TANK-BINDING KINASE 1 (*TBK1*) GENE AND OPEN-ANGLE GLAUCOMAS (AN AMERICAN OPHTHALMOLOGICAL SOCIETY THESIS)

By John H. Fingert MD PhD, Alan L. Robin MD, Todd E. Scheetz PhD, Young H. Kwon MD PhD, Jeffrey M. Liebmann MD, Robert Ritch MD, and Wallace L.M. Alward MD

ABSTRACT

Purpose: To investigate the role of TANK-binding kinase 1 (*TBK1*) gene copy-number variations (ie, gene duplications and triplications) in the pathophysiology of various open-angle glaucomas.

Methods: In previous studies, we discovered that copy-number variations in the *TBK1* gene are associated with normal-tension glaucoma. Here, we investigated the prevalence of copy-number variations in cohorts of patients with other open-angle glaucomas—juvenile-onset open-angle glaucoma (n=30), pigmentary glaucoma (n=209), exfoliation glaucoma (n=225), and steroid-induced glaucoma (n=79)—using a quantitative polymerase chain reaction assay.

Results: No *TBK1* gene copy-number variations were detected in patients with juvenile-onset open-angle glaucoma, pigmentary glaucoma, or steroid-induced glaucoma. A *TBK1* gene duplication was detected in one (0.44%) of the 225 exfoliation glaucoma patients.

Conclusions: *TBK1* gene copy-number variations (gene duplications and triplications) have been previously associated with normal-tension glaucoma. An exploration of other open-angle glaucomas detected a *TBK1* copy-number variation in a patient with exfoliation glaucoma, which is the first example of a *TBK1* mutation in a glaucoma patient with a diagnosis other than normal-tension glaucoma. A broader phenotypic range may be associated with *TBK1* copy-number variations, although mutations in this gene are most often detected in patients with normal-tension glaucoma.

Trans Am Ophthalmol Soc 2016;114:T6[1-11].

INTRODUCTION

By the year 2040, more than 110 million people will be affected with glaucoma worldwide.[1] In the United States, glaucoma is the second leading cause of irreversible blindness and the leading cause among Americans with African heritage.[2] Glaucoma is a heterogeneous group of diseases that share two defining clinical features: structural defects (eg, optic disc cupping) and functional defects (eg, visual field loss). Primary open-angle glaucoma (POAG) is the most common form of glaucoma worldwide.[1] Many clinical risk factors for POAG have been described, including older age, African or Latino ancestry, family history, higher intraocular pressure (IOP), and more recently, central corneal thickness (CCT).[3] Higher IOP confers greater risk for developing glaucoma.[4] However, glaucoma can occur at any IOP.[5] Primary open-angle glaucoma that occurs with IOPs that never rise above an arbitrary threshold of 21 mm Hg is frequently termed normal-tension glaucoma (NTG).

The concept that POAG is heritable is supported by epidemiologic studies, twin studies, and reports of families in which the condition is transmitted as a heritable trait.[6-19] Formal studies to quantify the role of genes in the development of POAG are extremely limited. One twin study, in which a diagnosis of glaucoma was detected by review of a government registry, estimated the heritability of POAG to be 0.13 (13%).[20] However, this is likely a significant underestimate owing to small sample size and the methods used to identify cases of glaucoma.

Many of the component features of glaucoma, or "endophenotypes," such as IOP, cup-to-disc ratio, and CCT, are also known to be heritable traits. Several studies of IOP have shown that this risk factor for glaucoma also has a genetic basis. For example, early studies of normal subjects by Armaly[21] indicated that the magnitude of IOP has a heritability of 0.23 to 0.47. The Beaver Dam Eye Study and the Salisbury Eye Evaluation Study also showed a correlation between IOP in family members (heritability of 0.29 to 0.36).[22,23] Similar studies of cup-to-disc ratio have reported heritability of 0.48 to 0.56.[22,23] More recently, CCT has been identified as another highly heritable risk factor for glaucoma. A strong correlation of CCT has been detected between first-degree relatives (heritability of 0.6 to 0.7)[24] and between twins (heritability of 0.95).[24,25] These data show that both POAG and its component features are highly heritable and have a strong genetic basis.

