

Title: Leber Hereditary Optic Neuropathy

Short Title: LHON

Patrick Yu Wai Man, Douglass M. Turnbull, Patrick F. Chinnery

Department of Neurology, School of Neurosciences and Psychiatry, The Medical School, University of Newcastle Upon Tyne, UK.

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Corresponding Author:

Dr Patrick F. Chinnery

Department of Neurology

School of Neurosciences and Psychiatry

The Medical School

University of Newcastle Upon Tyne

Tel: 0191-222-8334, Fax: 0191-222-8553

P.F.Chinnery@ncl.ac.uk

Abstract

Leber Hereditary Optic Neuropathy (LHON) is a mitochondrial genetic disease that preferentially causes blindness in young adult males, affecting about 1 in 25,000 of the British population. It is characterised by bilateral subacute loss of central vision due to focal degeneration of the retinal ganglion cell layer and optic nerve. Over 95% of LHON cases are primarily due to one of three mitochondrial DNA (mtDNA) point mutations: G3460A, G11778A and T14484C, which all involve genes encoding complex I subunits of the respiratory chain. An intriguing feature of LHON is that only ~ 50% of males and ~ 10% of females who harbour a pathogenic mtDNA mutation actually develop the optic neuropathy. This marked incomplete penetrance and gender bias imply that additional mitochondrial and/or nuclear genetic factors must be modulating the phenotypic expression of LHON. It is also likely that environmental factors contribute to the onset of visual failure. However, these secondary precipitating factors remain poorly defined at the present time. In this review, we describe the natural history of this optic nerve disorder and highlight issues relating to clinical diagnosis, management and genetic counselling. We also discuss the findings of recently published studies and the light they shed on the complex aetiology and pathophysiology of LHON.

Keywords:

Mitochondrial DNA, optic atrophy, optic nerve, Lebers hereditary optic neuropathy, Lebers optic atrophy, optic neuropathy.

History

Leber Hereditary Optic Neuropathy (LHON, OMIM 535000) was first described as a distinctive clinical entity in 1871 by the German ophthalmologist Theodore Leber (1840-1917) [1]. He described a characteristic pattern of visual loss among members of four families and his observations were subsequently confirmed in pedigrees from different populations [2-6]. These early studies highlighted several of the salient features of LHON including the maternal transmission of the disease, the predilection of males to lose vision and the almost exclusive involvement of the optic nerve. The non-mendelian pattern of inheritance was to remain a mystery until more became known about the mitochondrial genome. The hypothesis that a mitochondrial DNA (mtDNA) mutation was the causative factor in LHON then became firmly established.

Pathogenic Mutations

In one retrospective study, over 95% of LHON pedigrees harboured one of three mtDNA point mutations: G3460A, G11778A and T14484C, which all involve genes encoding complex I subunits of the mitochondrial respiratory chain [7]. These primary LHON mutations have so far not been found in a large sample of normal controls, without a family history of visual failure (> 1,000). However, the relative frequency of each of these pathogenic mutations varies considerably worldwide. A meta-analysis involving 159 pedigrees from Northern Europe and Australia showed that G11778A was the most prevalent LHON mutation (Table 1). The predominance of G11778A is even more marked in the Far East where it accounts for ~ 90% of total LHON cases [8]. Although T14484C is relatively rare in most countries [8, 9], it is the most common mutation found among French Canadians (87%). This has recently been convincingly ascribed to a founder event [10, 11].

Primary mutations have not been identified in a small minority of diagnosed LHON cases, the most likely explanation being that rare pathogenic mtDNA variants are segregating in these pedigrees. Some of these have recently been identified and most seem to cluster in the gene encoding the ND6 subunit. It has been suggested by some investigators that the latter represent a mutational hot spot for LHON and should be investigated in pedigrees where none of the three primary mutations are present [12].

