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Leber Hereditary Optic Neuropathy: How Do Mitochondrial DNA Mutations Cause Degeneration of the Optic Nerve?

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Leber hereditary optic neuropathy (LHON) is an inherited form of bilateral optic atrophy in which the primary etiological event is a mutation in the mitochondrial genome. The optic neuropathy involves a loss of central vision due to degeneration of the retinal ganglion cells and optic nerve axons that subserve central vision. The primary mitochondrial mutation is necessary—but not sufficient—for development of the optic neuropathy, and secondary genetic and/or epigenetic risk factors must also be present although they are poorly defined at the present time. There is broad agreement that mutations at nucleotides 3460, 11778, and 14484 are primary LHON mutations, but there may also be other rare primary mutations. It appears that the three primary LHON mutations are associated with respiratory chain dysfunction, but the derangements may be relatively subtle. There is also debate on whether there are mitochondrial mutations that have a secondary etiological or pathogenic role in LHON. The specific pattern of the optic neuropathy may arise from a "chokepoint" in the optic nerve in the region of the nerve head and lamina cribosa, and which may be more severe in those LHON family members who become visually affected. It is hypothesized that the respiratory chain dysfunction leads to axoplasmic stasis and swelling, thereby blocking ganglion cell function and causing loss of vision. In some LHON patients, this loss of function is reversible in a substantial number of ganglion cells, but in others, a cell death pathway (probably apoptotic) is activated with subsequent extensive degeneration of the retinal ganglion cell layer and optic nerve.

KEY WORDS: Mitochondrial DNA; Leber hereditary optic neuropathy (LHON); complex I; neurodegeneration; optic nerve; maternal inheritance.

INTRODUCTION

In 1871, Theodor Leber described an inherited form of blindness that has since been designated Leber hereditary optic neuropathy (LHON). LHON is manifested as a bilateral, acute or subacute, loss of central vision in young adults, predominately males (reviewed in Nikoskelainen, 1994; Sherman and Kleiner, 1994; Riordan-Eva *et al.*, 1995; Nikoskelainen *et al.*, 1996; Howell, 1997). It was first observed by Leber (1871), and subsequently confirmed in numerous studies, that the risk of vision loss in LHON families is inherited exclusively from the mother. It is now recognized that this pattern of maternal inheritance signifies that the primary etiological event in LHON is a mutation within the mitochondrial genome (mtDNA). A number of other mitochondrial genetic diseases have been identified during the last few years (see the reviews elsewhere within this issue), but LHON is the most prevalent mitochondrial genetic disease and it is a significant cause of blindness in otherwise healthy males. The simple story is that mtDNA mutations lead to degeneration of the optic nerve. LHON, however, is more complex and challenging than this simple story, and it is the purpose here to highlight the unresolved issues that will form the impetus for future investigations.

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THE OPTIC NEUROPATHY IN LHON

Typically, the initial symptom in LHON is a blurring or clouding of vision, first in one eye and then in the other, that progresses, usually without pain, over a period of weeks or months to its nadir (rare, atypical presentations have been reported). Both eyes are often affected simultaneously and, with very rare exceptions, vision loss is bilateral. The initial field defect is an enlargement of the blindspot that progresses to an absolute central or cecocentral scotoma. Loss of visual acuity is typically severe and complete blindness can occur; males and females are affected to similar extents. Dyschromatopsia is invariably present. The mean age of onset is in the mid-20's for both sexes, although the range is remarkably broad, ranging from children under the age of 10 years to adults in their 70's. Both the electrophysiology (reviewed in Sherman and Kleiner, 1994) and the neuropathology (reviewed in Howell, 1997) indicate a selective degeneration of the ganglion cell layer and optic nerve with sparing of the photoreceptors, bipolar cell layer, and retinal pigment epithelium. One of the most peculiar features of LHON is the frequent preservation of pupillary responses (Sherman and Kleiner, 1994; Wakakura and Yokoe, 1995; Nikoskelainen *et al.*, 1996).

An early change in the acute phase is a pseudoedema of the nerve fiber layer and hyperemia of the optic disk; the disk subsequently "flattens" and becomes pale during the atrophic phase. The peripapillary nerve fiber layer disappears, initially in the papillomacular bundle. A characteristic feature of LHON is a peripapillary microangiopathy, first described by Leber (1871), which involves tortuous vessels in the central retina and telangiectatic capillaries (reviewed in Nikoskelainen, 1994; Riordan-Eva *et al.*, 1995; Nikoskelainen *et al.*, 1996). The microangiopathy is present both in presymptomatic individuals and in affected individuals during the acute phase, but it resolves during the atrophic phase.

