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Review

Clinical, biochemical and molecular genetic features of Leber's hereditary optic neuropathy

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Abstract

Leber's hereditary optic neuropathy (LHON) has traditionally been considered a disease causing severe and permanent visual loss in young adult males. In nearly all families with LHON it is associated with one of three pathogenic mitochondrial DNA (mtDNA) mutations, at bp 11778, 3460 or 14484. The availability of mtDNA confirmation of a diagnosis of LHON has demonstrated that LHON occurs with a wider range of age at onset and more commonly in females than previously recognised. In addition, analysis of patients grouped according to mtDNA mutation has demonstrated differences both in the clinical features of visual failure and in recurrence risks to relatives associated with each of the pathogenic mtDNA mutations. Whilst pathogenic mtDNA mutations are required for the development of LHON, other factors must be reponsible for the variable penetrance and male predominance of this condition. Available data on a number of hypotheses including the role of an additional X-linked visual loss susceptibility locus, impaired mitochondrial respiratory chain activity, mtDNA heteroplasmy, environmental factors and autoimmunity are discussed. Subacute visual failure is seen in association with all three pathogenic LHON mutations. However, the clinical and experimental data reviewed suggest differences in the phenotype associated with each of the three mutations which may reflect variation in the disease mechanisms resulting in this common end-point.

Keywords: Leber's hereditary optic neuropathy; Mitochondrial DNA

Contents

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1. Introduction

The first descriptions of subacute, bilateral visual loss in young adult males date back to the early 19th century but the majority of these are poorly documented. In reviewing these and providing a description of three brothers affected by the same disease, Von Graefe (1858) recognised this as a form of hereditary optic neuropathy [1]. However, Leber (1871) was the first to provide an accurate description of the clinical features and inheritance of the condition that now bears his name [2]. He reported four families which included members who developed subacute, sequential visual failure. He noted that the disease was hereditary, could be transmitted by an unaffected mother and that more males were affected than females. Further descriptions of families with the same phenotype followed in kindreds from diverse ethnic backgrounds [3–6] but there remained considerable debate as to the mode of inheritance of the disease.

The demonstration that classical Leber's hereditary optic neuropathy (LHON) is only inherited through females [7] and the identification of exclusive maternal transmission of mitochondrial DNA (mtDNA) raised the hypothesis that an mtDNA defect might be responsible [8].

2. Mitochondrial DNA mutations in LHON

2.1. Pathogenic mtDNA mutations associated with LHON

MtDNA involvement in LHON was confirmed when Wallace and colleagues identified a point mutation in mtDNA in nine out of 11 families with LHON from Finland and the USA [9]. This mutation, at bp 11 778, produces an amino acid change in NADH dehydrogenase subunit 4 (ND4) of complex I of the respiratory chain, and it was present in all maternally related members of the LHON families, regardless of whether they were affected or not. Subsequent work has identified two further mtDNA mutations in LHON patients at bp 3460 in ND1 [10,11] and bp 14 484 in ND6 [12] of complex I. These three mutations are thought to be pathogenic (primary) mutations in LHON. They are found exclusively in families with LHON and never in control subjects. In many patients with the 11 778, 3460 and 14 484 mutations, and particularly in their unaffected relatives, there is a degree of heteroplasmy in mtDNA from blood [12–15]. Heteroplasmy is rarely described in relation to harmless polymorphisms, but is an almost universal finding in individuals with the mtDNA mutations that are associated with other mitochondrial encephalomyopathies. In one study of 159 LHON

families from Europe and Australasia, 153 (97%) carried one of the three pathogenic LHON mutations [16].

Other mtDNA bp changes, that have not been demonstrated in controls, have been described in a small number of cases in which LHON is the presumed diagnosis. These include changes at bp 5244, bp 9101, bp 9804, bp 14 482 and bp 14 498 [17–21]. Further evidence is required before the pathogenic significance of these base pair changes can be determined but, in at least some populations, mtDNA mutations other than the 11 778, 3460 and 14 484 mutations are presumed to be responsible for a significant proportion of LHON cases [22].

