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Molecular genetic basis of primary inherited optic neuropathies

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Abstract

Aim **To review the molecular genetic basis of primary inherited optic neuropathies.**
Methods **Medline and Embase search.**
Results Inherited optic neuropathies are a genetically diverse group of disorders that present with reduced visual acuity and the clinical appearance of optic atrophy. The inherited optic neuropathies may be sporadic or familial, in which case the mode of inheritance may be Mendelian (autosomal dominant, autosomal recessive, X-linked recessive) or non-Mendelian (mitochondrial). Two genes for dominantly inherited optic atrophy have been mapped (*OPA1* and *OPA4*), of which the gene has been identified in one (*OPA1*). A gene for recessive optic atrophy (*OPA3*) has also been identified. X-linked optic atrophy (*OPA2*) has been mapped but to date no gene has been identified. Mutations in mitochondrial DNA have been identified in Leber's hereditary optic neuropathy.
Conclusions **Mutations in genes from both the nuclear and mitochondrial genomes appear to be responsible. Mitochondrial dysfunction, in the broadest sense, is emerging as central to the pathogenesis of this group of conditions.**
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Introduction

The inherited optic neuropathies comprise a group of disorders in which there is cell death confined to the retinal ganglion cells. The *primary* hereditary optic neuropathies comprise autosomal dominant, autosomal recessive and X-linked recessive optic atrophy, and the maternally inherited Leber's hereditary optic neuropathy. The optic atrophy seen in some forms of glaucoma may be an inherited optic neuropathy and appears to have a genetic basis, at least in part.[1,2] The main clinical features of optic neuropathies are summarized in this volume by Newman *et al*. Ganglion cell loss in the hereditary optic neuropathies leads to central visual loss, dyschromatopsia and central visual field defects, with the papillomacular bundle preferentially affected. The visual loss is bilateral, symmetrical, and irreversible once ganglion cell death has occurred. The visual loss is usually gradual with the exception of Leber's hereditary optic neuropathy, where visual loss is sudden and severe and occurs asynchronously in the two eyes.[3] In keeping with a conduction deficit, visually evoked responses are delayed, the amplitude is reduced, the flash electroretinogram (ERG) is normal but the pattern ERG shows an N95 component reduction, a feature of typical of ganglion cell dysfunction.[4]

Leber's hereditary optic neuropathy

Leber's hereditary optic neuropathy (LHON) is transmitted by non-Mendelian, mitochondrial inheritance and is due to mutations in mitochondrial DNA. As mitochondria are maternally inherited[5] there is no male to male transmission in an LHON pedigree. Human mitochondrial DNA (mtDNA) is a closed, circular molecule of 16569 base pairs. There are thousands of copies per cell and they segregate randomly at meiosis and mitosis.[6] Each cell can contain different proportions of both wild type and mutant mtDNA. With multiple cell divisions the population of mtDNA in a cell can drift towards either pure mutant or wild type (homoplasmy) or stay mixed (heteroplasmy). This may be important, as the magnitude of a defect in oxidative phosphorylation (OXPHOS)

can relate to the proportion of mutant mtDNA. The mitochondrial genome is essential for aerobic metabolism, as it encodes 37 of the genes in the OXPHOS system and 13 of the protein subunits. Complexes I–V reside in the mitochondrial inner membrane. The vast majority of cellular adenosine triphosphate (ATP) is generated by this system as complexes I to IV are key components of the electron transport chain. This chain generates an electrochemical gradient across the mitochondrial inner membrane, which is used by complex V to synthesize ATP. The retina, optic nerve, and extra-ocular muscles are among the most ATP-dependent tissues in the body although each body tissue has different OXPHOS requirements. There are broader ophthalmic manifestations of mtDNA abnormalities including optic neuropathy, pigmentary retinopathy, ophthalmoplegia, and ptosis, but LHON appears to be the most prevalent mitochondrial genetic disorder.[7]

