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Optineurin in primary open angle glaucoma

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Glaucoma is a progressive optic neuropathy characterized by a particular pattern of visual field loss and optic nerve head damage resulting from a number of different disorders that affect the eye. Approximately 2.47 million people in the United States are affected with glaucoma [1], and more than 100,000 Americans are expected to develop this condition every year. Furthermore, more than 67 million people worldwide are estimated to suffer from glaucoma [2]. The most common form of this condition is primary open-angle glaucoma (POAG). Glaucomatous optic nerve damage and characteristic visual field loss are the two major clinical signs of this condition [3–5]. Elevated intraocular pressure (IOP) is the most common known risk factor for glaucomatous damage, but it is not equivalent to the disease itself, and numerous other risk factors are presently under investigation. Approximately one third to one half of patients with POAG consistently have IOP values within the statistically normal range of less than 22 mm Hg [6–9]. These patients have been considered to have low- or normal-tension/pressure glaucoma (LTG or LPG, NTG, or NPG) and exhibit typical glaucomatous cupping of the optic nerve head and visual field loss [10]. The actual number of patients with low-pressure glaucoma is likely to have been underestimated. In fact, if one integrates previously published epidemiologic data from a 30-year period (1966–1996) for different ethnicities and geographic regions of the world, the overall estimate of LPG may represent 50% of all open-angle glaucoma cases (Table 1).

During the last decade, nine different genetic loci have been identified for several inherited forms of glaucoma. Three loci have been reported for primary congenital glaucoma (PCG), one for juvenile-onset (JOAG), and another five for adult-onset POAG [11]. The causative gene has only been identified for two types of this condition, PCG [12] and JOAG/POAG [13]. The authors' ongoing study shows that cytochrome P4501B1 (*CYP1B1*) is the major gene for PCG (ie, for approximately 85% of familial and 33% of sporadic cases) [14–16]. Mutations in the Myocilin gene (*MYOC*) are primarily involved in 3% to 4% of persons with either JOAG or POAG [13]. Most Myocilin mutations are identified in JOAG cases (ie, 2.0% to 2.5%), although other JOAG families do not have a mutation in this gene [17]. Until recently, no other gene had been identified for the adult-onset POAG phenotype.

Optineurin: a new adult-onset glaucoma gene

Recently, the authors' group reported a new gene for adult-onset POAG [18] that they named "Optineurin" (*OPTN*). This gene was identified by genetic linkage analysis of a large family with adult-onset LPG/POAG that led to the mapping of a new locus (GLC1E) on chromosome 10p14 [19]. This linkage analysis was followed by saturation mapping and screening of another 60 LPG and POAG families [20] for linkage to the GLC1E locus that eventually reduced its critical candidate region from 21 cM to 5 cM. A number of genes were selected and screened for mutation in the affected members of the original GLC1E-linked family. Optineurin was selected as a good candidate gene because (1) it was mapped within

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Table 1
Prevalence of low-pressure glaucoma relative to all open-angle glaucoma (1966–1996)

Study	LPG / Total OAG patients	Reference
Wales	7 / 20 = 35%	[44]
Bedford	3 / 45 = 6.7%	[45]
Des Moines, Iowa	129 / 189 = 68%	[46]
Framingham	21 / 40 = 52%	[47]
Sweden	16 / 33 = 48%	[48]
Japan	99 / 151 = 66%	[49]
Texas	60 / 200 = 30%	[50]
St. Lucia, West Indies	52 / 147 = 36%	[51]
Baltimore	114 / 194 = 58%	[52]
Beaver Dam	33 / 104 = 32%	[53]
Rotterdam	7 / 18 = 39%	[54]
Blue Mountains	76 / 108 = 70%	[55]
Totals	617 / 1249 = 49.40%	

Abbreviations: LPG, low-pressure glaucoma; OAG, open-angle glaucoma.

the newly reduced candidate region; (2) it is expressed in the retina; (3) its interaction with a number of other proteins has already been established; and (4) the gene was implicated as possibly being involved in apoptosis. Optineurin was originally identified as a tumor necrosis factor-alpha (TNF-α)– inducible protein [21] and was named FIP-2 (for adenovirus E3-14.7K–interacting protein). Subsequently it was also identified as HYPL (Huntingtin–interacting protein L) [22], NRP (NEMO-related protein) [23], TFIIIA-INTP (transcription factor IIIA interacting protein) [24], and RAB8-interacting protein [25]. Because the first series of mutations in this gene were responsible for the clinical phenotype of glaucoma, the name "Optineurin" (for optic neuropathy–inducing protein) was suggested by the authors' group and was approved by the HUGO committee as a new name for this molecule.

Optineurin structure

This gene maps to the GLC1E locus on 10p14 [19] and contains three noncoding exons at its 5′ untranslated region (UTR) and another 13 exons [18] that encode for a total of 577 amino acids (aa). Alternative splicing at the 5′ UTR generates at least three different isoforms (accession numbers AF420371 to AF420373), but none alter the coding exons. Optineurin is a coiled-coil protein with an estimated molecular weight of 66 kd. This cellular protein contains two putative bZIP transcription factor basic motifs, several leucine-zipper domains, and a C2H2 type zinc finger. This acidic protein (pI = 5.15) is rich in both glutamate (15.8%) and leucine (11.8%).