Epidemiology

LHON is by far the most common of the mitochondrial genetic diseases, with an estimated prevalence of ~ 1 in 25,000 in the North East of England [23]. No prevalence data is currently available for other populations, although 2% of people on the blind register in Australia are reported to suffer from LHON [24]. The reported median age of onset in LHON varies somewhat between various case series but 95% of those who lose their vision do so by their early 50's (Table 2). However, visual deterioration can occur anytime during the first to the seventh decade of life. Except for one report which found a slight increase in the age of onset in females carrying the G11778A mutation [9], it is generally accepted that neither gender nor mutational status significantly influences the timing and severity of the initial visual loss [10, 25-27]. LHON is also characterised by a marked gender bias, with males more likely to become affected than females. Finally, up to 60% of LHON probands will give a reliable history of other maternal relatives being affected. The remainder most likely represent cases where family history is difficult to trace back, given that *de novo* mutation is rare in LHON [28].

Clinical Features

Acute Phase

LHON carriers remain asymptomatic until they experience blurring or clouding of vision in one eye. In the vast majority of cases, visual dysfunction is bilateral, the fellow eye becoming affected either simultaneously (25%) or sequentially (75%), with a median inter-eye delay of 8 weeks [9]. Visual acuity usually reaches a nadir 4-6 weeks after the first start of symptoms and is severely reduced to 6/60 or less. The characteristic field defect in LHON is a centrocaecal scotoma. Other clinical features include the early impairment of colour perception but more importantly, pupillary reflexes are preserved and patients usually report no pain on eye movement.

Fundoscopy provides other diagnostic clues and in classical cases the following abnormalities can be observed: vascular tortuosity of the central retinal vessels, a circumpapillary telangiectatic microangiopathy and swelling of the retinal nerve fibre layer (Figure 1). However, it must be stressed that in ~ 20% of LHON cases, the optic disc looks entirely normal in the acute phase [29].

Chronic Phase

The retinal nerve fibre layer gradually degenerates and after 6 months, optic atrophy is a universal feature of LHON [30]. If a patient is only seen at this stage, it can be difficult to exclude other possible causes of optic atrophy, especially if there is no clear maternal family history. In these cases, molecular genetic testing is warranted. The extent of final visual recovery depends greatly on the patient's mutational status, with G11778A carrying the worst overall prognosis (Table 2). There is also some evidence that patients with the T14484C mutation are more likely to show improvement if visual loss occurs before the age of 20 [27, 31]. However, LHON is a

devastating disorder with the majority of patients showing no functional improvement and remaining within the legal requirement for blind registration.

Associated Features

A significant minority of Caucasian LHON carriers, especially females with the G11778A mutation, develop clinical and neuroimaging features indistinguishable from multiple sclerosis (MS), including unmatched oligoclonal bands in the cerebrospinal fluid [32-36]. The prevalence of this MS-like illness in LHON is higher than expected due to chance only and some investigators have argued for a potential role of autoimmunity in the pathophysiology of this mitochondrial disorder [32, 37]. It is of note that higher levels of antibodies to the optic nerve protein tubulin have been found among LHON carriers compared to controls [38]. However, other reports have failed to detect any significant association between LHON and either class I or class II major histocompatibility complex (MHC) genotypes [39, 40]. Overall, the “autoimmunity” hypothesis has not yet been convincingly substantiated. Other clinical abnormalities have also been reported to be more common in LHON compared to controls. These include postural tremor, peripheral neuropathy, non-specific myopathy, movement disorders and cardiac arrhythmias [31, 41-46]. The jury is still out as to whether these represent real or spurious associations.

However, it is now well recognised that a group of LHON pedigrees does exist characterised by optic neuropathy and additional severe neurological deficits (spastic dystonia, ataxia and juvenile onset encephalopathy). These “LHON +” syndromes have been linked to various mtDNA mutations in isolated pedigrees from Holland, Australia and North America: A11696G and/or T14596A [22], T4160C [47] and

G14459A [19] respectively. So far, none of the three primary LHON mutations have been linked to such severe phenotypes.

