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Clinical Expression of Leber Hereditary Optic Neuropathy Is Affected by the Mitochondrial DNA–Haplogroup Background

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Leber hereditary optic neuropathy (LHON) is due primarily to one of three common point mutations of mitochondrial DNA (mtDNA), but the incomplete penetrance implicates additional genetic or environmental factors in the pathophysiology of the disorder. Both the 11778G→A and 14484T→C LHON mutations are preferentially found on a specific mtDNA genetic background, but 3460G→A is not. However, there is no clear evidence that any background influences clinical penetrance in any of these mutations. By studying 3,613 subjects from 159 LHON-affected pedigrees, we show that the risk of visual failure is greater when the 11778G→A or 14484T→C mutations are present in specific subgroups of haplogroup J (J2 for 11778G→A and J1 for 14484T→C) and when the 3460G→A mutation is present in haplogroup K. By contrast, the risk of visual failure is significantly less when 11778G→A occurs in haplogroup H. Substitutions on *MTCYB* provide an explanation for these findings, which demonstrate that common genetic variants have a marked effect on the expression of an ostensibly monogenic mtDNA disorder.

Leber hereditary optic neuropathy (LHON [MIM 535000]) is a common cause of maternally inherited visual failure that affects at least 1 in 14,000 males.[1] LHON typically presents during young adulthood with dyschromatopsia followed by a subacute painless loss of vision in one eye, with symptoms developing in the other eye 6–12 wk after the initial onset.[2,3] In >95% of cases, LHON is due primarily to one of three point mutations of mtDNA that affect genes coding for different subunits of complex I of the mitochondrial respiratory chain: 3460G→A in *MTND1,* 11778G→A in *MTND4,* and 14484T→C in *MTND6.*[4] However, not all individuals who inherit a primary LHON mtDNA mutation will develop the optic neuropathy, which indicates that additional environmental or genetic factors are important in the etiology of the disorder.[3,5,6]

Numerous anecdotal reports have linked the onset of blindness with various environmental insults, including excess alcohol consumption and smoking.[7,8] In addition, the analysis of large pedigrees followed over decades suggests that the penetrance of the primary LHON mutations may be decreasing in some families, possibly through an improved diet and reduced tobacco and alcohol consumption.[9] However, rigorous cross-sectional epidemiological studies reached different conclusions,[10] and the role of environmental agents has yet to be established.

By contrast, evidence supporting an additional genetic influence is more compelling. Although the majority of individuals with LHON inherit mutated mtDNA only from their mother and thus are homoplasmic for the primary mtDNA LHON mutation, some subjects harbor a mixture of mutated and wild-type mtDNA (heteroplasmy).[1,4] Retrospective studies suggest that the risk of visual failure is reduced in family members with <60% mutated mtDNA in their blood.[11,12] Heteroplasmy cannot, however, explain the marked sex bias in LHON, which affects predominantly males. Segregation analysis is consistent with an interacting recessive X-chromosomal locus in some families,[13] which is supported by the results of genetic linkage analysis implicating an ~6-cM region of the X chromosome that is likely to contain an interacting nuclear modi-

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fier.[14] Finally, there is a well-established strong association between the mtDNA genetic background and both the 11778G→A and 14484T→C mutations but not 3460G→A.[15,16]

mtDNA is inherited through the maternal line, is highly polymorphic, and can be divided into haplogroups on the basis of the presence of specific combinations of distinguishing mutations scattered throughout its entire sequence. mtDNAs belonging to the same haplogroup derive by descent from the same ancestral female, as revealed by the sharing of the distinguishing mutational motif.[17,18] Meta-analysis of the available data has shown that individuals with the 14484T→C mutation are >27-fold more likely to belong to western Eurasian haplogroup J than are control subjects and that individuals with the 11778G→A mutation are >3-fold more likely to belong to haplogroup J than are control subjects.[19] This has been observed in different western Eurasian populations in genetically distinct pedigrees, which indicates that this is not a founder effect.[15,20] The reasons for the association are not clear, but the association may relate to functional variants in the *MTCYB* gene interacting synergistically with the primary LHON mutation, leading to further compromise of complex I function.[21] If this is the case, then the clinical penetrance of both the 11778G→A and the 14484T→C mutations should be increased on a haplogroup J background—but this hypothesis has not been formally tested. Intriguingly, in one pedigree, 14484T→C had a low penetrance on a haplogroup H background,[9] which raises the possibility that other mtDNA haplogroups might alter penetrance of the primary LHON mutations in a mutation-specific manner.

