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Pedigree Analysis in Leber Hereditary Optic Neuropathy Families with a Pathogenic mtDNA Mutation

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Summary

Eighty-nine index patients from 85 families were defined as having Leber hereditary optic neuropathy (LHON) by the presence of one of the mtDNA mutations at positions 11778 (66 families), 3460 (8 families), or 14484 (11 families). There were 62 secondary cases. Overall, 64% of index cases had a history of similarly affected relatives. The ratios of affected males to affected females were 3.7:1 (11778), 4.3:1 (3460), and 7.7:1 (14484). The 95th centile for age at onset of symptoms was close to 50 years in index, secondary, male, and female patients. There were no differences in the distributions of age at onset between different mutation groups, between index and secondary cases, or between males and females, apart from this being slightly later in all female patients than in male 11778 patients. There was no significant correlation between age at onset in index cases and that in their affected siblings or cousins. Heteroplasmy (<96% mutant mtDNA) was detected in 4% of affected subjects (67%–90% mutant mtDNA) and in 13.6% of 140 unaffected relatives (<5%–90% mutant mtDNA). Analysis of all pedigrees, excluding sibships <50 years of age and index cases, indicated recurrence risks of 30%, 8%, 46%, 10%, 31%, and 6%, respectively, to the brothers, sisters, nephews, nieces, and male and female matrilineal first cousins of index cases. Affected females were more likely to have affected children, particularly daughters, than were unaffected female carriers. The pedigree data were entirely compatible with the previously proposed X-linked susceptibility locus, with a gene frequency of .08, penetrance of .11 in heterozygous females, and 40% of affected females being homozygous, the remainder being explained by heterozygosity and disadvantageous X inactivation.

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Introduction

Leber hereditary optic neuropathy (LHON) usually causes severe and permanent visual loss, most commonly in young adult males. It is associated with one of three pathogenic mutations of mtDNA, at position 11778, 14484, or 3460 (Wallace et al. 1988; Howell et al. 1991; Huoponen et al. 1991; Mackey and Howell 1992; Johns et al. 1993*a*), in nearly all families in the United Kingdom (Riordan-Eva et al. 1995). Other, so-called secondary, mtDNA mutations have been reported in LHON pedigrees, particularly those at 13708 and 15257 (Johns and Berman 1991; Brown et al. 1992; Johns et al. 1993*c*), but their significance is unclear, since they also occur in combination with the 11778, 3460, and 14484 mutations and in the normal population (Kellar-Wood et al. 1994; Oostra et al. 1994*a*, 1994*b*); mtDNA is highly polymorphic.

The availability of mtDNA analysis has made the diagnosis of LHON easier, particularly in atypical cases, females, and patients without affected relatives. Recent studies of LHON defined at a molecular level in Caucasian populations (Newman et al. 1991; Johns et al. 1992; Mackey and Howell 1992; Ooostra et al. 1994*a*; Riordan-Eva et al. 1995) have shown that the age at onset of the disorder may be earlier or later than traditionally thought, and they have generally indicated that the proportion of affected females is higher than the previously quoted figures of 10%–15%, reported in other series of Caucasian pedigrees (van Senus 1963; Nikoskelainen 1984; Nikoskelainen et al. 1987).

Genetic counseling in LHON is not straightforward, since transmission of the mutation is not always associated with transmission of the disease. There have been no published data concerning recurrence risks in genetically defined LHON. Although mtDNA heteroplasmy may be a factor determining penetrance of the disease (Holt et al. 1989), most patients and their unaffected relatives from LHON families have very high amounts of mutant mtDNA (>95%), and many males at risk remain unaffected in this context. This and the excess of males are not accounted for by mitochondrial inheritance. The existence of an interacting X-linked visual loss–susceptibility locus was suggested by analysis of 31 pedigrees from published series (Bu and Rotter 1991)

and linkage studies in the Finnish population (Vilkki et al. 1991). In this model, affected females were explained by being either homozygous for the susceptibility allele, which could be common in the general population, or heterozygous and the subject of skewed X inactivation (Bu and Rotter 1991). The pedigree data analyzed were largely published prior to the possibility of molecular diagnosis of LHON and may not be representative of the disease; the reviewed reports inevitably presented large kindreds containing multiply affected members. The linkage data of Vilkki et al. (1991), suggesting a susceptibility locus close to DXS7, were not confirmed in British, Italian, and German families or on reanalysis of an expanded Finnish data set (Carvalho et al. 1992; Sweeney et al. 1992; Juvonen et al. 1993).