2.2. 'Secondary' mtDNA base pair changes

A number of other mitochondrial DNA substitutions have been described in families with LHON and some investigators have termed these 'secondary' mutations (e.g. see [23]), although they are found in the normal population. Many of these were first described in families lacking the 11 778 mutation, prior to the identification of the 3460 and 14 484 mutations, and it is not known how many of these families have one of these pathogenic LHON mutations. It has been suggested that these 'secondary' mutations cause visual loss when associated with other 'secondary' mutations in the same individual. This hypothesis is difficult to test, given the high degree of polymorphism seen in mtDNA. Some authors, however, have suggested that these changes are merely harmless polymorphisms of mtDNA. This hypothesis is supported by three observations. First, none have ever been seen in heteroplasmic form. Second, they occur in combination with pathogenic LHON mutations [24–26]. Third, when this occurs they do not alter the phenotype of the disease [24–26]. Further, it is clear that the frequency of these base pair changes in some populations may be higher than previously recognised [27].

2.3. MtDNA haplotype analysis in LHON

A possible role for these 'secondary' base pair changes in the development of visual failure in LHON has been suggested by haplotype analysis. A number (including base pair changes at 4216, 4917, 13 708, 15 257 and 15 812) are found in specific combinations, which have been used to define haplotype groupings and hence an evolutionary pattern for mtDNA. Haplotype analysis of patients with LHON from a number of populations suggests that the 3460 mutation is distributed randomly among haplotypes. However, the 14 484 mutation and, to a lesser extent the 11 778 mutation, occur on one specific haplotype (termed haplotype J) considerably more frequently than expected [22,28–30].

A number of explanations for this association have been advanced. First, the pathogenic LHON mutation may have occurred once early in the evolution of haplotype J. However, analysis of the evolution of haplotype J subgroups suggests that this would then require loss of the pathogenic LHON mutation in some subgroups. Second, haplotype J may be more susceptible to mutational events producing the pathogenic LHON mutation, although there is no other evidence to support this. Third, it is possible that haplotype J may increase the probability of an individual carrying the pathogenic LHON mutation developing visual failure, thus creating a sampling error. This explanation requires that some families who carry the pathogenic LHON mutation should be asymptomatic (particularly those families without haplotype J mtDNA) and this is not supported by available data. In addition, it is clear that in some cases the 14 484 mutation can be associated with visual failure in the absence of any of the haplotype J base pair changes [31]. Further research may clarify these unresolved issues.

3. Clinical features of LHON associated with pathogenic mutations

3.1. Male predominance and age at onset

Early series of Caucasian pedigrees reported that 80–90% of affected members were male [32–35], but a recent study in the UK found that only 73% of genetically proven cases were male [36]. This difference may reflect increased recognition of LHON in females as a result of the identification of pathogenic mtDNA mutations, particularly in isolated cases. The ability to confirm the diagnosis of LHON in atypical cases has also demonstrated that the range

of age at onset is wider than previously recognised. Whilst the majority of patients do develop visual loss in the second and third decades of life and 95% of patients are affected by the age of 50 years [15], age at onset can range between the first and seventh decades [35–37].

3.2. Visual failure

The visual disturbance in LHON is typically sequential with a median inter-eye delay of 8 weeks. Acuities decline over a period of 4–6 weeks to 6/60 or less, colour vision is lost early, and visual field loss initially consists of an enlarged blind spot that progresses to involve central vision producing a large centrocaecal scotoma. Some patients report pain in an affected eye or on eye movements, and a small proportion experience Uthoff's phenomenon [35,36, 38,39].

Fundoscopy classically reveals a circumpapillary telangiectatic microangiopathy with swelling of the peripapillary retinal nerve fibre layer. Fluorescein angiography usually does not show staining or leakage from the telangiectatic vessels. The combination of these features is said to be pathognomic of LHON [40]. A few weeks after the onset of visual loss, small vessels on the temporal side of the disc become attenuated. Axonal loss continues over several months, such that frank optic disc pallor is virtually universal after 6 months [36].

Visual evoked potentials (VEPs) can be normal in patients with early visual impairment. In more severe cases, VEPs are reduced in amplitude, delayed or desynchronised and are generally less prolonged in latency and smaller than VEPs recorded in patients with known demyelinating optic neuropathy [41,42]. In patients with severe visual loss of long duration, VEPs are usually absent.