Mutation screening of Optineurin in adult-onset glaucoma

The authors' group used a group of 54 families with adult-onset glaucoma for mutation screening of the *OPTN* gene [18]. Because their original GLC1E-linked kindred [19] had affected members with both LPG and high-pressure glaucoma (HPG), they carefully reviewed the clinical presentation of more than 400 families with adult-onset POAG that were available in their laboratory and identified only 54 that had at least one member affected with LPG. A number of these 54 families presented only with LPG members (ie, IOP ≤ 22 mm Hg), whereas others had mixed clinical manifestations of both LPG and HPG in different affected members. These 54 glaucoma families consisted of a total of 147 living affected individuals, including LPG/HPG members of a large family that was originally used to map the GLC1E locus to 10p14-p15 [19]. Additionally, 124 subjects, predominantly with sporadic LPG, were used for mutation screening of only one of the *OPTN* exons.

Mutation screening of *OPTN* in these 54 families identified two missense mutations (E50K and R545Q) and one truncating mutation (2-bp AG insertion after Asp127; 691_692insAG) in 9 of 54 families (16.7%) [18]. The most common mutation of E50K was segregated in 124 members of seven families (13.0%) (Fig. 1). Extensive genotyping and inspection of a total of 18 *OPTN* flanking and intragenic DNA markers (Fig. 2) did not reveal a common haplotype in these seven families. As shown in Fig. 2, the first two families share a common haplotype, the second two share a different haplotype, but the remaining three families do not seem to share a common haplotype. Therefore, the E50K mutation in these seven families is not inherited from a common ancestor but has spontaneously reoccurred at least five times (Fig. 2). Altogether, this mutation was present in 38 affected and 16 asymptomatic gene carriers, but it was absent in another 50 unaffected and 20 spouse members of these seven families (Fig. 1) [18]. Of the 38 affected subjects with E50K mutation, 7 (or 18%) had IOP measurements recorded between 22 and 26 mm Hg. The remaining individuals had IOP values ranging between 10 and 22 mm Hg (Table 2). Five of these families had members affected only with LPG (Table 3), but two families had members affected with both LPG and HPG in different branches of their respective pedigrees (Fig. 1 and Table 2). It is therefore