Diagnosis

A tentative diagnosis of LHON can usually be made based solely on the patient's clinical history. A series of baseline investigations are usually carried out. These include a formal assessment of visual fields with static or kinetic perimetry and fluorescein angiography which in LHON reveals no leakage, implying that the microvasculature is not compromised. Electrophysiological studies, including electroretinograms and visual evoked potentials (VEPs), can be carried out to exclude retinal pathology and confirm optic nerve dysfunction. A more in-depth review of the electrophysiological features in LHON is provided by Sherman and Kleiner [48]. CT and MRI scans are usually normal in LHON although there are some case reports of non-enhancing high signals within the optic nerve and sheath distension [49-54]. The latter is thought to represent slight oedema or gliosis in the atrophic phase. Cranial imaging is sometimes required in those situations where other inflammatory or structural causes of acute optic neuropathy need to be excluded. In singleton cases with atypical clinical features and no clear maternal history of blindness, molecular genetic testing for the three primary LHON mutations will usually clarify matters.

Neuropathology

To date, there are no pathological data on the acute phase of LHON. Post mortem studies have been carried out mostly on elderly patients who had experienced visual loss several decades earlier [55, 56]. In some of the early reports, the mutational status of the patient is also unknown although the clinical history was highly suggestive of LHON [49, 50, 57, 58]. These limitations notwithstanding, the

neuropathology in LHON seems to be limited to the retinal ganglion cell layer with sparing of the retinal pigment epithelium and photoreceptor layer. There is marked cell body and axonal degeneration, with associated demyelination and atrophy observed from the optic nerves to the lateral geniculate bodies. Apoptosis is thought to be involved although this has yet to be formally demonstrated [59].

Biochemical Features

Since all three primary LHON mutations involve complex I subunits, one would expect respiratory chain function to be compromised, leading to deficient ATP production and subsequent degeneration of retinal ganglion cells as a consequence of energy failure. However, both *in vitro* and *in vivo* biochemical studies have produced conflicting results regarding the extent of respiratory chain dysfunction in LHON (Table 3). These variations could be partly due to the different protocols used. For example, a wide range of cell types was assayed in these experiments, including platelets, leukocytes, fibroblasts and skeletal muscle, to name just a few. For obvious technical reasons, it is not possible to investigate retinal ganglion cells directly. A striking feature of these studies is that none found any significant difference between affected and unaffected LHON carriers. Balancing the current weight of evidence, it seems that LHON is associated with a rather more subtle respiratory chain defect when compared to other pathogenic mtDNA mutations (such as the A3243G in the syndrome of mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS)).

Pathophysiology

These functional studies also raise important issues regarding the pathophysiology of LHON (Figure 2). The most obvious one relates to the focal degeneration of the optic nerve. One could argue that only retinal ganglion cells are affected because they require a sustained level of ATP for normal function. This is supported by a recent study that found a high level of mitochondrial enzyme activity in retinal ganglion cells and the nerve fiber layer [78]. However, two points mitigate against this assertion. Firstly, it has been shown quite conclusively that photoreceptors have a much higher oxidative demand than retinal ganglion cells [79, 80]. Secondly, it is difficult to reconcile why other mitochondrial disorders characterised by more severe complex I defects do not universally cause optic atrophy (Jan Smeitink, personal communication). Although purely speculative, it is possible that retinal ganglion cells are preferentially involved because they are somehow exquisitely sensitive to subtle imbalances in cellular redox state or increased level of free radicals [81].

The clinical picture of LHON shows some overlap with that of autosomal dominant optic atrophy (ADOA) and in both disorders, optic nerve dysfunction results from the selective degeneration of the retinal ganglion cell layer [82]. Therefore, it is of great interest that the causative gene in ADOA has recently been identified as being a dynamin-related GTPase, located on chromosome 3q28-29 [83, 84]. This protein has a highly basic amino-terminal domain that targets it to the mitochondria and preliminary studies in yeast indicate important roles in vesicular transport and outer membrane integrity [85-87]. These findings add further weight to the long-held view that the maintenance of retinal ganglion cells is heavily dependent upon normal mitochondrial function. The development of faithful animal models will hopefully provide us with a better insight into the still obscure pathophysiology of LHON.

