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A Variant of Leber Hereditary Optic Neuropathy Characterized by Recovery of Vision and by an Unusual Mitochondrial Genetic Etiology

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Summary

The Tas2 and Vic2 Australian families are affected with a variant of Leber hereditary optic neuropathy (LHON). The risk of developing the optic neuropathy shows strict maternal inheritance, and the ophthalmological changes in affected family members are characteristic of LHON. However, in contrast to the common form of the disease, members of these two families show a high frequency of vision recovery. To ascertain the mitochondrial genetic etiology of the LHON in these families, both (*a*) the the nucleotide sequences of the seven mitochondrial genes encoding subunits of respiratory-chain complex I and (*b*) the mitochondrial cytochrome *b* gene were determined for representatives of both families. Neither family carries any of the previously identified primary mitochondrial LHON mutations: ND4/11778, ND1/3460, or ND1/4160. Instead, both LHON families carry multiple nucleotide changes in the mitochondrial complex I genes, which produce conservative amino acid changes. From the available sequence data, it is inferred that the Vic2 and Tas2 LHON families are phylogenetically related to each other and to a cluster of LHON families in which mutations in the mitochondrial cytochrome *b* gene have been hypothesized to play a primary etiological role. However, sequencing analysis establishes that the Vic2 and Tas2 LHON families do not carry these cytochrome *b* mutations. There are two hypotheses to account for the unusual mitochondrial genetic etiology of the LHON in the Tas2 and Vic2 LHON families. One possibility is that there is a primary LHON mutation within the mitochondrial genome but that it is at a site that was not included in the sequencing analyses. Alternatively, the disease in these families may result from the cumulative effects of multiple secondary LHON mutations that have less severe phenotypic consequences.

Introduction

Leber hereditary optic neuropathy (LHON) is a mitochondrial genetic disease in which there is a bilateral loss of central vision, usually during early adulthood. A number of studies have described the ophthalmological changes at the presymptomatic, acute, and atrophic stages of the disease (Smith et al. 1973; Nikoskelainen et al. 1983, 1984). As a general rule, the loss of vision is permanent, although improvement in some affected individuals has been reported, beginning with the original work of Leber (1871). Recovery of vision to varying degrees in LHON patients has been subsequently described by several groups (Bell 1931; Constantine 1955; Lessell et al. 1983; Holt et al. 1989; Stone et al. 1992). There is one study of a French-Canadian LHON family in which 20% of the affected individuals showed improvement in visual acuity to 20/50 in at least one eye (Brunette and Bernier 1970). Thus far, this is the only report of a LHON family in which there is a consistent and frequent improvement in vision after the acute phase.

Previous molecular genetic analyses of numerous LHON pedigrees have shown that the risk of developing the neuroretinopathy is usually associated with

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one of three primary mutations in the mitochondrial genes encoding subunits of respiratory-chain complex I (NADH–ubiquinone oxidoreductase). Beginning with the studies of Wallace et al. (1988), it has been established that 50%–70% of all LHON pedigrees carry a mitochondrial mutation at nucleotide 11778, which replaces the conserved ARG residue at position 340 of the ND4 protein by HIS. The ND4 protein is one of the seven mitochondrially encoded subunits of respiratory-chain complex I (NADH–ubiquinone oxidoreductase). Second, the disease in 15%–25% of all LHON pedigrees (about one-half of the non-ND4/11778 pedigrees) has been found to be caused by a mutation at nucleotide 3460, which results in the substitution of THR for the conserved ALA at position 52 of the ND1 protein (Howell et al. 1991*a*; Huoponen et al. 1991). Third, the members of a large Queensland LHON family carry a mutation at nucleotide 4160, which results in the replacement of the LEU at ND1 position 285 by PRO (Howell et al. 1991*b*). The disease in the Queensland family is an extreme form of LHON in which severe neurological abnormalities accompany the characteristic optic neuropathy (Wallace 1970). Most recently, two groups have concluded that a primary LHON mutation occurs within the mitochondrial cytochrome *b* gene in a small subset of LHON families (Johns and Neufeld 1991; Brown et al. 1992*a*).

There is accumulating evidence that the mitochondrial genetic etiology of LHON is more complicated than the occurrence of a single primary mutation. Johns and Berman (1991) reported three substitution polymorphisms, or secondary LHON mutations, within the mitochondrial complex I genes, which occurred at much higher frequencies among LHON patients than they did among normal controls. In contrast to primary LHON mutations, secondary LHON mutations result in conservative amino acid changes and occur in less well-conserved regions of the affected protein. Thus far, there has been no indication of the etiological or pathogenetic role played by these secondary LHON mutations.

