reductase in organisms other than Mtb, perhaps involving AhpD-equivalents with a thioredoxin-like active site. Others have suggested an antioxidant function for Lpd related to the role of free lipoic acid as an antioxidant (*21*).

Finally, immunochemistry and bioinformatics suggested that SucB appears to be the only lipoylated protein in Mtb H37Rv. If so, then SucB presumably sustains both the PDH and KGDH activities that were detected in Mtb 30 to 40 years ago but were only partially purified (*22*). *E. coli* has organized into operons its genes encoding PDH (*aceE*, *aceF*, and *lpd*) (*23*) and KGDH (*sucA*, *sucB*, *sucC*, and *sucD*) (*24*). No such gene clusters are evident in Mtb. Near *sucB* lie *lipB* (lipoate protein ligase) and *lipA* (lipoate synthase), which may lipoylate SucB. Mtb's *sucA* (E1 homolog of KGDH) is transcribed divergently elsewhere.

If SucB or Lpd could be inhibited in Mtb without affecting their human counterparts, the Krebs cycle in Mtb as well as the bacillus's ability to synthesize acetyl–coenzyme A (CoA) from endogenous precursors could both be vulnerable. Acetyl-CoA is essential for the glyoxylate shunt that helps sustain persistence of Mtb (*25*) and for formation of the fatty acid–rich cell wall, which constitutes both a barrier and target for chemotherapy. This may be the first known instance in which essential metabolic enzymes also engage in antioxidant defenses.

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Adult-Onset Primary Open-Angle Glaucoma Caused by Mutations in Optineurin

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Primary open-angle glaucoma (POAG) affects 33 million individuals worldwide and is a leading cause of blindness. In a study of 54 families with autosomal dominantly inherited adult-onset POAG, we identified the causative gene on chromosome 10p14 and designated it *OPTN* (for "optineurin"). Sequence alterations in *OPTN* were found in 16.7% of families with hereditary POAG, including individuals with normal intraocular pressure. The *OPTN* gene codes for a conserved 66-kilodalton protein of unknown function that has been implicated in the tumor necrosis factor–α signaling pathway and that interacts with diverse proteins including Huntingtin, Ras-associated protein RAB8, and transcription factor IIIA. Optineurin is expressed in trabecular meshwork, nonpigmented ciliary epithelium, retina, and brain, and we speculate that it plays a neuroprotective role.

Glaucoma affects over 67 million people worldwide (*1*) and is the second largest cause of bilateral blindness in the world, after cataracts (*2*). The most common form is POAG, which includes a subgroup termed normal-pressure (NPG) or low-tension glaucoma (LTG) (*3–7*). Glaucoma is genetically heterogeneous (*8*). At least eight loci have been linked to the disorder, and two genes have been identified: *CYP1B1*, encoding cytochrome P4501B1 enzyme, is mutated in primary congenital glaucoma (*9*, *10*), and *MYOC*, encoding myocilin, is mutated in juvenile-onset POAG (*11*). Here we identify one of the genes responsible for adult-onset POAG. With approval from the Human Genome Organization (HUGO) nomenclature committee, we designate the gene *OPTN* and its protein product optineurin (for "optic neuropathy inducing" pro-

Table 1. *OPTN* sequence alterations in hereditary adult-onset POAG.

Exon	cDNA change*	Predicted protein change	Number of observed mutations/families (%)	Number of observed mutations/normal chromosomes†	*P* values‡
			Disease-causing alterations		
4	c.458G>A	E50K	7/52 (13.5)	0/540	2.74×10^{-8}
6	c.691_692insAG	Premature stop	1/46 (2.2)	0/200	0.187
16	c.1944G>A	R545Q	1/46 (2.2)	0/100	0.315
	Totals		9/54§ (16.7)	0.0	5.03×10^{-5}‖
			Risk-associated alteration		
5	c.603T>A	M98K	23/169 (13.6)¶	9/422 (2.1)	2.18×10^{-7}

*New nomenclature is used (*22*). Nucleotides are numbered as in GenBank accession number AF420371. †Normal chromosomes were from Caucasian individuals with a similar age group. ‡*P* values for Fisher's exact test. §These 54 glaucoma families were screened by SSCP analysis for the entire of *OPTN*. ‖Only 100 shared chromosomes were used for calculation of this *P* value. ¶Within this group of 169 subjects, M98K was observed in 8 of 45 (17.8%) familial and 15 of 124 (12.1%) sporadic individuals with glaucoma. Most of these individuals have normal IOP and were screened for sequence changes only in exon 5.

