J Hum Genet (2006) 51:298–304
DOI 10.1007/s10038-006-0361-1

ORIGINAL ARTICLE

Nopasak Phasukkijwatana
Wanicha L. Chuenkongkaew · Rungnapa Suphavilai
Bhoom Suktitipat · Sarinee Pingsuthiwong
Ngamkae Ruangvaravate · La-ongsri Atchaneeyasakul
Sukhuma Warrasak · Anuchit Poonyathalang
Thanyachai Sura · Patcharee Lertrit

The unique characteristics of Thai Leber hereditary optic neuropathy: analysis of 30 G11778A pedigrees

Received: 22 September 2005 / Accepted: 13 December 2005 / Published online: 14 February 2006

Abstract Leber hereditary optic neuropathy (LHON) is characterized by acute or subacute bilateral visual loss, and affects mostly young males. The most common mitochondrial DNA mutation responsible for LHON worldwide is G11778A. Despite different genetic backgrounds, which are believed to influence the disease expression, most features of LHON are quite common in different populations. However, there seem to be a few ethnic-specific differences. Analyses of our 30 G11778A LHON pedigrees in Thailand showed some characteristics different from those of Caucasians and Japanese. In particular, our pedigrees showed a lower male to female ratio of affected persons (2.6:1) and much higher prevalence of G11778A blood heteroplasmy (37% of the pedigrees contained at least one heteroplasmic G11778A individual). Heteroplasmicity seemed to influence disease manifestation in our patients but did not appear to alter the onset of the disease. The estimated overall penetrance of our G11778A LHON population was 37% for males and 13% for females. When each of our large pedigrees were considered separately, disease penetration varied from 9 to 45% between the pedigrees, and also varied between different branches of the same large pedigree. Survival analysis showed that the secondary LHON mutations G3316A and C3497T had a synergistic deleterious effect with the G11778A mutation, accelerating the onset of the disease in our patients.

Keywords Mitochondria · Mitochondrial DNA · Mitochondrial genetics · Mitochondrial disease · Leber hereditary optic neuropathy

N. Phasukkijwatana · R. Suphavilai · B. Suktitipat
S. Pingsuthiwong · P. Lertrit (✉)
Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700 Thailand
E-mail: lertrito@yahoo.com
Tel.: +66-2-4197582
Fax: +66-2-4111428

W. L. Chuenkongkaew · N. Ruangvaravate · L. Atchaneeyasakul
Department of Ophthalmology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700 Thailand

S. Warrasak · A. Poonyathalang
Department of Ophthalmology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, 10400 Thailand

T. Sura
Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, 10400 Thailand

W. L. Chuenkongkaew · P. Lertrit
Siriraj Neurogenetics Network, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700 Thailand

Introduction

Leber hereditary optic neuropathy (LHON) is a maternally inherited disease characterized by acute or subacute bilateral painless loss of central vision resulting from optic atrophy (Nikoskelainen et al. 1987). The three most common mitochondrial DNA (mtDNA) mutations responsible for >95% of LHON pedigrees worldwide are G3460A, G11778A and T14484C (Mackey et al. 1996; Man et al. 2002). Of these, G11778A is the most common worldwide; however, the frequency of each of these three mutations varies markedly in different populations.

Only ~50% of males and ~10% of females harbouring LHON mutations develop optic neuropathy (Harding et al. 1995; Riordan-Eva et al. 1995). In addition, about 80% of affected individuals are males (Nikoskelainen et al. 1987). The incomplete penetrance as well as the male preponderance indicates that there must be other unknown factors, apart from mtDNA mutations, responsible for disease manifestation.

In Southeast Asia, including Thailand, where the genetic background is different, only a few reports of LHON families have been published (Chuenkongkaew

et al. 2001; Sudoyo et al. 1998, 2002). We report here an analysis of our 30 unrelated G11778A LHON pedigrees. The purposes of this study were to define the mitochondrial genetics and the pedigree characteristics in multiple Thai G11778A LHON families. The analyses of LHON in the Southeast Asia together with other regions around the world will have implications regarding how distantly related genetic backgrounds both in the mitochondrion and the nucleus might contribute to the phenotypic expression and the complexity of LHON.

Materials and methods

Pedigrees and sample collection

Blood samples of patients clinically similar to LHON were sent to our laboratory following informed consent. Pedigree information of the patients who were positive for the mutation was investigated and blood samples from their family members were also collected with informed consent.