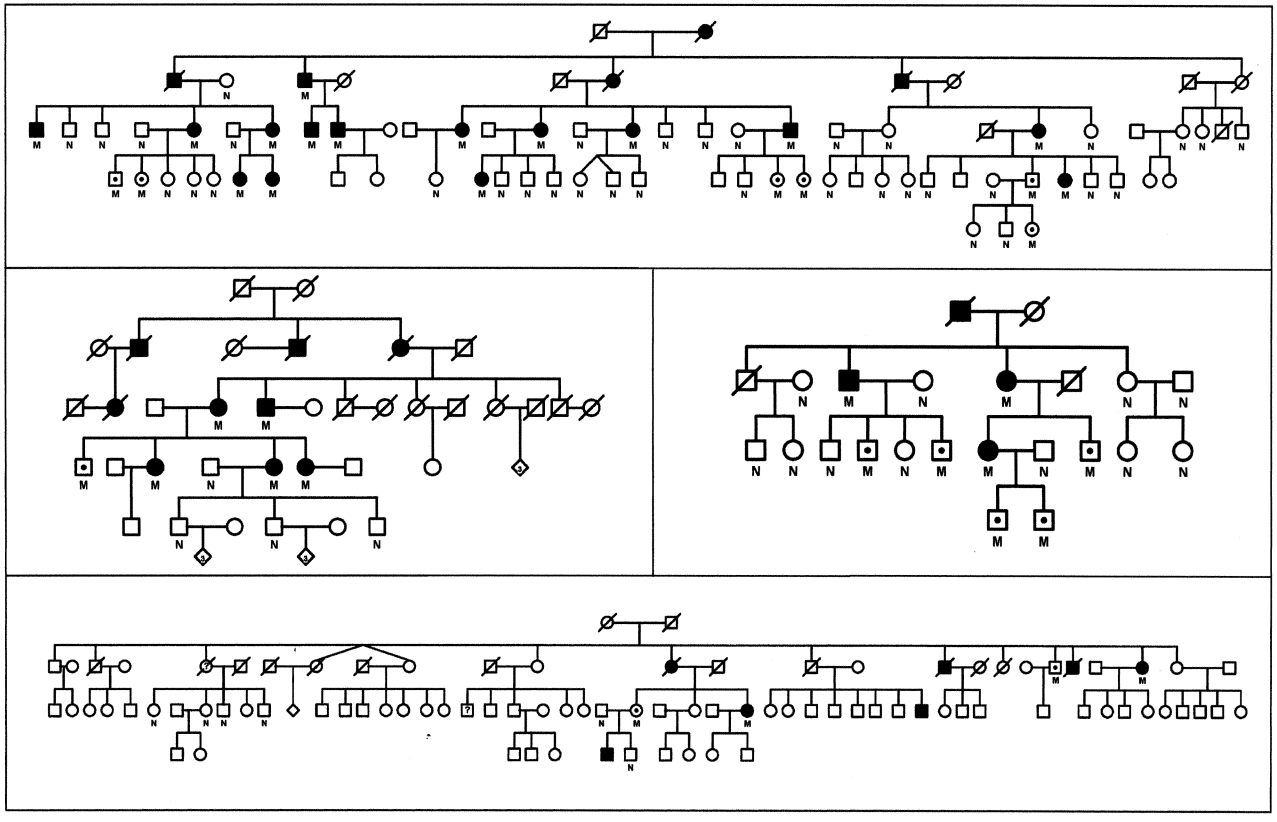

Fig. 1. Pedigrees of four glaucoma families with *OPTN*-E50K mutation. The top pedigree is the original LPG/POAG that was used to map the GLC1E locus [19]. The affected members of the remaining pedigrees all have LPG. Squares and circles represent males and females, respectively. Filled-in symbols represent individuals affected with glaucoma. All other persons are unaffected. A slashed line across the symbol represents an asymptomatic person bearing the affected haplotype who also carries the E50K mutation. The letter M below a symbol indicates the presence of E50K mutation. The letter N indicates absence of this mutation. DNA from all other subjects was not available for mutation screening.

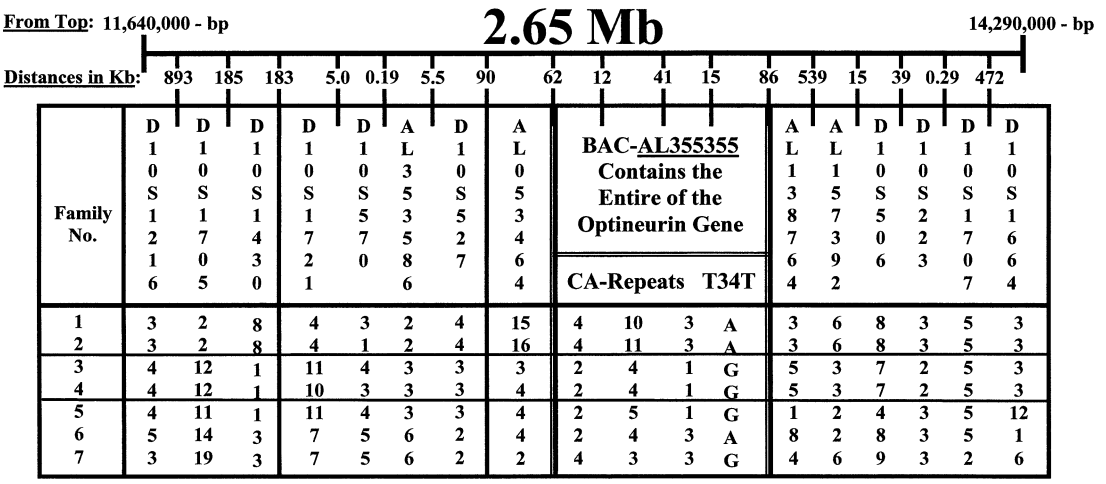

Fig. 2. Haplotype analysis of seven adult-onset primary open angle glaucoma families with optineurin E50K mutation.

conceivable that mutations in the *OPTN* gene are involved mainly in the LPG cases (> 80%) and certain other HPG cases (< 20%) with IOP values of predominantly 26 mm Hg or lower. Another mutation in exon 6 (691_692insAG) has shifted the open reading frame after the point of insertion (ie, Asp127) and translated into 22 new amino acids before finally terminating with a new premature stop codon. This stop codon truncates the remaining 76% of the normal protein. A further mutation in exon 16 (R545Q) was identified in another unrelated LPG pedigree that was not present in more than 100 normal chromosomes. Finally, a sequence change in exon 5 (M98K) was documented in 23 (13.61%) of 169 index cases (ie, 45 families and 124 other sporadic and predominantly LPG subjects screened only for this exon). Only 3 of these 23 subjects had IOP values recorded above normal average (ie, 23, 26, and 40 mm Hg). The remaining 20 subjects had been previously diagnosed as having LPG. The M98K change was also present in 9 of 422 normal control chromosomes (2.13%). Nevertheless, because the observed difference between affected (13.61%) and normal control (2.13%) frequencies is highly significant ($\chi^2 = 30.99$; $df = 1$; $P < 0.00001$), and because the altered amino acid is conserved in macaque, the M98K may indeed represent a risk-associated factor for glaucoma.

New mutations and sequence alterations in optineurin

At the 2003 meeting of the Association for Research in Vision and Ophthalmology (ARVO), the authors and other investigators reported that mutations in *OPTN* do not play a major role in the etiology of HPG patients. The authors have recently screened 22 HPG families and did not observe any mutations in *OPTN*. The E50K mutation has now been identified in few other LPG and HPG subjects, however. At least five other new *OPTN* mutations and a few other DNA polymorphisms were reported in a mixed group of subjects with LPG, JOAG, and POAG. So far the authors' study alone shows that mutations in the *OPTN* gene are present in 10 of 76 familial LPG/HPG cases

Table 2
Mean and range of intraocular pressure variations in two mixed low-pressure glaucoma/primary open-angle glaucoma families with OPTN-E50K mutation

Family	POAG subjects (IOP > 22 mm Hg)	Range of IOP (mm Hg)	Mean IOP (mm Hg)	LPG subjects (IOP ≤ 22 mm Hg)	Range of IOP (mm Hg)	Mean IOP (mm Hg)
1	3	22–25	23.0	12	14–22	17.6
2	4	22–26	23.9	4	10–20	16.7
Total	7	22–26	23.5	16	10–22	17.2