The three primary LHON mutations were identified almost 2 decades ago, but, despite major advances in our understanding of the molecular pathophysiology of LHON, clinical management has remained largely the same. Molecular genetic testing and genetics counseling based on empirical recurrence risks have changed little in the past decade. Given the potential role of mtDNA haplotypes in the penetrance of LHON, we performed a multicenter study of 3,613 subjects from 159 different families transmitting the 3460G→A, 11778G→A, and 14484T→C mutations. By defining the role of each haplogroup in LHON, we hoped to improve the accuracy of genetics counseling for LHON and also to advance our understanding of mtDNA genetic variation and its role in complex disease. Using this approach, we also formally explored the possibility that the penetrance of LHON is changing over time.

Material and Methods

Pedigrees were identified through a pan-European collaboration and were anonymously entered into a central database. To minimize ascertainment bias, we did not analyze singleton cases (i.e., pedigrees with one affected individual). Extensive pedigree analysis was performed at each center. Sibships were included only if there was one affected individual and/or the mother harbored a primary LHON mtDNA mutation. The clinical phenotype was determined by a local ophthalmologist. Unaffected individuals had no visual symptoms. Previous studies have included unaffected subjects only if they had no symptoms after reaching a specific age (typically 30 years, given the median age of ~24 years for visual failure in LHON). This would, however, introduce an ascertainment bias into a study of this type, since only affected individuals aged <30 years old would be included, elevating the penetrance value. We therefore included all subjects, irrespective of age, to assess the lifetime penetrance of the disorder. The primary aim of this study was to determine the effect of mtDNA haplogroups on clinical penetrance, and we had no a priori reason to think that this would be influenced by the age of subjects.

The diagnosis was confirmed in all studied affected individuals by direct sequencing of the *MTND* genes or by PCR-RFLP analysis. For the purposes of this study, we did not accurately quantify heteroplasmy, and we classified sibships only as either (1) homoplasmic, if the mother was shown to be homoplasmic by an established technique, or (2) heteroplasmic, if one member of the nuclear family had been shown to be heteroplasmic. Retrospective analysis omitting the heteroplasmic sibships did not influence the overall conclusions. mtDNA-haplogroup analysis was performed either by PCR-RFLP analysis or by direct sequencing of the mtDNA coding or control regions.[16] Haplogroup J subanalysis was performed by direct sequencing across nucleotide positions 3010 (3010A in J1) and 15257 (15257A in J2), as described elsewhere.[21]

We identified 3,613 individuals, including affected and unaffected subjects, harboring a primary LHON mutation (11778G→A [n = 2,104, 58.2%]; 14484T→C [n = 851, 23.6%]; 3460G→A [n = 658, 18.2%]) from 159 genealogically independent pedigrees (11778G→A [n = 90, 56.6%]; 14484T→C [n = 33, 20.8%]; 3460G→A [n = 36, 22.6%]). Of the subjects, 48.9% were male (95% CI = 47.3%–50.6%, including affected and unaffected males). These proportions are in concordance with those of other published smaller series.[4] The mtDNA-haplogroup distribution for each mtDNA LHON mutation is shown in table 1 and reflects the well-established overrepresentation of haplogroup J in European LHON-affected pedigrees. The complete data set is shown in table 2. The sex was not known for four subjects who all belonged to the same 11778G→A haplogroup H family. Of the sibships, 4.6% harbored at least one heteroplasmic individual.

Binary logistic regression was used to determine, simultaneously across the whole cohort, which variables influence the risk of developing visual failure. This approach reduces the chance of

Table 1. Haplogroup Frequencies for the 3,613 Subjects with the 11778G→A, 14484T→C, and 3460G→A Mutations

mtDNA Haplogroup[a]	No. (%) of Subjects with			
	11778G→A	14484T→C	3460G→A	Total
H	927 (44)	2 (<1)	307 (47)	1,236 (34)
J	593 (28)	840 (99)	169 (26)	1,602 (44)
K	101 (5)	…	75 (11)	176 (5)
M	3 (<1)	…	…	3 (<1)
T	87 (4)	…	…	87 (2)
U (without K)	184 (9)	…	27 (4)	211 (6)
V	…	…	41 (6)	41 (1)
W	11 (<1)	…	…	11 (<1)
X	135 (6)	9 (1)	…	144 (4)
Other	63 (3)	…	39 (6)	102 (3)
Total	2,104	851	658	3,613

[a] There were no haplogroup I families.