The optic neuropathy in LHON families shows incomplete penetrance. Approximately 50% of the males and 10% of the females in large European LHON families become affected (e.g., Nikoskelainen, 1994; Riordan-Eva *et al.*, 1995; Mackey *et al.*, 1996). The incomplete penetrance indicates that, although the primary mtDNA mutation is the primary etiological factor in LHON, there must be *secondary* genetic and/or epigenetic factors which are necessary for manifestation of the optic neuropathy. The marked disparity in penetrance between males and females suggested an X-linked susceptibility locus that modifies pathophysiology (Vilkki *et al.*, 1991). More recent studies, however, have failed to locate such a locus (e.g., Juvonen *et al.*, 1993; Pegoraro *et al.*, 1996), and male predominance may be due to gender-based physiological and/or anatomical differences. There is evidence that heavy alcohol and/or tobacco use increases the risk of the optic neuropathy in LHON family members (Johns, 1994; Riordan-Eva *et al.*, 1995; Chalmers and Harding, 1996), but other environmental or physiological insults have also been associated with the onset of the acute stage. One of the difficulties in "pinning down" the secondary risk factors in LHON is that it is not clear if they must necessarily act immediately prior to the onset of the acute phase.

PATHOGENIC MITOCHONDRIAL LHON MUTATIONS

Wallace *et al.* (1988) were the first investigators to identify a pathogenic LHON mutation when they showed that several LHON families carried a mutation at nucleotide 11778 which changed a highly conserved arginine residue to histidine at amino acid position 340 (designated ND4/R340H) of the ND4 subunit of complex I (NADH-ubiquinone oxidoreductase). The mitochondrial genomes from numerous LHON pedigrees and sporadic patients have been analyzed since then to identify other pathogenic mutations, and there is now broad agreement that mutations at nucleotides 3460 (ND1/A52T) and 14484 (ND6/M64V) are also primary LHON mutations (Brown and Wallace, 1994; Howell 1994a,b; Brown *et al.*, 1995). However, one view is that there are many other mitochondrial mutations, affecting different respiratory chain complexes, that have a primary, secondary, or intermediate pathogenic role. Thus, Brown and Wallace (1994) list 16 mitochondrial mutations associated with LHON (their Table 1).

Another view is that the mutational spectrum of LHON is relatively narrow. Mackey *et al.* (1996) have analyzed 159 LHON families (totaling over 11,000 members, more than 1,400 of whom have been affected) from Northern Europe, the United Kingdom, and Australia. It was found that 153 families (96%) carried either the 3460 (21 families), the 11778 (109 families), or the 14484 (23 families) primary LHON mutations. Both the Johns and Wallace groups continue to vouchsafe a primary role for a mutation at nucleotide 15257 that alters an amino acid residue (D150N) in

the protonmotive cytochrome *b* subunit of complex III (see the discussion in Howell 1994a; Brown *et al.*, 1995; Mackey *et al.*, 1996). However, none of the 159 LHON families carried the 15257 mutation in isolation of one of the three "canonical" primary mutations. The disparity between these opposing viewpoints largely reflects the weight that different investigators place on sequencing studies of sporadic patients or small families where maternal inheritance is more problematic. It is likely that there are rare primary LHON mutations in addition to the three major ones (e.g., Howell *et al.*, 1993b; Leo-Kottler *et al.*, 1996), but their pathogenic status is much more difficult to establish.

In most LHON patients, the primary mutation is homoplasmic; that is, every mtDNA molecule carries the mutant allele. However, in a small proportion of LHON patients (approximately 15%), the primary mutation is heteroplasmic and there are two populations of mitochondrial DNA molecules, one carrying the wildtype allele and the other carrying the mutant allele (e.g., Ghosh *et al.*, 1996). Heteroplasmy of the primary mutation cannot account for the incomplete penetrance (see above) in LHON families because there are many large, well-characterized LHON families in which the primary mutation is homoplasmic.