A preliminary screening of multigeneration Australian LHON pedigrees revealed two families in which the disease was not associated with the ND4/11778, ND1/3460, or ND1/4160 mitochondrial mutations. They also had a neuroretinopathy that was atypical in showing a high frequency of vision recovery. The ophthalmological and molecular genetic analyses of these two families are the subject of the present investigation.

Experimental Procedures

Assessment of vision in affected patients included measurements of Snellen visual acuities, Humphrey visual fields, and Farnsworth Munsell 100 hue tests. Fundoscopic and general neurological examinations were also performed. Patients were examined during the late or atrophic stage of the neuroretinopathy, except for one member of the Tas2 LHON family who was followed throughout the acute phase. The affected members of the Vic2 LHON family have been examined at yearly intervals. The affected members of the Tas2 family were most recently examined in 1990, 1–20 years after the initial loss of vision. No member of the Tas2 or Vic2 LHON families who was examined in this study had any neurological abnormality beyond the ophthalmological changes.

The nucleotide sequencing analyses of the mitochondrial complex I genes were carried out as detailed in previous publications (Howell and McCullough 1990; Howell et al. 1991*a*). In brief, DNA was isolated from the white blood cell/platelet fraction of whole blood samples obtained with informed consent. Twenty-four pairs of synthetic oligonucleotide primers were used to PCR-amplify fragments of the mitochondrial genome that cumulatively span the seven complex I genes. Each fragment was cloned into an M13 sequencing vector, and the nucleotide sequence was determined by the dideoxy chain-termination method. The nucleotide sequence of the mitochondrial cytochrome *b* gene was determined using the same approach.

In these studies, the DNA sequence of the seven complex I genes and of the cytochrome *b* gene were determined in their entirety for one affected representative of each of the two families. These genes represent slightly more than 50% of the total length of the human mitochondrial genome. Gene regions containing nucleotide changes of interest were sequenced for an additional two or three family members. The same sequence changes were found in all family members. It should also be noted that 6–10 independent M13 clones were sequenced for each mitochondrial gene region of each individual who was analyzed.

Results

Genealogy of the Tas2 and Vic2 LHON Families

The Tas2 LHON family has a large pedigree that can be traced through 12 generations and includes 659 maternally related descendants (Mackey and Buttery,

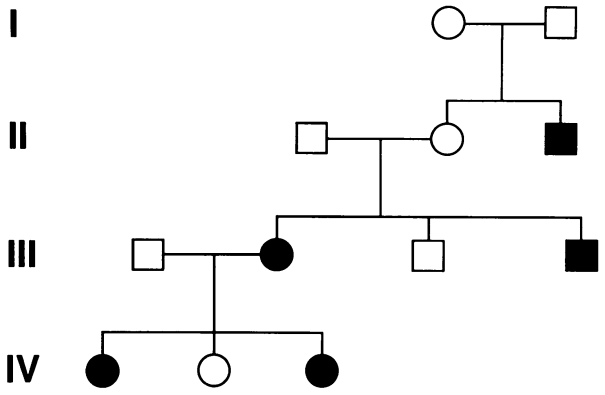

Figure 1 Pedigree of the Vic2 LHON family. The blackened symbols indicate the individuals affected with the optic neuropathy.

in press). Of these, 58 family members are known to have lost vision in a manner consistent with LHON. In a previous publication, the Tas2 family was designated "pedigree I" (Howell et al. 1991*a*). It has also been determined that an affected member of this family was examined while living in the United Kingdom and was previously designated "pedigree G" (Howell et al. 1991*a*). The Vic2 LHON family is much smaller, with 10 maternally related descendants spanning four generations, five of whom have lost vision (fig. 1). This family was previously designated "pedigree K" (Howell et al. 1991*a*).

Ophthalmological Studies

Thirteen affected members of the Tas2 LHON family and 4 affected members of the Vic2 family have been examined in the present study. The visual acuities for these individuals are summarized in table 1. Significant visual improvement in at least one eye was found in all 4 members of the Vic2 family and in 7 of 13 members of the Tas2 family. In an earlier study, Hamilton (1938) examined 3 other members of the Tas2 LHON family and observed recovery of vision in 2 of the 3. Further analysis of the data in table 1 indicates that recovery of vision is more likely to occur if vision is initially lost at a younger age. Thus, the mean age of those who recovered visual acuity to better than 6/36 in one eye was 17 years, while it was 34 years for those who did not recover visual acuity beyond 6/36. This difference is statistically significant ($P \approx .001$). Among the LHON patients who showed some degree of vision recovery, nearly one-half recovered vision to 6/15 or better, which compares with the 20% of the affected individuals in the Canadian LHON family who recovered vision to a similar level (Brunette and Bernier 1970).