In each pedigree, clinical data were obtained by direct examination by an ophthalmologist, or indirectly, by interviews with one or more of family members. Affected status in unseen maternal relatives was based on a history of acute visual loss without other known causes.

Mitochondrial genetic analysis

Total leukocyte DNA was extracted from at least 5 ml whole blood sample containing ethylenediaminetetraacetic acid (EDTA) or the anticoagulant citrate dextrose solution A (ACD-A) using a standard phenol/chloroform method. The G11778A mutation was tested in all available patients and family members (247 blood samples from both maternal and non-maternal relatives). One sample in the maternal lineage of each family was tested for other primary and secondary mutations (nt 3316, 3394, 3460, 3496, 3497, 3635, 4136, 4160, 4171, 4261, 4917, 5244, 7444, 9738, 9804, 13708, 13730, 14459, 14482, 14484, 14495, 14498, 14568, 14596, 15257 and 15812) by either restriction fragment length polymorphism (RFLP) analyses or direct sequencing of the mtDNA as detailed in Lertrit et al. (1998) and Sudoyo et al. (2002), respectively. Degrees of heteroplasmy of the G11778A mutation were quantitated using a radioactive restriction analysis method modified from that of Moraes et al. (1992). In order to be certain that all 30 pedigrees are genetically unrelated, the hypervariable segment 1 (HVS-1) in mtDNA D-loop (nt 16024–16383) from the proband of each family was sequenced.

To avoid the confounding effects of multiple risk factors on one another, we studied the effects on the age-dependent penetrance of the G11778A mutation of sex, secondary mutation and degree of heteroplasmy simultaneously. From the 166 samples positive for the G11778A mutation, 13 samples with only one blood sample per family were omitted to reduce ascertainment bias, resulting in 152 samples with known phenotype and mtDNA profile. We performed a survival analysis using Cox's proportional hazards model to fit our data. This model assumes that, for all individuals, the hazard function $h(t)$ (the probability that a person gets LHON at a particular age t) has the same basic shape, but that certain factors (sex, secondary mutation and degree of heteroplasmy) may change the risk of LHON by multiplying $h(t)$ by a fixed factor. The analysis was performed using R v1.8.1 statistical software (R Development Core Team 2003).

Results

Pedigrees

Thirty G11778A LHON pedigrees were identified in this study. All these pedigrees are of Thai or Chinese ethnic origin except for one pedigree of Indian ethnic origin. Six were large pedigrees comprising four to seven generations. From these 30 families, 27 HVS-1 mitochondrial haplotypes were detected. However, when the mitochondrial genome was subject to high resolution screening of polymorphic restriction sites and screened for the 9-bp deletion, they all carried distinct mtDNA haplotypes. Therefore, these 30 families are not closely genetically related.

From these pedigrees, 166 samples (81 males and 85 females) consisting of 65 affected, 2 possibly affected (the affected status was difficult to assign owing to cataract in both eyes) and 99 unaffected individuals, were positive for the G11778A mutation. One family (F19) was found to have two genetic diseases simultaneously: LHON, a mitochondrial disease and facioscapulohumeral dystrophy (FSHD), an autosomal dominant disorder (Chuenkongkaew et al. 2005).

Age of onset and male:female ratio

The 65 affected patients consisted of 47 males and 18 females, and the male:female ratio was 2.6:1. In other words, 72% of patients were male (Table 1). In affected persons directly evaluated, 58 were documented with their age of onset. The mean age of onset was 22.6 ± 11.7 years (range 6–53, median 20 years) for all patients, 20.7 ± 10.0 years ($n = 44$, range 6–44, median 19 years) for males and 28.6 ± 14.6 years ($n = 14$, range 10–53, median 30 years) for females. It appeared that the mean age of onset in females was higher than that in males in our patients, although the difference was not statistically significant ($P = 0.073$; Mann–Whitney U test).