Some cases of POAG are likely caused by the combined actions of several genetic and environmental factors that each contribute a small risk for glaucoma. Such genetic risk factors are commonly observed in both glaucoma patients and normal individuals, but are statistically more frequently observed in POAG patients. Each factor is incapable of causing disease on its own, but the cumulative effects of many of these relatively weak genetic and environmental risk factors may lead to development of POAG.[26] This form of POAG does not have an obvious inheritance pattern as a result of the many independently inherited factors that combine to cause disease. However, because families (and ethnic groups) share many genetic factors, complex genetic forms of glaucoma may be more common within families or specific ethnic groups. Recently, large genome-wide association studies have begun to detect several of these POAG genetic risk factors, including caveolin 1/2 (*CAV1*/*CAV2*),[27-29] cyclin-dependent kinase inhibitor 2B antisense RNA1

From the Department of Ophthalmology and Visual Sciences, Carver College of Medicine, University of Iowa, Iowa City, Iowa (Dr Fingert, Dr Scheetz, Dr Kwon, Dr Alward); the Stephen A. Wynn Institute for Vision Research, University of Iowa, Iowa City, Iowa (Dr Fingert, Dr Scheetz, Dr Kwon, Dr Alward); the Departments of Ophthalmology and International Health, School of Medicine and the Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland (Dr Robin); the Department of Ophthalmology, University of Maryland, Baltimore, Maryland (Dr Robin); Columbia University Medical Center, New York, New York (Dr Liebmann); the Einhorn Clinical Research Center, New York Eye and Ear Infirmary of Mount Sinai, New York, New York (Dr Ritch).

(*CDKN2B-AS1*),[30-36] transmembrane and coiled-coil domains (*TMC01*),[30,35] sine oculis homeobox 1/6 (*SIX1*/*SIX6*),[34,36-38] atonal homolog 7 (*ATOH7*),[31] S1 RNA binding domain 1 (*SRBD1*),[39] elongation of long-chain fatty acids family member 5 (*ELOVL5*),[39] toll-like receptor 4 (*TLR4*),[40,41] and a chromosome 8q22 locus.[34] It is likely that many more of these factors remain to be discovered.

Other cases of glaucoma have a relatively simple genetic basis and are caused primarily by mutations in single genes. These glaucomas have been characterized through genetic studies (linkage analysis) of large POAG pedigrees. Studies of POAG pedigrees have identified and confirmed glaucoma-causing mutations in three POAG genes: myocilin (*MYOC*),[42] optineurin (*OPTN*),[43] and TANK-binding kinase 1 (*TBK1*).[44] Each of these genes has been reported to cause glaucoma with little contribution from other genes or from environmental factors. The vast majority of individuals that inherit a mutation in one of these genes develop glaucoma, and mutations are very rarely seen in healthy individuals. Glaucoma due to these genes is passed down through families in simple recognizable patterns (ie, autosomal dominant) as described by Mendel. As a result, single gene forms of disease are often described as Mendelian.

Mutations in the known Mendelian glaucoma genes are responsible for approximately 5% of POAG cases.[26] The first glaucoma gene to be discovered was myocilin, and mutations in this gene are responsible for 3% to 4% of POAG cases.[45] Myocilin-associated glaucoma is characterized by elevated IOP and a strong family history of disease with dominant inheritance patterns.[42,45] Mutations cause abnormal accumulation of intracellular myocilin protein that is toxic to trabecular meshwork cells, which leads to dysfunction of the aqueous fluid drainage system and ultimately to high IOP and glaucoma.[3] Transgenic animal studies of myocilin glaucoma have recapitulated human glaucoma and suggested promising novel therapies.[46,47]

Mutations in optineurin, the second Mendelian glaucoma gene to be discovered, are associated with 1% to 2% of POAG cases that occur at lower IOP, ie, NTG.[26,43,48] Optineurin encodes a protein that has roles in several important cellular processes, including autophagy and NF-κB signaling. Additional glaucoma genes have been reported (*WDR36*, *NTF4*, and *ASB10*), but their role in the development of glaucoma has not been confirmed in all studies.[49-56]

IDENTIFICATION OF TANK-BINDING KINASE 1 AS A NEW NORMAL-TENSION GLAUCOMA GENE

We recently identified a second NTG gene, TANK-binding kinase 1 (*TBK1*), with studies of a large four-generation African American family (pedigree GGO-441) in which 10 family members were diagnosed with NTG.[44] Affected family members had clinical features consistent with severe NTG. The mean maximum IOP in the affected family members was 18.2 ± 4.1 mm Hg, and mean cup-to-disc ratio at the time of diagnosis was greater than 0.9. Moreover, there was an early onset of glaucoma in this pedigree, with a mean age at diagnosis of 36 ± 8.2 years.[44] Linkage-based studies of this family and mutation screening identified a large duplication spanning the *TBK1* gene as the cause of NTG in this pedigree.[44]