Table 3
Mean and range of intraocular pressure IOP variations in seven families with OPTN-E50K mutation

Family	Total number of affected subjects	Number of living affected subjects	Range of IOP values (mm Hg)	Mean IOP (mm Hg)
1	18	15	14–25	18.7
2	12	8	10–26	20.8
3	9	5	11–17	13.8
4	6	3	15–18	16.5
5	6	1	19.0	19.0
6	5	3	13–19	15.8
7	4	3	15–17	16.0
Total	60	38	10–26	17.2

(13%) but in only 6 of 206 sporadic cases (3%). The M98K risk-associated factor has now been observed in 12% (8 out of 66) of familial and 16% (32 out of 200) of the authors' sporadic cases. Most patients with M98K sequence alteration are of LPG type. Taken together, these data indicate that mutations in the *OPTN* gene are mainly observed in familial rather than sporadic cases and are more common in patients with LPG than in patients with HPG. This observation is also in general agreement with a previously reported tendency for *CYP1B1* mutations in PCG [15,16] and for *MYOC* mutations in JOAG/POAG index cases. As yet, however, there is no clear biological evidence explaining why mutations identified in the three glaucoma-causing genes *MYOC*, *CYP1B1*, and *OPTN* are more prevalent in familial than in sporadic cases. One possible explanation is that an undetermined percentage of sporadic glaucoma cases result from environmental factors or simply exhibit as a secondary phenotype to other undiagnosed ocular and nonocular clinical conditions. Note that this group of glaucoma patients could be classified as primary and become indistinguishable from those with other classic forms of secondary or syndromic disease. Furthermore, of the remaining glaucoma cases caused by genetic factors, a certain percentage is caused by mutations in numerous genes that are thought to be involved in the etiology of glaucoma. Over the last decade, evidence from various molecular genetics studies of glaucoma families (and sporadic cases) that had led to identification of only a handful number of genetics loci clearly testifies that glaucoma is a highly heterogeneous genetic condition and that a large number of genes are involved in the etiology of this blinding disease. Therefore, because glaucoma is a relatively common condition, the possibility of identifying a single gene that would be responsible for a significant proportion of either familial or sporadic cases seems to be diminishing.

Optineurin in other species

The authors have also cloned *OPTN* orthologue genes in mouse (AY071834) and rhesus monkey (AY228373-4). Mouse and monkey genes encode for 584 (67 kd) and 571 (65 kd) amino acids and show 80% and 97% identity to human Optineurin, respectively (unpublished data). Both these two genes are also divided into 13 coding exons, and their boundaries are fully conserved with human *OPTN*. Inspection of public databases also identified complete cDNA sequences for rat (586 aa; NM_145081), chicken (556 aa; AF380358 [26]) and other partial sequences for rat [24], pig, and cow. Overall, human optineurin protein has 78% to 85% identity with its homologues in mouse, rat, pig, and cow and 97% identity with macaque. Both E50K and M98K mutations observed respectively in 7 and 23 index cases of the authors' original study [18] are conserved between human and macaque. The M98K evolutionary conservation further corroborates the theory that this mutation is a risk factor for glaucoma. The E50K mutation is further conserved in mouse, rat, chicken, and cow.

Expression studies of human optineurin

After identifying *OPTN* as a new gene for adult-onset LPG/POAG cases, the authors studied expression of this gene both at RNA and protein levels. They documented expression of Optineurin in samples prepared from human trabecular meshwork (HTM), nonpigmented ciliary epithelium (NPCE), retina, brain, adrenal cortex, liver, fetus, lymphocyte, and both normal human dermal fibroblasts (NHDF) and mutant (E50K-DF) dermal fibroblasts [18]. By Northern blotting they observed a major band of approximately 2.0-kilobase (kb) message in both HTM and NPCE cell lines that was three to four times more abundant

than a 3.6-kb message. By Western analysis they showed an approximately 66-kd protein in whole-cell extracts from different lines including HTM, NPCE, E50K-DF, NHDF, and HeLa. Because Optineurin was detected in both HTM and NPCE, and because NPCE is a component of transport and secretory epithelium, the authors decided to determine its expression in aqueous humor. They examined a Zoo Western blot prepared from aqueous humors of human and seven other species and were able to show the presence of this protein in all the samples tested. They further confirmed the presence of this protein in eye tissue homogenates prepared from a selected group of these animals. Their data suggest that Optineurin is a secretory protein that is highly conserved during evolution. The antibody used in all of the authors' protein studies was raised in chicken.