Vision loss is usually permanent, but some LHON patients show objective improvement, sometimes to a dramatic degree. Recovery is rare in affected individuals who carry the 11778 primary mutation; for example, Johns (1994) cites a frequency of 4%. In marked contrast, approximately one-half of all 14484 LHON patients show improvement; the frequency of improvement is particularly high in those who lose vision before the age of 30 years (Mackey and Howell, 1992; Johns, 1994; Riordan-Eva *et al.*, 1995). Recovery of vision in 3460 LHON patients has an intermediate frequency (Johns, 1994).

Johns and Berman (1991) observed that sequence changes ND1/Y304H (at nucleotide 4216), ND2/N150D (4917), and ND5/A458T (13708) were present more frequently in 11778 LHON patients than in normal controls. On the basis of this statistically-significant association, they proposed that these sequence changes may function as *secondary* LHON mutations. In contrast to primary mutations, secondary mutations tend to encode more conservative amino acid changes and/or to alter less stringently conserved residues. It has been found subsequently that there is a particularly strong association of the 14484 primary mutation with the 4216 and 3708 secondary LHON mutations (Johns *et al.*, 1993; Brown *et al.*, 1995; Torroni *et al.*, 1996), although there is no evidence that the 3460 primary mutation shows any preferential association with these putative secondary LHON mutations.

What is the etiological and/or pathogenic significance of these secondary LHON mutations? This is an important question because 10–15% of all Europeans carry at least one of these putative secondary pathogenic mutations. Our preliminary phylogenetic analyses (Howell *et al.*, 1995) may provide a starting point for further investigation of this issue. In the first place, we showed that mtDNA haplotypes which carry the 4216, 4917, or 13708 secondary mutations form a single monophyletic cluster and that each mutation has arisen once within this cluster (Fig. 1). Furthermore, the putative pathogenic mutation at nucleotide 15257 has also arisen once within this cluster (also, see Brown *et al.*, 1995). One obvious conclusion, therefore, is that the 11778 and 14484 primary LHON mutations cannot be increasing the frequency at which the secondary mutations arise or are fixed within the population. Furthermore, it was also ascertained that the 11778 and 14484 primary mutations had arisen multiple times within this phylogenetic cluster (Howell *et al.*, 1995), and the association of these secondary mutations with the primary LHON mutations is thus not due to a marked "founder" effect (see Torroni *et al.*, 1996). It remains a possibility that the association results from the population history of the cluster (see the discussion in Howell *et al.*, 1995), although the multiple origins of the primary LHON mutations complicate such an interpretation. As a third possibility, the secondary mutations may increase the *penetrance* of the optic neuropathy and thereby create a sampling bias (Brown *et al.*, 1995; Torroni *et al.*, 1996). This proposed explanation seems to require that there are haplotypes in which the 11778 and 14484 primary mutations would be phenotypically (i.e., clinically) cryptic. Among Australian 11778 LHON families, we noted that the penetrance of the optic neuropathy was somewhat higher in families whose mitochondrial genomes carry the 4216 and 13708 secondary LHON mutations than in those who do not (Howell *et al.*, 1993a), but other studies have found no pathogenic effect of these secondary mutations (Oostra *et al.*, 1994). Penetrance is difficult to ascertain with certainty for several reasons, but this possible explanation merits further investigation. Finally, it is possible that one or more of these secondary mutations increases the origin or fixation of the 11778 and 14484 primary LHON mutations, although a plausible molecular mechanism is difficult to formulate.

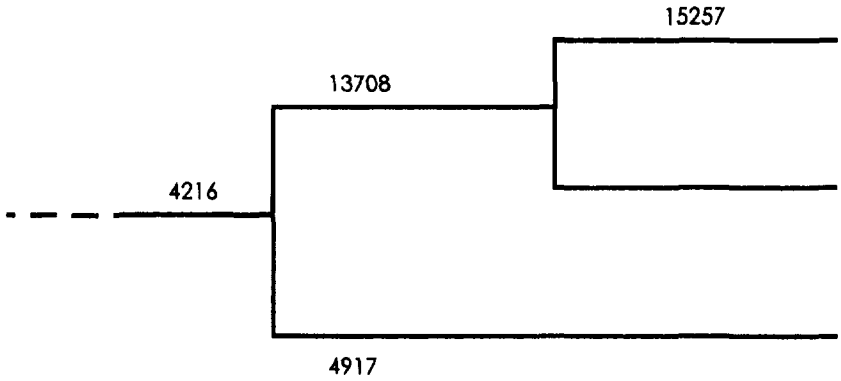

Fig. 1. The secondary LHON mutations arise within a single phylogenetic cluster. This figure shows a simplified phenogram of the phylogenetic cluster that carries the secondary LHON mutations (see Howell *et al.*, 1995 for further details). The 4216 mutation arose early and is present in all lineages within the cluster. In one sub-branch, the 4917 secondary mutation arose whereas the 13708 mutation arose within another main sub-branch. Within the 13708 sub-branch, there is a further "split" that is distinguished by the presence of the putative pathogenic mutation at nucleotide 15257.