Disease penetration

Excluding the two possibly affected persons, the directly evaluated 164 samples harbouring the G11778A

Table 1 Comparison of G11778A Leber hereditary optic neuropathy (LHON) pedigree characteristics of the present study with previous reports

	Asian			Caucasian				
	Present study	Hotta et al. (1995)	Yen et al. (1999)	Newman et al. (1991)	Smith et al. (1993)	Oostra et al. (1994)	Harding et al. (1995)	Man et al. (2003)
Ethnic population	Thai	Japanese	Taiwanese Chinese	American	American	Dutch	British	British
No. of families	30	79	17	49	68	15	66	9
No. of affected cases	65	90	24	72	75	146	109	49
Cases with positive family history (%)	50	62	–	43	–	–	56	–
Male patients (%)	72	92.1	88	82	77	88	79	84
Heteroplasmy								
Heteroplasmic families (%)	37	–	0	14	18	13	7.6	33
Heteroplasmic persons (%)	28	19	0	–	14	–	–	–
Heteroplasmic patients (%)	14	14	0	–	6.7	–	0	16
Average age at onset (years)								
Males	20.7 (6–44)	–	21.85 (10–39)	26.2 (8–60)	–	28.55 (6–61)	21.0 (6–62)	–
Females	28.6 (10–53)	–	13, 56[a]	34.0 (9–54)	–	31.47 (8–69)	28.0 (10–58)	–
Both	22.6 (6–53)	23.4 (7–59)	20.52 (10–56)	27.6 (8–60)	–	28.87 (6–69)	24.0 (6–62)	–

Figures within the brackets represent ranges of the age at onset, – not available

[a]Only two females in this series with age at onset of 13 and 56 years old

mutation consisted of 40% (65/164) affected and 60% (99/164) unaffected individuals. When male and female groups were analyzed separately, 58% (47/81) of males and 22% (18/83) of females carrying the mutation expressed the disease. It should be noted that 34% of the currently unaffected persons who were directly evaluated were aged less than 24 years (the average age of onset of LHON in Thailand), thus some of them might become affected later and would affect our penetrance calculation. In addition, these proportions of affected persons, calculated using the directly evaluated individuals, could be overestimated because affected people were more likely to be ascertained than the unaffected.

To avoid the above ascertainment bias, the proportion of affected person was calculated using all individuals in the maternal lineages of the pedigree structures. Therefore, 295 maternal family members whose disease status was known (either directly or indirectly) were analysed, assuming (based on the principles of mitochondrial genetics) that the unseen maternal members should carry the mutation. However, we had to compromise on certainty regarding the disease status in the unseen persons. It was found that 24% (70/295) of all individuals, 37% (50/135) of males and 13% (20/160) of females, develop optic neuropathy. The ages of 90% of the unaffected persons analysed could be determined and, again, it should be noted that 30% of those individuals were less than 24 years old, and some of them might become affected in later life.

The penetrance for each individual pedigree was calculated. We considered only ten large pedigrees with more than ten maternal relatives spanning at least three generations in order to avoid the effect of differences in the size of the pedigrees and in the degree of ascertainment. Disease penetrance varied from 9 to 45% with a mean $\pm$ SD of $19 \pm 11\%$. In addition, our preliminary observations showed that the proportion also varied between different branches of the same large pedigree.

With the criterion that all the unaffected persons were at least 24 years of age, 19 sibships (and their mothers) were identified, comprising 13 sibships with unaffected mothers and 6 sibships with affected mothers. Fifty-six percent (9/16) of males born to affected mothers became affected, compared with 34% (10/29) of those born to unaffected mothers; whereas 33% (3/9) of females born to affected mothers developed optic neuropathy, compared with only 17% (4/23) of those born to unaffected mothers. Statistically, there was not enough evidence to show that affected mothers were more likely to have affected children than unaffected mothers [odds ratio (OR) = 2.51, $P = 0.12$; Chi-square test].

Heteroplasmy of the G17778A mutation

Eleven (37%) of our 30 LHON pedigrees contained at least one individual with the heteroplasmic G11778A mutation (heteroplasmic pedigree). Of the 166 individuals positive for the G11778A mutation, 28% (46/166) were heteroplasmic and 72% (120/166) were homoplasmic. Considering only the patients (affected persons), only 14% (9/65) were heteroplasmic (mutation load ranged from 44 to 93%, median 78%), while in the unaffected group, 35% (35/99) were heteroplasmic (mutation load ranged from 1 to 94%, median 46%). It was found that 20% (9/44) of heteroplasmic persons manifested the disease, compared with 47% (56/120) of the homoplasmic group (OR = 3.40, $P = 0.004$; Chi-square test). When sex was considered in the analysis, similar results were obtained. Our results supported the belief that heteroplasmy influences the expression of LHON, and the prevalence of heteroplasmy was higher in the unaffected group compared with the affected group.