Table 2. Haplogroup Distribution of Affected and Unaffected Subjects with the 11778G→A, 14484T→C, and 3460G→A Mutations

Sex and mtDNA Haplogroup	No. of Subjects with Mutation					
	11778G→A[a]		14484T→C		3460G→A	
	Unaffected	Affected	Unaffected	Affected	Unaffected	Affected
Male:						
H	289	155	1	…	112	36
J1	127	79	206	172	35	20
J2	34	36	38	18	15	16
K	30	17	…	…	18	15
T	21	25	…	…	…	…
U (without K)	51	36	…	…	8	4
V	…	…		…	19	3
W	2	1	…	…	…	…
X	48	21	3	2	…	…
Other	21	11	…	…	16	4
Total	623	381	248	192	223	98
Female:						
H	437	42	1	…	141	18
J1	185	27	292	25	53	6
J2	90	15	86	3	22	2
K	47	7	…	…	31	11
T	40	1	…	…	…	…
U (without K)	86	11	…	…	14	1
V	…	…	…	…	17	2
W	5	3	…	…	…	…
X	62	4	4	…	…	…
Other	28	6	…	…	15	4
Total	980	116	383	28	293	44

[a] The sex was not known for four subjects (not included in the table), all belonging to the same 11778G→A haplogroup H family.

type I error (false-positive result) and controls for differences in the frequency of key variables among the different groups. Visual failure was the dependent variable in the model, with the following independent categorical variables: sex, primary LHON mtDNA mutation (modeled as a single categorical variable), presence of heteroplasmy, and mtDNA haplogroup. Since each haplogroup is an independent categorical variable associated with a different cluster of haplotype-specific polymorphisms, we introduced each haplogroup separately into the regression equation while including all of the other potential confounding variables. We studied only haplogroups present at >1% frequency across the whole study group, in keeping with the "rule of thumb" whereby logistic regression should be performed only when the number of study subjects is 1 order of magnitude greater than the number of parameters under study. To test the hypothesis that the penetrance of primary LHON mutations has decreased over the generations, we coded the present generation 1 and previous generations 2, 3, 4 … *n,* thus allowing us to include sibship generation as a continuous variable in the logistic-regression model. If the penetrance of LHON were changing with subsequent generations, the model would identify a significant direct correlation between penetrance and generation number.

Results

Before studying the mtDNA haplogroups, we initially investigated the effects of the other variables on the risk of visual failure (table 3). As expected, the strongest predictor of visual failure was sex, which was associated with a 5.41-fold increased risk of blindness for males compared with females. In addition, mtDNA heteroplasmy was associated with a 0.37-fold reduced risk of visual failure when compared with homoplasmic pedigrees. By contrast, there was no difference in the risk of visual failure for the different LHON mtDNA mutations ($P = .70$) nor any evidence to support a change in penetrance in different generations ($P = .26$).

Given the well-established mutation-specific association with mtDNA haplogroup J,[19] we studied separately the effect of common mtDNA haplogroups on the risk of visual failure for each LHON mtDNA mutation (see table 4 for the results of the logistic regression and table 2 for the complete data set). For 11778G→A, the risk of visual failure was increased for pedigrees with a haplogroup J background ($P = .02$; odds ratio [OR] = 1.31; 95% CI = 1.03–1.65) but was reduced in pedigrees with a haplogroup H background ($P = .04$; OR = 0.79; 95% CI =

Table 3. Major Factors Influencing the Clinical Penetrance of the 11778G→A, 14484T→C, and 3460G→A Mutations in 3,613 Subjects

Variable	*P*	OR	95% CI
Sex	9.27×10^{-76}	5.41	4.52–6.48
LHON mutation	.70		
Heteroplasmy	1.37×10^{-4}	.37	.22–.62
Pedigree generation	.26	.97	.90–1.01

Table 4. Effect of mtDNA Haplogroups on the Clinical Penetrance of the 11778G→A, 14484T→C, and 3460G→A Mutations