Subsequent studies of many large populations of NTG patients worldwide established that copy-number variations (CNVs), including duplications or triplications of the *TBK1* gene, cause approximately 1% of NTG cases (Figure 1).[44,57-59] In these studies, another large NTG pedigree was discovered to have a triplication spanning the *TBK1* gene.[44,59,60] Overall, the frequency of *TBK1* CNVs in NTG patients (8 of 1105, 0.72%) in these studies is statistically higher ($P = .0018$) than in control subjects (0 of 1320, 0%). The CNVs ranged in size from 295,000 base pairs (GGJ-414) to 690,000 base pairs (GGO-441), and all of the identified CNVs overlap the entire *TBK1* gene. Moreover, *TBK1* is the only gene that is spanned by the overlap of the CNVs (Figure 1). Duplications and triplications of *TBK1* are associated with between 0.4% and 1.3% of NTG cases in various populations and have a prevalence of 0.72% in NTG patients overall.

FUNCTIONAL STUDIES OF *TBK1* DUPLICATION ON GENE EXPRESSION

We investigated the functional effect of an extra copy of the *TBK1* gene by comparing gene expression in a fibroblast cell culture system. Fibroblast cells were obtained from six NTG patients from pedigree GGO-441 who were known to have a *TBK1* gene duplication and from six control subjects. The expression level of *TBK1* mRNA in these cells was measured using three independent methods: Northern blot analysis, quantitative reverse transcriptase polymerase chain reaction (PCR) assays, and microarray analysis. In all cases, *TBK1* gene duplications were found to result in increased *TBK1* mRNA expression. In the microarray-based studies, *TBK1* mRNA was found to be 1.60-fold higher in fibroblast cells from patients with a *TBK1* gene duplication than in controls ($P = .00074$), demonstrating a functional consequence of the gene duplication.[44] Increased transcription of *TBK1* mRNA suggests that duplication of the *TBK1* gene dysregulates its expression and may lead to increased production and/or kinase activity of *TBK1* as a step in the pathogenesis of glaucoma.

Although increased *TBK1* gene expression was detected in cells carrying a *TBK1* gene duplication, the expression levels of other genes of interest in autophagy, including PTEN-induced putative kinase 1 (*PINK1*), parkin RBR E3 ubiquitin protein ligase (*PARK2*), optineurin (*OPTN*), and microtubule-associated protein 1 light chain 3 alpha (*LC3*), beclin (*BECN1*), and sequestosome (*SQSTM1*) were not significantly altered.

TBK1 GENE DISCOVERY PROVIDES INSIGHTS INTO NORMAL-TENSION GLAUCOMA PATHOGENESIS

The human *TBK1* gene is composed of 21 exons and encodes a 729 amino acid, 84 kDa protein that has several known functional domains, including a kinase domain (AA 1-308), a ubiquitin-like domain (AA 309-383), a scaffold/dimerization domain (AA 407-657), and a TANK-binding domain (AA 688-729).[61] Several key structures in the *TBK1* protein have been identified that regulate its function. Kinase function is activated by phosphorylation of Ser172[62] and ubiquination of Lys30 and Lys401.[61] *TBK1* participates in several signaling pathways (NF-κB,[63] autophagy,[64] and others) by phosphorylating downstream target proteins. Several substrates for

TBK1's kinase activity have been identified,[65] including interferon regulatory factor 3 (IRF3),[66] interferon regulatory factor 7 (IRF7),[66] p62,[67] and most notably, OPTN.[64]