Table 1

Visual Acuities in TAS2 and VIC2 LHON Patients

Patient (sex)	Age at Onset (years)	Visual Acuity[a]		Comment
		Right	Left	
TAS2 LHON family:				
BW (M)	39	2/36	2/60	No recovery
MF (M)	28	2/60	2/60	No recovery
NF (M)	9	6/5	6/5	Recovery over several years
MB (F)	48	1/60	1/36	No recovery
NG (M)	10	6/6	6/5	Recovery over 6 mo
HW (M)	28	6/60	6/60	No recovery
GK (M)	19	2/60	1/60	No recovery
SG (M)	16	6/4	6/4	Recovery over several years
AG (F)	14	6/4	6/4	Recovery over 2 years
ML (F)	26	6/24	6/9	Recovery over several years
AP (M)	40	<6/60	<6/60	No recovery
KL (M)	25	6/24	CF	Recovery
FM (M)	27	6/12	HM	Recovery
VIC2 LHON family:				
SP (F)	14	6/24	6/24	Recovery over 6 mo
CP (F)	6	6/7.5	6/36	Slow improvement throughout
MP (F)	17	6/18	6/60	Minimal improvement
MJ (M)	18	6/9	4/60	Improvement after 4 mo

[a] Expressed in meters. CF = patient can count fingers; and HM = patient can detect hand movements.

For the purposes of comparison, affected individuals from other Australian LHON families have been examined ophthalmologically. These families included those in whom the disease was associated with the primary mutations at nucleotide ND4/11778, ND1/3460, or ND1/4160. Visual improvement was observed in only one patient carrying the ND4/11778 mutation, although he had a minute island of vision rather than field recovery. The same pattern of vision recovery has been reported for two of the five ND4/11778 LHON patients described by Stone et al. (1992). Visual acuity better than 6/60 was also seen in one patient, of the five who were examined, with the ND1/3460 mutation. In the ND1/4160 Queensland LHON family, six of eight members who were examined had visual acuities better than 6/60 in at least one year. However, it is not known whether this finding for the Queensland LHON family represents recovery of vision or, alternatively, a milder loss of vision at the acute stage.

The standard Humphrey 30-2 threshold strategy was used to measure the visual fields in the Tas2 and Vic2 LHON patients. By use of an averaging program, the fields of 14 of these patients at the atrophic stage are shown in figure 2. For comparative purposes, the averaged fields of 20 unaffected controls and 5 ND4/11778 LHON patients are also included in figure 2. As a result of the frequent recovery of vision in the Tas2 and Vic2 LHON patients, the averaged visual field impairment is clearly less than that in the ND4/11778 LHON patients.

One member of the Tas2 LHON family (patient NG in table 1) was followed during the acute phase and into the atrophic phase. Visual field and acuity data for this patient are shown in figure 3. There is definite evidence that the centrocecal scotomas enlarge and then contract in parallel with the loss and then the recovery of vision.

Previous fundoscopic examination of a member of the Tas2 LHON family (patient SG in table 1) showed some peripapillary telangiectatic vessels associated with mild disk hyperemia. His younger brother (patient NG) showed disk hyperemia and swelling of the nerve fiber layer around the disk but had no telangiectatic vessels. Both of these affected individuals showed recovery of vision. At least for the Tas2 LHON family, therefore, these results do not support those of Lessell et al. (1983), who concluded that LHON patients who recover vision lack the microvascular abnormalities.

Farnsworth Munsell 100 hue tests (Munsell) were performed on both eyes of 13 patients. In all cases, there was a generalized decrease in color discrimination, with a slightly greater defect found in the Tritan axis. This finding is in contrast to the greater color defect in LHON patients with the ND4/11778 mutation and in whom the defect is more in the protan/deutan axis (D. Mackey, unpublished data).

Molecular Genetic Analyses

Preliminary sequencing studies demonstrated that the members of the Tas2 and Vic2 LHON families did not carry any of the previously identified primary LHON mutations: ND4/11778, ND1/3460, or ND1/4160. The complete DNA sequences of the seven mitochondrial complex I genes and of the cytochrome *b* gene were then determined for representatives of both LHON families. Relative to the standard Cambridge human mtDNA sequence (Anderson et al. 1981), a total of 16 nucleotide changes were found in these two LHON pedigrees; 6 of these result in silent polymorphisms (table 2). Also included in table 2 are the sequence changes occurring in the mitochondrial complex I genes and in the cytochrome *b* genes of the P1 LHON family reported recently by Brown et al. (1992*a*).