NEUROLOGICAL ABNORMALITIES AND LHON

In addition to the optic neuropathy, LHON family members often display additional abnormalities including peripheral neuropathy, tremor, CNS signs, and heart conduction defects (e.g., Nikoskelainen *et al.*, 1995; Riordan-Eva *et al.*, 1995; Chalmers and Harding, 1996). Also, there appears to be an increased incidence of an MS-like demyelinating disorder in LHON families (e.g., Harding *et al.*, 1992; Flanigan and Johns, 1993). These observations raise an important question: are the ophthalmological and/or neurological abnormalities in LHON patients the consequence of an autoimmune pathophysiology? There is no evidence of inflammation in the retina and optic nerve from LHON patients (reviewed in Howell, 1997), and the electrophysiological studies establish the differences between the LHON optic neuropathy and the optic neuritis in MS patients (Sherman and Kleiner, 1994), both sets of results arguing against an autoimmune mechanism for the degeneration of the optic nerve (however, see Smith *et al.*, 1995 for the opposite viewpoint). On the other hand, it is possible that primary LHON mutations predispose certain individuals to a secondary, MS-like autoimmune disorder.

In addition to these "classical" LHON families, there is another group of families who show maternal inheritance of a LHON-like optic neuropathy combined with severe neurological abnormalities. For example, Wallace and coworkers have analyzed "LHON plus dystonia" families who carry a pathogenic mutation at nucleotide 14459 (ND6/A72V) which is associated with a defect in complex I (Shoffner *et al.*, 1995; Jun *et al.*, 1996). The proximity of this mutation to the primary LHON mutation at nucleotide 14484 is striking. The Queensland QLD1 LHON family, in which there is an array of severe neurological abnormalities (Wallace, 1970), has a different etiology. The optic neuropathy in this family is apparently caused by the 14484 primary LHON mutation whereas the neurological abnormalities are caused by a *second* pathogenic mutation at nucleotide 4160 (ND1/Y285P; Howell, 1994b). De Vries *et al.* (1996) have recently shown that a large Dutch family, in which the LHON-like optic neuropathy is associated with maternally transmitted hereditary spastic dystonia, carries both a heteroplasmic mutation at nucleotide 11696 (ND4/V312I) and a homoplasmic mutation at nucleotide at nucleotide 14596 (ND6/M26I). Either or both of these mutations may be the primary pathogenic cause of the disorder in this family.

MITOCHONDRIAL RESPIRATORY CHAIN FUNCTION IN LHON

Assays of mitochondrial respiratory chain function in LHON family members have yielded results that, at least superficially, are somewhat paradoxical. Larsson *et al.* (1991) isolated mitochondria from affected and unaffected members of an 11778 LHON

family and found no decrease in complex I specific activity (or in any other respiratory chain complex). However, flux through the entire chain with NADH-linked substrates was decreased to about one-half of the values found in mitochondria from normal controls, but there was no decrease in flux with succinate as substrate, which enters the chain through complex II. The authors suggested that complex I function was impaired at the level of its association with the proximal dehydrogenases. Essentially the same biochemical results were obtained by Majander *et al.* (1991) in studies with EB-virus transformed lymphocytes from 11778 LHON patients. Smith *et al.* (1994) detected a small decrease (25%) in platelet mitochondrial complex I specific activity when non-smoking 11778 LHON patients were compared to non-smoking controls; no difference was found when smokers were included in the two groups.

Vergani *et al.* (1995) have constructed cybrid lines in which mitochondria from control or 11778 LHON patients were transferred to cells that lack their own mtDNA (designated rho-zero lines). The 11778 primary LHON mutation caused decreased cellular respiration and lower mitochondrial complex I specific activity, although the latter decrease was not statistically significant. Similarly, a 40% decrease in complex I-linked mitochondrial respiration was measured by Hofhaus *et al.* (1996) in their 11778 cybrid lines although no decrease in the specific activity of complex I was detected.