The aim of the present study was to define recurrence risks to the relatives of patients with LHON and to determine if these differ depending on the mutation. We have also studied heteroplasmy in our families, and we have analyzed the pedigree data to assess whether they are compatible with the X-linked–susceptibility hypothesis.

Patients and Methods

Index patients and their relatives were identified from the records of the DNA laboratory and the Department of Neuro-Ophthalmology, National Hospital for Neurology and Neurosurgery, London. Patients were considered as clinical index cases if they or their DNA were referred independently to the National Hospital; there were 89 index cases from 85 families. All affected subjects had a worst visual acuity of ≤6/60 in their most severely affected eye (Riordan-Eva et al. 1995). mtDNA analysis using standard PCR-based methods (Riordan-Eva et al. 1995) showed one of the mutations at position 11778, 3460, or 14484 in all families reported here. The presence of each mutation was confirmed by either restriction analysis with two enzymes (*Sfa*NI and *Mae*III) for the 11778 change or by sequence analysis for those at 3460 and 14484 (Riordan-Eva et al. 1995). mtDNA analysis was also performed in a total of 35 secondary cases (affected relatives ascertained through index cases) and 140 unaffected matrilineal relatives. Of the latter, 27 were mothers, 30 sisters, and 17 brothers of patients. The degree of heteroplasmy (defined conservatively as $>\sim 5\%$ normal mtDNA) was assessed as described by Sweeney et al. (1992), by using densitometric analysis of Southern blots for the 11778 and 3460 mutations, and more approximately (within 10%) for the 14484 mutation by visual analysis of PCR products.

Detailed pedigree information was obtained from the index patient and relatives or, occasionally, from a referring physician. Confirmation of the diagnosis in unseen relatives was obtained from hospital records wherever possible. When these were not available, affected status was based on a history of bilateral visual loss, occurring over days to months, without other credible cause.

Table 1

Sex Ratios and Incidence of Affected Relatives in Patients with Genetically Defined LHON

	11778 Mutation	3460 Mutation	14484 Mutation
Index cases:			
All	68	9	12
Male	51	8	11
Female	17	1	1
Male:female ratio	3:1	8:1	11:1
All cases:			
All	109	16	26
Male	86	13	23
Female	23	3	3
Male:female ratio	3.7:1	4.3:1	7.7:1
No index cases with affected relatives:			
Total (%)	38 (56)	7 (78)	12 (100)
Male (%)	26 (51)	6 (75)	11 (100)
Female (%)	12 (70)	1 (100)	1 (100)
Not known	2	...	...

For the purpose of counting proportions of affected sibs and nieces/nephews, either the clinical index case or the oldest affected male (or female, if there were no affected males) in each sibship was considered as a sibship index case. The proportions of affected matrilineal first cousins were derived in relation to a generation index case, i.e., the oldest affected male (or female, if there were no affected males) in each generation. The clinical index case was not included in any of these calculations, and sibships were only included if all members were >50 years of age (see Results).

Between-group comparisons of ages at onset were performed by using the Mann-Whitney U test. Correlation analysis used the Spearman rank method. Proportions were compared by using χ^2 analysis with Yates's correction for 2×2 tables. The 95% confidence limits (95% CL) were calculated by the formula $\pm 1.96 \times \sqrt{p(1-p)/n}$, where p is the proportion of affected subjects and n is the total number of subjects.

Results

There were 89 clinical index cases ascertained independently from 85 families, and reliable information, including age at onset, was available concerning them and 62 secondary cases. Sex ratios and details of family history are shown in table 1, with patients divided into three groups depending on the mtDNA mutation. The 11778 mutation was by far the most frequent, detected

Table 2

Age (Years) at Onset of Visual Loss

	MALE		FEMALE		
	Range	Median (No. of Cases)	Range	Median (No. of Cases)	MEDIAN (No. of Cases)
11778 Mutation (66 families):					
Index cases	8–62	20 (51)	10–50	28 (17)	24 (68)
All cases	6–62	21 (86)	10–58	28 (23)	24 (109)
14484 Mutation (11 families):					
Index cases	14–59	23 (11)	...	6 (1)	23 (12)
All cases	3–59	20 (23)	8–25	7 (3)	20 (26)
3460 Mutation (8 families):					
Index cases	18–48	19 (8)	...	20 (1)	19 (9)
All cases	17–48	20 (13)	8–62	20 (3)	20 (16)