Abnormalities of colour vision, fundal changes and atypical VEPs [41–43] have been reported in a proportion of asymptomatic male and female members of families with LHON in whom they may predict the later development of visual loss [33,43]. These data predate genetic confirmation of the diagnosis of LHON and further studies are needed to clarify their significance.

Computerised tomography (CT) of the brain and optic nerves is usually normal in LHON, although distension of the optic nerve sheaths was reported in one case [44]. Standard magnetic resonance imaging (MRI) of the optic nerves is normal [45], but scans using short time inversion recovery (STIR) sequences show increased signal in the mid and posterior intraorbital sections that is thought to represent gliosis [46]. Two cases studied using fast spin echo (FSE) showed bright signal in the optic nerve associated with small optic nerve sheath complexes and absence of CSF surrounding the nerves [47].

There are no pathological data on patients with early optic nerve disease. Autopsy studies of patients with long-standing visual loss show marked neuronal cell body and axonal degeneration with associated loss of myelin, mainly involving the retinal ganglion cell layer, the optic nerves, optic chiasm, optic tracts and lateral geniculate bodies [48,49].

3.3. Variation in the visual failure associated with pathogenic LHON mutations

Leber and other early workers recognised that some patients with LHON have a good visual prognosis [2], and the identification of pathogenic LHON mutations has clarified this phenotypic variability. Large series of patients with each of the three pathogenic mutations have been studied in the USA [35,38,39] and in the UK [36].

First, such series show that the incidence of fundal appearances characteristic of LHON during the acute phase of visual failure varies between mutation groups. Thus, the majority (approximately 75%) of patients with the 11 778 or 3460 mutations examined during the acute phase of LHON had typical fundal appearances, but this was true of only a minority ($\sim$40%) of patients with the 14 484 mutation.

Second, although the degree of initial visual loss is similar between mutation groups, there is clear variation in the final visual outcome between patients with the 11 778 and 3460 mutations and patients with the 14 484 mutation. This is well illustrated by the UK study [36], which found that the proportions of patients with final visual acuities worse than 6/60 in the better eye (classified as partially blind) were high ($\sim$75%) for the 11 778 and 3460 mutations and considerably lower ($\sim$30%) for the 14 484 mutation. The degree of visual recovery in patients with the 14 484 mutation is inversely correlated with age, a

feature that is not seen with the 11 778 or 3460 mutations.

3.4. Additional features

Harding and colleagues reported six females with a clinical illness indistinguishable from multiple sclerosis (MS), as defined by the Poser criteria, occurring in the context of a family history of LHON and the demonstration of the 11 778 mutation [50]. In all cases visual failure was a prominent, early feature and patients subsequently developed a progressive or relapsing illness indistinguishable from MS. The clinical diagnosis of MS was supported by the demonstration of widespread white matter lesions on MRI, as seen in MS, in the five patients scanned and by abnormalities of CSF analysis and evoked potentials. Two women with LHON and the 11 778 mutation, but without additional signs, had similar MRI abnormalities.

Other reports of this association in women with the 11 778 mutation have followed [51,52], and in UK families 45% of females with LHON and the 11 778 mutation have an MS-like illness [36]. In contrast, it has been reported rarely in males [51,53] or in patients with the 3460 mutation [27], and it has not been reported in patients with the 14 484 mutation.

Other neurological abnormalities have been described in patients with LHON and a pathogenic mtDNA mutation at 11 778, 3460 or 14 484. In a series of Finnish patients additional signs included movement disorders, kyphosis, polyneuropathy, epilepsy, dementia and brain stem involvement [52]. Such additional abnormalities are unusual in other large series – in one series of 72 patients with the 11 778 mutation only five patients had other disorders (neuropathy, non-specific muscle weakness, and a previous diagnosis of poliomyelitis) and these were considered to be coincidental [35]. A case-control study of UK patients identified an increased incidence of tremor in patients with LHON, but no other neurological features were identified at higher frequency in patients than in controls [54]. There have been individual case reports of patients with the 11 778 mutation and LHON together with a variety of additional features [55,56]. Similarly, in large series of patients with the 3460 and 14 484 mutations, additional neurological features were thought to be coincidental or alcohol related [36,38,39], although there have been case reports of individuals with additional neurological features [57,58].