The authors used immunocytochemistry analysis to study the intracellular localization of Optineurin protein in a variety of cell lines [18]. They observed granular staining for the endogenous protein that was associated with vesicular structures near the nucleus (Fig. 3). This observation was supported by specific staining for Golgi indicating a perinuclear localization of Optineurin protein that extended to structures on the Golgi complex and on vesicles (Fig. 3) [18]. When the authors studied the intracellular localization of Optineurin in the optic nerve head astrocytes from both normal (Fig 4A) and glaucoma (Fig. 4B) subjects, they observed similar protein localization with no major significant differences between them. They also noted that, although both normal and E50K-mutant fibroblast cultures grew naturally and equally, the amount of endogenous protein product was substantially lower in the E50K mutant cells (Fig. 4D) than in the normal cells (Fig. 4C). Only 10% to 20% of the E50K cells were positive for the Optineurin protein as compared with 70% to 80% of the normal cells. Additionally, within the very limited E50K-positive cells, Optineurin protein seemed to be less perinuclear and more disorganized. Therefore, E50K Optineurin mutation may lower synthesis and also redistribute protein products in the affected cells. In certain other cells examined in the same panel, Optineurin was poorly detectable in the cytoplasm. The predicted theoretical instability of this protein, together with its heterogeneous intracellular distribution, suggests that Optineurin is expressed transiently and is either rapidly secreted out of the mature cells or is removed from the cells, probably through degradation signals in its 3′ UTR. The observation that, with time, the concentration of this protein accumulated in the cell culture medium at much higher levels than observed intracellularly supports this supposition.

More recently, by immunohistochemistry the authors examined the Optineurin protein distribution in the anterior segment of the normal human eye. As shown in Fig. 5, the endogenous Optineurin is strongly stained for the ciliary muscle, NPCE, and vascular endothelium. Although previously the authors had shown the presence of Optineurin mRNA in the trabecular meshwork by reverse-transcription polymerase chain reaction (RT-PCR) and Northern blotting, no specific staining was observed in this site by immunohistochemistry. This result is perhaps not surprising, because certain cell types can express visible amounts of Optineurin in culture and invisible amounts in vivo. The in vivo expression of Optineurin depends on the life span of the protein before it is degraded, the functional status of cell types, the quiescent status of Optineurin, and its antigenicity that may have been affected by postmortem sampling and tissue fixing. Additional analysis showed that Schlemm's canal and the aqueous channels are also positive for

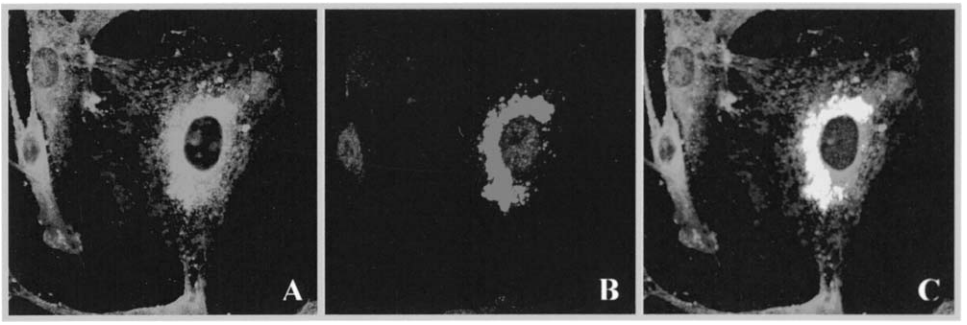

Fig. 3. Colocalization of optineurin with the Golgi apparatus. Dermal fibroblast specific staining for the Golgi apparatus (*A*), endogenous Optineurin (*B*), and composite picture of the two (*C*) are shown. Note localization of Optineurin with the Golgi apparatus (yellow staining in *C*). Also note that both cells are positive for Golgi (*A*), but only the cell stained yellow is positive for Optineurin. (See also Color Plate 1.)

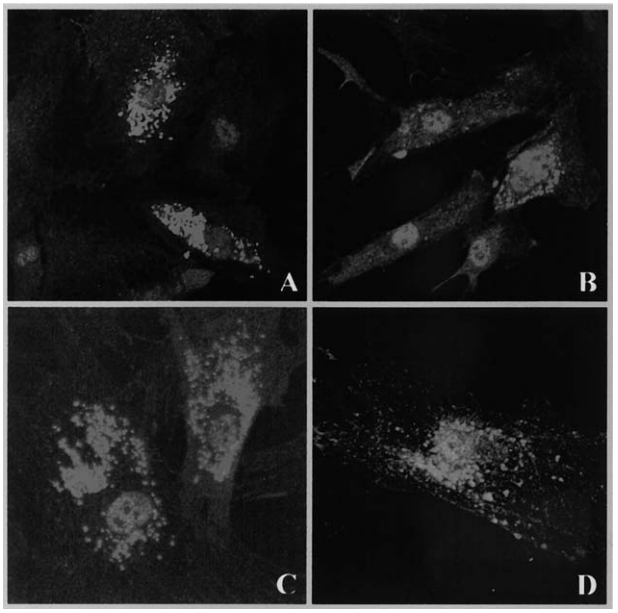

Fig. 4. Immunocytochemistry assay and cellular expression of Optineurin protein in different human cell lines. (*A*) Normal optic nerve head astrocytes; (*B*) glaucoma optic nerve head astrocytes; (*C*) normal human dermal fibroblasts; and (*D*) *OPTN* mutant dermal fibroblasts (E50K). (See also Color Plate 2.)