Of the 10 nucleotide substitutions that result in amino acid replacements, 5 are found in both the Vic2 and Tas2 LHON pedigrees, 3 occurred only in the Vic2 pedigree, and 2 occurred only in the Tas2 family. These mutations have the following points of interest:

a) The ND1/4216 and ND5/13708 mutations are two of the three substitution polymorphisms or secondary mutations that Johns and Berman (1991) found to occur with greater frequency in LHON pedigrees. They reported an increased frequency of both polymorphisms in ND4/11778 and non-ND4/11778 LHON pedigrees: the combination was detected in 4/36 (11%) and 9/28 (32%) pedigrees, respectively. We have detected the combination of these two secondary LHON mutations in 2/38 (5%) normal individuals (N. Howell, unpublished data). The 13708 secondary mutation has also been found in frequent association with the LHON mutation at nucleotide 15257 of the cytochrome *b* gene (Johns and Neufeld 1991). Johns and Neufeld (1991) found the 13708 mutation in three of eight ND1/3460 LHON patients, but we have not found it in any of the eight ND1/3460 LHON pedigrees that have been analyzed in this laboratory (Howell et al. 1991*a*; N. Howell, unpublished data).

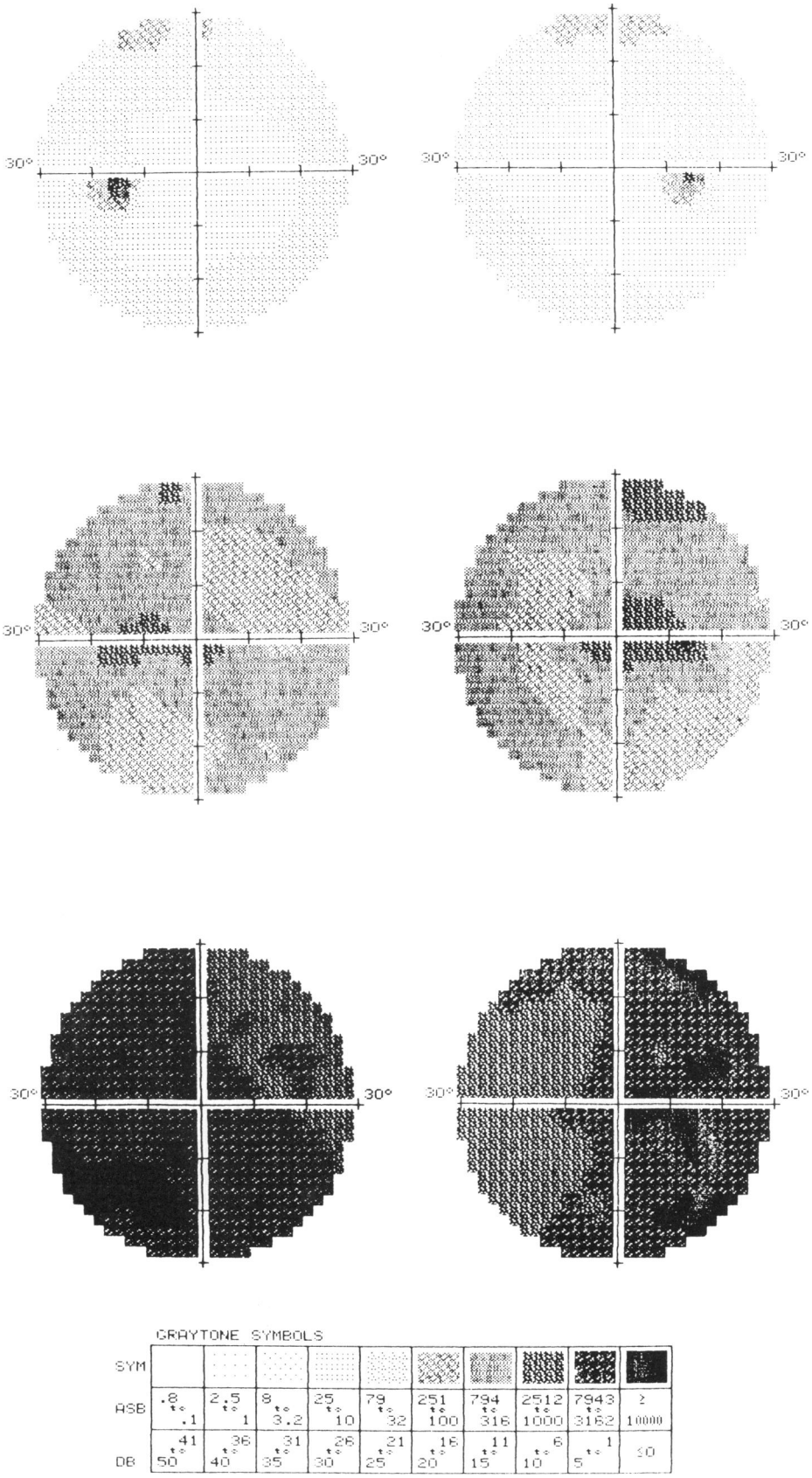

Figure 2 Visual field data for unaffected and affected LHON family members. The top set of results shows the averaged visual fields for 20 unaffected members of the Tas2 and Vic2 LHON families; the middle set shows the averaged results for 14 affected members of these two families; and the bottom set shows the averaged results for 5 affected individuals from ND4/11778 LHON families.

