Acknowledgments

We thank the members of the family described in this report for their cooperation. We gratefully acknowledge the support of the Ligue Nationale Contre le Cancer and the Programme Hospitalier de Recherche Clinique.

References

Claus EB, Risch NJ, Thompson WD (1991) Genetic analysis of breast cancer in the Cancer and Steroid Hormone Study. Am J Hum Genet 48:232–242

Easton DF, Bishop DT, Ford D, Crockford GP, the Breast Cancer Linkage Consortium (1993) Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. Am J Hum Genet 52:678–701

Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC (1990) Linkage of early onset familial breast cancer to chromosome 17q21. Science 250: 1684–1689

Miki Y, Swensen J, Shattuck-Eidens D, Futreal AP, Harshman K, Tavtigian S, Liu Q, et al (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266:66–71

Narod SA, Feunteun J, Lynch HT, Watson P, Conway T, Lynch J, Lenoir GM (1991) Familial breast-ovarian cancer locus on chromosome 17q12-23. Lancet 338:82–83

Narod S, Ford D, Devilee P, Barkardottir RB, Eyfjord J, Lenoir G, Serova O, et al (1995) Genetic heterogeneity of breast-ovarian cancer revisited. Am J Hum Genet 57: 957–958

Neuhausen SL, Swensen J, Miki Y, Liu Q, Tavtigian S, Shattuck-Eidens D, Kamb A, (1994) A P1-based physical map of the region from D17S776 to D17S78 containing the breast cancer susceptibility gene BRCA1. Hum Mol Genet 3:1119–1126

Sobol H, Birnbaum D, Eisinger F (1994) Evidence for a third breast-cancer susceptibility gene. Lancet 344: 1151–1152

Struewing JP, Abeliovitch D, Peretz T, Avishai N, Kaback MM, Collins F, Brody LC (1995) The carrier frequency of the BRCA1 185delAG mutation is approximately 1 per cent in Ashkenazi Jewish individuals. Nat Genet 11: 198–200

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789–792

Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, et al (1994) Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-1. Science 265:2088–2090

Address for correspondence and reprints: Dr. D. Stoppa-Lyonnet, Unité de Génétique Oncologique, Institut Curie, 26 rue d'Ulm, F75231 Paris cedex 05, France.

© 1996 by The American Society of Human Genetics. All rights reserved.

0002-9297/96/5902-0030$02.00

Am. J. Hum. Genet. 59:481–485, 1996

Primary Pathogenic mtDNA Mutations in Multigeneration Pedigrees with Leber Hereditary Optic Neuropathy

To the Editor:

Leber hereditary optic neuropathy (LHON) is an inherited form of bilateral optic atrophy in which the primary etiologic factor is a mutation in the mitochondrial genome (reviewed by Brown and Wallace [1994] and Howell [1994]). The optic neuropathy in LHON shows incomplete penetrance (the ophthalmologic abnormalities in LHON have been reviewed recently by Riordan-Eva et al. [1995] and Nikoskelainen et al. [1996]), and there are additional genetic and environmental etiologic factors, which are rather poorly defined at the present time, that influence the onset of the disorder (reviewed by Johns [1994]).

Although it has been recognized for more than a century that the risk of LHON shows strict maternal transmission (Leber 1871), it has been much more recently that this inheritance pattern has been associated with the mitochondrial genome (Wallace 1995). Wallace et al. (1988) first identified a mutation at nucleotide 11778 of the mitochondrial genome as a primary etiologic factor in LHON. This mutation results in a histidine-for-arginine substitution at amino acid position 340 (R340H) of the ND4 subunit of complex I (NADH–ubiquinone oxidoreductase). Subsequently, mutations at nucleotide 3460 (Howell et al. 1991; Huoponen et al. 1991) and 14484 (Johns et al. 1992; Mackey and Howell 1992) were detected in a number of LHON families. These mutations produce A52T and M64V substitutions in the ND1 and ND6 subunits of complex I, respectively.

There is now a consensus that these three sequence changes are the most prevalent and the most strongly supported *primary* pathogenic LHON mutations. Beyond this area of agreement, however, there exists considerable debate and uncertainty about the etiologic and/or pathogenic role of other candidate mitochondrial mutations in LHON. The unresolved issues include the following:

1. Is the mutational spectrum wide or narrow? For example, Brown and Wallace (1994) list 16 LHON-associated mutations. The pathogenesis of LHON should become more accessible when it is established whether the etiology involves a nonspecific respiratory-chain defect due to mutations affecting any of complexes I, III, and IV or—alternatively—whether it is limited to mutations that specifically derange complex I. Although the three established primary LHON mutations affect subunits of complex I, two groups have proposed that

a mutation at nucleotide 15257 has a primary pathogenic role (Johns and Neufeld 1991; Brown et al. 1992). This mutation produces a D171N substitution in the mitochondrial gene that encodes the proton-motive cytochrome *b*, a key redox component of complex III (ubiquinone–cytochrome *c* oxidoreductase). Furthermore, Johns and Neufeld (1993) have suggested a pathogenic role for mutations that affect subunits of cytochrome oxidase (complex IV).