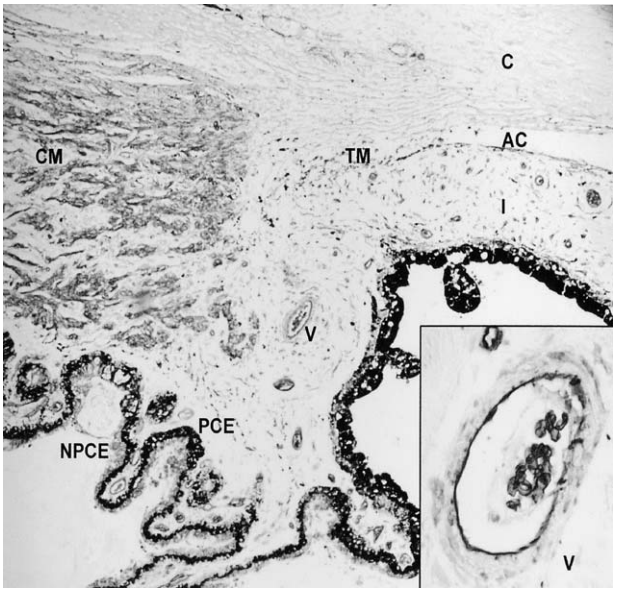

Fig. 5. Immunohistochemistry localization of the optineurin protein in anterior segment of human normal eye. AC, anterior chamber; C, cornea; CM, ciliary muscle; I, iris; NPCE, nonpigmented ciliary epithelium; PCE, pigmented ciliary epithelium; TM, trabecular meshwork; V, blood vessels. (See also Color Plate 3.)

Optineurin. In the normal optic nerve head, Optineurin is colocalized with von Willenbrand's factor in the small vessels, thus further confirming vascular localization of Optineurin protein (data not shown). Localization of Optineurin to the vascular endothelium is consistent with its role in LPG phenotype.

Discussion

The authors originally identified three mutations in the Optineurin gene in nine adult-onset LPG/POAG families and a risk-associated change in 23, mainly LPG, index cases [18]. It is not currently known exactly how these mutations function to produce the clinical presentation of glaucoma in these patients. The authors, however, reported that one particular mutation (E50K) resulted in significant reduction of this protein in cultured cells from these patients and, therefore, a dominant-negative mechanism may have been the causative effect in these patients. Furthermore, the E50K mutation is conserved in chicken, mouse, cow, and macaque and alters basic region of the first putative bZIP transcription factor domain. Because bZIP domains have a basic region for sequence-specific DNA-binding, it is likely that E50K mutation is abrogating binding of Optineurin to its partners. Evidence for this possibility was recently presented at the ARVO 2003 meeting in Florida. One of the proteins that previously had been reported to interact with Optineurin is RAB8 [25]. Interaction of Optineurin with RAB8 occurs at its N-terminus and within a region that overlaps with E50K mutation. Iwata et al [27] studied Optineurin–RAB8 protein interaction by using quartz-crystal microbalance (QCM). They were able to show although the wild-type Optineurin interacts with RAB8, the E50K mutation completely abolishes normal interaction of Optineurin with the RAB8 protein. Because RAB8 is involved in protein endocytosis and exocytosis, it is not clear how altered Optineurin–RAB8 interaction can lead to glaucoma.

Another mutation was an AG insertion in exon 6 (after Asp127; 691_692insAG) that shifted the open reading frame and truncated the normal protein by 76%. This truncated protein is expected to forfeit the normal interaction of Optineurin with RAB8, TFIIIA, Huntingtin, and E3-14.7K proteins. Another mutation (R545Q) identified in *OPTN* exon 16 is not part of a known protein domain, but it is located close to the only C2H2 zinc finger motif in the Optineurin molecule [18]. Because this protein domain is usually found in transcription factors, it is likely that the observed mutation interferes with this potential function of Optineurin. The authors also observed another alteration (M98K) in exon 5 of *OPTN* that was present in 13.61% of mainly LPG index cases and 2.13% of normal controls ($P < 0.00001$). Because this sequence change located within the second putative bZIP transcription factor basic domain and is also conserved in macaque, it is likely to be another risk factor for this condition. Functional significance of 691_692insAG, R545Q and M98K has recently been investigated. By using QCM, Iwata et al [27] were also able to show that truncated 691_692insAG mutation causes Optineurin to react very weakly with RAB8. M98K at first showed an interaction similar to that of wild-type Optineurin, but this interaction was less stable than the wild type. As one would expect, the interaction of R545Q mutation with RAB8 was similar to that of wild-type Optineurin, because this mutation is located at the C-terminus of this protein and distal to the predicted RAB8-binding site.

Optineurin and tumor necrosis factor-α pathway

Although Optineurin has no significant homology to any known protein, its interaction with a number of other proteins has already been established. Fig. 6 provides a pictorial illustration for Optineurin interaction with other proteins and its potential involvement in alternative pathways. It has previously been reported that adenovirus E3-14.7K interacts with the last 172 amino acids of Optineurin [21]. This specific interaction can block the protective effect of E3-14.7K on TNF-α cell killings induced by its receptors (ie, TNFR1 and RIP). TNF-α can also directly induce Optineurin expression in a time-dependent manner [21]. This finding suggests that Optineurin is a component of the TNF-α signaling pathway that can shift the equilibrium towards induction of apoptosis. Furthermore, it has been documented that TNF-α markedly increases the severity of damage in optic nerve heads of subjects with POAG/LTG [28,29]. Generation of this cytokine by reactive optic nerve head astrocytes and glial cells can induce excessive nitric oxide and render the cells neurotoxic to axons of the retinal ganglion cells [28,29]. Therefore, it is possible that normal endogenous Optineurin, either directly or through its interaction with other proteins, can restrain TNF-α production, possibly through a feedback mechanism, and thereby plays a neuroprotective role for this group of optic neuropathies. Consequently, the mutant forms of Optineurin in glaucoma patients may steadily provide an inadequate neuroprotection over decades of normal life, thus leading to the late-onset presentation of this optic neuropathy.