Incomplete Penetrance

An intriguing feature of LHON is that only ~ 50% of males and ~ 10% of females who harbour one of the three primary mutations actually develop the optic neuropathy [5, 88-90]. This incomplete penetrance and predilection for males to lose vision imply that additional genetic and/or environmental factors must modulate the phenotypic expression of LHON. Alternatively, the gender bias could also result from a combination of subtle anatomical, hormonal and/or physiological variations between males and females [25, 56, 91].

Mitochondrial Genetic Factors

Heteroplasmy

In most LHON pedigrees, the primary mutation is homoplasmic (every mtDNA molecule harbours the mutant allele). By contrast, 10-15% of LHON carriers are thought to be heteroplasmic, with one mtDNA sub-population carrying the wild type allele [9, 92]. It has been suggested that heteroplasmy might influence the expression and inheritance pattern of LHON but there have been no rigorous prospective studies to address this possibility [93-97]. Preliminary data suggest that heteroplasmy might contribute to incomplete penetrance, with the risk of blindness being minimal if the mutational load is less than 60% [98].

MtDNA Haplogroups

There still exists some controversy surrounding the pathogenic role of so-called “secondary” mtDNA mutations in LHON: T4216C, A4917G, G9804A, G9438A, G13708A, G15257A and G15812A [99-101]. These nucleotide substitutions are found at a higher frequency in LHON patients relative to controls and some

investigators argue that they act in synergy with the primary mutations, increasing the risk of disease expression. Based on phylogenetic analysis, it has been shown that T4216C, G13708A, G15257A and G15812A all cluster on a specific mtDNA background, haplogroup J (reviewed in Torroni and Wallace [102]). Several studies have subsequently found that LHON pedigrees, T14484C and to a lesser extent G11778A, are not randomly distributed along the phylogenetic tree but show a strong preferential association with haplogroup J [103-105]. This could be due to an early founder effect whereby the G11778A and T14484C mutations arose early in the evolution of haplogroup J, leading to its over-representation on that mitochondrial lineage [106]. However, this explanation is unlikely given that it has been shown convincingly that all three primary LHON mutations have arisen multiple times on different mitochondrial backgrounds [104]. Moreover, up to now, none of these mtDNA mutations have been found in normal controls belonging to haplogroup J. The most compelling explanation is that the risk of visual loss in patients harbouring a primary LHON mutation is increased by haplogroup J and by extension one or more of the polymorphisms that defines it [107]. If this specific mtDNA background does have a deleterious effect, one would expect haplogroup J to result in a more pronounced respiratory chain defect. Cybrid cell lines carrying the G11778A mutation and haplogroup J were shown to have a lower oxygen consumption and a longer doubling time compared to cell lines with the G11778A mutation alone [67]. However, a recently published study using *in vivo* MRS failed to detect any deleterious effect in brain and skeletal muscle, with haplogroup J not further impairing mitochondrial oxidative metabolism in patients harbouring the G11778A mutation [108]. The influence of haplogroup J on the biochemical features of the T14484C mutation has not yet been determined. This would be interesting to clarify

given the much stronger association of haplogroup J with T14484C compared to G11778A. However, as already mentioned, these biochemical studies are not without their own limitations and require cautious interpretation.

Haplogroup J is one of nine European-specific haplogroups and therefore one would expect LHON to be more common in populations of European extraction. However, this hypothesis is difficult to assess given the current paucity of data regarding the prevalence of this mitochondrial disorder in different ethnic groups. Haplogroup J is not thought to influence age of onset or final visual outcome in LHON but this requires further confirmation in a larger LHON cohort [30, 109]. The question also remains as to why G3460A pedigrees do not show a skewed haplogroup distribution. The hypothesis that haplogroup J increases penetrance for the G11778A and T14484C mutations is not widely accepted and further studies are required to clarify matters.

