	Family history	Visual acuity		Colour vision		Fundal abnormalities	Visual field defect
					R	L	R	L		
1	M	17	Simultaneous	−	LP	CF 1	0/12	0/12	Bilateral optic atrophy	Right total loss, left dense central scotoma
2	F	28	Sequential (5 years)	−	nLP	nLP	0/12	0/12	Bilateral optic atrophy	Bilateral total loss
3	M	21	Simultaneous	−	CF 1	CF 1	0/12	0/12	Bilateral optic atrophy	Bilateral dense central scotoma
4	M	52	Simultaneous	+	nLP	nLP	0/12	0/12	Bilateral hyperaemic optic discs, peripapillary microangiopathy	Bilateral total loss
5	M	15	Simultaneous	−	20/200	20/200	4/12	6/12	Bilateral optic atrophy	Bilateral central scotoma
6	M	18	Sequential (3 days)	−	CF 1	CF 1	0/12	0/12	Bilateral optic atrophy	Bilateral dense scotoma
7	M	17	Sequential (3 months)	−	20/800	20/400	1/12	1/12	Bilateral optic atrophy	Bilateral dense central scotoma
8	M	18	Sequential (2 weeks)	−	20/200	CF 1	8/12	0/12	Bilateral optic atrophy	Bilateral central scotoma
9	M	11	Simultaneous	−	20/200	20/200	0/12	0/12	Bilateral optic atrophy	Bilateral central scotoma
10	M	14	Simultaneous	+	20/200	20/200	0/12	0/12	Bilateral optic atrophy	Bilateral central scotoma
11	M	44	Simultaneous	−	CF 1	CF 1	0/12	0/12	Bilateral optic atrophy	Bilateral dense central scotoma
12	M	21	Simultaneous	−	LP	LP	0/12	0/12	Bilateral optic atrophy	Bilateral dense scotoma
13	M	18	Simultaneous	−	CF 1	CF 1	0/12	0/12	Bilateral optic atrophy	Bilateral dense scotoma
14	M	20	Simultaneous	−	CF 1	CF 1	0/12	0/12	Bilateral optic atrophy	Bilateral dense central scotoma
15	M	13	Simultaneous	−	CF 1	CF 1	0/12	0/12	Bilateral optic atrophy	Bilateral dense central scotoma
16	F	15	Simultaneous	−	CF 1	CF 1	0/12	0/12	Bilateral hyperaemic optic discs, peripapillary microangiopathy	Bilateral dense central scotoma
17	F	45	Sequential (1 month)	−	LP	20/800	0/12	0/12	Bilateral hyperaemic optic discs, peripapillary microangiopathy	Right total loss, left central scotoma
18	F	46	Simultaneous	−	nLP	nLP	0/12	0/12	Bilateral hyperaemic optic discs, peripapillary microangiopathy	Bilateral total loss
19	F	18	Sequential (2 weeks)	−	20/200	20/400	4/12	1/12	Bilateral optic atrophy	Bilateral central scotoma
20	F	42	Simultaneous	−	20/200	20/200	6/12	6/12	Bilateral optic atrophy	Bilateral central scotoma
21	M	14	Sequential (3 weeks)	−	20/200	20/800	4/12	0/12	Bilateral hyperaemic optic discs, peripapillary	Bilateral dense central scotoma
22	M	15	Sequential (1 year)	−	20/400	20/400	0/12	0/12	Bilateral optic atrophy	Bilateral dense central scotoma
23	F	51	Sequential (3 months)	−	20/200	20/200	4/12	4/12	Bilateral optic atrophy	Bilateral central scotoma
24	M	38	Sequential (2 years)	−	20/200	20/200	5/12	4/12	Bilateral optic atrophy	Bilateral central scotoma
25	M	41	Sequential (4 months)	−	LP	nLP	0/12	0/12	Bilateral optic atrophy	Bilateral total loss
26	F	23	Sequential (5 months)	+	CF 1	20/800	0/12	0/12	Bilateral optic atrophy	Bilateral dense central scotoma
27	M	18	Sequential (3 months)	−	20/200	20/200	1/12	1/12	Bilateral hyperaemic optic discs, peripapillary microangiopathy	Bilateral central scotoma
28	F	16	Simultaneous	−	20/200	20/200	5/12	5/12	Bilateral hyperaemic optic discs, peripapillary microangiopathy	Bilateral central scotoma
29	F	36	Sequential (1 year)	−	20/200	nLP	0/12	0/12	Bilateral optic atrophy	Right central scotoma, left total loss
30	M	15	Simultaneous	−	20/400	20/200	0/12	0/12	Bilateral optic atrophy	Bilateral central scotoma
31	M	16	Sequential (1 month)	−	20/400	20/400	0/12	0/12	Bilateral optic atrophy	Bilateral dense central scotoma
32	M	24	Simultaneous	−	CF 1	CF 1	0/12	0/12	Bilateral optic atrophy	Bilateral dense central scotoma