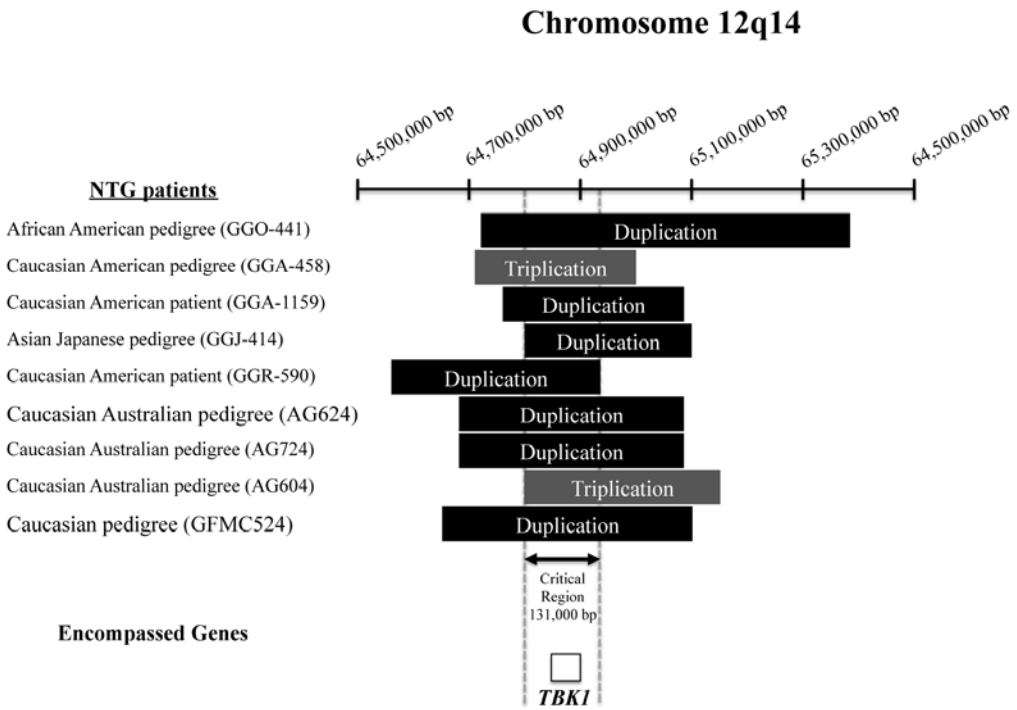

FIGURE 1

Extent of *TBK1* copy-number variations (CNVs) on chromosome 12q14 detected in normal-tension glaucoma (NTG) patients and pedigrees. The overlap of these CNVs spans a single gene, *TBK1*.

Two genes, *TBK1* and *OPTN*, are known to cause high-penetrance, autosomal dominant forms of NTG.[26] Copy-number variations in *TBK1* are associated with approximately 1% of NTG cases, whereas missense mutations in *OPTN* are associated with 1% to 2% of NTG cases.[43,44] Remarkably, these genes encode proteins that have been shown to directly interact with one another. Early studies by Morton and coworkers[68] and by others[69] used the yeast two-hybrid system, glutathione-S-transferase pull-down assays, and co-immunoprecipitation to show that *TBK1* interacts with OPTN. The *TBK1*-binding domain of OPTN was subsequently localized to its N-terminus (amino acid residues 78-121), and the OPTN-binding domain of *TBK1* was mapped to the C-terminus (amino acid residues 688-729).[68] In addition to interacting with OPTN, *TBK1* phosphorylates and activates OPTN.[64] Not only have both of the two known Mendelian NTG genes been shown to interact with each other, but glaucoma-causing mutations have also been shown to alter this interaction. The E50K mutation in OPTN enhances binding with *TBK1*,[68] and recent cell culture studies have suggested that the pathogenicity of OPTN mutations is dependent on interactions with *TBK1*.[70] These data suggest a common biological pathway and mechanism of disease for NTG caused by mutation of *TBK1* or *OPTN*.

TBK1 FUNCTION AND INTERACTION WITH OPTINEURIN SUGGESTS A ROLE FOR AUTOPHAGY IN NORMAL-TENSION GLAUCOMA PATHOGENESIS

Genetic studies of NTG have identified mutations or variations in three genes known to participate in autophagy, an important catabolic biological process. In addition to the *TBK1* and *OPTN* genes, which have been linked with autosomal dominant, Mendelian forms of NTG, the toll-like receptor 4 (*TLR4*) gene has also been associated with NTG. In some,[40,41] but not all,[71] association studies of NTG, *TLR4* has been associated with complex genetic forms of NTG. The known roles of *TBK1*, OPTN, and TLR4 in autophagy (described below) and their association with NTG suggest that autophagy has an important role in this form of glaucoma.

Macroautophagy, hereafter referred to as autophagy, is a catabolic process used to digest proteins and cellular structures such as organelles for energy in times of nutritional deprivation. In autophagy, a double membrane structure (the autophagosome) forms around materials in the cytoplasm. Autophagosomes then fuse with lysosomes and their contents are degraded into basic building blocks (ie, amino acids), which support cell survival in times of starvation.[72] The catabolic mechanisms of autophagy have been shown to serve a variety of disparate purposes beyond adapting to nutritional stress. Autophagy may support cellular homeostasis by degrading proteins that accumulate from basal metabolism.[72] The innate immune system may also utilize autophagy to capture and eliminate intracellular pathogens.[64,73] Finally, autophagy has a key role in the pathophysiology of many neurodegenerative diseases, such as amyotrophic lateral sclerosis,[74] Parkinson disease, Huntington disease, and Alzheimer disease.[75] The role of autophagy in the neuronal cell death in these diseases suggests that it may be involved in other neurodegenerative diseases, such as glaucoma.