Three primary mtDNA mutations account for 95% of LHON cases in populations of European descent. These mutations are G3460A (13% of cases), G11778A (69%) and T14484C (14%),[8] and they are homoplasmic in the large majority of families. Mutations at np 11778,[9] 3460,[10] and 14484,[11,12] are 'primary', as the identification of one of these pathogenic mutations establishes the diagnosis even in the absence of a family history. Mutations at np 3460 and 14484 produce A52 T and M64 V substitutions in the NADH dehydrogenase (ND) 1 and ND6 subunits of complex I, respectively, and mutation at 11778 produces an R340H substitution in the respiratory chain complex I subunit ND4. Other rare primary mutations, including T14596A, C14498T, G13730A, G14459A, C14482G, and A14495G have been identified.[13–15] LHON shows variable penetrance and a primary mtDNA mutation is essential but not sufficient to manifest optic neuropathy. So-called 'secondary' mutations may also be involved in the pathogenesis of LHOH but these also occur at a lower prevalence in control populations, where they may represent polymorphisms. The secondary mutations usually occur in association with a primary mutation or other secondary mutations if implicated in LHON. They generally cause the mutation of a less highly conserved amino acid. Secondary pathogenic mutations include np G13708A, G15812A, A4917G, T4216C, G9804A, G9438A, and G15257A.[16–18]

The role of the European haplotype J (G15812A, G15257A, G13708A, and T4216C) in disease expression is still unclear.[19] In particular, approximately 75% of patients with the 14484 mutation have this haplotype, while it is only found in ~10% of mtDNAs from the general European population. It may be that having a polymorphism within the haplotype J increases the penetrance of the 14484 mutation. It is not clear why men are clinically affected more often than women. The estimated male to female ratio is between 3 : 1 and 5.6 : 1, and up to 80–90% of cases in some series are male.[20] The suggestion that an X-linked factor predisposes to disease[21] has received little support in the literature and has been questioned by a re-evaluation of the original data,[22] leaving many unanswered questions. A given LHON family will have one primary mutation and may harbour several secondary mutations. A nuclear modifier, such as a functional polymorphism in a tissue-specific protein, could be involved in the variable penetrance seen. However, there are a few well-defined families in which a primary mutation has not yet been found. There are also four reported families that carry two primary mutations: that is, the 11778 and 14484 mutations.[23]

Genotype and phenotype

The clinical differences in the optic neuropathy seen as a result of the three primary mutations are subtle. The only real distinguishing feature appears to be in the spontaneous recovery of acuity. The T14484C mutation is associated with the best visual outcome (6/24 or better in 71% of patients).[12,24,25] In all, 50% of reported patients with the T14484C mutation have some recovery of vision. A younger age of onset of visual loss with this mutation and other mutations is also associated with a better visual outcome, especially if the onset is before the age of 20 years.[26] Visual recovery can occur more than a year later. If visual acuity improves it does not deteriorate again. Mutations at G11778A and G3460A appear to be associated with a similar visual outcome (1/60 to 3/60), but mutation at np 11778 is particularly severe in one-third of female patients.[27] Mutation load may be significant in the ~15% of LHON patients in whom the mtDNA is heteroplasmic. Males with less than 60% load of 11778 mutation in peripheral blood have a lower risk of visual loss.[28]

Pathophysiology of LHON

Since the identification of the first mtDNA mutation in LHON in 1988[9] over 15 years of research have yet to give us a full understanding of the pathogenesis of visual loss. Histopathology from LHON patients with long-standing visual loss shows axonal degeneration in the optic nerve and loss of myelin, and there is evidence that the small axons of the papillomacular bundle, found centrally in the optic nerve, are particularly vulnerable.[29,30] The respiratory dysfunction may lead to axoplasmic stasis and swelling, with evidence of demyelination. This may be reversible, as evidenced by the possibility of recovery of vision, but if the mitochondrial apoptotic pathway is

activated, as it is most likely,[31] ganglion cells are permanently lost.

The relatively high prevalence of tobacco and alcohol consumption in affected individuals, reported by some authors,[26] may suggest a clinical interaction between the reduction in ATP generating capacity caused by the mutations that lead to complex I dysfunction and environmental factors. There is however, mounting evidence that the optic neuropathy in LHON is not a simple result of decreased complex I activity in mitochondrial electron transfer.[32] Analysis of *in vitro* lymphoblastoid lines from LHON patients and transmitochondrial cybrid lines into which the 3460, 11778, 14484, or 14459 mutations were introduced, does not show a convincing correlation between mutation and complex I activity.[32] The equivalent *in vivo* work, using 31P magnetic resonance spectroscopy to assess energy metabolism in skeletal muscle of LHON patients, has also failed to show convincing reduction in bioenergetic function in muscle,[33] although a marked defect in brain has been observed.[34] However, LHON mutations may increase mitochondrial reactive oxygen species and this could lead to oxidative stress and the trigger for retinal ganglion cell dysfunction and apoptosis. This may be particularly important in neural cells.[35]

Autosomal dominant optic atrophy

Dominantly inherited optic atrophy (ADOA), also known as Kjer's optic atrophy, is the most common isolated optic neuropathy. A gene for dominant optic atrophy mapping to chromosome 3q28-qter, *OPA1*[36] has been identified.[37,38] A large number of families have been reported to map to the locus on chromosome 3q28-qter, suggesting that it may be the predominant locus. A second dominant optic atrophy locus (*OPA4*) has been mapped in one pedigree to chromosome 18q12.2-q12,[39] although the gene has not been identified to date. The available evidence suggests that the phenotype of *OPA4* may be similar to *OPA1*.