LP, light perception; nLP, no light perception; CF 1, Counting fingers at 1 m.

mutations may act synergistically with each other and with the primary mutations or nuclear factors and increase the risk of disease expression.[11] The role of Complex III-apocytochrome *b* gene, 15257 mutation, in the pathogenesis of LHON remains is a matter of debate. It is postulated to be of primary pathogenetic importance in LHON on account of the replacement of an evolutionarily highly conserved amino acid by the mutation.[12] On the other hand the 4917 mutation also changes a highly conserved amino acid but certainly has no primary pathogenetic importance, if any phenotypic importance at all.[9,13,14] Furthermore, it is found in 0.3% of normal controls and in combination with each of the 3460, 11778 and 14484 mutations, and Oostra *et al.*[15] have suggested that these are the points against the mtDNA mutation at np 15257 being of primary pathogenetic significance.

All these LHON-associated mtDNA mutations have been found in LHON patients around the world. The purpose of this study was to define the prevalence of the LHON-associated mutations in the Turkish LHON population.

Patients and methods

Patients

Thirty-two patients (22 males, 10 females) were defined clinically as having LHON on the basis of painless, subacute bilateral optic neuropathy and the exclusion of other causes of subacute optic neuropathy. The results of an extensive evaluation including complete blood count, coagulation studies, serum folic acid and vitamin B_{12} levels, analysis of cerebrospinal fluid and brain MRI were normal in all patients. Blood tests for syphilis, HIV and other viral infections were negative. Except for optic neuropathy, neurological and systemic examinations were normal. The age of onset was 11–51 years for males and 15–51 years for females. The two eyes were affected sequentially (3 days to 5 years) in 15 patients and simultaneously in 17 patients. No family history of visual loss was obtained in 29 patients. In 2 patients, one had a maternal uncle and the other had a brother with a history of visual loss. In 1 proband, a maternal uncle, a maternal aunt and a brother were visually affected. Colour vision was affected severely (with Ishihara colour plates), visual acuity was documented 20/200 or worse (with a Rosenbaum near vision chart) and visual fields demonstrated central defects (with Goldmann perimetry) in all patients. Fundoscopic examination demonstrated characteristic findings of LHON (circumpapillary telangiectatic microangiopathy and swelling of the optic disc with the absence of leakage on fluorescein angiography) in 7 patients who presented in the early stage of visual loss, and optic atrophy in 25 patients.

Clinical details are summarised in Table 1.

Methods

Molecular genetic analysis for a panel of three well-known (at np 11778, 3460 and 14484) and two recently reported (at np 9438 and 9804) primary and 11 secondary (at np 3394, 4160, 4216, 4917, 5244, 7444, 7706, 13708, 13730, 15257 and 15812) LHON-associated mtDNA point mutations was performed on blood samples of 18 of the 32 Turkish LHON probands. The remaining 14 probands were tested for only four common LHON mutations (three primary: at np 11778, 3460, 14484; one secondary: at np 15257) by polymerase chain reaction (PCR)-based methods. DNA was extracted by standard proteinase K/SDS methods, and PCR amplification was performed with 4 μl template DNA, 35 μl dH20, 5 μl 10× PCR buffer, 4 μl dNTPs, 2 μl forward primer, 2 μl reverse primer and 0.5 μl *Taq* polymerase. Mutation detection was performed by means of restriction endonuclease digestion. Amplified DNA was digested with restriction enzymes and the fragments were analysed by polyacrylamide gel electrophoresis and ethidium bromide staining.