Autophagy has been implicated as a contributor to retinal ganglion cell death in several experimental animal models of glaucoma. Activation of autophagy in some of these animal models increases the rate of cell death. For example, increased autophagy has been linked with retinal ganglion cell death observed in a rat glaucoma model with chronic elevation of IOP[76] and in a rat model of retinal ischemia following high IOP.[77] In contrast, in other animal models of glaucoma, activation of autophagy appears to be neuroprotective. In one mouse model of glaucoma that employs an acute, severe injury to the optic nerve (optic nerve transection), induction of autophagy appears to be protective to retinal ganglion cells.[78] The range of influences that autophagy may exert on retinal ganglion cell survival suggests complex interactions that depend heavily on biological context. In some circumstances autophagy may promote retinal ganglion cell survival (such as following optic nerve transection), perhaps by clearing damaged or toxic cellular components.[79,80] However, in other circumstances, activation of autophagy may promote apoptosis and retinal ganglion cell death, possibly by excessive degradation of cellular structures necessary for survival.[76] These data also raise the possibility that dysregulation of autophagy by either overstimulation (too much autophagy) or by defects that block its function (too little autophagy) may be pathologic. Tight regulation of autophagy may be necessary for cell survival. Moreover, mitophagy is a subset of autophagy in which the mitochondria are targeted for degradation, and recent studies have suggested that *TBK1* has a role in this specific type of autophagy.[81,82] Although the evidence linking *TBK1*-related NTG to autophagy is compelling, it is possible that mutations in *TBK1* cause disease via another mechanism. *TBK1* also has important roles in NF-κB signaling and other biological processes that could also be involved in the pathogenesis of NTG.[26]

CURRENT STUDIES OF *TBK1* AND JUVENILE-ONSET OPEN-ANGLE GLAUCOMA AND SECONDARY FORMS OF GLAUCOMA

Family-based genetic studies of glaucoma have successfully identified glaucoma-causing genes such as myocilin and optineurin and provided important insights into the pathophysiology of glaucoma. Here we present additional research into the mechanisms of NTG using research that began with genetic studies of large glaucoma pedigrees and has been confirmed with population-based experiments. It is our general hypothesis that genetic studies of open-angle glaucoma pedigrees will facilitate identification of new glaucoma-causing genes and will provide novel insights into the biological pathways that are important in the pathogenesis of glaucoma. With this approach, we have identified *TBK1* as a gene that causes NTG. Frequently, a gene discovered to cause one form of a disease is later shown to cause similar forms of disease as well. For example, mutations in myocilin were initially discovered to be a common cause of juvenile-onset open-angle glaucoma (JOAG)[42] and were later shown to also cause 3% to 4% of POAG.[42,45] We have already searched for *TBK1* CNVs in some forms of glaucoma, ie, POAG. However, no *TBK1* CNVs were detected in patients with POAG that have IOP > 21 mm Hg.[44,59,83] Here we extend this research by hypothesizing that mutations in *TBK1* might cause other forms of open-angle glaucoma, such as JOAG, pigmentary glaucoma (PG), exfoliation glaucoma (XFG), or steroid-induced glaucoma (SIG). To test this hypothesis, cohorts of patients with JOAG, PG, XFG, and SIG were investigated for the presence of *TBK1* mutations.

METHODS AND MATERIALS

STUDY DESIGN

Patients from Iowa diagnosed with JOAG, PG, XFG, or SIG were tested for CNVs in the *TBK1* gene in a prospective case-control study. Glaucoma patients were tested for *TBK1* CNVs in a prospective manner, and 608 matched control subjects from Iowa that had been previously tested for *TBK1* mutations were also used as controls for this study.[44,58]

ENROLLED STUDY PATIENTS

All participants provided informed consent for this research project, which was conducted under the approval of the University of Iowa's Internal Review Board for human subjects research. All patients received a comprehensive eye examination by glaucoma fellowship–trained, board-certified ophthalmologists that included visual acuity, gonioscopy, tonometry, perimetry, and ophthalmoscopy. Most patients also underwent optic nerve and retinal nerve fiber layer examination with spectral domain optical coherence tomography. In some cases, family members who were unavailable for examination shared records from community ophthalmologists that were reviewed by the research team. Patients were judged to have open-angle glaucoma based on standard criteria as we have previously described.[44,84] Briefly, patients with glaucomatous cupping of the optic disc and corresponding visual field defects with a maximum IOP > 21 mm Hg were diagnosed with POAG. Patients with POAG diagnosed at age < 40 years were diagnosed with JOAG. All JOAG patients had been previously tested for myocilin mutations, and those with myocilin mutations were not included in this study. A total of 30 JOAG patients from Iowa were included in the study.