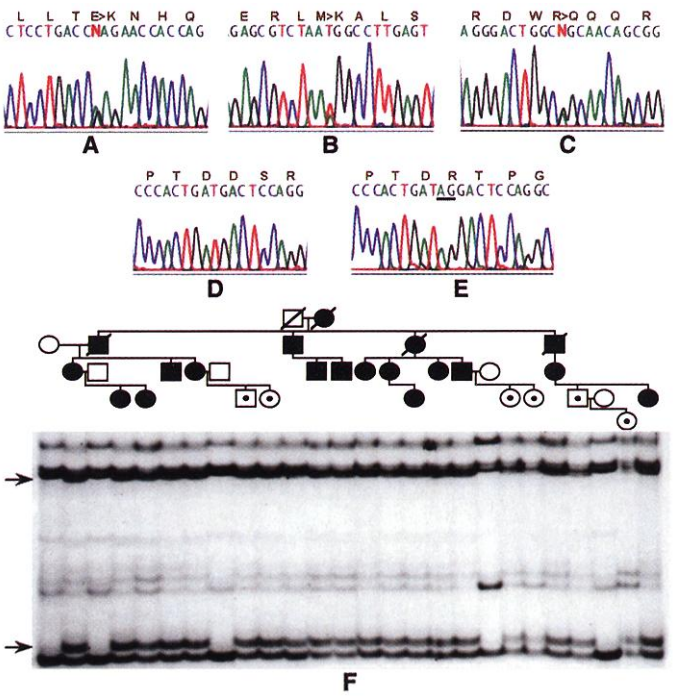

Fig. 1. (**A** to **E**) DNA sequence analyses showing four of the *OPTN* mutations detected in patients with POAG. (A) E50K, (B) M98K, (C) R545Q, (D) wild-type, and (E) mutant (AG insertion) cloned sequences. (**F**) Single-strand conformational polymorphism segregation of the E50K mutation in our original GLC1E-linked family (*13*). For simplicity, 28 normal family members with no mutation are excluded. Asymptomatic individuals are indicated with a dot inside their symbols. Arrows indicate the E50K mutant band.

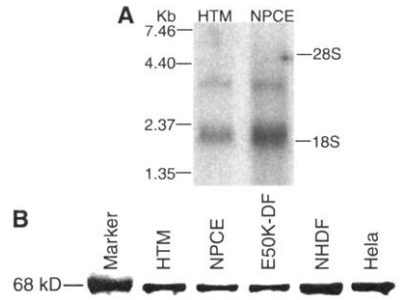

Fig. 2. (**A**) Northern blot analysis of *OPTN* mRNA in HTM and NPCE cell lines. RNA size markers are shown on the left, and the positions of 28*S* and 18*S* ribosomal RNAs are indicated on the right. (**B**) Western blot (*18*) showing optineurin protein expression in HTM, NPCE, dermal fibroblast from a patient with E50K mutation (E50K-DF), normal human dermal fibroblast (NHDF), and HeLa cell lines.

tein). The gene was previously identified as *FIP-2* (*12*).