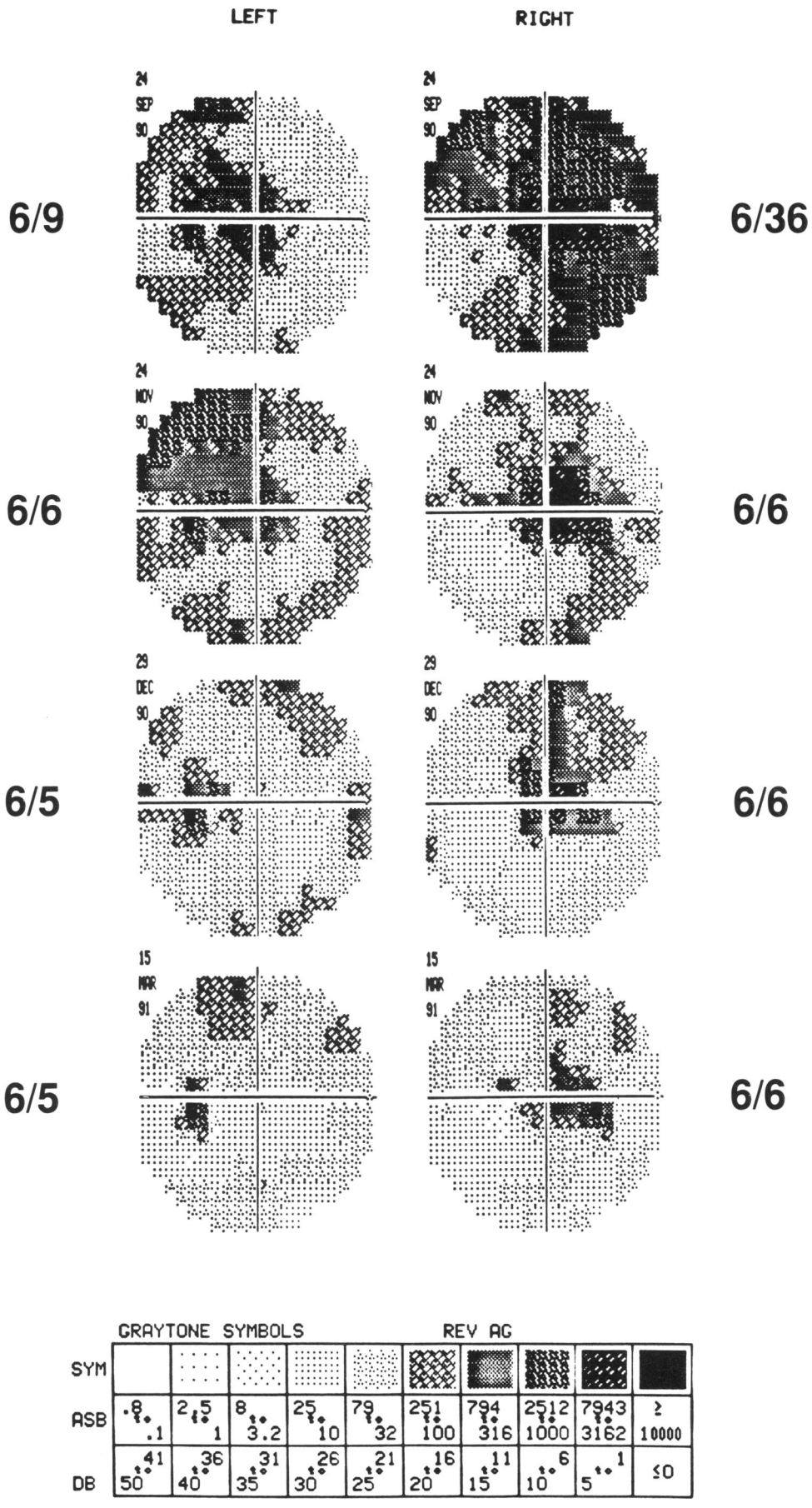

Figure 3 Visual field and acuity data for one member of the Tas2 LHON family. The patient was followed through the acute phase and into the period of recovery.