Patients were judged to have pigment dispersion syndrome (PDS) if Krukenberg spindle, iris transillumination defects, pigment on zonules or lens equator, or dark pigmentation of the trabecular meshwork were identified on examination, and they were further diagnosed with PG if they met criteria for glaucoma as described above. A total of 297 patients with PDS were enrolled (221 from Iowa and 76 from New York), and of these PDS patients, 209 also had PG (165 from Iowa and 44 from New York). Patients were judged to have exfoliation syndrome (XFS) if they had signs of exfoliation material on the anterior surface of the lens, iris margin, or corneal endothelium; peripupillary iris transillumination defects; or brown sugar–colored pigmentation of the trabecular meshwork. Patients were further judged to have XFG if they had signs of XFS and also met criteria for glaucoma as described above. A total of 267 patients with XFS were enrolled (167 from Iowa and 100 from New York), and of these XFS patients, 225 had XFG, 167 from Iowa and 58 from New York. Patients were judged to have SIG if they had glaucoma associated with ocular hypertension following

administration of topical, oral, periocular, or intraocular steroids (79 from Iowa). Normal control subjects had a complete eye examination and were judged not to have glaucoma by an ophthalmologist. Control subjects in this report were part of previous studies.[44,58] DNA samples were prepared from blood samples using standard techniques.[85]

Power calculations were conducted to assess our ability to detect *TBK1* CNVs in each cohort. Given the individual cohort sizes, we had 80% power to detect CNVs if they occurred with a frequency of 7% or greater in the JOAG population (n=30), 0.94% or greater in the PG population (n=209), 0.86% or greater in the XFG population (n=225), and 2.5% or greater in the SIG population (n=79).

GENETIC STUDIES (MUTATION DETECTION)

A TaqMan Copy Number Assay, a quantitative polymerase chain reaction (qPCR) assay (TaqMan, Probe Hs06980763_cn specific for *TBK1* exon 3, Applied BioSystems, Carlsbad, California) was used to assess *TBK1* gene dosage in JOAG, PG, XFG, and SIG patients as well as in previously tested control subjects.[44,58] The TaqMan assay was conducted using the manufacturer's protocol on a BioRad C1000 thermocycler with the CFX96 real-time PCR adapter, and a positive control was used in every experiment. All experiments were conducted in triplicate. Confirmation of positive tests for *TBK1* CNVs was conducted with two additional independent Taqman primer sets (Probe Hs02974732_cn specific for *TBK1* exon 13 and Probe Hs07396069_cn specific for *TBK1* exon1, Applied BioSystems). In all CNV experiments, an RNAseP probe was used as a control.

RESULTS

TESTING COHORTS OF JUVENILE-ONSET OPEN-ANGLE GLAUCOMA PATIENTS FOR COPY-NUMBER VARIATIONS THAT SPAN THE *TBK1* GENE

Juvenile-onset open-angle glaucoma patients have not been evaluated for *TBK1* mutations in prior reports. In this study, a cohort of 30 patients diagnosed with JOAG was tested for CNVs that span the *TBK1* gene using a qPCR assay that measures copies of the 5' end of the gene, exon 3. In this novel assessment of JOAG patients, no *TBK1* CNVs were detected, suggesting that *TBK1* gene dosage variants are not commonly involved in the pathogenesis of JOAG.

TESTING COHORTS OF SECONDARY GLAUCOMA PATIENTS FOR COPY-NUMBER VARIATIONS THAT SPAN THE *TBK1* GENE

The role of *TBK1* CNVs in secondary forms of glaucoma has not previously been investigated. Here we tested patients with three different types of secondary open-angle glaucomas, including PG, XFG, and SIG (Table 1), for mutations that alter *TBK1* gene dosage using the same qPCR assay described above.

TABLE 1. PREVALENCE OF *TBK1* COPY-NUMBER VARIATIONS IN SECONDARY GLAUCOMA PATIENTS

TBK1 CNV	TYPE OF SECONDARY GLAUCOMA			
	PIGMENTARY n = 209	EXFOLIATION n = 225	STEROID-INDUCED n = 79	CONTROLS n = 608
TBK1 duplications	0	1 (0.44%)	0	0
TBK1 triplications	0	0	0	0
Total	0	1 (0.44%)	0	0

CNV, copy-number variation.

Pigmentary Glaucoma

A cohort of 297 patients with PDS, 209 of which also had secondary PG, was tested for deletions or duplications of the *TBK1* gene. No *TBK1* deletions or duplications were detected.