Nuclear Genetic Factors

Segregation Analysis

The predominance of affected males in LHON cannot be explained by mitochondrial inheritance. Segregation analysis of a large number of pedigrees from diverse ethnic groups suggests the existence of a recessive X-linked susceptibility gene acting in synergy with the mtDNA mutation [9, 110, 111]. In the *Bu and Rotter* model, development of blindness in males is consistent with the simultaneous inheritance of an X-linked visual loss susceptibility allele and the LHON mutation. Females are affected either if they are homozygous at the susceptibility locus (40%) or heterozygous with skewed X-chromosome inactivation (60%). The gene frequency for the susceptibility locus was proposed to be 0.08 and the estimated penetrance in a heterozygous female 0.11.

Linkage Analysis

Attempts to identify this X-linked susceptibility locus by standard linkage analysis have so far been unsuccessful [112-116]. According to the *Bu and Rotter* model, a proportion of heterozygous female will be affected as a result of unfortunate lyonization of the “normal” X-chromosome. Mathematical modelling suggests that visual loss in women will only occur if at least 60-83% of retinal ganglion cells harbour the visual loss susceptibility allele [117]. However, a number of studies have failed to demonstrate skewed X-chromosome inactivation in the leukocyte fraction of affected female carriers [115, 118-120]. Despite these negative results, it would be premature to conclude that there are *no* additional nuclear genetic factors modulating the expression of the primary LHON mtDNA mutations. The situation may be highly complex, with the existence of genetic heterogeneity and the epistatic interaction of multiple nuclear susceptibility loci.

Environmental Factors

Five pairs of monozygotic twins harbouring a primary LHON mutation have been reported in the literature [9, 25, 28, 123-125]. In two cases, the twins remained discordant, although there is always the possibility that the unaffected sibling will lose vision later on in life. The existence of discordant monozygotic twins does not exclude the possibility of nuclear genetic factors in LHON but strongly suggests that environmental factors also contribute to penetrance. Anecdotal evidence suggests that smoking, alcohol, nutritional deprivation, psychological stress or acute illness can precipitate the onset of blindness in LHON [41, 126-131]. However, a recently published case-control study failed to confirm the association between heavy smoking or alcohol intake and an increased risk of visual loss [132].

Potential environmental triggers have not been extensively investigated because of the logistic problems inherent to the proper conduct of case control studies for a rare disease. The most obvious limitation of these types of retrospective studies is the possibility of recall bias given that most patients are interviewed several years after they lost vision. This makes it very difficult to obtain reliable data regarding not only possible exposure to environmental triggers but also to quantify their duration and intensity. A possible solution round this problem will be to set up a longitudinal study involving the long term and regular follow-up of a large cohort of unaffected LHON carriers. Although an attractive option, this will almost certainly require a multi-centre collaborative effort in order to collect sufficient number of subjects.

Clinical Management

Prevention

No generally accepted measures have been shown to either prevent or delay the onset of blindness in LHON. In spite of this, for general health reasons, it would be wise to advice unaffected LHON carriers to moderate their alcohol intake and stop smoking. There is therefore no need for long-term follow up of asymptomatic carriers in the clinic.

Treatment

There is currently no treatment available that improves the final visual outcome in LHON. One small, non-randomised trial claimed that oral administration of a quinone analogue (idebedone) and vitamin supplementation (B₁₂ and C) can speed up visual recovery [133]. However, more rigorous studies are required before such a

regimen can be advocated during the acute phase. The long-term management of visually impaired patients is mainly supportive.