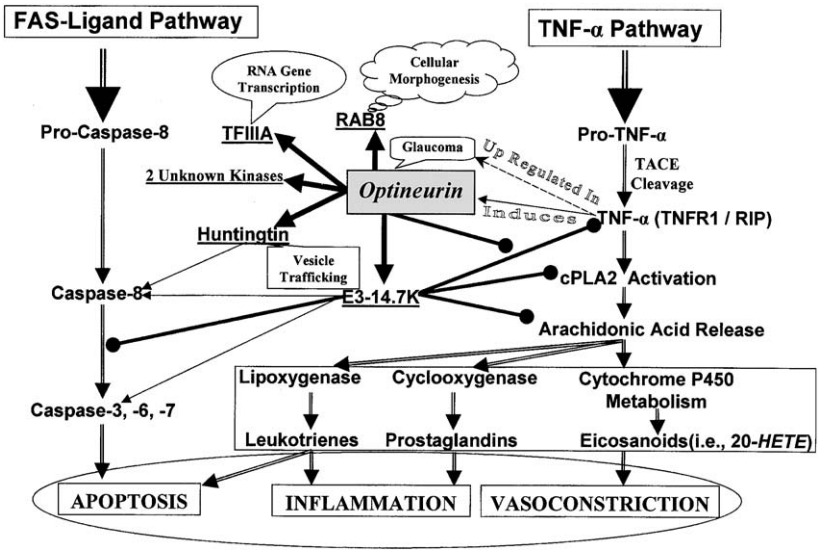

Fig. 6. A hypothetical model showing interaction of optineurin with other known proteins. Potential involvement of optineurin in two alternative pathways of Fas ligand (*left*) and TNF-α (*right*) are shown. Each arrow shows the interaction (*solid arrow*) or downstream effect (*open arrow*) of one element with another. Arrows ending with a circle show the blocking effect of one protein on another.

As illustrated in Fig. 6, TNF-α induces activation of cytosolic phospholipase A2 (cPLA2) to release arachidonic acid (AA) and its potent products, the mediators of inflammation [30]. Because E3-14.7K can block this inflammatory response [30], it is likely that Optineurin interaction with this protein may also reverse its blocking ability. Therefore, Optineurin involvement in TNF-α pathway could potentially lead to either apoptosis or inflammation (Fig. 6). In a third alternative pathway, cytochrome P450 can metabolize AA into biologically active molecules that may be directly relevant to the glaucoma phenotype. In support of this suggestion, the authors' laboratory has previously shown that mutations in cytochrome P4501B1 are responsible for PCG [12,14,16]. One AA metabolite that is directly implicated in blood vessel constriction and ion transport [31] is 20-hydroxy-eicosatetraenoic acid (20-HETE). Because recurrent vasospasm is frequently reported in LTG patients [32,33] and vasoconstriction leads to reduced aqueous humor production [34], and because the authors have recently shown a very strong expression of Optineurin protein in blood vessels and vascular endothelium, it is likely that Optineurin mutations through the AA–P450 pathway play a role in the structural damage reported in LTG patients [35]. The effectiveness of calcium-channel blockers in the treatment of LTG [36] and the authors' observations of Optineurin protein expression in vascular endothelium and human coronary arterial cell cultures further support this hypothesis. Vasospasm is present in LTG and also in Raynaud's disease and migraine [32], two conditions frequently reported with high-pressure POAG.

Optineurin and Fas ligand pathway

Because E3-14.7K interaction with Caspase-8 (CASP8) can efficiently block Fas ligand–induced apoptosis [37], it is likely that Optineurin either forms a protein complex with E3-14.7K and CASP8 to inhibit apoptosis. Alternatively, this interaction may reverse the protective effect of E3-14.7K and thereby induce apoptosis, as previously reported for TNF-α [21] (Fig. 6). Therefore, interaction of Optineurin with E3-14.7K may regulate signaling pathways downstream of both TNF receptors and Fas.

In addition to E3-14.7K, the C-terminal part of Optineurin also interacts with Huntingtin [22], the defective protein in Huntington's disease. Huntingtin is reported to have an antiapoptotic effect [38]. Because Huntingtin and E3-14.7K both bind to the C-terminal of Optineurin, it is conceivable that binding of Huntingtin to Optineurin could neutralize apoptotic signals normally mediated through Optineurin [21].

Likewise, E3-14.7K interacts with CASP8 to inhibit Fas ligand–induced apoptosis [37]. Because CASP8 is required for cell death induced by expanded polyglutamine repeats in Huntington's disease [39], a potential multidimensional protein complex formation between Optineurin-Huntingtin-CASP8-E3-14.7K may play a common role in neurodegeneration of both Huntington's disease and LPG/POAG. Furthermore, Optineurin also indirectly links Huntingtin to RAB8 [25], a small GTPase protein that binds to the N-terminal region of Optineurin. Because reorganization of actin and microtubules by RAB8 dictates drastic changes in the cell shape, it is likely that a complex molecule formed by interaction of RAB8-Optineurin-Huntingtin plays a central role in controlling cellular morphogenesis, membrane (through RAB8) or vesicle (through Huntingtin) trafficking. The authors' immunocytochemistry localization of Optineurin to the Golgi apparatus (Figs. 3,4) [18] suggests that protein trafficking is another likely function for this molecule. Recently, in a new Xenopus transgenic model, it was shown that mutant forms of Rab8 protein cause retinal degeneration [40]. The authors also showed that this protein is involved in docking of post-Golgi membranes in rods. Future investigations will determine if the interaction of Optineurin with RAB8 plays a significant role in degeneration of retinal ganglion cells as typically presented in patients with glaucoma.