	RANGE	MEDIAN (No. of Cases)
All index cases	6–62	23 (89)
Secondary cases	3–62	20.5 (62)
Index males	8–62	20 (70)
Index females	6–50	30 (19)
All males	3–62	20.5 (122)
All females	6–62	27 (29)

in 78% of families. One index patient, without affected relatives, had both the 11778 mutation and the 14484 mutation (Riordan-Eva et al. 1995). He is only included in the 11778 group in tables 1 and 2. Two families in our patient base, not included in this study or in the above numbers, have a diagnosis of probable LHON (presenting with subacute optic neuropathy and a positive family history compatible with mitochondrial inheritance in more than one generation) but have none of the three mtDNA mutations investigated. Neither has the 15257 mutation, which has been associated with LHON (Johns et al. 1993*c*).

Overall, 64% of index cases had a history of similarly affected relatives. This was true of only 56% of 11778 patients but the proportion was higher for the 3460 (78%) and 14484 (100%) mutations. In several patients with a positive family history, this was not elicited at the time of presentation. The existence of affected relatives was sometimes not known to patients in younger generations, or blindness had been attributed to other causes. The ratios of affected males to affected females (index patients and affected relatives) were 3.7:1, 4.3:1, and 7.7:1 for the 11778, 3460, and 14484 mutations, respectively. These ratios were slightly higher in index cases alone. There was no statistically significant difference between the male:female ratios in each mutation group, for either index cases or all cases ($\chi^2 = 2.31$, $P = .31$; and $\chi^2 = 1.73$, $P = .42$, respectively), but the numbers of 3460 and 14484 patients were relatively small.

Data concerning age at onset of visual loss are shown in table 2, again divided by sex and mutation group. Mutation did not influence distribution of age at onset, either in index cases (11778 vs. 3460, $P = .84$; 11778 vs. 14484, $P = .86$; and 3460 vs. 14484, $P > .05$) or in all cases (11778 vs. 3460, $P = .54$; 11778 vs. 14484, $P = .13$; and 3460 vs. 14484, $P = .61$). The distributions did not differ significantly between index and secondary cases ($P = .11$). Age at onset was significantly later in females when all 11778 patients were analyzed ($P = .035$), but there was no significant difference between the distribution of ages at onset in male 11778 index patients versus female 11778 index patients ($P = .08$), and there were too few female patients to compare these with males in the other two mutation groups. In all three groups combined, there was no difference between males and females ($P = .40$).

Cumulative age-at-onset curves for index and secondary cases were constructed by using data from patients of both sexes and members of all three mutation groups, and they are also shown separately for males and females (fig. 1). The 95th centile for age at onset was 50.5 years in index cases, 49.5 in secondary cases, 51 years for all males, and 56 for all females (90th centile was 49.6 years). In order to obtain maximum information with a minimum risk of falsely assigning unaffected status, a minimum age of 51 years was chosen for inclusion of sibships in the pedigree analysis. Correlation coefficients for age-at-onset data were calculated for patients with all three mutations grouped together, comparing