Table 2

TAS2 and VIC2 LHON Pedigrees: Nucleotide Changes in the Mitochondrial Complex I and Cytochrome *b* Genes

Gene/Nucleotide[a]	Amino Acid[b]	LHON Pedigree[c]
ND1/4216/T→C	Y304H	VIC2 + TAS2 + P1
ND2/4659/G→A	A64T	VIC2
ND2/5460/G→A	A331T	VIC2
ND3/10172/G→A	E38E	P1
ND3/10398/A→G	T114A	VIC2 + TAS2 + P1
ND4L/10685/G→A	A72A	TAS2
ND4/10966/T→C	T69T	P1
ND4/11251/A→G	L164L	VIC2 + TAS2 + P1
ND4/11719/G→A	G320G	TAS2 + P1
ND4/12007/G→A	415W	VIC2
ND5/12441/T→C	Y35Y	P1
ND5/12612/A→G	V92V	VIC2 + TAS2 + P1
ND5/13281/T→C	V315V	TAS2
ND5/13708/G→A	A458T	VIC2 + TAS2 + P1
ND5/13879/T→C	S515P	VIC2
ND5/13933/A→G	T533A	TAS2
ND6/14484/T→C	M64V	VIC2[d] + TAS2 + P1
CYB/14798/T→C	F18L	TAS2
CYB/15257/G→A	D171N	P1
CYB/15452/C→A	L236I	VIC2 + TAS2 + P1
CYB/15812/G→A	V357M	P1

[a] The nucleotide substitution is that which occurs in the L-strand sequence; this is the noncoding strand for all complex I genes except ND6.

[b] With the one-letter code, the first amino acid listed is the one that occurs in the wild-type protein. The number is the position of the residue within the protein. The second amino acid results from the indicated nucleotide change. Silent polymorphisms are those in which there is no change in the amino acid.

[c] Results for the P1 LHON family are taken from Brown et al. (1992*a*).

[d] Mutation was heteroplasmic in the VIC2 family (see Results).

b) The ND3/10398 polymorphism is unlikely to be of etiologic importance, as it occurs frequently both in non-LHON control individuals and in LHON pedigrees (Johns and Neufeld 1991; Noer et al. 1991).

c) The ND6/14484 mutation is heteroplasmic in the Vic2 LHON family. The affected female in the third generation (fig. 1) carried the mutation in 11 of 22 independent clones. One of her affected daughters contained the mutation in 27 of 31 clones, while the frequency for the unaffected daughter was 15 of 21. At first glance, the ND6/14484 mutation appears to be a simple polymorphism of no importance for the development of the disease. The substitution of VAL for MET at amino acid position 64 of the ND6 protein is a conservative amino acid substitution and one that occurs in the most rapidly evolving mitochondrial complex I gene. Among vertebrate mitochondrial ND6 genes, MET is also found in cows, but LEU occurs at this position in the protein from mice, rats, and *Xenopus* (Howell et al. 1991*b*). However, this sequence change has not been found in 36 normal individuals, 15 patients with non-LHON neurological or mitochondrial diseases, 22 ND4/11788 LHON pedigrees, or 8 ND1/3460 LHON pedigrees (N. Howell, unpublished data). Evidence indicating an etiological role for this mutation is discussed in a later section of this report.

d) Both the Tas2 and Vic2 LHON pedigrees carry substitution mutations altering the amino acid sequence in the carboxy-terminal region of the mitochondrial ND5 protein (table 2). Both the ND5/13879 (Vic2) and ND5/13933 (Tas2) mutations produce conservative amino acid substitutions in poorly conserved regions of the protein. These results argue against a primary etiological role for these changes. On the other hand, these specific mutations have not been found among more than 30 normal controls and 20 patients with other neurological or mitochondrial diseases, so a secondary etiological role cannot be ruled out.

e) The ND2/4659 and ND2/5460 nucleotide alterations produce conservative amino acid changes. However, the latter substitution, which occurs in the Vic2 LHON family, is interesting because it has recently been reported to occur at high frequency in patients with Alzheimer disease and in patients with amyotrophic lateral sclerosis (Lin et al. 1992).

f) The sequence change at nucleotide 14798 is unlikely to be of etiological significance, as the resulting amino acid change is conservative and occurs within the very poorly conserved N-terminal region of the cytochrome *b* protein (Howell 1989). Similarly, the sequence change at nucleotide 15452 produces a conservative amino change at a position where a number of aliphatic and aromatic residues are found (Howell 1989, and unpublished results).