Mutation and mtDNA Haplogroup	*P*	OR	95% CI
11778G→A:			
H	.04	.79	.63–.98
J	.02	1.31	1.03–1.65
K	.97	.99	.60–1.63
T	.31	1.30	.79–2.14
U (without K)	.59	1.11	.76–1.60
X	.05	.62	.39–1.0
14484T→C:			
H	1.0	1.49×10^{-9}	0
J	.40	2.06	.40–10.66
X	.60	.64	.12–3.53
3460G→A:			
H	.14	.72	.47–1.11
J	.34	1.24	.80–1.90
K	2.35×10^{-3}	2.37	1.36–4.13
U (without K)	.60	.76	.27–2.10

NOTE.—Binary logistic-regression model with visual failure as the dependent variable. The following independent variables were included in each model: sex, presence of heteroplasmy, and pedigree generation (see table 1). The effect of the major European mtDNA haplogroups was modeled in turn, adding each sequentially to the logistic-regression equation. No 14484T→C pedigrees belonged to haplogroups K, T, or U, and no 3460G→A pedigrees belonged to haplogroup T or X.

0.63–0.98). For 3460G→A, the risk of visual failure was increased in pedigrees with a haplogroup K background ($P = 2.35 \times 10^{-3}$; OR = 2.37; 95% CI = 1.36–4.13).

We were initially surprised that the analysis did not identify an association between 14484T→C and haplogroup J (table 4). However, the lack of increased risk of visual failure among the 840 subjects with the 14484T→C mutation on haplogroup J is most likely to be a consequence of the virtual absence of non-J pedigrees (only 11 subjects, ~1%) (table 1). Given the fact that haplogroup J frequencies are in the range of 3%–15% in European populations,[22] the 99% frequency of 14484T→C on J mtDNAs observed in this study (table 1) strongly suggests that the clinical penetrance of the 14484T→C mutation is close to zero when occurring on European mtDNA backgrounds other than J. The 14484T→C mutation and the pedigrees in which it occurs remain undetected simply because LHON does not show up in the family when the mtDNA belongs to a non-J haplogroup. This scenario is supported by the finding of a U8b mtDNA harboring the 14484T→C mutation in the course of a random survey of U mtDNAs from unaffected subjects.[23] Moreover, recent work has also shown that the preferential association between LHON-affected pedigrees with the 14484T→C mutation and J is attributable largely to J1, a specific subgroup of haplogroup J.[21] We therefore compared the clinical penetrance of the 14484T→C mutation on the J1 background with that on the J2 background. We observed a 1.85-fold increased risk of visual failure for J1 relative to J2 ($P = 2.3 \times 10^{-2}$; 95% CI = 1.09–3.15), in agreement with the observation that 28.3% of the J1 subjects versus only 14.5% of the J2 subjects are clinically affected (table 1). The same kind of comparison (J2 vs. J1) was performed for the 11778G→A mutation. In that case, the situation was just the opposite, with J2 harboring a 1.73-fold increased risk of visual failure ($P = 1.7 \times 10^{-2}$; 95% CI = 1.10–2.72) relative to J1.

Discussion

By studying 3,613 individuals from 159 pedigrees, we provide the first clear evidence that different mtDNA haplogroups influence the clinical penetrance of the three primary LHON mtDNA mutations. We made four principal observations: (1) the penetrance of 14484T→C is increased on a haplogroup J background, most prominently on the J1 subhaplogroup; (2) the penetrance of 11778G→A is also increased on a haplogroup J background, but, for this mutation, the effect is most prominent on the J2 subhaplogroup; (3) the penetrance of 11778G→A is reduced on a haplogroup H background; and (4) the penetrance of 3460G→A is increased on a haplogroup K background.

The increased penetrance of 14484T→C and 11778G→A on different J subhaplogroups is in keeping with previous haplogroup-association studies reporting an increased frequency of haplogroup J1 in 14484T→C pedigrees and an increased frequency of J2 in 11778G→A pedigrees.[21] It is, however, most intriguing that closely related subhaplotypes appear to have different effects on the two most common LHON mtDNA mutations. For 3460G→A, the most striking novel finding was the marked increased risk of visual failure when the mutation was on the haplogroup K background. This was not apparent in 11778G→A pedigrees, despite a greater number of subjects (table 1). In keeping with this, haplogroup K subjects were overrepresented in the 3460G→A group (11%, which is greater than the frequency in most published European data sets[22]). The 3460G→A mutation was the least common in our sample (18% of subjects), and some haplogroups were not represented (table 1), which limited statistical power and our ability to confidently interpret a slight tendency toward increased penetrance of 3460G→A on haplogroup J2 and a reduced penetrance on haplogroup H. Finally, although the reduced penetrance on a haplogroup H background has been described in a single 14484T→C family,[9] this has not been described elsewhere for 11778G→A. Together, these findings demonstrate that the clinical penetrance of LHON mtDNA mutations depends on the background mtDNA haplotype, implicating epistatic genetic mechanisms that modulate the biochemical defect of complex I that underpins the pathophysiology. How can we explain these observations?