We previously mapped an adult-onset POAG locus (*GLC1E*) to a 21-centimorgan (cM) region on chromosome 10p14-p15 (*13*) and subsequently reduced the critical region to 5 cM. After excluding four genes, we selected *OPTN* as a candidate gene on the basis of its physical location in this region and its expression in retina. The *OPTN* gene has previously been identified as *FIP-2* (*12*) and *NRP* (*14*) and its product as an interacting protein for Huntingtin (*15*), transcription factor IIIA (*16*), and RAB8 (*17*). After identifying a missense mutation [$Glu^{50} \rightarrow$ Lys (E50K)] in our original kindred (*13*), we studied 54 families with autosomal dominant adult-onset glaucoma with at least one member having NPG. The majority of these families presented with normal intraocular pressure (IOP) (<22 mm Hg), whereas others had mixed clinical pictures of both normal and moderately raised IOP (23 to 26 mm Hg) in the same family. Our analysis revealed four sequence alterations in *OPTN* (Table 1 and Fig. 1). A recurrent E50K mutation (Fig. 1, A and F) was identified in seven families; it segregated in 124 members, including 38 affected, 16 asymptomatic gene carriers, 50 unaffected, and 20 spouses. Of the 38 affected subjects, 7 (18.4%) had elevated IOP (23 to 26 mm Hg) and the remaining individuals had normal IOP (11 to 21 mm Hg). Two additional mutations [2–base pair (bp) "AG" insertion and $Arg^{545} \rightarrow$ Gln (R545Q)] were identified in two other families with normal IOP (Table 1) (Fig. 1, C to E). The recurrent E50K is located within a putative bZIP motif, conserved in the mouse, bovine, and macaque genomes, and may have a dominant-negative effect. The bZIP motif is a transcription factor domain that is normally involved in DNA binding and protein dimerization. The 2-bp "AG" insertion truncates the protein by 76% and presumably leads to loss of function or haploinsufficiency. Although the R545Q mutation is not part of a known protein domain, it is situated near the only zinc finger motif within optineurin. This motif is normally seen in transcription factors.

A fourth sequence change [$Met^{98} \rightarrow$ Lys (M98K)] (Fig. 1B) was initially detected in both glaucoma families and in normal control subjects (Table 1). To determine the significance of this change, we screened 124 sporadic cases of glaucoma with predominantly normal IOP. M98K was identified in 23 of 169 (13.6%) glaucoma subjects and 9 of 422 (2.1%) normal control chromosomes. Only 3 of these 23 (13.0%) subjects had IOP values above normal (23, 26, and 40 mm Hg). The average age of the normal control subjects with the M98K sequence change was 54.3 years, so it remains possible that a subset will eventually develop glaucoma. Nevertheless, because the difference in frequency between the affected (13.6%) and normal (2.1%) chromosomes is highly significant ($P = 2.18 \times 10^{-7}$) and because M98K is located within a putative bZIP domain and also conserved in macaque, it may represent a risk-associated factor or a dominant susceptibility allele.

Our data suggest that mutations in *OPTN* may be responsible for 16.7% of hereditary forms of normal-tension glaucoma with an additional attributable risk factor of 13.6% in both familial and sporadic cases (Table 1). However, because we sequenced the entire *OPTN* gene in only one family (*13*) and thereafter used single-strand conformational polymorphism (SSCP) analysis to screen the remaining glaucoma cases, it is possible that additional mutations have been missed. We also identified additional non–disease-causing sequence alterations in the *OPTN* gene (Web table 1) (*18*).

OPTN contains three noncoding exons in the 5′-untranslated region (UTR) and 13 exons (Web fig. 1) (*18*) that code for a 577–amino acid protein. Alternative splicing at the 5′-UTR generates at least three different isoforms, but all have the same reading frame (GenBank accession numbers AF420371 to AF420373). The mouse *Optn* gene codes for a 584–amino acid protein (67 kD) that has 78% identity with human optineurin (*19*). The public databases contain partial or complete sequences for *OPTN* homologs from macaque, rat (*16*), pig, and bovine, all showing a substantial degree of similarity to human *OPTN*.

Expression of human *OPTN* transcript has previously been reported in heart, brain, placenta, liver, skeletal muscle, kidney, and pancreas (*12*). Our analysis of *OPTN* by reverse tran-

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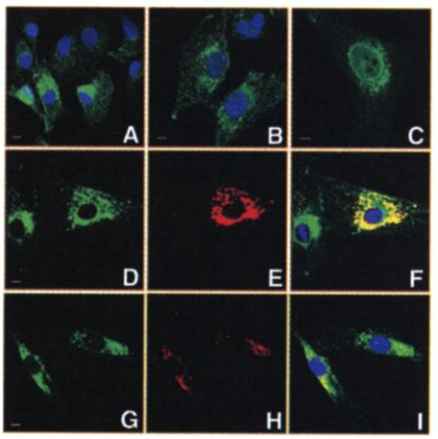