2. It has been proposed that there are both *primary* and *secondary* pathogenic LHON mutations. Thus, Johns and Berman (1991) identified putative secondary mutations at nucleotides 4216, 4917, and 13708 (also see Brown et al. 1992). Secondary mutations are found in normal individuals, but at lower frequencies than in LHON patients, and they produce more conservative amino acid substitutions.

There are several factors that have confounded a more complete understanding of the mitochondrial genetic etiology of LHON. In the first place, the human mitochondrial genome evolves rapidly, and etiologically important mutations are typically "buried" in a background of polymorphisms (the signal-to-noise problem). Second, different groups have used different methodologies. For example, some investigators favor the screening of patient populations, including a large proportion of singleton optic atrophy patients, whereas others rely more heavily on analysis of large LHON pedigrees (the approach used here). Finally, inclusion criteria for analysis are relatively clear-cut in regard to the ophthalmologic characteristics of LHON but are much less so for the prevalence and severity of neurological abnormalities among family members. In simplest terms, there seem to be two broad groups of LHON families. In the first group, the optic neuropathy is the most prominent, if not the exclusive, clinical deficit, although there may be extra-ophthalmologic abnormalities, including peripheral neuropathy, heart conduction defects, and a multiple sclerosis–like condition, in a small proportion of family members (Leber 1871; Nikoskelainen et al. 1995; Riordan-Eva et al. 1995). In contrast, there are other families that present severe neurological abnormalities, which also are maternally inherited and which are more prominent than the optic neuropathy. These "LHON-plus" families involve pathogenic mutations different from those that produce the more ophthalmologically limited disorder. For example, a mutation at nucleotide 4160 apparently causes the neurological abnormalities (e.g., dysarthria, spasticity, and juvenile encephalopathy) in a Queensland LHON family, whereas the optic neuropathy is caused by the 14484 primary LHON mutation (Howell 1994). A mutation at nucleotide 14459 causes a "LHON-plus-dystonia" disorder (Jun et al. 1994). More recently, De Vries et al. (1996) have suggested that mutations at nucleotides 11696 and 14596 are associated with the LHON plus spastic dystonia in a large Dutch family.

The purpose of the present report is, first, to survey a large assembly of LHON pedigrees to determine the spectrum of primary mutations and, second, to assess the pathogenic role of the 15257 mutation by this approach. Each collaborative-research group pooled their LHON pedigrees and the results of mtDNA screening. The LHON families were northern European in origin and are from Australia and New Zealand, the United Kingdom (including Ireland), the Netherlands, Denmark, and Finland. Some previously published LHON families are not included in the present report, because they are no longer available for DNA screening. To avoid inclusion of pedigrees with autosomal dominant or autosomal recessive optic atrophy, the only pedigrees that were analyzed were those in which there were at least two affected individuals related through an unaffected woman. Furthermore, most of the pedigrees spanned several generations, and strict maternal transmission was confirmed. The genealogical analyses of these LHON families have been extensive, and we are confident that, as a general rule, they have not shared a maternal relative during the previous 200 years. Finally, the LHON families were limited to those in which optic neuropathy was the major, if not exclusive, clinical deficit. Thus, we exclude LHON-plus-dystonia (Jun et al. 1994; De Vries et al. 1996) and the Queensland LHON (Howell 1994) families. This winnowing of LHON-plus families is unlikely to skew our results, because they account for $\leq 5\%$ of all matrilineal pedigrees with an LHON-like optic neuropathy.

A total of 159 LHON families, selected by use of the inclusion criteria described above, were analyzed here; the results are summarized in table 1. These pedigrees comprise >12,000 maternally related individuals and >1,500 affected individuals. Of these 159 families, 153 (97%) carried one of the three previously identified primary LHON mutations at nucleotides 3460 (13% of the 159 LHON families), 11778 (69%), or 14484 (14%). Conversely, the results in table 1 also indicate that 5 (3%) of these 160 LHON families in this survey do not carry one of these mutations and thus are predicted to carry as-yet-unidentified primary mutations.