Age of onset was compared between heteroplasmic and homoplasmic patients. In eight heteroplasmic patients with known age of onset, the mean age of onset was 21.1 ± 10.3 years (range 10–42, median 19 years), whereas in 50 homoplasmic patients, the mean age of onset was 22.9 ± 11.9 years (range 6–53, median 20 years). There was no difference in the age of onset of the heteroplasmic and the homoplasmic groups ($P = 0.77$; Mann–Whitney U test).

Other primary and secondary LHON mutations in the G11778A LHON pedigrees

Another 27 LHON secondary mutations were screened, and two families were found to possess mutations other than the G11778A; one (F11) carried a C3497T and the other (F19) carried a G3316A mutation. The mean age of onset in patients carrying G11778A mutation plus either secondary mutation ($n = 10$) was 16.4 ± 8.9 years (range 8–33, median 14.5 years), while in the patients carrying only G11778A ($n = 43$), the mean age of onset was 23.5 ± 11.8 years (range 6–53, median 20 years). The difference in the mean age of onset between the patients with and without the secondary mutations was statistically significant ($P = 0.036$; Mann–Whitney U test).

From survival analysis using Cox's proportional hazard model, we found that male sex, secondary mutation, and high mutation load each had a significant effect on the age-dependent penetrance of LHON. The model predicted that males were 2.8 times more likely than females to develop LHON ($P = 0.00062$). People with secondary mutations (G3316A or C3497T) were 3.5 times more likely to express the disease than people without the mutations ($P = 0.0069$). Moreover, the model predicted that each 1% drop in degree of heteroplasmy reduced the rate of getting LHON by a factor of 0.97 ($P = 0.0053$). We also tested for interactions between these three risk factors but none were significant. Examples of survival curves for 6 individuals using this fitted model are plotted in Fig. 1.

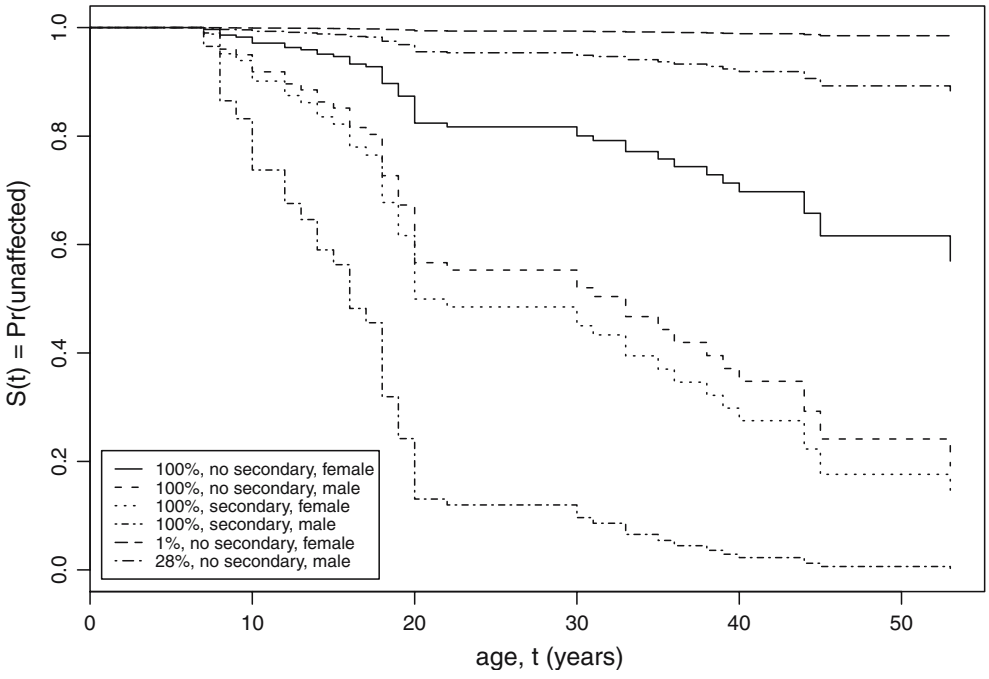

Fig. 1 Survival curves fitted using Cox's proportional hazards model from 152 samples positive for the G11778A Leber hereditary optic neuropathy (LHON) mutation in Thailand. The curves represent six samples with different sex, secondary LHON mutation status, or mutation load. $S(t)$ Probability of a person being unaffected at age t