Fig. 3. Colocalization of optineurin with the Golgi apparatus. Immunocytochemistry assay and intracellular expression of optineurin in different human cell lines: (**A** to **C**) transformed cell lines and (**D** to **I**) primary dermal fibroblast cells. (A) HTM, (B) NPCE, and (C) HeLa. (D to F) Normal dermal fibroblast specific staining for (D) Golgi apparatus, (E) endogenous optineurin, and (F) superimposition of the two (yellow staining). (G to I) Dermal fibroblast of a glaucoma patient with an E50K mutation stained for (G) Golgi apparatus, (H) endogenous protein, and (I) superimposition of the two. Scale bars, 5 μm.

scription–polymerase chain reaction showed further expression in human trabecular meshwork (HTM), nonpigmented ciliary epithelium (NPCE), retina, brain, adrenal cortex, liver, fetus, lymphocyte, and fibroblast. Northern blotting revealed a major 2.0-kb transcript in HTM and NPCE and a minor 3.6-kb message that was three to four times less abundant (Fig. 2A).

Two different 18–amino acid peptides from the NH_2- and COOH-termini of optineurin were used to immunize chickens and to obtain antibodies to optineurin (*18*). The selected peptides are 100% conserved within human, mouse, and macaque. One of these antibodies cross reacted (*18*) with an ~66-kD protein in whole-cell extracts from a variety of cell lines (Fig. 2B). We also detected optineurin expression in aqueous humor samples of human, macaque, bovine, pig, goat, sheep, cat, and rabbit, suggesting that it is a secreted protein.

We next investigated the intracellular localization of optineurin by immunocytochemistry (*18*) using both primary and transformed cell lines. Endogenous optineurin showed granular staining that was associated with vesicular structures near the nucleus (Fig. 3, A to C, E, and H). The staining colocalized (Fig. 3, F and I) with a marker specific for the Golgi apparatus (Fig. 3, D and G). In a dermal fibroblast culture from a patient with an E50K mutation, optineurin was present at much lower levels than in a similar culture from a normal subject.

Optineurin has no significant homology to any protein, but it is known to interact with adenovirus E3-14.7K (*12*), Huntingtin (*15*), transcription factor IIIA (*16*), RAB8 (*17*), and two unknown kinases (*14*). Optineurin's ability to block the protective effect of E3-14.7K on tumor necrosis factor–α (TNF-α)–mediated cell killing suggests that this protein may be a component of the TNF-α signaling pathway that can shift the equilibrium toward induction of apoptosis (*12*). TNF-α can markedly increase the severity of damage in optic nerve heads of both POAG and LTG subjects (*20*, *21*). We speculate that wild-type optineurin, operating through the TNF-α pathway, plays a neuroprotective role in the eye and optic nerve, but when defective, it produces visual loss and optic neuropathy as typically seen in normal and high-pressure glaucoma.

Identification of *OPTN* as an adult-onset glaucoma gene provides an opportunity to study the biochemical pathways that may be involved in the pathogenesis of this group of optic neuropathies. In addition, because *OPTN* mutations are a contributing factor in patients with NPG, the gene may be a useful tool for presymptomatic screening of the general population.

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Methyltransferase Recruitment and DNA Hypermethylation of Target Promoters by an Oncogenic Transcription Factor

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DNA methylation of tumor suppressor genes is a frequent mechanism of transcriptional silencing in cancer. The molecular mechanisms underlying the specificity of methylation are unknown. We report here that the leukemia-promoting PML-RAR fusion protein induces gene hypermethylation and silencing by recruiting DNA methyltransferases to target promoters and that hypermethylation contributes to its leukemogenic potential. Retinoic acid treatment induces promoter demethylation, gene reexpression, and reversion of the transformed phenotype. These results establish a mechanistic link between genetic and epigenetic changes during transformation and suggest that hypermethylation contributes to the early steps of carcinogenesis.

Cancer cells present global hypomethylation of the genome and hypermethylation of islands of CpG dinucleotide clusters within specific DNA regions (*1*–*3*). Although overexpression of DNA methyltransferases (Dnmt's) has been proposed as a mechanism for aberrant genome methylation, it does not explain the specific regional hypermethyl-