It was further observed here that the 15257 mutation occurs in 6 of these 159 LHON families. However, in every one of these instances, it is associated with one of the three established primary LHON mutations: 11778 (4 of 78 families), 3460 (1 of 14 families), and 14484 (1 of 23 families). Because it does not occur in isolation of an established primary LHON mutation, the present results do not support a primary pathogenic role for the 15257 mutation. This conclusion, however, must be tempered with caution, for the following reasons. In the

Table 1

Primary Mutations in LHON Families

Mutation	No. of Families	No. of Affected Males	No. of Affected Females	Total No. of Matrilineal Progeny
Australia/New Zealand:				
11778	12	208	51	3,193
3460	1	4	2	38
14484	3	89	29	885
Unidentified	0	0	0	0
United Kingdom/Northern Ireland:				
11778	48	146	40	1,029[a]
3460	10	38	14	171[a]
14484	8	25	0	96[a]
Unidentified	1	2	0	Unknown
The Netherlands:				
11778	25	203	35	2,690
3460	5	28	10	294
14484	11	211	36	1765
Unidentified	1	2	1	10
Denmark:				
11778	19	171	41	1,154[a]
3460	2	26	0	170[a]
14484	1	12	1	206[a]
Unidentified	0	0	0	0
Finland:				
11778	6	48	14	303
3460	3	8	5	95
14484	0	0	0	0
Unidentified	3	4	6	61
Totals:				
11778	110	776	181	8,369[a]
3460	21	104	31	768[a]
14484	23	337	66	2,952[a]
Unidentified	5	8	7	71

[a] Pedigree data are incomplete; the numbers shown are the minimum estimates and are based on previously published studies.

first place, this study was specifically designed to assess which primary LHON mutations cause optic neuropathy with a penetrance sufficiently high to produce a clear-cut, multigenerational pattern of maternal inheritance. Thus, it cannot be ruled out that the 15257 mutation is a primary mutation but with a very low penetrance, thus obscuring maternal inheritance. Furthermore, the 15257 mutation has been detected in normal controls, in contrast to the three established primary mutations (Brown et al. 1992; E. Stone, personal communication), a finding that is compatible with no (or very low) pathogenicity. Alternatively, this mutation may have a secondary etiologic role, and its association with optic neuropathy would thus require other risk factors. A secondary role, however, has not been supported in previous studies (Howell et al. 1993; Oostra et al. 1994).

Second, how much bias was introduced into our study by exclusion of singleton LHON cases? Among the patient populations evaluated for this survey, there were 111 males and 37 females who were singleton cases of bilateral optic atrophy and who carried one of the three established primary LHON mutations. This figure represents ~10% of the total affected family members (table 1). Put another way, within these populations, an affected individual who carries one of the three primary mutations has a 90% chance of a positive family history. In a similar fashion, only ~8% of Australian bilateral optic atrophy patients without a positive family history carry one of the three primary LHON mutations (Chan et al. 1996). These findings provide further support for the conclusion that the three established primary LHON mutations produce a high-penetrance disorder (in the sense of a positive history of maternal transmission). On the other hand, they also indicate that there are relatively large populations of patients who *might* carry rare, unidentified pathogenic mitochondrial mutations. We would submit, however, that it will be extremely diffi-

cult, if not impossible, to distinguish benign polymorphisms from primary or secondary pathogenic mutations that have very low penetrance (see below). That ambiguity is why we favor the use of more stringent, more conservative criteria for pathogenicity.

Third, it is possible that our survey, although the largest of its kind, was biased and that other population samples would include LHON families who carry only the 15257 mutation and no other primary mutation. In this regard, there is a report of a German LHON family that carries the 15257 mutation but that lacks any of the three established primary mutations (Obermaier-Kusser et al. 1994). However, there is also the possibility of an unidentified primary mutation in the German LHON family, as is the probable case for five of the LHON families in the present study. It has been shown that LHON patients in Japan have a very high incidence of the 11778 primary mutation (Mashima et al. 1993); one inference to be drawn from this result is that there will also be a dearth of cases in which the 15257 could occur in isolation of a recognized primary LHON mutation.

One final point merits comment. The pathogenic role of the 15257 mutation was adduced on the basis of its increased frequency among LHON patients relative to its frequency among normal controls (Johns and Neufeld 1991; Brown et al. 1992). At first glance, this criterion is intuitively attractive. However, the 15257 mutation is almost always found in association with the putative secondary LHON mutations at nucleotides 4216 and 13708, and analysis indicates that the 15257 mutation arose once within a branch of this phylogenetic cluster (Howell et al. 1995; N. Howell, unpublished data). One consequence of this situation is that the increased frequency of the 15257 mutation among LHON patients can be explained solely in terms of population history and genetics, rather than as a reflection of its pathogenic role (Howell et al. 1995).