The OPA1 gene

The *OPA1* gene is 6031 nucleotides long and is composed of 31 exons spanning >114 kb of genomic DNA. It encodes a mitochondrial dynamin-related GTP protein of 960 amino acids. The *OPA1* gene is ubiquitously expressed with most abundant expression in retina and brain.[37,38] *OPA1* is widely expressed in many regions of mouse brain.[40] Two additional exons, 4b and 5b, generate eight isoforms by alternative splicing, and two of these splice variants are particularly highly expressed in fetal brain, retina, and heart.[41]

There is a wide spectrum of mutations throughout the gene, with over 70 reported to date.[37,38,41–46] There is a concentration of mutations in the GTPase and dynamin central region (coded for by exons 8 to 28), but to date no mutations have been found in exons 4, 4b, and 5b, which are alternately spliced. Since the majority of mutations result in protein truncation, and most mutations probably represent null alleles, dominant inheritance of the disease may result from haploinsufficiency of *OPA1*. A 560–860 kb microdeletion on chromosonme 3q28 that results in the complete loss of one copy of the *OPA1* gene has been reported.[47] Missense mutations may cause disease by a dominant-negative mechanism. One family has been reported showing semi-dominance,[43] with heterozygous mutations in *OPA1*. The estimated penetrance figure of 98% in dominant optic atrophy has been revised downwards recently in the light of molecular studies showing that penetrance may vary from family to family and mutation to mutation. Penetrance has been reported as high as 100% (IVS12 + 1g > t mutation resulting in exon 12 skipping[46]) and as low as 43% (2708del(TTAG) mutation in exon 27[42]).

OPA1 protein and mitochondria

The OPA1 protein is a dynamon-related GTPase, comprising a highly basic amino-terminal that forms a mitochondrial targeting sequence, a dynamin-GTPase domain, and a C-terminus of unknown function. The C-terminus of OPA1 differs from other dynamin family members in lacking a proline-rich region, a dynamin GTPase effector domain and a pleckstrin homology domain, and may therefore determine the specific functions of the OPA1 protein. Primary structure predictions suggest a high probability of coiled-coil formations in domains encoded by exons 5b–7 (100%) and exons 27–28 (95%), and these may be involved in the formation of homodimers or polymers or the binding of as yet unknown cellular partners. OPA1 shows 33% homology to Mgm1 and 31% homology to Msp1, both members of a subfamily of dynamins found in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* where they have been shown to play a role in the maintenance of the mitochondrial genome and mitochondrial morphology. Recent immuno-fluorescence and biochemical evidence suggests that the highly basic amino-terminal extension is essential for OPA1 targeting to mitochondria[40,48,49] and for mitochondrial inner membrane structure and integrity.[50]

The Bst/+ mouse, which has a variable reduction in the size of the optic nerves, reduced or complete absence of the pupillary light reflex and atrophy of the optic nerves[51] (and also shows subretinal neovascularization

with age,[52] has recently been excluded as a model for ADOA.[53]

Recessive optic atrophy

Isolated recessive optic atrophy, of which Behr syndrome is an example, may present at birth or soon after[54] with profound visual deficit, nystagmus, and marked optic nerve pallor. Type III 3-methylglutaconic aciduria (MGA), also known as optic atrophy plus syndrome, or Costeff optic atrophy syndrome, is a recessive neuro-ophthalmological syndrome that consists of early-onset bilateral optic atrophy and later-onset spasticity, extrapyramidal dysfunction, and cognitive deficit. The *OPA3* gene (chromosome 19q13.2–q13.3) has been found to be mutated in patients with MGA.[55] *OPA3* consists of a 5′UTR of 150 bp, an open reading frame of 179 amino acids, and >970 nucleotides of 3′ untranslated sequence. Northern blot analysis demonstrates a primary transcript of approximately 5.0 kb that is ubiquitously expressed, most prominently in skeletal muscle, kidney, and brain. The protein product is predicted to be a 20-kD peptide, containing a mitochondrial targeting peptide, NRIKE, at amino acid residues 25–29. The protein is predicted, with a probability of 0.87, to be exported to the mitochondrion, where it may have a significant, but totally unknown, role in mitochondrial processes. This fact is most intriguing in view of the work to date on LHON and the OPA1 protein. Recently, the first locus for isolated autosomal recessive optic atrophy (ROA1), has been mapped to chromosome 8q21–q22.[56]