The results for the 3460 primary mutation have been more clear-cut. In our initial studies (Howell *et al.*, 1991), platelet mitochondria were isolated from 3460 LHON family members. When specific activities of the respiratory chain complexes were normalized to the specific activity of mitochondrial citrate synthase, there was an approximately 80% reduction in complex I specific activity, whereas there was no reduction in those of complex II or complex IV (cytochrome oxidase). The complex I dysfunction was equally severe in both affected and unaffected LHON family members, and in both males and females (Howell *et al.*, 1991). Similar marked reductions were obtained by Majander *et al.* (1991) and by Smith *et al.* (1994).

The effect of the 14484 primary LHON mutation upon respiratory chain function has been particularly problematic. Parker *et al.* (1989) measured an approximately 80% reduction in complex I specific activity in platelet mitochondria from QLD1 LHON patients; there were statistically insignificant decreases in the activities of complexes III and IV. The interpretation of these results, however, is complicated by the simultaneous presence of the 14484 LHON and 4160 mutations. Cock *et al.* (1995) found no respiratory chain dysfunction in fibroblast mitochondria from "14484 only" LHON patients. Our preliminary results, however, indicate a marked complex I defect in 14484 fibroblast mitochondria (Bindoff *et al.*, manuscript in preparation). Finally, Oostra *et al.* (1995), in assays of mitochondria from leucocytes of 14484 LHON patients, observed that complex I specific activity was reduced more than 50% when normalized to the activity of citrate synthase or to that of complex IV. However, complex I-driven ATP synthesis was reduced only 20%.

We have measured the release of lactate and pyruvate from intact, dividing fibroblasts in an effort to avoid the problems that are associated with isolation of mitochondria and the assay of respiratory chain function. The lactate:pyruvate ratio is a sensitive indicator of the NADH/NAD balance and it will be, in large part, "set" by flux through the mitochondrial respiratory chain. When the respiratory chain is blocked, the NADH generated by the citric acid cycle and/or glycolysis accumulates and the lactate:pyruvate ratio increases. Our preliminary results indicate that all three primary LHON mutations are associated with mitochondrial respiratory chain dysfunction, although the defect associated with the 11778 mutation appears to be less severe (Howell *et al.*, manuscript in preparation).

Taken together, these results are paradoxical because the 11778 primary mutation is the most severe in terms of the optic neuropathy, but possibly the least severe in terms of the severity of the respiratory chain defect. One must be cautious in the interpretation of these biochemical studies, not least because they utilized cell types that are not those pathologically affected, but they indicate that the mechanistic pathway linking the primary mtDNA mutations and the optic neuropathy may not produce a simple correlation between clinical severity and complex I specific activity. Furthermore, the causal relationship between mitochondrial mutations and a focal degeneration of the ganglion cell layer and optic nerve suggests that LHON involves neurodegeneration of those cells (viz., retinal ganglion cells) that have the highest demand for mitochondrially-produced energy. However, previous studies of the bioenergetics of vision have not established that ganglion cell function is markedly dependent upon mitochondrial energy production. In fact, it has been recognized for 40 years that the photoreceptor layer

of the retina is the richest in the enzymes of oxidative metabolism (Lowry *et al.*, 1956). Furthermore, Ames *et al.* (1992) showed that phototransduction in the rabbit retina was dependent upon energy generated by the mitochondrial respiratory chain, whereas neurotransmission through the inner retina was fueled almost exclusively by glycolysis (also, see Rungger-Brandle *et al.*, 1996). On the other hand, chloramphenicol—an inhibitor of mitochondrial protein synthesis—produces an optic neuropathy in humans which is remarkably similar to that in LHON (reviewed in Howell, 1997). Those results indicate that the ganglion cell layer and optic nerve are exquisitely sensitive to the disruption of mitochondrial biogenesis. It is possible that mitochondrial function in the ganglion cell layer may be necessary to maintain the proper NAD/NADH redox balance, rather than for ATP biosynthesis *per se*. The fact that primary LHON mutations affect complex I subunits, and that complex I is the site of NADH reoxidation, appears more than coincidental when viewed within this metabolic context.