We have suggested that no single criterion should be used to establish the pathogenic role of a mutation in LHON (Howell 1994). Nevertheless, the available data suggest that the strongest support occurs for those mutations that are associated with an unambiguous pattern of maternal transmission and that have arisen multiple times within the human population. The pathogenicity of mitochondrial mutations in LHON patients is an important issue because of its relevance for diagnosis, for genetic counseling, and for elucidation of the pathway connecting the mutation to degeneration of the optic nerve. Furthermore, the possible role of mitochondrial mutations in the pathogenesis of the late-onset neurodegenerative diseases is being explored (Brown et al. 1996), and LHON will serve as a useful model system for these pathogenically complex disorders in which maternal inheritance is not an overt etiologic feature.

DAVID A. MACKEY,[1] ROELOF-JAN OOSTRA,[2] THOMAS ROSENBERG,[4] EEVA NIKOSKELAINEN,[5] JOAN BRONTE-STEWART,[7] JOANNA POULTON,[8] ANITA E. HARDING,[9] GREGOR GOVAN,[9] PIETER A. BOLHUIS,[3] SOREN NORBY,[10] ELISABETH M. BLEEKER-WAGEMAKERS,[2] MARJA-LIISA SAVONTAUS,[6] CHRISTOPHER CHAN,[1] AND NEIL HOWELL[11]

[1]*The Murdoch Institute and the Department of Ophthalmology, The University of Melbourne, Melbourne;* [2]*The Netherlands Ophthalmic Research Institute, and* [3]*Academic Medical Centre, Laboratory Neurozintuigen, Amsterdam;* [4]*National Eye Clinic for the Visually Impaired, Hellerup, Denmark;* [5]*Department of Ophthalmology, University of Turku, and* [6]*Department of Medical Genetics, Turku University Central Hospital, Turku, Finland;* [7]*Tennent Institute, Glasgow;* [8]*Department of Paediatrics, The John Radcliffe Hospital, Oxford;* [9]*The Institute of Neurology, London;* [10]*Department of Forensic Pathology, The University of Copenhagen, Copenhagen; and* [11]*Departments of Radiation Therapy and Human Biological Chemistry and Genetics, The University of Texas Medical Branch, Galveston*

Acknowledgments

The research reported here has been supported in part by grants from the Wellcome Trust (to D.A.M., J.P., A.E.H., and G.G.) and from the National Eye Institute (to N.H.).

References

Brown MD, Shoffner JM, Kim YL, Jun AS, Graham BH, Cabell MF, Gurley DS, et al (1996) Mitochondrial DNA sequence analysis of four Alzheimer's and Parkinson's disease patients. Am J Med Genet 61:283–289

Brown MD, Voljavec AS, Lott MT, Torroni A, Yang CC, Wallace DC (1992) Mitochondrial DNA complex I and III mutations associated with Leber's hereditary optic neuropathy. Genetics 130:163–173

Brown MD, Wallace DC (1994) Spectrum of mitochondrial DNA mutations in Leber's hereditary optic neuropathy. Clin Neurosci 2:138–145

Chan C, Mackey DA, Byrne E (1996) Sporadic Leber hereditary optic neuropathy in Australia and New Zealand. Aust NZ J Ophthalmol 24:7–14

De Vries DD, Went LN, Bruyn GW, Scholte HR, Hofstra RMW, Bolhuis PA, van Oost BA (1996) Genetic and biochemical impairment of mitochondrial complex I activity in a family with Leber hereditary optic neuropathy and hereditary spastic dystonia. Am J Hum Genet 58:703–711

Howell N (1994) Primary LHON mutations: trying to separate "fruyt" from "chaf." Clin Neurosci 2:130–137

Howell N, Bindoff LA, McCullough DA, Kubacka I, Poulton J, Mackey D, Taylor L, et al (1991) Leber hereditary optic

neuropathy: identification of the same mitochondrial ND1 mutation in six pedigrees. Am J Hum Genet 49:939–950

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, Kubacka I, Halvorson S, Mackey D (1993) Leber's hereditary optic neuropathy: the etiological role of a mutation in the mitochondrial cytochrome *b* gene. Genetics 133: 133–136

Huoponen K, Vilkki J, Aula P, Nikoskelainen EK, Savontaus M-L (1991) A new mtDNA mutation associated with Leber hereditary optic neuroretinopathy. Am J Hum Genet 48: 1147–1153