Relative to the root of the superhaplogroup R, haplogroup H is defined by the synonymous T7208C and A11719G, the nonsynonymous T14766C in the cytochrome b gene (*MTCYB,* cyt b I7T), and the 12S rRNA gene substitution G2706A.[24] Variation in the mtDNA rRNA genes can alter susceptibility to the organ-specific nonsyndromic deafness through a gene-environment interaction,[25] and it is con-

ceivable that different polymorphisms in the same gene might reduce susceptibility to environmental precipitants of the acute visual failure caused by LHON. Alternatively, genetic variation in the *MTCYB* gene might be responsible. Recent phylogenetic analysis has shown that *MTCYB* mutations are overrepresented on both J1 and J2 subhaplogroups and that J1c and J2b are the principal clades responsible for the association with 11778G→A, whereas J1 is associated with 14484T→C.[21] In addition to the 15452C→A substitution (*MTCYB* L236I) common to all haplogroup J mtDNAs, J1c harbors an additional *MTCYB* substitution (14798T→C/F18L), and J2b also carries two additional *MTCYB* substitutions (15257G→A/D171N and 15812A→G/V356M).[21]

Is this explanation consistent with the increased risk of visual failure in 3460G→A families on a haplogroup K background? The *MTND3* 10398 reversion is shared by both haplogroups J and K (and I) and has been proposed as the functional variant responsible for the decreased risk of Parkinson disease associated with these two haplogroups.[26,27] However, like J1c, haplogroup K is defined by *MTCYB* 14798T→C/F18L, in keeping with the *MTCYB* hypothesis explaining the haplogroup associations for LHON.[21] Although the association with *MTCYB* substitutions could be a chance finding,[21] recent evidence supporting the existence of supercomplexes, consisting of complex I and dimeric complex III,[28] raises the possibility that genetic variation in *MTCYB* might alter complex I function, possibly by destabilizing the assembled supercomplex.[29] Differences in the amino acid sequence of cyt b (through subhaplogroup-specific polymorphisms), ND1, ND4, and ND6 (through primary LHON mutations and subhaplogroup-specific polymorphisms) could alter the known physical interaction between complexes I and III,[30] leading to the loss of complex I activity, as has been observed in a patient with an *MTCYB* mutation.[31–34] Likewise, it is also conceivable that the I–III supercomplex could be relatively stabilized by specific cyt b substitutions (in haplogroup H), although there is no experimental data to support this possibility at present. An alternative explanation is that specific substitutions in *MTCYB* lead to a subtle biochemical defect that adds to the systemic complex I deficiency because of the primary LHON mutations affecting complex I (*MTND*) genes.

Differences in the size of individual pedigrees are one possible confounding factor in a study of this kind. To minimize ascertainment bias, we excluded singleton cases from this study. In addition, for the major haplogroup effects, we obtained the same result when the study population was subdivided into small pedigrees (2–5 generations) or large pedigrees (>5 generations). The lack of a clear relationship between penetrance and the pedigree generation is surprising, especially given the size of this study and the extensive clinical data from a large number of generations. The major improvement in the socioeconomic scene in Europe and a corresponding improvement in nutritional status imply that a simple improvement in these variables is unlikely to reduce the penetrance of LHON. This and other studies[10] highlight the difficulty of establishing a link between LHON and major environmental precipitants.

Given the marked geographic variation in specific mtDNA subhaplogroups, it is conceivable that an association may be important in one country but not another. For example, the absence of J1c from the Iranian population probably explains why the association between LHON and haplogroup J is not seen in Iran.[21] This poses a challenge when it comes to genetics counseling and highlights the importance of performing further studies to determine the basis of the varied penetrance. We have shown that a number of variables modulate the clinical expression of LHON, and smaller studies should be interpreted with great caution, because of the possibility of confounding factors. Defining these factors will, however, have a major impact on the clinical management of families transmitting this disorder.

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Web Resource

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for LHON)

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