Discussion

Like in most countries worldwide, the G11778A mutation is the most prevalent LHON mutation in Thailand. So far, the G3460A mutation has never been reported in Thai or Southeast Asian individuals. The prevalence of these mutations in Thai LHON is consistent with most LHON families from several Asian countries (87–95% for the G11778A mutation, 0–9% for the T14484C mutation and 0–8% for the G3460A mutation, Mashima et al. 1998; Sudoyo et al. 2002; Yen et al. 2002; Chuenkongkaew et al. 2004). In contrast, among most Caucasian LHON pedigrees, the prevalence is lower for the G11778A and higher for the 3460 and the14484 mutations when compared with Asian LHON families (69% for the G11778A mutation, 14% for the T14484C mutation and 13% for the G3460A mutation, Mackey et al. 1996). The marked difference in the prevalence of each of the classical LHON mutations between Asian and Caucasian LHON families might reflect the effects of different genetic backgrounds (nuclear and/or mitochondrial) on the generation and clinical expression of these LHON mutations.

In the present study, the estimated overall penetrance of our G11778A LHON population was 37% for males and 13% for females. These figures were comparable to those in G11778A Finnish LHON (39% for males and 14% for females, Nikoskelainen et al. 1996) but were different from G11778A British LHON (51% for males and 8.5% females, Man et al. 2003). When each large pedigree was considered separately, penetrance varied from 9 to 45% between pedigrees. In Caucasians, as a rule of thumb, ~50% of males and 10% of females in LHON families lost vision (Man et al. 2002; Newman 1993; Howell 1997, 1998). However, more extensive data regarding penetrance are needed for Asian LHON.

However, some pedigree features in our series were different from most G11778A LHON in the literature (Table 1). The most striking point was the high prevalence of blood leukocyte heteroplasmy of the G11778A mutation in Thailand. Thirty-seven percent (11/30) of our 30 LHON pedigrees contained at least one individual heteroplasmic for the mutation, while this proportion is generally considered to be 15% in most studies (Chinnery et al. 2001; Newman et al. 1991; Smith et al. 1993). Moreover, the proportion of our heteroplasmic pedigrees might be underestimated owing to the fact that in 11 of our 19 homoplasmic pedigrees, blood samples from probands only were obtained. Therefore, other family members whose blood samples were not available could be heteroplasmic for the mutation. If heteroplasmy reflects a recent mutational event (Savontaus 1995), it is interesting to reflect how recent mutational events could occur with such a high incidence in our population in the 10 years (1994–2003) of our sample collection. A recent epidemiological study in the northeast of England also shows a higher proportion (33%) of heteroplasmic families than the general figure of 15% (Man et al. 2003).

Our analyses of heteroplasmy supported the belief that heteroplasmy influences the penetrance of LHON but it did not appear to alter the age of onset of the disease in our patients. However, this result should be interpreted with caution because the number of heteroplasmic people who were affected in our age of onset analysis was small.

It should be noted that in two of our heteroplasmic families, eight samples of maternal lineages were found to be negative for the G11778A mutation. This provided

evidence that the heteroplasmic G11778A mutation could segregate to pure wild type. This supports the importance of molecular mtDNA testing in family members seeking genetic counselling, as suggested by Man et al. (2003).

Another different feature of our G11778A LHON patients was that the male to female ratio (2.6:1 or 72%) appeared to be smaller than that of most G11778A LHON patient series worldwide, especially in Japan (Hotta et al. 1995) where 92% of LHON patients are male (Table 1).

Several secondary LHON mutations have been found (Wallace and Lott 2003); however, in most cases, their pathogenicity is still uncertain and several studies have yielded conflicting evidence regarding the roles of secondary mutations (Brown et al. 2002; Howell 1997; Howell et al. 1995; Lodi et al. 2000; Oostra et al. 1994). Two secondary LHON mutations (G3316A and C3497T) were found, one each in two pedigrees. Our analysis of age at onset indicated that the secondary LHON mutations G3316A and C3497T seemed to have a synergistic deleterious effect with the G11778A mutation, accelerating the onset of the disease.