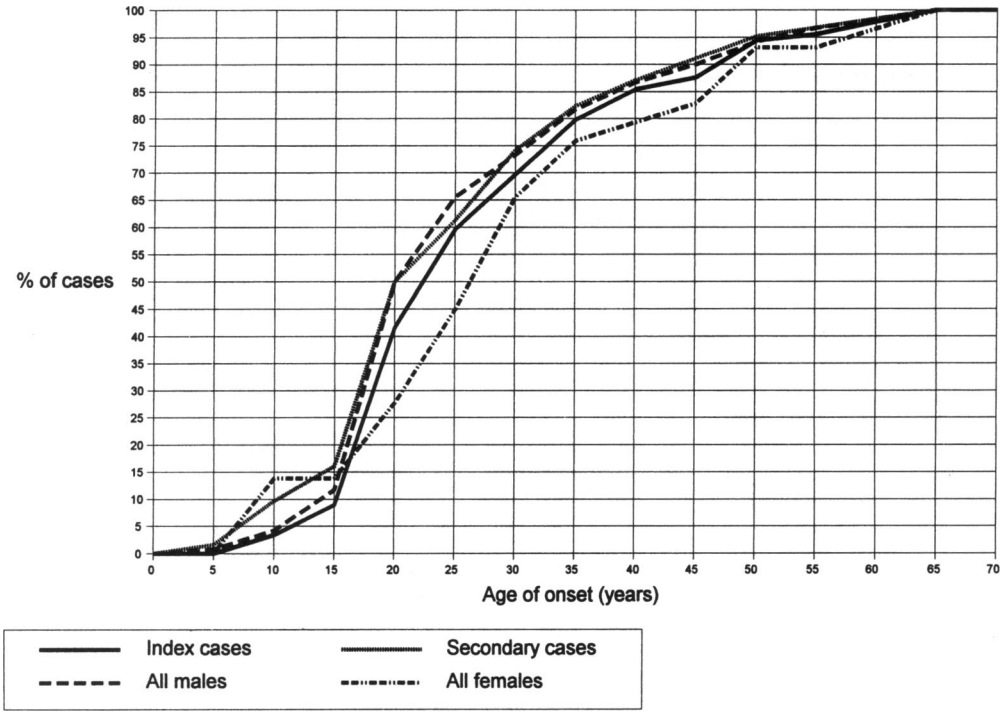

Figure 1 Cumulative age-at-onset curves for index (n = 89), secondary (n = 62), all male (n = 122), and all female (n = 29) patients with LHON and mutations at positions 11778, 3460, and 14484.

index cases with their sibs (n = 22, r = .28, P = .21) (fig. 2) and cousins (n = 11, r = −.50, P = .11).

The proportions of affected sibs, nieces/nephews, and matrilineal first cousins of pedigree index cases, with 95% CL, are shown in table 3. Most data are available for the 11778 families and imply recurrence risks of 25%, 8%, 41%, 17%, 30%, and 7%, respectively, to the brothers, sisters, nephews, nieces, and male and female first cousins of patients. The difference between brothers and nephews is not significant (χ^2 = 1.7, P = .19). There were few 3460 families in this study, and the resulting data—particularly the proportions of affected females in all categories—should be viewed with caution. There were no significant differences, between mutations, in the segregation ratios in any group of relatives (table 3). The series included four pairs of male twins, one monozygous and three dizygous; all were concordant and affected.

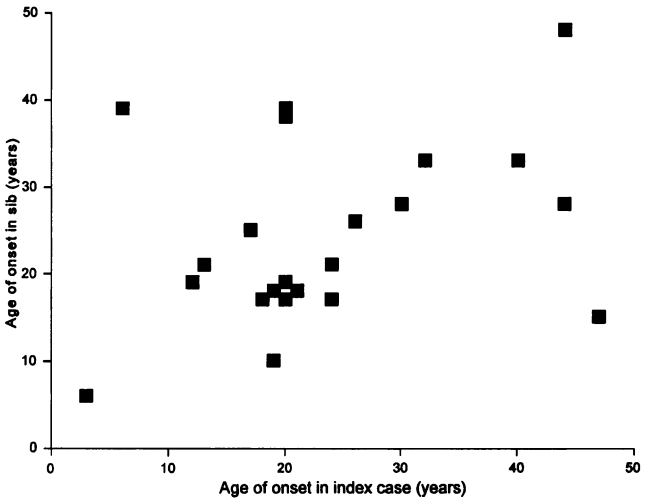

Figure 2 Scattergram showing relationship between age at onset in index cases and their affected siblings.

The proportions of affected and unaffected sons and daughters of affected and unaffected carrier females are shown in table 4. This analysis included all affected women (on the basis that they must be carriers); unaffected carriers were defined as women with either two affected offspring or one affected child and an affected sibling, aunt/uncle, or maternal cousin. Only sibships >50 years of age were analyzed. The proportion of affected offspring of affected women was higher than that of unaffected carriers, and this was just significant when the whole data set was analyzed (χ^2 = 8.04, P = .045). If the proportions of affected sons and daughters were analyzed separately, the excess of affected daughters of affected women remained significant (χ^2 = 4.14, P = .041), but that of affected sons did not (χ^2 = 1.3, P = .24). It is worth noting in this context that, of the 26 families containing affected females, there was more

Table 3

Proportions of Affected Relatives

	11778 Mutation		3460 Mutation		14484 Mutation				All Mutations	
	No. Affected/ No. Unaffected	p (95% CL)	No. Affected/ No. Unaffected	p (95% CL)	No. Affected/ No. Unaffected	p (95% CL)	χ^2 [a]	P	No. Affected/ No. Unaffected	p (95% CL)
Sibs:										
Males	16/48	.25 (.14–.36)	3/8	.27 (.08–.53)	4/8	.33 (.06–.60)	.36	.80	23/64	.30 (.21–.39)
Females	5/57	.08 (.01–.15)	0/9	0	3/30	.09 (0–.19)	.85	.65	8/96	.08 (.03–.13)
Sisters' children:										
Males	12/17	.41 (.23–.59)	1/5	.17 (0–.47)	9/4	.69 (.44–.94)	5.1	.07	22/26	.46 (.32–.60)
Females	5/25	.17 (.10–.24)	0/5	0	0/16	0	4.4	.11	5/46	.10 (.01–.19)
Matrilineal first cousins:										
Males	10/23	.30 (.22–.38)	3/5	.37 (0–.70)	5/11	.31 (.08–.54)	.15	.92	18/39	.31 (.19–.43)
Females	2/27	.07 (0–.16)	0/4	0	1/17	.05 (0–.15)	.31	.86	3/48	.06 (0–.12)