Table 2. *Oligonucleotide primers for PCR amplifications and restriction endonucleases*

	Oligonucleotide primers[a]		
mtDNA point mutations	Forward	Reverse	Restriction endonucleases
Primary mutations			
11778	11429–11449	11929–11909	*Sfa*NI (New England BioLabs)
3460	3787–3801	3081–3064	*Bsa*HI (New England BioLabs)
14484	14519–14538	14483–14463	*Sau*3AI (New England BioLabs)
15257	15731–15748	15115–15101	*Acc*I (New England BioLabs)
9804	9756–9775	9866–9848	*Mae*III (Boehringer Mannheim)
9438	9151–9169	9581–9561	*Stu*I (New England BioLabs)
Secondary mutations			
3394	3064–3081	3650–3636	*Hae*III (New England BioLabs)
4160		4351–4337	*Alu*I (New England BioLabs)
4216	4060–4074	4351–4337	*NIa*III (New England BioLabs)
4917	4850–4864	5120–5103	*Bfa*I (New England BioLabs)
5244	4704–4721	5480–5463	*Hpa*II (New England BioLabs)
7444	6924–6938	8079–8061	*Xba*I (New England BioLabs)
7706	7407–7425	8079–8061	*Acc*L (New England BioLabs)
13708	13900–13917	13586–13569	*Bst*NI (New England BioLabs)
15812	15101–15115	16074–16060	*Rsa*I (New England BioLabs)

[a]Primer pairs are numbered according to the canonical Cambridge human mtDNA sequence with the 5' → 3' coordinates.[16]

Table 3. *mtDNA analysis of the 32 Turkish LHON patients for the known LHON mutations*

Mutations \ Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19[a]	20[a]	21[a]	22[a]	23[a]	24[a]	25[a]	26[a]	27[a]	28[a]	29[a]	30[a]	31[a]	32[a]
11778																										+						
3460																												+				
15257																													+			
14484																				+							+			+		
9804																		+														
9438																																
3394																																
4160																																
4216							+		+			+																				
4917									+																							
5244																																
7444																																
7706																																
13708	+											+																				
15812																																

[a]The last 14 patients were analysed only for the primary LHON mutations at nucleotide positions 11778, 3460, 15257 and 14484.

The forward and reverse primers and restriction endonucleases for each LHON associated mutation are listed in Table 2.

All nucleotide positions are numbered according to the canonical Cambridge human mtDNA sequence.[16]

Results

The results of the LHON mutation analysis of the Turkish LHON patients are shown in Table 3.

The 11778, 3460, 14484 (three well-known primary LHON mutations) and 15257 mutation analysis in 32 patients

The primary LHON mutations were identified in 5 of 32 (16%) patients. Three of them (patients 20, 27 and 30) carried the 14484 mutation, 1 carried the 3460 mutation (patient 28) and 1 carried the 11778 mutation (patient 26). None of the patients with the 14484 and 3460 mutations had a family history of visual loss. The patient with the 11778 mutation had a visually affected brother, maternal uncle and maternal aunt.

One patient harboured the 15257 LHON mutation (patient 29). This patient did not have a family history of LHON.

The Complex IV–cytochrome c oxidase subunit III gene LHON mutation and secondary LHON mutation analysis in 18 patients

One LHON proband harboured the 9804 primary LHON mutation. The 9804 mutation was verified in the patient's PCR-amplified mtDNA by using a forward amplification primer at nucleotide positions 9756–9775 (5' TCTCCCTTCACCATTTCCGA 3') and a reverse amplification primer at nucleotide positions 9848–9866 (5' GATGAAGCAGATAGTGAGG 3') with the creation of a *Mae*III (Boehringer, Mannheim, Germany) restriction site (GTNAC) caused by the mutation.

Molecular analysis of this mutation among the family members showed that the proband's asymptomatic close maternal relatives harboured the mutation and it was present in a heteroplasmic state in the family members including the proband.

Family analysis. Blood samples were obtained from the proband's sister (5 years older than the proband) and the proband's two children (a daughter and a son). None of these relatives has shown any signs of optic neuropathy. They were analysed in a similar fashion for the cytochrome *c* oxidase/9804 mutation. We observed that the mutation was also carried in the proband's asymptomatic daughter, son and older sister.