The G3316A mutation changes a nonpolar alanine to a polar threonine at the fourth amino acid in the ND1 protein. Although no definite conclusion regarding pathogenicity of the 3316 mutation can yet be drawn, evidence from several independent studies indicates that the mutation might cause a mild defect in mitochondrial function and, thus, precipitate type 2 diabetes (McCarthy et al. 1996; Nakagawa et al. 1995) as well as LHON (Matsumoto et al. 1999) in appropriate genetic backgrounds. For our "11778 + 3316" LHON pedigree, it was difficult to interpret the contribution of the 3316 mutation to the manifestation of the 11778 mutation since this family also suffered from FSHD, which might confound the expression of the mitochondrial disease. At least there is evidence indicating that FSHD is associated with a deficiency in the mitochondrial respiratory chain complex III (Slipetz et al. 1991).

The C3497T mutation changes an alanine to a valine at the 64th amino acid of the ND1 protein. This was proposed by Matsumoto et al. (1999) to be a secondary LHON mutation since it is found in 5% (1/19) of Japanese LHON patients and 1.9% (2/108) of Japanese normal controls. We observed that our "11778 + 3497" LHON family displayed the highest proportion of affected individuals (77%) in our pedigree series, which might be partly due to the effect of the 3497 mutation.

Note that, from our survival analysis using Cox's proportional hazard model, while the proportion of men with LHON was about 50%, which is similar to other studies (Man et al. 2003), the 'life-time' risk of LHON for homoplasmic men without secondary mutations as predicted by this model is around 80%. A long-term prospective cohort study is required to verify this lifetime risk.

It is clear that there have to be factors other than the primary LHON mutations, which are responsible for LHON features that cannot be explained by mitochondrial inheritance. These features include incomplete penetrance, male predominance, and optic nerve specific disease expression. Currently, genetic backgrounds in the mitochondria and/or in the nucleus are strongly suggested to play a role in disease expression of LHON (Brown et al. 2000, 2002; Carelli et al. 2003; Cock et al. 1998; Howell et al. 2003; Qi et al. 2003; Sadun et al. 2002; Sudoyo et al. 2002). Despite the different genetic backgrounds, most features that constitute the picture of LHON are quite common between different populations; however, there seem to be a few ethnic-specific differences. Deep looking into these differences may provide some clues to the discovery of other factors modifying the disease, its pathophysiology and eventually lead to an effective therapeutic intervention for this devastating disease.

Acknowledgements The authors would like to thank Drs. Jim Stankovich for the survival analysis used in this paper and Prida Malasit for his critical comments on this paper. We would like to also thank Komon Luangtrakool, Bussaraporn Khunhaphan, Pattamon Tharaphan and Thitima Sanpachudayan for their great assistance in the field trip investigation, and Benjamas Intharabut and Treenud Suntisiri for their help in DNA extraction. This work was supported by the Thailand Research Fund (TRF): grant No. BRG4580018 to Lertrit P and grant No. PHD/0031/2546 though the Royal Golden Jubilee Ph.D. Program to N.P and P.L.

References

Brown MD, Trounce IA, Jun AS, Allen JC, Wallace DC (2000) Functional analysis of lymphoblast and cybrid mitochondria containing the 3460, 11778, or 14484 Leber's hereditary optic neuropathy mitochondrial DNA mutation. J Biol Chem 275:39831–39836

Brown MD, Starikovskaya E, Derbeneva O, Hosseini S, Allen JC, Mikhailovskaya IE, Sukernik RI, Wallace DC (2002) The role of mtDNA background in disease expression: a new primary LHON mutation associated with Western Eurasian haplogroup. J Hum Genet 110:130–138

Carelli V, Giordano C, d'Amati G (2003) Pathogenic expression of homoplasmic mtDNA mutations needs a complex nuclear-mitochondrial interaction. Trends Genet 19:257–262

Chinnery PF, Andrews RM, Turnbull DM, Howell N (2001) Leber hereditary optic neuropathy: does heteroplasmy influence the inheritance and expression of the G11778A mitochondrial DNA mutation? Am J Med Genet 98:235–243

Chuenkongkaew WL, Lertrit P, Poonyathalang A, Sura T, Ruangvaravate N, Atchaneeyasakul L, Suphavilai R (2001) Leber's hereditary optic neuropathy in Thailand. Jpn J Ophthalmol 45:665–668

Chuenkongkaew W, Lertrit P, Suphavilai R (2004) Case report: a Thai patient with Leber's hereditary optic neuropathy linked to mitochondrial DNA 14484 mutation. Southeast Asian J Trop Med Public Health 35:167–168