Johns DR (1994) Genotype-specific phenotypes in Leber's hereditary optic neuropathy. Clin Neurosci 2:146–150

Johns DR, Berman J (1991) Alternative, simultaneous complex I mitochondrial DNA mutations in Leber's hereditary optic neuropathy. Biochem Biophys Res Commun 174:1324–1330

Johns DR, Neufeld MJ (1991) Cytochrome b mutations in Leber hereditary optic neuropathy. Biochem Biophys Res Commun 181:1551–1557

——— (1993) Cytochrome c oxidase mutations in Leber hereditary optic neuropathy. Biochem Biophys Res Commun 196:810–815

Johns DR, Neufeld MJ, Park RD (1992) An ND-6 mitochondrial DNA mutation associated with Leber hereditary optic neuropathy. Biochem Biophys Res Commun 187:1551–1557

Jun AS, Brown MD, Wallace DC (1994) A mitochondrial DNA mutation at nucleotide pair 14459 of the NADH dehydrogenase subunit 6 gene associated with maternally inherited Leber hereditary optic neuropathy and dystonia. Proc Natl Acad Sci USA 91:6206–6210

Leber T (1871) Über hereditäre und congenital-angelegte Sehnervenleiden. Graefes Arch Clin Exp Ophthalmol 17(2): 249–291

Mackey D, Howell N (1992) A variant of Leber hereditary optic neuropathy characterized by recovery of vision and by an unusual multistep mitochondrial genetic etiology. Am J Hum Genet 51:1218–1228

Mashima Y, Hiida Y, Oguchi Y, Kudoh J, Shimizu N (1993) High frequency of mutations at position 11778 in mitochondrial ND4 gene in Japanese families with Leber's hereditary optic neuropathy. Hum Genet 92:101–102

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

Nikoskelainen EK, Marttila RJ, Huoponen K, Juvonnen V, Lamminen T, Sonninen P, Savontaus M-L (1995) Leber's "plus": neurological abnormalities in patients with Leber's hereditary optic neuropathy. J Neurol Neurosurg Psychiatry 59:160–164

Obermaier-Kusser B, Lorenz B, Schubring S, Paprotta A, Zerres K, Meitinger T, Meire F, et al (1994) Features of mtDNA mutation patterns in European pedigrees and sporadic cases with Leber hereditary optic neuropathy. Am J Hum Genet 55:1063–1066

Oostra RJ, Bolhuis PA, Zorn-Ende I, De Kok-Nazaruk MM, Bleeker-Wagemakers EM (1994) Leber's hereditary optic neuropathy: no significant evidence for primary or secondary pathogenicity of the 15257 mutation. Hum Genet 94: 265–270

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 118:319–338

Wallace DC (1995) Mitochondrial DNA variation in human evolution, degenerative disease, and aging. Am J Hum Genet 57:201–223

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AMS, Elsas LJ, et al (1988) Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242:1427–1430

Address for correspondence and reprints: Dr. Neil Howell, Biology Division 0656, Department of Radiation Therapy, The University of Texas Medical Branch, Galveston, TX 77555-0656.

© 1996 by The American Society of Human Genetics. All rights reserved.

0002-9297/96/5902-0031$02.00

Am. J. Hum. Genet. 59:485–486, 1996

Even a Deficit of Shared Marker Alleles in Affected Sib Pairs Can Yield Evidence for Linkage

To the Editor:

The affected-sib-pair method has been widely used in linkage analysis, especially for mapping genes involved in complex diseases, for which the mode of inheritance is difficult to specify. By this method, the observed distribution for the number of marker alleles shared identical by descent (ibd) is compared to the expected distribution in case of no linkage. It is generally believed (see, e.g., Lander and Schork 1994) that only an observed *excess* of alleles shared ibd should be considered as indicative for linkage between a marker locus and the disease. The following example demonstrates that this view may not be completely correct:

Suppose that in a sample of n affected sib pairs, $^2/_3$ of the sib pairs share zero marker alleles and $^1/_3$ share two marker alleles ibd. The likelihood for this observation in case that there is no linkage between marker and disease is $L_{H_0} = (^1/_4)^n$. Now consider the diallelic single locus model, which is specified by the penetrances f_i ($i = 0, 1, 2$) of the genotypes at the disease locus and the disease allele frequency p. Let z_j ($j = 0, 1, 2$) denote the probability that affected sib pairs share i marker alleles ibd. For $f_2 = \sqrt{^1/_2}$ and $p = f_1 = f_0$, these probabilities become $z_2 = ^1/_3$, $z_1 = ^4/_9$, and $z_0 = ^2/_9$, when the