Optineurin in other pathways

The central leucine-rich domain of Optineurin interacts with the N-terminal portion of TFIIIA [24]. The latter binds to the internal control region of 5S ribosomal DNA and then in association with TFIIIB and TFIIIC forms a stable preinitiation complex for gene transcription by RNA polymerase III. It is likely that Optineurin interaction with TFIIIA transforms this molecule from an inactive to an active state and thereby activates its transcription.

Optineurin has also been cloned as an NF-κB essential modulator or FIP3-related protein (NRP) but has been shown to have no effect on NF-κB signaling [23]. These authors reported that phorbol esters induce Optineurin phosphorylation but at the same time reduce its half-life. This phosphorylation is reported not to affect the subcellular localization of endogenous Optineurin [23]. Although no specific kinase activity responsible for this phosphorylation has been identified, the authors showed that Optineurin could function in the assembly and activity of two unknown kinases with molecular weights of 85 and 180 kd.

Optineurin gene expression in trabecular meshwork

Recently, two different groups studied the *OPTN* gene expression in trabecular meshwork in response to increased perfusion pressure. One group [41] used anterior chamber perfusion model of human eye and elevated IOP for 1, 3, and 24 hours. They did not observe any significant increased expression of *OPTN* in trabecular network and therefore concluded that *OPTN* gene expression is not involved in the regulation of aqueous humor outflow. Another study [42] evaluated the effect of glaucomatous insults on the expression of *OPTN* in human eyes maintained in organ culture. These investigators elevated IOP for 6 hours and for 2, 4, and 7 days. They observed significantly increased expression of *OPTN* after 2, 4, and 7 days. They also reported that TNF-α exposure induces *OPTN* expression by 2.3 times, whereas prolonged treatment with dexamethasone induces *OPTN* expression by 2.6 times. Therefore, the authors concluded that *OPTN* is inducible in trabecular meshwork and may have a protective role in trabecular meshwork. Another group [43] recently studied the effect of the expression of prostaglandin $F_{2\alpha}$ analogue genes in human ciliary muscle and trabecular meshwork. They were unable to show any changes in *OPTN* gene expression, but *MYOC* expression in the trabecular meshwork was slightly decreased.

Summary

The authors' initial estimate indicated that mutations in Optineurin are responsible for a significant proportion of LPG/POAG families [18]. Currently, there are up to 1.2 million persons with LPG and up to 2.47 million persons with POAG in the United States alone. Perhaps twice as many individuals are already affected with this condition without any identifiable clinical signs or symptoms. Investigators are eagerly awaiting confirmation of *OPTN* mutations in other glaucoma populations. Although additional mutations have already been identified in the sporadic cases of LPG, the significance of this gene in high-pressure POAG requires more intensive investigation. Limited data on partial screening of this gene indicate that *OPTN* mutations are responsible for a limited number of cases of high-pressure POAG. If the reported mutation rates of *OPTN* in the LPG group can be confirmed in other LPG or POAG patients, then this gene would be useful in diagnosing presymptomatic persons many decades before they develop this

silent and blinding eye condition. Early identification of such at-risk patients would provide an opportunity for immediate targeted medical treatments and specific glaucoma therapy that might significantly delay or completely stop the gradual progression of this condition. Therefore, identification of glaucoma-causing genes such as Myocilin, Optineurin, and others could provide molecular diagnostic tools for this category of optic neuropathy. Although patients with advanced glaucoma will not directly benefit from the use of such molecular diagnostic tools, their immediate family members could certainly benefit from the identification of the cause of the glaucoma decades before the first manifestation of the disease.

In summary, a series of mutations in the Optineurin gene have been shown to be the principal cause of adult-onset LPG/POAG phenotype in certain pedigrees [18]. The exact mechanisms through which these mutations lead to the development of glaucoma require additional functional study. The existing evidence suggests that direct interaction of Optineurin with E3-14.7K protein probably utilizes TNF-α [21] or Fas-ligand [22] pathways to mediate apoptosis, inflammation, or vasoconstriction (Fig. 6). Optineurin also functions through its interactions with other proteins in cellular morphogenesis and membrane trafficking (RAB8) [25], vesicle trafficking (Huntingtin) [22], transcription activation (TFIIIA) [24], and assembly or activity of two unknown kinases [23]. Identification of Optineurin as an adult-onset glaucoma gene and its known interaction with a group of proteins provides the first opportunity to study biochemical pathways that are thought to be involved in causation of this group of eye disorders. Furthermore, identification of this gene as a contributing factor to the development of glaucoma gives a useful tool for screening of this disorder in the elderly population and other high-risk individuals. The exact impact of *OPTN* in the development of all glaucoma phenotypes requires future study.

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