MODELS FOR OPTIC NERVE DEGENERATION IN LHON

The cumulative results reviewed here have established that the primary pathogenic event in LHON is a mtDNA mutation. Furthermore, but with more caution, the results indicate that there is a mechanistic linkage between respiratory chain dysfunction and the pathological end-stage, degeneration of the optic nerve and ganglion cell layer. What is the biochemical pathway that links a generalized respiratory chain dysfunction to a relatively specific pattern of neurodegeneration? Rizzo (1995) has recently hypothesized that the common mechanism in several optic neuropathies, including LHON, is a deficiency in mitochondrial ATP production. He further hypothesizes that the P (parvo or small) ganglion cells, which are primarily located in the central retina, are particularly susceptible to ATP depletion because of their high firing rate, compared to that of M (magno or large) ganglion cells, and to their sustained action potentials. However, the neurodegeneration in LHON appears to involve *both* M and P ganglion cells (Bindoff, Howell *et al.*, manuscript in preparation), and it appears that LHON involves a selective degeneration of the ganglion cells that subserve central vision.

Burde (1993) has developed the "disk at risk" concept in which he identifies the anatomical features that are common to several optic neuropathies including LHON: a relatively small optic nerve head, absent or small cup, increased branching of the central retinal vessels within the disk, and a crowding or "heaping up" of the nerve fiber layer. He further postulated that the region in and around the lamina cribosa is particularly vulnerable to ischemia because of the limited vascularization. Furthermore, the septae of the lamina cribosa are more closely packed and thicker walled along the horizontal meridian of the optic nerve (Fechtner and Weinreb, 1994), which are the areas that "channel" the fibers that subserve the central and temporal portion of the visual field, the regions that are more severely affected in LHON (also, see Sadun and Dao, 1994). There is also an accumulation of mitochondria in the unmyelinated segments of the prelamellar optic nerve axons before they transverse the lamina cribosa (Kageyama and Wong-Riley, 1984; Hollander *et al.*, 1995). This accumulation of mitochondria probably indicates impaired or labile axoplasmic transport, a "chokepoint," in this region of the optic nerve. Burde envisages that the respiratory chain defect in LHON patients produces compromised axoplasmic transport and axon swelling, which in turns leads to compression of the vessels and nerve fibers in this region, which produces an even more severe ischemia, ultimately causing an acute and major episode of neurodegeneration.

Riordan-Eva *et al.* (1995) have proposed that the LHON family members who become visually affected may have congenital crowding of the ganglion cell axons at the disk and in the peripapillary region, consequently leading to the degeneration of the smaller diameter M and P ganglion cells. It is interesting that Kageyama and Wong-Riley (1984) found that parafoveal ganglion cells in the monkey retina tended to be less intensely stained for cytochrome oxidase than those in the periphery and, furthermore, that the larger ganglion cells tended to be more intensely stained than those of medium and small size. Their results suggest that parafoveal ganglion cells may be "poorer" in mitochondrial content, and—one may extrapolate—more susceptible to disruption of the electron transfer chain.

One can thus envisage that the primary LHON mutation produces a respiratory chain defect that compromises axoplasmic transport, particularly in the lamina cribosa "chokepoint," but not so severely that there is a frank loss of retinal ganglion cell function (Fig. 2). Further physiological or environmental stress, particularly in those individuals that are anatomically "vulnerable," triggers a more profound slowing of axoplasmic transport to a level that precludes normal functioning of the smaller-diameter ganglion cells. It is not