The suggestion that Finnish patients with LHON and their relatives are at increased risk of cardiac pre-excitation syndromes [59] has not been supported by studies of UK, Australian and US families [36,60,61]. Other ECG abnormalities have been reported in LHON [34,61].

4. Clinical features of LHON-like illness seen in association with other mtDNA mutations

Other mtDNA mutations are associated with the development of subacute visual failure and additional prominent neurological features. Members of families who carry the heteroplasmic mtDNA mutation at bp 14 459 develop a LHON-like illness associated with dystonia [62,63]. This was first identified in an Hispanic family with clinical manifestations ranging from normal, through late-onset optic atrophy, to early-onset dystonia associated with bilateral basal ganglia degeneration. When the mutation approaches homoplasmy, the penetrance is high, with 48% of maternal relatives manifesting childhood-onset dystonia, 10% a LHON-like illness, and 3% visual failure and dystonia. A Dutch family with a heteroplasmic mtDNA mutation at bp 11 696 (together with a homoplasmic bp change at 14 596) also manifest a combination of an LHON-like illness and dystonia [64]. In a large Queensland family with a homoplasmic bp 4160 mutation in addition to the 14 484 LHON mutation, nearly half the patients had a severe encephalitic illness in childhood [65,66]. Patients who recovered had optic atrophy but little additional residual neurological deficit, whilst other cases had dysarthria, mild spasticity and athetoid movements of the face and limbs. MRI scans demonstrated bilateral putaminal lesions.

5. Pedigree analyses in LHON

The male predominance and reduced penetrance of LHON are central difficulties in the understanding of LHON and, in addition, confound accurate predic-

tions of recurrence risks to relatives of index cases. A number of pedigree analyses have attempted to address these problems.

The hypothesis that inappropriate X-inactivation in the optic nerve could explain the transmission pattern of LHON was first proposed by Bu and Rotter who analysed 31 pedigrees from published series [67]. They suggested that the development of LHON in males is consistent with simultaneous inheritance of an X-linked visual loss susceptibility locus (VLSL) and a pathogenic mtDNA mutation. The development of LHON in females is also consistent with this model, provided that a female can be affected either by being homozygous at the X-linked locus (40%) or by being heterozygous with inappropriate X-inactivation (60%). The X-linked gene frequency was proposed to be 0.08 and the estimated penetrance in a heterozygous female 0.11. A similar analysis of 79 Japanese pedigrees with LHON supported the model of Bu and Rotter, although the predicted penetrance for a heterozygous female was somewhat higher [68].

An analysis of 85 UK families with a pathogenic mtDNA mutation was entirely compatible with the model of Bu and Rotter [15]. As predicted by the model, recurrence risks were higher in children of affected females than in those of unaffected females. Most of the families in this study carried the 11 778 mutation and recurrence risks for this mutation are shown in Table 1. Similar risks were noted for families with the 3460 and 14 484 mutations, but only small numbers of families were available for study and confidence limits were therefore wide.

Table 1
Recurrence risks to relatives of index case with the 11 778 mutation [15] or the 14 484 mutation [30]

	11 778 mutation	14 484 mutation
Sibs		
Males	0.25	0.28
Females	0.08	0.05
Sister's children		
Males	0.41	0.30
Females	0.17	0.03
Matrilineal first cousins		
Males	0.30	0.19
Females	0.07	0.04

The majority of French Canadian LHON families carry the 14 484 mutation and pedigree analysis of this group shows some differences [30]. First, the male:female ratio is higher than that seen with the 3460 and 11 778 mutations. Second, this study found no difference in the number of affected children born to affected and unaffected mothers, contrary to the predictions of Bu and Rotter. Recurrence risks from this study are given in Table 1.