Exfoliation Glaucoma

A cohort of 267 patients with XFS, 225 of which also had secondary XFG, was tested for deletions or duplications of the *TBK1* gene. A *TBK1* gene duplication was detected in one (0.44%) of 225 XFG patients, patient GGR-623-1. This CNV was subsequently confirmed by two additional qPCR probes specific for additional segments of the *TBK1* gene (exon 1 and exon 13).

Steroid-Induced Glaucoma

A cohort of 79 patients with steroid-induced ocular hypertension and SIG were tested for deletions or duplications of the *TBK1* gene. No *TBK1* deletions or duplications were detected.

A *TBK1* gene duplication was detected in one of 225 patients with XFG, and no such mutations were identified in PG or steroid-induced ocular hypertension/glaucoma patients. These data indicate that mutations involving *TBK1* gene dosage may be involved in a subset of XFG cases**.**

CLINCAL FEATURES OF A PATIENT WITH EXFOLIATION GLAUCOMA AND A *TBK1* GENE DUPLICATION

Patient GGR-623-1 tested positive for a *TBK1* gene duplication. He was diagnosed with XFG at 59 years of age based on the bilateral pattern of exfoliation material observed on his anterior lens capsules and gonioscopy, which showed heavy pigmentation in the trabecular meshwork and Sampaolesi lines in both eyes. He has markedly thin central corneas (450 μm in the right eye and 460 μm in the left eye), severe optic nerve damage with large cup-to-disc ratios (Figure 2), and corresponding visual field defects consistent with end-stage glaucoma. Both a trabeculectomy with mitomycin C and a Baerveldt implant were ultimately required in both eyes for control of IOP. The maximum recorded IOPs prior to filtering surgeries, but on topical medications, were 44 mm Hg in the right and 40 mm Hg in the left eye. Patient GGR-623-1 had a positive history of glaucoma in his mother, but no information about XFS was available.

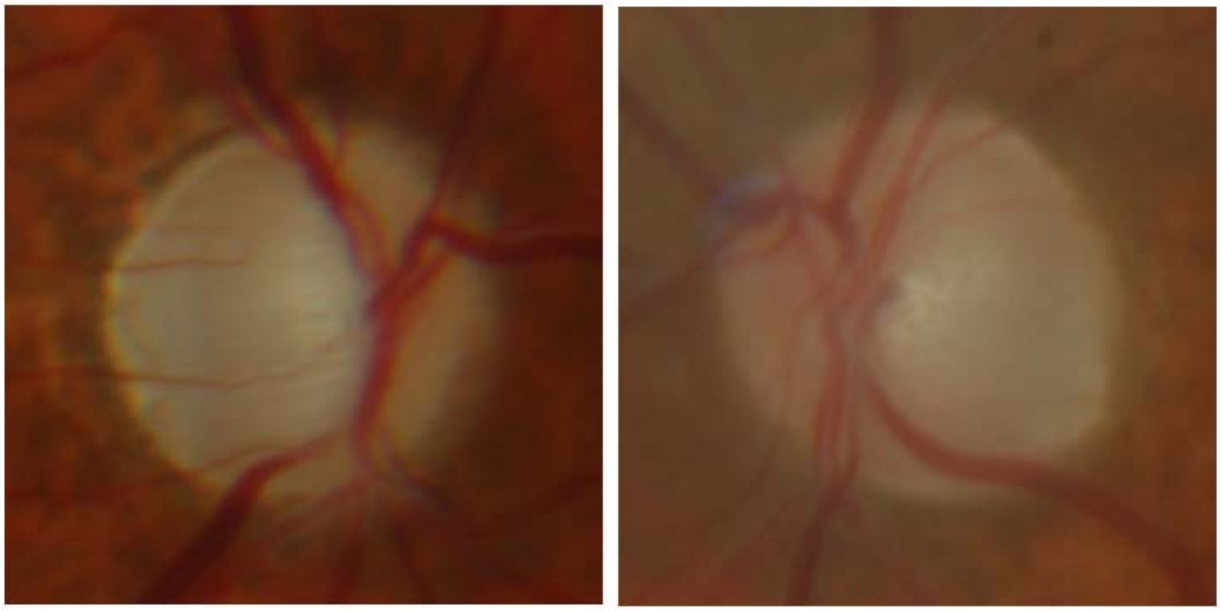

FIGURE 2

Optic disc photos of right eye (on left) and left eye (on right) of exfoliation glaucoma patient GGR-623-1 with a *TBK1* gene duplication, taken when patient was 74 years old. Glaucomatous cupping is seen.