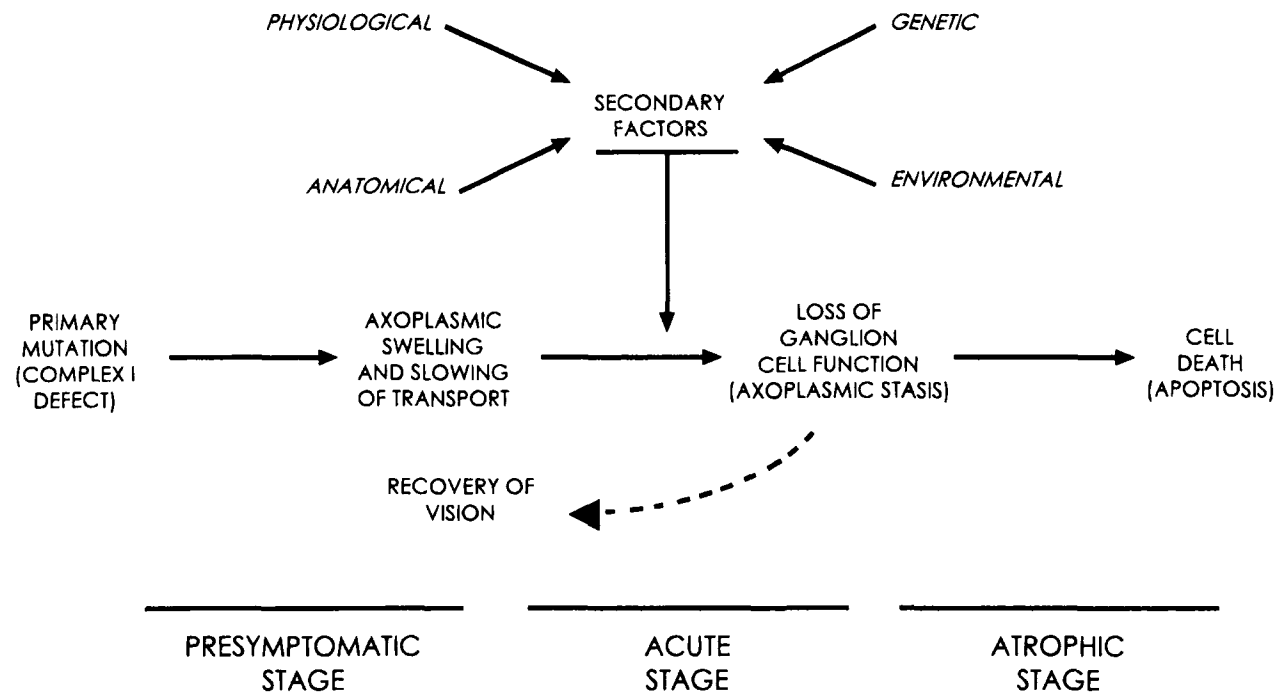

Fig. 2. A proposed scheme of the stages of the optic neuropathy in LHON. The central panel shows one possible pathway that links the primary LHON mutations and the degeneration of the optic nerve. The bottom panel shows the previously identified clinical stages of LHON. The top panel depicts the possible secondary etiological factors that are necessary for the development of the optic neuropathy.

yet understood, in terms of metabolism or cell biology, why some retinal ganglion cells, such as those that control the pupillary response, are relatively refractory. According to this hypothetical scheme, the peripapillary microangiopathy in presymptomatic LHON patients may be a *secondary* consequence of the swelling of the optic nerve axons in the anatomically constricted chokepoint that resolves during the acute phase because the neurodegeneration relieves the crowding in this region. The biochemical signal for neurodegeneration is presumably triggered at the stage of axoplasmic stasis. In the case of the 11778 primary mutation, for reasons which are not yet known, the "commitment to death" is made with only rare exception: the inactive cells die, probably through an apoptotic pathway. At the opposite extreme, the commitment to death is much less strong or less frequent for the 14484 primary mutation, and a substantial proportion of the retinal ganglion cells can remain inactive but viable for prolonged periods of time. If this hypothesis is correct, recovery of vision in 14484 LHON patients might occur as the result of a small amount of neurodegeneration, thus reversing the anatomical block to axoplasmic transport in the viable but inactive neurons. This suggested mechanism can also explain the peak incidence of LHON in the mid-20's: starting in the second decade, there is a slow loss of foveal ganglion cells (Gao and Hollyfield, 1992), thus widening the chokepoint and commensurately diminishing the subsequent risk of vision loss. It is tempting to speculate that the neurodegenerative pathway, at the biochemical level, proceeds through some type of chronic excitotoxicity, culminating in a wave of apoptosis in the ganglion cell layer (reviewed in Beal, 1995). One possible explanation for the differences among primary LHON mutations, in terms of recovery of vision, is that they differ in the levels of free radicals that are produced at the level of complex I, a key component in some excitotoxicity pathways. Further investigation will elucidate the intermediate steps between the primary LHON mutations and endpoint of neurodegeneration. This additional information will undoubtedly provide many fundamental insights, but—more importantly—it will allow the development of therapeutic strategies to prevent or minimize the devastating ophthalmological consequences of these mitochondrial mutations.

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