6. Proposed explanations for male predominance and reduced penetrance in LHON

6.1. X-linked visual loss susceptibility locus

Attempts to demonstrate the existence of an X-linked VLSL have been unsuccessful. Initial studies of Finnish families showed linkage to DXS7 ($z_{max} = 2.32$ at $\theta = 0.00$) [69], but analysis of the same small region of Xp did not confirm this finding in UK, Italian or German families [70,71]. Reanalysis of an expanded Finnish data set, with altered affected status in some patients, did not show linkage to DXS7 [72]. A more recent study of UK families excluded the presence of a single VLSL acting according to the model of Bu and Rotter over 169 cM of the X chromosome in families with the 11 778 mutation [73]. Further, a number of studies have failed to demonstrate unbalanced X-inactivation in females from LHON pedigrees as required by the model of an X-linked VLSL [73,74].

These observations do not exclude the existence of an X-linked VLSL. First, exclusion mapping can be inaccurate if the analysis model is incorrect [75], and it is difficult to obtain pedigree data from LHON families in a way that does not favour large, multigeneration pedigrees thus biasing data used in formulating the model. A sib-pair study would eliminate some of the problems inherent in the previous linkage analyses. Second, the presence of two or more separate X-linked VLSLs in families with the 11 778 mutation has not been excluded, nor has the possibility that families carrying the 3460 or 14 484 mutations could develop LHON due to such a VLSL. Third, the absence of unbalanced X-inactivation in the leucocytes of females with LHON does not exclude its occurrence in the tissues affected by LHON.

Bu and Rotter proposed that the clinical manifestations of LHON might be dependent on X chromosome inactivation of a small number of embryonic precursor cells for optic nerve tissue. However, there is evidence that the degree of unbalanced inactivation in peripheral blood is generally similar to or greater than that seen in other tissues [76–78].

Thus, an X-linked VLSL in some cases of LHON cannot be excluded, and the recent demonstration of an X-linked optic atrophy gene [79] is of some interest in this context.

6.2. Impairment of mitochondrial respiratory chain activity

The three pathogenic LHON mutations all occur in genes encoding subunits of complex I of the mitochondrial respiratory chain and a number of studies have analysed the effects of such mutations on the activity of the respiratory chain.

The 3460 mutation produces a severe (approximately 65%) deficiency of mitochondrial complex I (NADH CoQ_1 reductase) activity without reduction in the activity of complexes II, III or IV. This effect has been documented in platelets [11,80], cultured lymphocytes [81] and fibroblasts [82]. Clonal expansion of fibroblasts from a patient heteroplasmic for the 3460 mutation produced clones with zero and 100% mutant mtDNA [82]. Complex I activities in these clones were normal in the zero percent mutant clones and 65% deficient in the 100% mutant clones. These results confirm that the presence of the 3460 mutation is an absolute requirement for the complex I deficiency. However, the construction of cybrids with varying nuclear backgrounds in association with the 3460 mutation suggests that the nuclear environment can strongly influence the expression of the biochemical defect [82].

The 11 778 mutation apparently produces a less severe deficiency of complex I activity. Studies of respiratory chain function in platelets [83] and cultured lymphocytes [81] did not show any reduction in complex I activity as determined spectrophotometrically. A small reduction (approximately 25%) in platelet complex I activity was observed when non-smoking patients were compared to non-smoking controls [80]. However, the rate of electron transport through the respiratory chain is reduced and this has led to suggestions that the association of complex I with other components of the respiratory chain may be impaired [81]. The construction of cybrids containing mitochondria with either wild type or mutant 11 778 mtDNA has confirmed a small reduction in complex I activity and a more marked reduction in complex I-linked mitochondrial respiration [84,85].

The 14 484 mutation has been less well studied. No mitochondrial respiratory chain dysfunction was found in fibroblasts [86] but a moderate reduction of complex I activity has been demonstrated in leucocytes [87]. The demonstration of a severe complex I deficiency in platelet mitochondria from the Queensland family with LHON and dystonia who carry the 14 484 mutation (see above) is complicated by the presence of additional mtDNA mutations [88].

The complex I deficiency associated with the pathogenic LHON mutations is identical in both affected and unaffected members of LHON pedigrees. On the available evidence, it is therefore difficult to propose a direct link between complex I deficiency and the development of visual failure. One interesting observation is that comparatively small amounts of wild type mtDNA can correct some of the biochemical defect [89], suggesting that small variations in the proportions of wild type mtDNA in affected tissues might influence the development of LHON.