[a] Comparing affected/unaffected proportions, between groups.

than one affected woman in 5 families, with 2 families each containing three and four affected females. There were 11 affected women who had at least one affected son. Three of these women also had unaffected sons, and the ages at onset in two of these women were 19 and 60 years (onset age in the third was unknown). Ages at onset were known in six of the eight affected women who only had affected sons; these ages were 7, 8, 9, 15, 20, and 41 years.

Heteroplasmy was detected in nine pedigrees, and these are shown in figure 3 in the present report and in figure 1 of Sweeney et al. (1992). All but 5 of 124 affected subjects (i.e., 96%) showed virtual homoplasmy (>95%) for mutant mtDNA. One affected male with the 14484 mutation had ~90% mutant mtDNA. His affected sister and her affected daughter were homoplasmic (family 14). Three affected members of a 3460 family (family 9) were heteroplasmic (67%–84% mutant mtDNA). The patient with both the 11778 mutation and the 14484 mutation was heteroplasmic for the latter (~70% mutant); his normal mother also had both mutations and was homoplasmic for the 11778 mutation but heteroplasmic (~30% mutant) for 14484 mutation (family 18).

All the 140 unaffected matrilineal relatives studied, from 37 families, had the same mtDNA mutation as did the index case, including all of 7 mothers of patients investigated who had no affected antecedents. Nineteen relatives (13.6%) showed substantial heteroplasmy (fig. 3 and table 5). It was present in 3 of the 8 mothers without affected antecedents, as opposed to 1 of 20 mothers with affected antecedents. The incidence of heteroplasmy appears highest (3 of 6) in the grandmothers or great-aunts/great-uncles of patients, although the numbers of these were small. Pedigree 11 (3460 mutation), reported by Sweeney et al. (1992), has been expanded (fig. 3) and shows that the grandmother with <5% (but detectable) mutant mtDNA and heteroplasmic descendants (one affected) had two cousins who were probably affected.

Discussion

This study of British families shows that the 11778 mutation is by far the most common cause of LHON,

Table 4

Proportions of Affected Offspring of Affected and Unaffected Carrier Females

	11778 Mutation			3460 Mutation			14484 Mutation			All Mutations		
	No. Affected/ No. Unaffected	p	No. of Mothers	No. Affected/ No. Unaffected	p	No. of Mothers	No. Affected/ No. Unaffected	p	No. of Mothers	No. Affected/ No. Unaffected	p	No. of Mothers
Unaffected carrier females:												
Male offspring	35/35	.5	36	6/4	.6	4	7/3	.7	6	48/42	.53	46
Female offspring	8/73	.09		1/4	.2		3/8	.27		12/85	.12	
Affected carrier females:												
Male offspring	7/2	.77	8	3/2	.6	2	1/0	1	2	11/4	.73	12
Female offspring	4/4	.5		0/3	.0		1/1	.5		5/8	.38	