Genetic Counselling

Once a primary LHON mutation has been identified in a proband, other family members can be offered molecular genetic testing to exclude the possibility of a *de novo* mutation. The latter is exceedingly rare and has only been previously demonstrated in the literature for the T14484C mutation [28]. We recently confirmed the occurrence of a *de novo* G3460A mutation in a pedigree from the North East of England (Patrick Yu Wai Man, unpublished data). Since LHON shows strict maternal inheritance, males can be reassured that none of their children will inherit the mtDNA mutation. On the other hand, females will transmit the pathogenic mutation to all of their offsprings. Since most mothers are homoplasmic, their children will only harbour the mutant allele and their lifetime risk of losing vision can be derived from established gender- and age -dependent penetrance figures, as detailed below. The situation is rather more complicated for a heteroplasmic mother given the theoretical possibility that she could transmit only a low level of mutant mtDNA to a particular offspring. However, genetic counselling is not straightforward for unaffected carriers who are found to be heteroplasmic for a primary LHON mutation. Although, there is a suggestion that a mutational threshold of ~ 60% is necessary for disease expression, it must be stressed these are only preliminary findings and require further confirmation [98].

Some indication of recurrence risks can be provided to maternal relatives of a LHON proband (Table 4). However, robust estimates for the G3460A mutation have not yet been determined in a large number of pedigrees and although these are unlikely to

differ significantly from the G11778A and T14484C mutations, any extrapolation should be done with caution. It is important for LHON carriers to be made aware that it is currently not possible to predict accurately whether or when they will become affected. Despite these caveats, the two main predictive factors for visual failure remain age and gender. Males have a 50% lifetime risk of blindness compared to only 10% for females but these approximate figures can be further refined based upon the patient's age. From published age-dependent penetrance data, we know that most patients experience visual loss in their late teens or early 20's and the probability of becoming affected is minimal once past the age of 50 [10, 31, 89].

Conclusions

LHON is a mitochondrial genetic disease characterized by bilateral subacute loss of central vision due to focal degeneration of the optic nerve. The vast majority of cases are due to one of three mtDNA point mutations: G3460A, G11778A and T14484C, which all involve genes encoding complex I subunits of the respiratory chain. With molecular genetic testing now routinely available, this has greatly facilitated clinical diagnosis, especially in atypical cases. However, many aspects of the complex aetiology of LHON remain poorly defined at the present time. The incomplete penetrance and sex bias clearly indicate that, although necessary, the mtDNA mutation is insufficient on its own for disease manifestation. The identification of the secondary factors modulating the phenotypic expression of LHON is currently an area of intense research. Better characterisation of the relationship between mtDNA mutations, mitochondrial biogenesis and optic nerve dysfunction is also needed to clarify the still unclear pathophysiology of LHON. Progress in all of these areas is a

prerequisite for both improved genetic counselling and the development of future therapeutic strategies.