The effects of pathogenic LHON mutations have also been studied in vivo using phosphorus magnetic resonance spectroscopy (MRS). The 11 778 mutation produces a marked impairment of brain and skeletal muscle mitochondrial ATP production [83,90,91] which is similar in degree to the in vitro reduction of the rate of oxidation of NADH-linked substrates seen with this mutation. There is a smaller decrease in skeletal muscle ATP production (phosphocreatine resynthesis) with the 14 484 mutation and no reduction with the 3460 mutation [91]. Once again, the impairment of respiratory chain function is similar in affected and unaffected family members.

6.3. MtDNA heteroplasmy

Some have suggested that mtDNA heteroplasmy may be a factor determining the penetrance of the disease. Families have been reported in which a rapid segregation of mitochondrial genotype towards mutant type homoplasmy in blood of either 11 778

[92,93] or 3460 [14,94] has been associated with development of LHON in later generations. This is a difficult theory to test as there is some evidence that the degree of heteroplasmy seen in blood is not the same as that found in other tissues in patients with LHON. Thus, a post mortem study of an affected female with the 11 778 mutation found that her blood contained 33% mutant DNA whilst optic nerve and retina contained 95 and 100%, respectively [95]. However, most patients and their unaffected relatives from LHON families have very high amounts of mutant mtDNA (>95%) in blood. With some experimental support [49,96] it is assumed that, in the majority at least, this is paralleled by near homoplasmy of mutant DNA in the tissues affected by LHON.

6.4. Environmental factors

A number of findings suggest that there may be an environmental precipitant to the development of LHON. First, there is no evidence of significant correlation between age of onset in index cases and affected siblings in families with LHON [15]. Only four pairs of monozygous twins have been reported in families with LHON [15,34,97,98] and two of these pairs were discordant (in one case occupational exposure to smoke and fumes was postulated to be the cause of visual loss in the affected twin). Second, there is evidence that alcohol and tobacco use may influence the course of LHON. This was first proposed by Bell [99] and a number of anecdotal reports note that the proportion of patients with the 3460 and 14 484 mutations who consume alcohol and tobacco is unusually high [15,38,39]. A case-control study suggested that there was no excess alcohol and tobacco consumption in patients with the 11 778 mutation, but that there was a significant increase in alcohol and tobacco consumption at age at onset in patients with the 3460 and 14 484 mutations in comparison to controls [54]. The mechanism by which alcohol and tobacco might precipitate visual failure in patients with the 3460 or 14 484 mutation is unclear, although the association between tobacco smoking and reduced complex I activity [100] is interesting in this context. Tobacco-alcohol amblyopia shares some clinical features with the acute phase of LHON. Thus, in tobacco-alcohol amblyopia visual loss is subacute, field examination characteristically reveals a centrocaecal scotoma, fundoscopy in the acute stage may reveal papillary microvascular abnormalities which do not leak on fluorescein angiography and optic atrophy appears in the later stages of the disease [101,102]. Two series reported patients who had pathogenic LHON mutations and had previously been misdiagnosed as having tobacco-alcohol amblyopia [36,103]. However, tobacco-alcohol amblyopia is clearly not the same disease as LHON [103] and its pathogenesis is not known.

Because tobacco smoke contains cyanide, Wilson (1965) suggested that patients with LHON might have an inborn error of cyanide metabolism [104]. However, studies of the activity of rhodanese (thiosulphate sulphurtransferase, which metabolises cyanide to thiocyanate) in LHON have been conflicting [104–107]. There is no evidence of impairment of the metabolism of potential environmental toxins by cytochrome P450 enzymes in LHON [108].

Other single case reports have postulated that LHON can be triggered by a variety of metabolic and toxic precipitants, including diabetes [35,109], B_{12} deficiency in Crohn's disease [110], carbon monoxide poisoning [111], and toxin exposure [35]. By extrapolation from the known effects of B_{12} deficiency in the pathogenesis of optic nerve disease and the possible role of cyanide intoxication, hydroxycobalamin has been advocated for the treatment of LHON [112]. There is, however, no evidence of clinical benefit of this therapy in LHON.