X-linked optic atrophy

An X-linked inheritance of optic atrophy was first described in 1974[57,58] in a pedigree in which affected males had mental retardation and dysarthria, tremor, dysdiadochokinesia, and abnormal reflexes. Female carriers were normal. The age of onset of optic atrophy was early childhood, no nystagmus was seen, and there was a very slow loss of visual acuity with age. The optic discs showed total pallor and defects in colour vision were reported and visually evoked potentials showed prolonged latencies. A gene for X-linked optic atrophy (*OPA2*) has been mapped to Xp11.4–p11.2[59] in the same pedigree, but so far the gene has not been identified.

Glaucoma

The glaucomas are a highly heterogeneous group of disorders in which the final common path for visual loss is also retinal ganglion cell death. Retinal ganglion cell death leads to optic nerve head excavation, or glaucomatous cupping, and a corresponding visual field loss. Intraocular pressure may be elevated above 21 mmHg, such as in primary open-angle glaucoma (POAG), or within the normal range, such as in normal tension glaucoma (NTG). Primary glaucoma is heterogeneous in both phenotype and in its heritable basis. The onset may be congenital, juvenile or adult, and there may be several different disease mechanisms. The genetic contribution to glaucoma is estimated to be as high as 50–60%,[60] although estimates vary.[61] While there is clearly a significant genetic basis to glaucoma, this could be due to single genes or multiple genes acting with or without environmental factors.[2] Although rare forms of juvenile glaucoma may be caused by mutations in a single gene,[62] the majority of cases are likely to have a complex aetiology, with environmental risk factors such as intraocular pressure, age, systemic hypertension, and vasospasm, leading to ganglion cell damage in individuals with a high-risk genotype.

There are currently eight mapped disease loci for nonsyndromic glaucoma (GLC1B 2cen-q13, GLC1C 3q21-24, GLC1D 8q23, GLC1F 7q35-36, GLC3B 1p36, PDS1 7q35-36, PDS2 18q11-21, and RIEG2 13q14). Three genes (*CYP1B1*,[62] *MYOC*,[63] and *OPTN*[64]) have been identified. There are four genes (*PAX6, PITX2, FOXC1*, and *LMX1B*) associated with complex glaucoma phenotypes involving anterior segment dysgenesis or ocular developmental anomalies. However, these loci and genes may account for only a small proportion of glaucoma seen in the general population. Studies of *MYOC* and *OPTN* mutations in the general glaucoma population have shown that they account for 3.4% of open angle glaucoma[65] and up to 15% of NTG,[64] respectively. The association of two intronic *OPA1* polymorphisms in the *OPA1* gene with normal tension glaucoma (IVS8 + 4C/T and IVS8 + 32T/C),[66,67] is of interest since NTG may account for 20–50% of open angle glaucoma.[68] Normal tension glaucoma and dominant optic atrophy share many overlapping clinical features,[69] and it may be difficult in some cases to distinguish the conditions by the appearance of the optic nerve.[70,71] By inference, there may also be a role for mitochondrial dysfunction in NTG, although data are currently lacking to support this hypothesis. Other genes may also play a role in NTG, including the inheritance of the apolipoprotein E allele ε4, which appears to be associated with elevated risk of NTG in a Tasmanian population.[72]

Discussion

The last decade has significantly deepened our understanding of the molecular genetics of inherited optic neuropathies. New data highlight a possible unifying role for mitochondrial dysfunction in inherited optic nerve disease, whether it be morphological or

physiological. This is being extensively investigated in LHON, and the recent research on ADOA and *OPA1*, and the suggestion that the OPA3 and OPTN proteins may also have a mitochondrial link, provides intriguing new insights. Loss of vision in the primary optic neuropathies is by the final common pathway of ganglion cell death, but it is still far from clear why mutations in such ubiquitously expressed proteins should give rise to such a restricted ocular phenotype. We have much to learn about the pathophysiology of this fascinating group of diseases before we can begin to design new therapeutic interventions. However, it is encouraging that there has been a recent increase in interest in mitochondrial targets for drug development, and this may lead to novel treatments in the future.

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