Chuenkongkaew WL, Lertrit P, Limwongse C, Nilanont Y, Boonyapisit K, Sangruchi T, Chirapapaisan N, Suphavilai R (2005) An unusual family with Leber's hereditary optic neuropathy and facioscapulohumeral muscular dystrophy. Eur J Neurol 12:388–391

Cock HR, Tabrizi SJ, Cooper JM, Schapira AH (1998) The influence of nuclear background on the biochemical expression of 3460 Leber's hereditary optic neuropathy. Ann Neurol 44:187–193

Harding AE, Sweeney MG, Govan GG, Riordan-Eva P (1995) Pedigree analysis in Leber hereditary optic neuropathy families with a pathogenic mtDNA mutation. Am J Hum Genet 57:77–86

Hotta Y, Fujiki K, Hayakawa M, Nakajima A, Kanai A, Mashima Y, Hiida Y, Shinoda K, Yamada K, Oguchi Y (1995) Clinical features of Japanese Leber's hereditary optic neuropathy with 11778 mutation of mitochondrial DNA. Jpn J Ophthalmol 39:96–108

Howell N (1997) Leber hereditary optic neuropathy: how do mitochondrial DNA mutations cause degeneration of the optic nerve? J Bioenerg Biomembr 29:165–173

Howell N (1998) Leber hereditary optic neuropathy: respiratory chain dysfunction and degeneration of the optic nerve. Vis Res 38:1495–1504

Howell N, Kubacka I, Halvorson S, Howell B, McCullough DA, Mackey D (1995) Phylogenetic analysis of the mitochondrial genomes from Leber hereditary optic neuropathy pedigrees. Genetics 140:285–302

Howell N, Oostra RJ, Bolhuis PA, Spruijt L, Clarke LA, Mackey DA, Preston G, Herrnstadt C (2003) Sequence analysis of the mitochondrial genomes from dutch pedigrees with leber hereditary optic neuropathy. Am J Hum Genet 72:1460–1469

Lertrit P, Imsumran A, Trongpanich Y, Karnkirawattana P, Devahasdin V, Atchaneeyasakul L, Chuenkongkaew W, Ruangvaravate N, Sangruchi T, Mungkornkarn C, Neungton N (1998) Mitochondrial genetics of mitochondrial diseases in Thailand. Siriraj Hosp Gaz 50:53–64

Lodi R, Montagna P, Cortelli P, Lotti S, Cevoli S, Carelli V, Barbiroli B (2000) Secondary 4216/ND1 and 13708/ND5 Leber's hereditary optic neuropathy mitochondrial DNA mutations do not further impair in vivo mitochondrial oxidative metabolism when associated with the 11778/ND4 mitochondrial DNA mutation. Brain 123:1896–1902

Mackey DA, Oostra RJ, Rosenberg T, Nikoskelainen E, Bronte-Stewart J, Poulton J, Harding AE, Govan G, Bolhuis PA, Norby S (1996) Primary pathogenic mtDNA mutations in multigeneration pedigrees with Leber hereditary optic neuropathy. Am J Hum Genet 59:481–485

Man PY, Turnbull DM, Chinnery PF (2002) Leber hereditary optic neuropathy. J Med Genet 39:162–169

Man PY, Griffiths PG, Brown DT, Howell N, Turnbull DM, Chinnery PF (2003) The epidemiology of Leber hereditary optic neuropathy in the North East of England. Am J Hum Genet 72:333–339

Mashima Y, Yamada K, Wakakura M, Kigasawa K, Kudoh J, Shimizu N, Oguchi Y (1998) Spectrum of pathogenic mitochondrial DNA mutations and clinical features in Japanese families with Leber's hereditary optic neuropathy. Curr Eye Res 17:403–408

Matsumoto M, Hayasaka S, Kadoi C, Hotta Y, Fujiki K, Fujimaki T, Takeda M, Ishida N, Endo S, Kanai A (1999) Secondary mutations of mitochondrial DNA in Japanese patients with Leber's hereditary optic neuropathy. Ophthalmic Genet 20:153–160

McCarthy M, Cassell P, Tran T, Mathias L, t Hart LM, Maassen JA, Snehalatha C, Ramachandran A, Viswanathan M, Hitman GA (1996) Evaluation of the importance of maternal history of diabetes and of mitochondrial variation in the development of NIDDM. Diabetes Med 13:420–428