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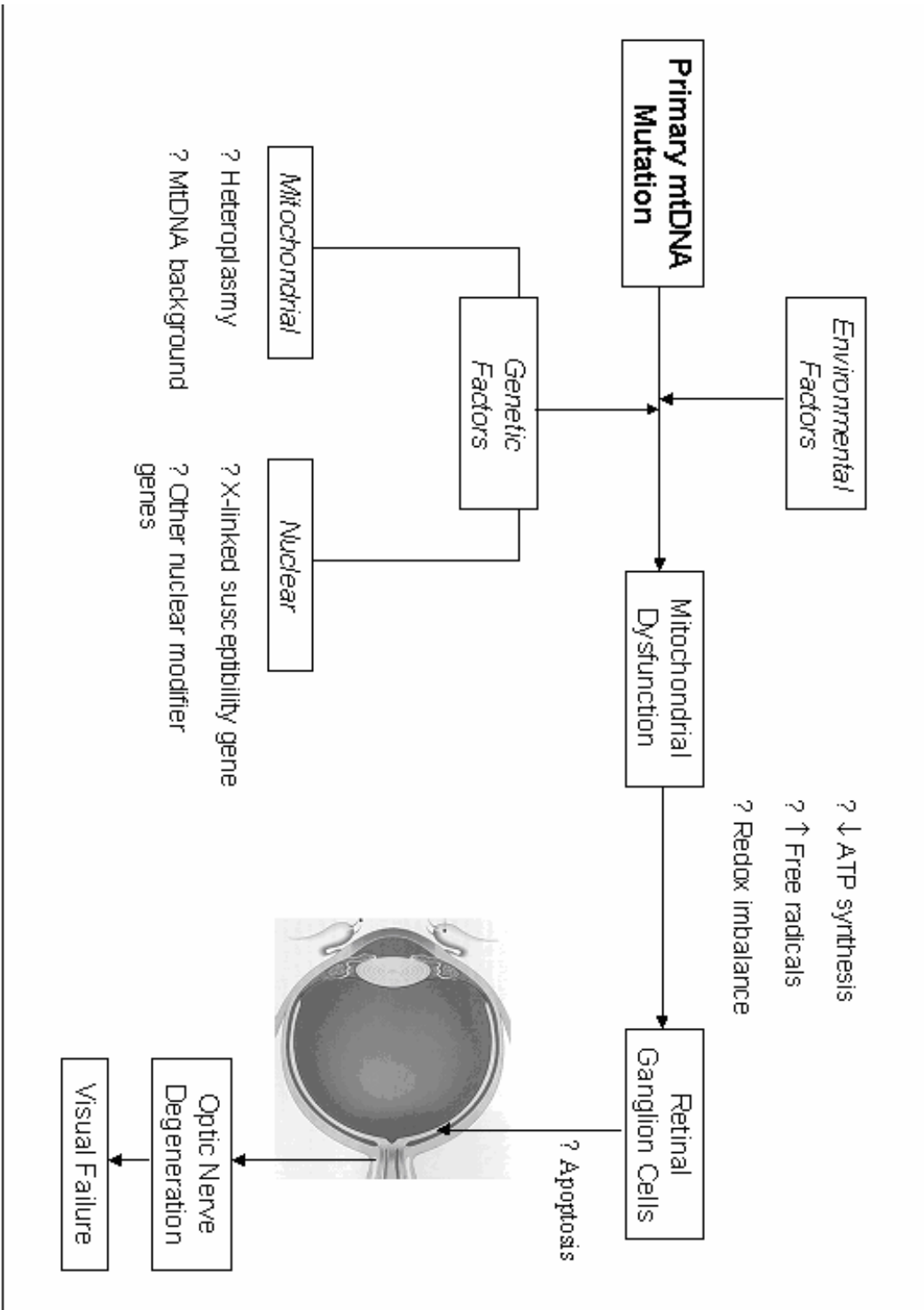
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Table 1 Reported pathogenic primary mtDNA mutations in LHON

	Mutation	Protein	Prevalence [7]	Reference
Primary			> 95%	
	G3460A	ND1	13%	[14, 15]
	G11778A	ND4	69%	[13]
	T14484C	ND6	14%	[16, 17]
Rare			< 5%	
	G13730A	ND5		[18]
	G14459A	ND6		[19]
	C14482G	ND6		[20]
	A14495G	ND6		[12]
	C14498T	ND6		[21]
	C14568T	ND6		[21]
	T14596A	ND6		[22]

Table 2 Summary of major reported case series in LHON

	Pedigrees N =	Median Onset	Male:Female Ratio	Visual Recovery	Reference
G3460A	9	29 yrs	2.3 : 1	22%	[26]
	8	20 yrs	4.3 : 1	25%	[9]
G11778A	49	28 yrs	4.5 : 1	4%	[25]
	66	24 yrs	3.7 : 1	25%	[9]
T14484C	17	27 yrs	2.1 : 1	37%	[27]
	23	19 yrs	7.7 : 1	58%	[10]

Table 3 Respiratory chain dysfunction in LHON

	In Vitro [60-74]		In Vivo [75-77]
	Complex I Activity*	Respiratory Rate *	MRS*
G3460A	60-80%	30-35%	0%
G11778A	0-50%	30-50%	75%
T14484C	0-65%	10-20%	50%

* : % Decrease relative to controls

Table 4 Recurrence risks for relatives of LHON probands

	Risk of Visual Loss (%)	
	G11778A [9]	T14484C [10]
Siblings		
Brother	25	28
Sister	8	5
Sister's Children		
Nephew	41	30
Niece	17	3
Maternal First Cousins		
Male	30	19
Female	7	4