6.5. Autoimmunity

An autoimmune aetiology of LHON is supported by a number of clinical observations. First, the subacute onset in young adult life is unusual for a genetic condition. Second, the optic disc appearance during the acute phase of LHON is similar to that seen in a number of inflammatory diseases [36]. However, there is no leakage of fluorescein on angiography [40], which suggests that the vascular endothelium remains intact. Third, some patients with LHON and optic nerve involvement alone have increased immunoglobulins in their CSF [36]. Finally, LHON is associated with a MS-like illness in some patients and MS is thought to have an autoimmune basis [50].

Antibodies directed against mitochondrial proteins have been described in human disease. In a patient with a pathogenic mtDNA mutation at bp 3243 and the MELAS phenotype, a specific antibody to a 41 kDa mitochondrial antigen was observed [113]. In patients with primary biliary cirrhosis (PBC), antimitochondrial antibodies are directed against a heterogeneous group of antigens, the most important of which are subunits of pyruvate dehydrogenase [114]. Although in each of these examples the antibodies are directed against mitochondrial proteins encoded by nuclear genes, it is clear from rodent studies that mtDNA-encoded peptides can restrict the immune response. The maternally transmitted factor (MTF) of mice was discovered as a component of an alloantigen that induced slow skin graft rejection and a vigorous cytotoxic T cell (CTL) response and MTF is encoded by mtDNA [115].

Some experimental data exist to support a role for autoimmunity in LHON. Antibodies to tubulin, an optic nerve protein, are present at high frequency in patients with LHON and their unaffected maternal relatives and at low frequency in healthy controls and patients with ischaemic or compressive optic neuropathies [116]. There is no association between LHON and major histocompatibility complex class I or class II genotypes [117,118].

7. Conclusions

Subacute, sequential visual failure is part of the phenotype seen in association with a number of mtDNA mutations. In families with the 14 459 or 11 696 mutations, additional neurological features are prominent and may be the sole manifestation in some affected individuals (often termed 'Leber's-plus'). In families with the 11 778, 3460 or 14 484 mutations, visual failure is the dominant feature and additional features are uncommon ('Leber's hereditary optic neuropathy').

Although patients with the 11 778, 3460 or 14 484 mutations are sometimes considered to have the same disease, an expanding body of evidence suggests that there are marked differences in the manifestations of these three pathogenic mutations. For example, patients with the 14 484 mutation show differences in acute fundoscopic appearances and final visual outcome in comparison to the 11 778 and 3460 mutation groups. Pedigree analyses delineate variation in sex ratios and in recurrence risks between the 14 484 and 11 778 mutation groups. In vitro biochemical data on complex I activity and MRS studies of mitochondrial ATP production show clear differences between mutation groups. Such observations suggest that the mechanisms underlying the development of visual failure may differ between patients carrying each of the pathogenic LHON mutations.

Whilst a number of hypotheses have been proposed, none satisfactorily explain the male predominance and reduced penetrance seen in families with LHON. The majority of LHON families carry the 11 778 mutation. Although pedigree analyses of these 11 778 families closely fit a model of an X-linked VLSL precipitating visual failure, there is no experimental evidence to support this model. Biochemical data cannot, at present, show differences between affected and unaffected family members. There is no clear evidence to support a central role for mtDNA heteroplasmy in the development of visual failure but analysis of this hypothesis is hampered by the limited availability of affected tissues (presumed to include optic nerve and retinal ganglion cells) for study. Exposure to alcohol and tobacco does not appear to precipitate visual failure in patients with the 11778 mutation.

Smaller numbers of patients carry the 3460 and 14 484 mutations and thus experimental data is more limited. Pedigree analysis of families carrying the 14 484 mutation provides evidence against an X-linked VLSL acting according to one proposed model but this has not been excluded in either mutation group. Biochemical data shows a marked complex I deficit for the 3460 mutation but conflicting results for the 14 484 mutation. Anecdotal evidence and one case-control study suggests a role for alcohol and tobacco exposure in the development of visual failure in both the 3460 and 14 484 mutation groups.

This review has outlined some of the clinical and experimental data in LHON. These data suggest differences between the phenotype associated with each of the three mtDNA mutations which may reflect variation in disease mechanisms, although there is no conclusive evidence to support such a contention. Further study will elucidate the means by which these mtDNA mutations lead to the common end-

point of subacute visual failure described by Theodore Leber.

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