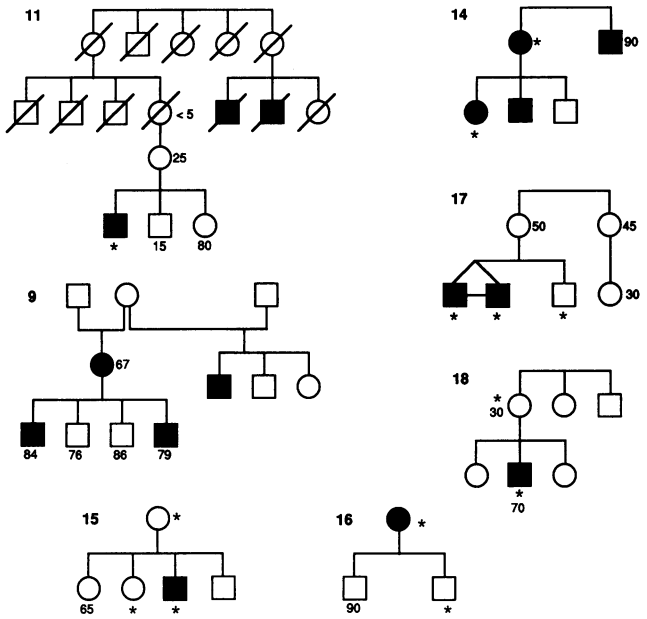

Figure 3 Pedigrees exhibiting heteroplasmy. Pedigrees of heteroplasmic families 2 and 5 are in fig. 1 of Sweeney et al. (1992), and pedigrees 9 (3460 branch) and 11 have been updated from this source. Families 9 and 11 have the 3460 mutation, families 15–17 have the 11778 mutation, family 14 has the 14484 mutation, and family 18 has both the 11778 mutation and the 14484 mutation. The numbers adjacent to pedigree symbols indicate proportion of mutant mtDNA (14484 in pedigree 18, with both 11778 and 14484 mutations), and asterisks indicate >95% mutant mtDNA (for 11778 in pedigree 18).

as is the case in other European, North American, and Japanese populations (Wallace et al. 1988; Holt et al. 1989; Vilkki et al. 1989; Poulton et al. 1991; Nakamura et al. 1992; Obermaier-Kusser et al. 1994). The clinical features of our families have been reported elsewhere (Riordan-Eva et al. 1995). We have only accepted mutations at 11778, 3460, and 14484 as being pathogenic in LHON. These are the only mutations that have been found exclusively in LHON families, and they were detected in all except two of our families with typical clinical features of LHON and a positive maternal family history. The role of so-called secondary mutations in LHON is controversial. It has been suggested that these contribute to disease pathogenesis in the presence of primary mutations (Johns and Berman 1991; Brown et al. 1992; Wallace 1994). This is a difficult hypothesis to prove or exclude, but there is no evidence that secondary mutations influence visual outcome (Oostra et al. 1994*a*, 1994*b*; Riordan-Eva et al. 1995). We have not studied secondary mutations extensively in our families. Any primary pathogenic role of the 15257 mutation in LHON (Brown et al. 1992; Johns et al. 1993*c*) has been disputed, since it occurs in 0.3% of control subjects and in association with other, primary mutations (Howell et al. 1993; Obermaier-Kusser et al. 1994; Oostra et al. 1994*b*). The presence of both the 14484 mutation and the 11778 mutation in one of our patients is novel. This may suggest that the 14484 mutation is not pathogenic, but it has not been described in controls and may be heteroplasmic, a feature of pathogenic mutations (Holt et al. 1990; Mackey and Howell 1992; present study). The heteroplasmy for the 14484 mutation seen in this patient and his mother may suggest that it has arisen fairly recently in this pedigree (Smith et al. 1993).

Most of the previous genetic studies of LHON were based on a small number of large pedigrees, with data collected from earlier generations ascertained through multiply affected sibships in younger generations (Seedorf 1968, 1985; Nikoskelainen et al. 1987). The study by Nikoskelainen et al. (1987) was complicated by inclusion of retinal microangiopathy as a criterion for affected or carrier status. This clinical sign is very subjective and of uncertain significance (Riordan-Eva et al. 1995). These surveys concluded that ~50% of males at

Table 5

Heteroplasmy in Unaffected Relatives

	No. Heteroplasmic/Total, by Relationship to Index Case								
	Mothers	Sisters	Brothers	Sons	Grandmothers	Great-Aunts/ Great-Uncles	Aunts/ Uncles	Nieces/ Nephews	Cousins
11778 Mutation (30 families)	2[a]/20	1/22	1/9	1/3	1/2	1/2	3/25	0/6	2/17
14484 Mutation (3 families)	1[a]/4	0/4	0/4	...	...	...	0/2	...	0/5
3460 Mutation (4 families)	1/3	1/4	3/4	...	1/2	...	0/4	...	0/2
Total mutation (37 families)	4/27	2/30	4/17	1/3	2/4	1/2	3/31	0/6	2/24

[a] All mothers of patients without affected antecedents (of a total of eight studied).

risk in LHON families develop the disease and that most, if not all, females related to males with LHON through females are "carriers," i.e., will all have affected descendants (if it is assumed that there are descendants at risk) eventually. This is difficult to exclude, as acknowledged by van Senus (1963) in a more population-based survey, who considered it unlikely that all such females were carriers. Apart from the fact that the diagnosis in these pedigrees was not made on a molecular genetic basis, these earlier surveys generally did not fully address the problems of ascertainment bias or age-dependent penetrance, and the conclusions cannot be used in genetic counseling. If LHON is defined on the basis of molecular genetic diagnosis, only 64% of index cases have affected relatives overall, reflecting the ease with which the diagnosis can now be made in patients without a family history.