Moraes CT, Ricci E, Bonilla E, DiMauro S, Schon EA (1992) The mitochondrial tRNALeu(UUR) mutation in mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS): genetic, biochemical and morphological correlations in skeletal muscle. Am J Hum Genet 50:934–949

Nakagawa Y, Ikegami H, Yamato E, Takekawa K, Fujisawa T, Hamada Y, Ueda H, Uchigata Y, Miki T, Kumahara Y (1995) A new mitochondrial DNA mutation associated with non-insulin-dependent diabetes mellitus. Biochem Biophys Res Commun 209:664–668

Newman NJ (1993) Leber's hereditary optic neuropathy. New genetic considerations. Arch Neurol 50:540–548

Newman NJ, Lott MT, Wallace DC (1991) The clinical characteristics of pedigrees of Leber's hereditary optic neuropathy with the 11778 mutation. Am J Ophthalmol 111:750–762

Nikoskelainen EK, Savontaus ML, Wanne OP, Katila MJ, Nummelin KU (1987) Leber's hereditary optic neuroretinopathy, a maternally inherited disease. A genealogic study in four pedigrees. Arch Ophthalmol 105:665–671

Nikoskelainen EK, Huoponen K, Juvonen V, Lamminen T, Nummelin K, Savontaus ML (1996) Ophthalmologic findings in Leber hereditary optic neuropathy, with special reference to mtDNA mutations. Ophthalmology 103:504–514

Oostra RJ, Bolhuis PA, Wijburg FA, Zorn-Ende G, Bleeker-Wagemakers EM (1994) Leber's hereditary optic neuropathy: correlations between mitochondrial genotype and visual outcome. J Med Genet 31:280–286

Qi X, Lewin AS, Hauswirth WW, Guy J (2003) Suppression of complex I gene expression induces optic neuropathy. Ann Neurol 53:198–205

R Development Core Team (2003) R: A language and environment for statistical computing. Foundation for Statistical Computing, Vienna

Riordan-Eva P, Sanders MD, Govan GG, Sweeney MG, Da Costa J, Harding AE (1995) The clinical features of Leber's hereditary optic neuropathy defined by the presence of a pathogenic mitochondrial DNA mutation. Brain 118:319–337

Sadun AA, Carelli V, Salomao SR, Berezovsky A, Quiros P, Sadun F, DeNegri AM, Andrade R, Schein S, Belfort R (2002) A very large Brazilian pedigree with 11778 Leber's hereditary optic neuropathy. Trans Am Ophthalmol Soc 100:169–178

Savontaus ML (1995) mtDNA mutations in Leber's hereditary optic neuropathy. Biochim Biophys Acta 1271:261–263

Slipetz DM, Aprille JR, Goodyer PR, Rozen R (1991) Deficiency of complex III of the mitochondrial respiratory chain in a patient with facioscapulohumeral disease. Am J Hum Genet 48:502–510

Smith KH, Johns DR, Heher KL, Miller NR (1993) Heteroplasmy in Leber's hereditary optic neuropathy. Arch Ophthalmol 111:1486–1490

Sudoyo H, Sitepu M, Malik S, Poesponegoro HD, Marzuki S (1998) Leber's hereditary optic neuropathy in Indonesia: two families with the mtDNA 11778G > A and 14484T > C mutations. Hum Mutat [Suppl 1]:S271–S274

Sudoyo H, Suryadi H, Lertrit P, Pramoonjago P, Lyrawati D, Marzuki S (2002) Asian-specific mtDNA backgrounds associated with the primary G11778A mutation of Leber's hereditary optic neuropathy. J Hum Genet 47:594–604

Wallace DC, Lott MT (2003) MITOMAP: a human mitochondrial genome database. http://www.mitomap.org

Yen MY, Lee HC, Wang AG, Chang WL, Liu JH, Wei YH (1999) Exclusive homoplasmic 11778 mutation in mitochondrial DNA of Chinese patients with Leber's hereditary optic neuropathy. Jpn J Ophthalmol 43:196–200

Yen MY, Wang AG, Chang WL, Hsu WM, Liu JH, Wei YH (2002) Leber's hereditary optic neuropathy—the spectrum of mitochondrial DNA mutations in Chinese patients. Jpn J Ophthalmol 46:45–51