None of the 18 LHON probands harboured the Complex IV, COXIII/9438 LHON mutation.

Secondary mutations at np 4216, 4917 and 13708 were found in 4 of the 18 LHON probands (22%). One carried the 13708 mutation (patient 1), 1 carried the 4216 mutation (patient 7), 1 carried the 4216 and 4917 mutations (patient 9) and 1 carried the 4216 and 13708 mutations (patient 12). None of the patients with the secondary LHON mutations had a family history of visual loss.

Discussion

The frequency of three well-known primary LHON mutations in Turkish LHON patients is significantly lower than in the other populations reported to date. In the United Kingdom, 9% of families tested had the 3460 mutation, 78% had the 11778 mutation and 13% had the 14484 mutation.[17] In the study by Vilkki *et al.*[18] in 19 Finnish families, 3 (16%) had the 3460 mutation and 10 (53%) had the 11778 mutation. The 11778 mutation accounts for 92% of families in the Japanese LHON population.[19]

In the Turkish LHON probands with the 11778, 3460 and 14484 mutations, 9% harboured the 14484 mutation, 3% had the 11778 mutation and 3% had the 3460 mutation. The 14484 mutation appears to be the most

frequent cause of LHON in our population, in contrast to the majority of previous reports in which 11778 is the most common mutation in the reported populations.[17–20] On the other hand, this is comparable to a previous study where 14484 had been the most common mutation in LHON families of French Canadian origin.[21]

We found the fourth 9804 mutation associated LHON proband in the literature and the occurrence of the 9804 mutation in a heteroplasmic form in this patient is consistent with a significant pathogenetic role.

One of our patients carried the Complex III, cytochrome *b* gene/15257 mutation. The patient with the 15257 mutation carried none of the 11778, 3460 and 14484 mutations. This finding is comparable with the results of those authors who indicate that the 15257 mutation has a primary pathogenetic importance in LHON, and contrary to the report of Oostra *et al*.[12–15] On the other hand, our 15257 positive patient who lacks the 3460, 11778 and 14484 mutations may harbour an as yet unidentified primary mutation.

Our LHON probands are singletons except for 3 patients. One of the LHON patients with a positive family history carried the 11778 mutation, and the remaining 2 harboured none of 16 LHON mutations. In 10 of 29 patients who lack a family history of visual loss, the occurrence of LHON mutations most probably reflects the fact that the mutations have occurred recently and the presence of the mutation in the unaffected family members of the patient with the 9804 mutation may verify this hypothesis.

The molecular basis of the Turkish LHON population appears to be significantly different from that of other LHON populations studied to date in four distinctive features. First, the primary LHON-associated mtDNA mutations at nucleotide positions 11778, 3460 and 14484 that account for the vast majority of LHON probands in previous reports was found only in the minority of the Turkish LHON probands. Second, the relative frequency of the 14484 mutation in our patients is higher than the other Complex I primary mutations, compatible with the study in French Canadian LHON patients. Third, in the absence of the common mutations, one LHON proband harboured the infrequent 9804 mutation. Fourth, the vast majority of our cases (91%) did not have a family history of visual loss.

There are three hypotheses for the mitochondrial gene aetiology of LHON in the Turkish probands. The first possibility is that there may be additional point mutations in the mtDNA which are yet to be discovered that account for the visual loss in the Turkish LHON probands not associated with the known mutations. This is currently being investigated. Alternatively, the Turkish LHON probands not associated with the known mutations have the primary mutations that could have not shown up due to heteroplasmy. A population of mutant mtDNA confined to the optic nerves, retina and their vasculature may account for the LHON probands with negative blood tests. Finally, the disease may result from the interaction of multiple secondary LHON mutations, none of which produces the risk of LHON when present in isolation from the others. If the last possible mechanism is the one that is operating, it may account for the development of visual loss only in 2 Turkish probands, one carrying the 4216 and 4917 mutations, and the other with 4216 and 13708 mutations.