The heteroplasmy of the mutation at nucleotide 14484 was striking, as this mutation is likely to play an etiological role in the LHON in these two families (see below). To ascertain whether any of the other potentially important sequence changes were also heteroplasmic, additional sequencing analyses were undertaken; the results are summarized in table 3. There is no evidence that any other sequence changes are heteroplasmic in this tissue source (WBC/patient fraction of whole blood), for either LHON family. Depending on the particular mutation that was analyzed,

Table 3

Sequencing Analyses of Complex I Mutations in the VIC2 and TAS2 LHON Families

Mutation[a]	LHON Family	No. of Family Members	Mutation Frequency[b]
ND1/4216	VIC2	Two	28/28
ND1/4216	TAS2	Four	34/34
ND5/13708	VIC2	Two	19/19
ND5/13708	TAS2	Four	39/39
ND5/13879	VIC2	Three	41/41
ND5/13933	TAS2	Four	58/58
ND6/14484	TAS2	Three	31/31
ND6/14484	VIC2	Three	53/74

[a] The complex I gene that was analyzed is followed by the nucleotide position.

[b] Results are expressed as the number of clones carrying the mutation (numerator) versus the total number of clones that were sequenced (denominator).

the frequency of undetected wild-type alleles can be no greater than 2%–5%.

A total of six silent polymorphisms in the seven mitochondrial complex I genes were found in the Vic2 and Tas2 LHON families (table 2). Two of these occur in both the Vic2 and Tas2 families, while one occurs only in the Vic2 family and three only in the Tas2 family. The silent polymorphisms at nucleotides 11251 and 12612 were found in the Vic2, Tas2, and P1 LHON families. The ND4/11719 silent polymorphism found in the Tas2 and P1 LHON families is not informative, as it occurs at high frequency in the human population (Noer et al. 1991; Ozawa et al. 1991; Howell et al. 1992).

In addition to the nucleotide changes listed in table 2, both the Vic2 and Tas2 LHON pedigrees carry the eight sequence changes that apparently represent errors in the original Cambridge sequence (Howell et al. 1992). In addition, both LHON families also carry the ND2/4769 and ND2/4985 silent polymorphisms, which appear to represent the most common alleles (>90%) in the human population.

Two studies have now reported that mutations within the mitochondrial cytochrome *b* gene are associated with LHON in a subset of families lacking primary mutations within the mitochondrial complex I genes (Johns and Neufeld 1991; Brown et al. 1992). Both studies found mutations at nucleotides 15257 (CYB/D171N) and 15812 (CYB/V356M), and both concluded that the former nucleotide alteration is likely to be the primary etiological mutation underlying the neuroretinopathy. Neither the Vic2 family nor the Tas2 LHON family carries either the mutation at nucleotide 15257 or that at nucleotide 15812 (table 2). Furthermore, neither LHON family carried any other mutations in these regions of the cytochrome *b* gene.

Discussion

Extensive ophthalmological studies of the Tas2 and Vic2 families establishes that both are affected with a LHON variant in which vision recovery is a frequent, though not universal, occurrence. Furthermore, recovery is more likely to occur if vision is lost at an early age, a phenomenon that was first noted by Evans (1917).

The increased recovery of vision observed in these two LHON families is associated with an unusual mitochondrial genetic etiology. The disease in the vast majority of LHON pedigrees can be associated with a single primary mitochondrial gene mutation (Wallace et al. 1988; Howell et al. 1991*a*, 1991*b*; Huoponen et al. 1991). In marked contrast, none of the mutations within either the seven complex I genes or the cytochrome *b* gene of the Vic2 or Tas2 LHON families is an obvious candidate for a primary LHON mutation: all the resulting amino acid changes are structurally/chemically conservative, occur at loosely conserved sites within the complex I subunit, or are found in normal individuals. As will be discussed below, establishing the etiology is further complicated by the evidence that the Tas2 and Vic2 LHON families are distantly related to each other, and, furthermore, both appear to be members of a phylogenetic cluster of LHON families (Brown et al. 1992*a*).