It is in fact difficult to analyze pedigree data from LHON families in a way that does not favor large, multigeneration pedigrees, containing multiple affected individuals. For genetic counseling purposes, the questions that we wished to answer, those often asked by members of LHON families, were as follows: (1) What are the risks that brothers and sisters of a patient will develop blindness? (2) What are the risks to the children of sisters of patients? and (3) What are the risks to the children of aunts of patients? Hardly any of these can be answered by using data from families containing only one relatively recently diagnosed patient, since all the sibs, nephews/nieces, and cousins have not reached an age at which they could safely be assigned unaffected status. The relevant data could only be obtained by analyzing affected:unaffected ratios in early generations related to a younger affected individual; in this study, they derived from 45 of the total of 85 families. This is likely to bias the data in favor of an excess of affected subjects. Also, families are more likely to be able to provide information about sibships in earlier generations or relatively distant relatives (cousins, second cousins, etc.) if these earlier generations contain affected individuals. The second problem was minimized by including only pedigrees in which complete information about matrilineal cousins was available. Our method of defining sibship and generation index cases was used to try and overcome the first problem, but this problem cannot be entirely circumvented, so the genetic risks derived from this study must be viewed as maximum, rather than as absolute.

For genetic counseling purposes, it seems appropriate to use the recurrence risks derived from all of our families, regardless of mutation, since (*a*) there were no significant differences between any of the ratios between mutation groups and (*b*) the numbers of 3460 and 14484 pedigrees are too small to allow individual figures to be used reliably. Given this, we have not entirely excluded a difference in transmission characteristics between the three mutations. The total data set implies risks of 30%, 8%, 46%, 10%, 31%, and 6%, respectively, to the brothers, sisters, nephews, nieces, and male and female matrilineal first cousins of index cases. These figures do not into account any possible effect of heteroplasmy, but this effect is likely to be small (see below). Recurrence risks can be modified by using both the cumulative age-at-onset curve for secondary cases (fig. 1) and Bayesian statistics.

One issue that frequently arises in clinical practice is whether detection of heteroplasmy is useful in determining either likelihood of developing the disease, particularly in males at risk, or transmission to offspring. Very few affected subjects have >5% normal mtDNA; this was confined to those in this series (4% of all affected subjects studied) who had either the 3460 mutation (three patients in one family) or the 14484 mutation (two patients, one with the 11778 mutation as well), but heteroplasmy was present in 5 of 75 patients with the 11778 mutation described by Smith et al. (1993). Zhu et al. (1992) reported a very high incidence of heteroplasmy, in all of six affected subjects with this mutation. The majority of these patients had <25% normal mtDNA, and it seems relatively unlikely that a subject at risk will develop LHON unless he or she has >75% mutant mtDNA. These data should be interpreted with caution, given (1) that the proportion of mutant mtDNA in blood may not reflect faithfully that in the optic nerve and (2) that differences in degree of heteroplasmy between blood and hair follicles are well documented (Lott et al. 1990). Furthermore, one patient in this study had only 67% mutant (3460 mutation) mtDNA, and other affected subjects with either undetectable or <75% mutant 11778 mtDNA in blood have been reported by Cormier et al. (1991), Zhu et al. (1992), and Howell et al. (1994). The last report is of particular interest because the patient had 33% mutant mtDNA in blood but analysis of several autopsy tissues showed higher amounts, specifically 95%, 100%, and 100%, respectively, in optic nerve, retina, and skeletal muscle. There was no obvious difference in disease severity between our heteroplasmic and homoplasmic patients.