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References

1. Wallace DC, Singh G, Lott MT, *et al*. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 1988;242:1427–30.
2. Huoponen K, Vilkki J, Aula P, Nikoskelainen EK, Savontaus M-L. A new mtDNA mutation associated with Leber hereditary optic neuroretinopathy. Am J Hum Genet 1991;48:1147–53.
3. Howell N, Bindoff LA, McCullough DA, *et al*. Leber hereditary optic neuropathy: identification of the same mitochondrial ND1 mutation in six pedigrees. Am J Hum Genet 1991;49:939–50.
4. Vilkki J, Savontaus M-L, Nikoskelainen EK. Segregation of mitochondrial genomes in a heteroplasmic lineage with Leber hereditary optic neuroretinopathy. Am J Hum Genet 1990;47:95–100.
5. Mackey D, Howell N. A variant of Leber hereditary optic neuropathy characterised by recovery of vision and by an unusual mitochondrial genetic etiology. Am J Hum Genet 1992;51:1218–28.
6. Johns DR, Neufeld MJ. Cytochrome *c* oxidase mutations in Leber's hereditary optic neuropathy. Biochem Biophys Res Commun 1993;196:810–5.
7. Johns DR, Neufeld MJ. Cytochrome *b* mutations in Leber hereditary optic neuropathy. Biochem Biophys Res Commun 1991;181:1358–64.
8. Brown MD, Voljavec AS, Lott MT, Torroni A, Yang C-C, Wallace DC. Mitochondrial DNA complex I and III mutations associated with Leber's hereditary optic neuropathy. Genetics 1992;130:163–73.
9. Johns DR, Berman J. Alternative, simultaneous complex I mitochondrial DNA mutations in Leber's hereditary optic neuropathy. Biochem Biophys Res Commun 1991;174:1324–30.
10. Brown MD, Yang C-C, Trounce I, Torroni A, Lott MT, Wallace DC. 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 1992;51:378–85.
11. Brown MD, Wallace DC. Spectrum of mitochondrial DNA mutations in Leber's hereditary optic neuropathy. Clin Neurosci 1994;2:138–45.
12. Johns DR, Smith KH, Savino PJ, Miller NR. Leber's hereditary optic neuropathy. Clinical manifestations of the 15257 mutation. Ophthalmology 1993;100:981–6.
13. Brown MD, Voljavec AS, Lott MT, McDonald I, Wallace DC. Leber's hereditary optic neuropathy: a model for mitochondrial neurodegenerative diseases. FASEB J 1992;6:2791–8.

14. Oostra RJ, Bolhuis PA, Wijburg FA, Zorn-Ende G, Bleeker-Wagemakers EM. Leber's hereditary optic neuropathy: correlations between mitochondrial genotype and visual outcome. J Med Genet 1994;31:280–6.
15. Oostra RJ, Bolhuis PA, Zorn-Ende I, de Kok-Nazaruk MM, Bleeker-Wagemakers EM. Leber's hereditary optic neuropathy: no significant evidence for primary or secondary pathogenicity of the 15257 mutation. Hum Genet 1994;94:265–70.
16. Anderson S, Bankier AT, Barrell BG, *et al*. Sequence and organisation of the human mitochondrial genome. Nature 1981;290:457–65.
17. Harding AE, Sweeney MG, Govan GG, Riordan-Eva P. Pedigree analysis in Leber hereditary optic neuropathy with a pathogenic mtDNA mutation. Am J Hum Genet 1995;56:72–6.
18. Vilkki J, Savontaus M-L, Nikoskelainen EK. Genetic heterogeneity in Leber hereditary optic neuropathy revealed by a mitochondrial DNA polymorphism. Am J Hum Genet 1989;45:206–11.
19. Nakamura M, Ara F, Yamada M, *et al*. High frequency of mitochondrial ND4 mutation in Japanese pedigrees with Leber hereditary optic neuropathy. Jpn J Ophthalmol 1992;36:56–61.
20. Nikoskelainen EK, Marttila RJ, Huoponen K, *et al*. Leber's 'plus': neurological abnormalities in patients with Leber's hereditary optic neuropathy. J Neurol Neurosurg Psychiatry 1995;59:160–4.
21. Macmillan C, Kirkham T, Fu K, *et al*. Pedigree analysis of French Canadian families with T14484C Leber's hereditary optic neuropathy. Neurology 1998;50:417–22.