In addition to sharing four complex I nucleotide changes and one cytochrome *b* nucleotide change that alter the amino acid sequence, the Vic2 and Tas2 LHON families have two rare, silent polymorphisms in common (table 2). Since the Vic2 and Tas2 families differ at eight sites within the complex I genes and at one site within the cytochrome *b* gene, they are clearly not the same family. The relatively high number of shared sequence changes, however, establishes that these two LHON families are distantly related or within the same phylogenetic cluster. Moreover, while the Vic2 and Tas2 LHON families do not carry the cytochrome *b* mutations at either nucleotide 15257 or nucleotide 15812, they appear to be members of the phylogenetic cluster described by Brown et al. (1992*a*). As shown in table 2, the Vic2, Tas2, and P1 LHON families have seven nucleotide changes in

common, while Tas2 and P1 also share an eighth nucleotide change. In addition, both the Vic2 and Tas2 LHON families carry a CG:TA transition at nucleotide 16069 in the noncoding or D-loop region (N. Howell, unpublished data), while Brown et al. (1992*a*) report the loss of a restriction site at nucleotide 16065 in their cluster of LHON families. It is not yet clear whether the LHON families described by Johns and Neufeld (1991) are also members of this cluster, as their work focused on the cytochrome *b* mutations and the ND5/13708 mutation. Phylogenetic clustering of LHON families has not been reported previously. For example, eight LHON families carrying the ND1/3460 primary mutation show no pattern of shared polymorphisms within the complex I genes (Howell et al. 1991*a;* N. Howell, unpublished data).

Although the Vic2 and Tas2 LHON families belong to the phylogenetic cluster of LHON families described by Brown et al. (1992*a*), neither family carries the mutations in the cytochrome *b* gene that have been concluded to be etiologically important in the development of the disease. There are two hypotheses for the mitochondrial gene etiology of LHON in the Vic2 and Tas2 families. The first possibility is that both families have a primary LHON mutation but that it is in a mitochondrial genome region not included within our sequencing analyses. This is currently being investigated. Alternatively, the disease in these two families may result from the interaction of multiple complex I mutations, none of which produces the risk of LHON when present in isolation from the others. In view of the relatively milder ophthalmological changes in the Vic2 and Tas2 LHON families, we lean toward this possibility.

If the second possible mechanism is the one that is operating, then which mitochondrial complex I mutations might have an etiological role in the optic neuropathy in the Vic2 and Tas2 LHON families? Immediate attention is focused on the ND1/4216 and ND5/13708 secondary LHON mutations (Johns and Berman 1991). However, this same combination also occurs in normal individuals, so additional mitochondrial mutations would seem to be required for development of the disease. Although at first consideration it is an unlikely candidate, the ND4/14484 nucleotide change is a strong possibility. In addition to its presence in the Vic2, Tas2, and P1 LHON families (table 2), this nucleotide change was also found in the Queensland LHON family (Howell et al. 1991*b*). More important, the sequence data indicate that it arose independently in the Queensland LHON family, relative to its origin in the Vic2 and Tas2 LHON families. The mitochondrial complex I genes of the Queensland LHON family and those of the Tas2 LHON family differ at 14 nucleotide positions, sharing only the common silent polymorphism at nucleotide 11719 and the mutation at nucleotide 14484. When the Vic2 LHON family is used for the comparison, 15 site differences are found, with no shared sequence changes other than that at nucleotide 14484. An etiological role for this mutation would be supported if it were found to occur in other CYB/15257 LHON families that were analyzed by Johns and Neufeld (1991) and Brown et al. (1992*a*)—but not in the *normal* control designated "CC" who is also a member of this phylogenetic cluster (Brown et al. 1992*a,* fig. 4). We have recently detected the ND6/14484 nucleotide change in a patient with bilateral optic atrophy but without a family history of LHON; the preliminary results also indicate that this mutation arose independently in this pedigree (N. Howell, unpublished data). One final point of interest: the heteroplasmy of the 14484 mutation in the Vic2 LHON family may indicate a relatively recent origin, a possibility compatible with the small size of this LHON family.

As a heuristic exercise, one possibility to consider is that the ND6/14484 nucleotide change has an etiological role in LHON—but only when it is accompanied by other mitochondrial mutations such as the ND1/4216 and ND5/13708 secondary LHON mutations. In addition to these three nucleotide changes, we found other nucleotide changes—notably those in the ND5 gene—leading to amino acid substitutions in the Tas2 and Vic2 LHON families (table 2), and these may also contribute to the etiology or pathogenesis. One obstacle to reaching definite conclusions as to which sequence changes are etiologically important arises from the phylogenetic clustering of this group of LHON families. As a consequence of this, the presence of a shared mutation is not unequivocal, since it may be etiologically neutral and represent a hitchhiking effect.

The Vic2 and Tas2 LHON families described here present some novel features with regard both to the ophthalmological features of the disease and to its mitochondrial genetic etiology. Sequencing analyses of the mitochondrial genome from additional LHON families will be necessary to resolve the issues raised in these studies.

Note added in proof. — Our most recent sequencing studies demonstrate that neither the Vic2 family nor the Tas2 family carries the putative LHON mutation at nucleotide 7444 of the mitochondrial cytochrome *c* oxidase subunit I gene (Brown et al. 1992*b*).

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