Anecdotal pedigree data suggest that women with substantial proportions of normal mtDNA might be less likely to have affected children (Holt et al. 1989), but some do (Zhu et al. 1992); this hypothesis has not been tested systematically, mainly because such women are uncommon. It has been proposed that heteroplasmy indicates that the mtDNA mutation has arisen relatively recently and that it segregates to homoplasmy over a few generations (Smith et al. 1993). Three of the eight mothers without affected antecedents in the present study were heteroplasmic, as opposed to only 1 of 20 mothers with a prior family history. However, data sug-

gesting an increase in the proportion of mutant mtDNA over successive generations (Bolhuis et al. 1990; Smith et al. 1993) could result solely from ascertainment bias (Howell et al. 1994). In some pedigrees the proportion falls (families 16 and 17, fig. 3; Vilkki et al. 1990; Zhu et al. 1992). Pedigree 11 in figure 3 also indicates that the random-drift model of mtDNA segregation is more likely (Howell et al. 1994), although it was difficult to confirm the diagnosis of LHON with absolute certainty in the affected members of the oldest generation, and it was impossible to analyze their mtDNA. This family and pedigree 17 indicate that the risks to children of mothers with $\leq 50\%$ mutant mtDNA are not negligible. The incidence of heteroplasmy in unaffected relatives in this series (13.6%, in 9 of 37 families) was comparable to that reported by others, 19% of 101 relatives (Smith et al. 1993) and 7 of 49 families (Newman et al. 1991).

It is clear that primary mtDNA mutations are necessary but not sufficient to cause LHON. The predominance of affected males is not explained by mitochondrial inheritance. The most compelling explanation for both of these observations is the existence of an X-linked visual loss–susceptibility locus, despite the negative data generated by linkage studies in this context (Carvalho et al. 1992; Sweeney et al. 1992; Juvonen et al. 1993). Our pedigrees can be analyzed in the light of the model put forward by Bu and Rotter (1991), in which there is such an X-linked gene with a frequency of .08 and a penetrance of .11 in heterozygous females. This predicts that 40% of affected females would be homozygous, the remainder being affected because of disadvantageous X inactivation. It is obvious that not all affected females are homozygous, since some (including those in this series) have unaffected sons. A similar analysis in Japanese pedigrees also supported the hypothesis of an X-linked susceptibility locus, but the predicted penetrance in heterozygous females was higher (.196; Nakamura et al. 1993). According to Bu and Rotter's model, the respective percentages of affected sons and daughters of unaffected females should be 50% and 13% those of affected females 74% and 41%. These predicted figures are in remarkable accordance both with the data shown in table 4 and with the observation that the risk to daughters of affected women is significantly more than that to sons. van Senus (1963) also observed this phenomenon. Bu and Rotter (1991) suggested that heterozygous women might have a milder disease course than do homozygous females and males, with later age at onset. We did not detect a significant difference in age at onset between males and females overall, but the age at onset was later in females in all 11778 patients. Our age-at-onset data for obligate-heterozygote women and possible homozygote women would be consistent with this hypothesis, although the numbers are small.

If the Bu-Rotter model is correct, why have linkage studies failed to find an X-linked susceptibility locus? The studies attempting (unsuccessfully) to confirm previous observations of linkage to DXS7 (Vilkki et al. 1991) only examined a small region of Xp (Carvalho et al. 1992; Sweeney et al. 1992) but used appropriate age-related liability classes. The tested penetrance in female heterozygotes was .01, as opposed to the .11 proposed in the Bu and Rotter model, and the same applies to the study by Vilkki et al. (1991). In this initial Finnish study, males were considered unaffected if they were >30 years of age, which is clearly too young. Affected status of some subjects had changed by the time of the reevaluation of these data, and more families were studied (Juvonen et al. 1993); improved age-related liability classes were used, but female heterozygote penetrance was still set at .01. These linkage data have not entirely excluded a susceptibility locus from parts of the X chromosome. The results of the present study indicate that the X-linked hypothesis is worth exploring further.

All four twin pairs in this series were concordantly affected. The difference, in age at onset, between the single pair of monozygous twins was 1 year. A further concordant pair of monozygous twins was reported by Nikoskelainen et al. (1987), but no age-at-onset data were given. Johns et al. (1993*b*) reported a pair of discordant monozygous twins, the unaffected twin being 6 years older than the age at onset in his affected brother, and proposed that this was due to environmental factors, specifically occupational exposure to smoke and fumes. Both twins were cigarette smokers. This does not exclude the possibility of one or more nuclear genetic factors in determining blindness in LHON, perhaps in addition to environmental factors. The presence of the latter would be compatible with the lack of significant correlation for age at onset between index cases and siblings in this study (fig. 2). A case-control study would be interesting in this context. There is anecdotal evidence that tobacco and alcohol use influences the course of LHON; in the present series this was particularly striking in families with the 3460 and 14484 mutations (Riordan-Eva et al. 1995). An autoimmune component in the etiology of this disease has also been proposed (Harding et al. 1992). LHON, in many ways the prototype of mitochondrial inheritance, may well turn out to be more etiologically complex than other human diseases associated with defects of mtDNA.

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