DISCUSSION

MUTATIONS INVOLVING *TBK1* GENE DOSAGE ARE MOST FREQUENTLY DETECTED IN PRIMARY OPEN-ANGLE GLAUCOMA THAT OCCURS AT LOW INTRAOCULAR PRESSURE

Our prior genetic study of a large African American pedigree demonstrated that duplication of the *TBK1* gene causes a familial, autosomal dominant NTG in rare extended pedigrees. Subsequent population studies of NTG further showed that *TBK1* CNVs (*TBK1* duplications or triplications) are responsible for between 0.4% and 1.3% of NTG cases worldwide.[44,57-59] Previous investigations of NTG with family-based studies had identified another glaucoma-causing gene, optineurin (*OPTN*).[43] Missense mutations in *OPTN* (ie, E50K mutation) have been shown to cause 1% to 2% of NTG cases. These data indicate that *TBK1* CNVs are one of the two most common known mutations that cause Mendelian forms of NTG (ie, after *OPTN* E50K mutations).

PHENOTYPE OF *TBK1*-RELATED NORMAL-TENSION GLAUCOMA

Eight unrelated NTG probands with *TBK1* CNVs had been previously identified.[44,57-59] Several features of NTG related to *TBK1* CNVs have been observed. Patients with *TBK1*-related NTG have four chief clinical features: early onset, strong family history of NTG, low IOP, and large cup-to-disc ratio at diagnosis. The average age at diagnosis of these patients is 37 years. The mean maximum observed IOP for patients with *TBK1*-related glaucoma was 17 mm Hg, although three NTG patients had IOP slightly above 21 mm Hg, which suggests that low IOP is not an absolute feature of this form of glaucoma. Six (75%) of the eight probands had a positive family history of NTG. In all *TBK1*-related NTG pedigrees, the inheritance pattern is consistent with autosomal dominant inheritance of glaucoma. Finally, many of the *TBK1*-related NTG patients had nearly completely cupped optic discs at the time of diagnosis, demonstrating that these patients have severe glaucoma. Overall, patients with *TBK1*-related NTG have an unremarkable mean CCT of 530 μm; however, some patients have CCT <500 μm, and one patient had a CCT >600 μm. The phenotype of the XFG patient with a *TBK1* mutation described in this report, patient GGR-623-1, differs from that of patients with

TBK1-related NTG. Patient GGR-623-1 had a later age at diagnosis and a higher maximum IOP. However, identification of more XFG patients with a *TBK1* mutation will be necessary to make any definitive comments about the phenotype of *TBK1*-related XFG.

Genetic testing for *TBK1* CNVs is not currently widely available for diagnosis of NTG. However, given the relatively low prevalence of *TBK1* CNVs (ie, 1% of NTG), testing for diagnostic purposes should be considered only in high-risk patients, that is, those patients with features of *TBK1*-related NTG (early onset, strong family history of NTG, low IOP, and large cup-to-disc ratio at diagnosis) or in relatives of NTG patients known to carry a *TBK1* CNV. Moreover, genetic testing should always be discussed with experienced physicians and/or genetic counselors to determine whether such investigations should be done and to help interpret results.[86]

COPY-NUMBER VARIATIONS OF THE *TBK1* GENE ARE NOT A COMMON CAUSE OF JUVENILE-ONSET OPEN-ANGLE GLAUCOMA, PIGMENTARY GLAUCOMA, OR STEROID-INDUCED GLAUCOMA

We also evaluated the role of *TBK1* CNVs in other open-angle glaucomas. In two prior studies, we examined 1,045 POAG patients and no *TBK1* CNVs were detected.[44,59] In the current study, we expanded our study of *TBK1* and open-angle glaucoma by investigating other types of glaucoma for *TBK1* CNVs, including JOAG, PG, XFG, and SIG. A single instance of a *TBK1* gene duplication was detected in a patient with XFG, and no such *TBK1* CNVs were identified in JOAG, PG, or SIG patients. This study suggests that *TBK1* CNVs are not commonly associated with JOAG, PG, or SIG. However, it is possible that with larger cohorts, rare instances of *TBK1* CNVs might be detectable among these types of glaucoma patients.

DETECTION OF A *TBK1* GENE DUPLICATION IN A CASE OF EXFOLIATION GLAUCOMA WITH HIGH INTRAOCULAR PRESSURE

The current study reports the first instance of a *TBK1* CNV in a patient with a secondary glaucoma, XFG. This is also the first case of a *TBK1* gene duplication detected in a patient with markedly elevated IOPs, ie, 44 mm Hg. Prior to this finding, mutations in *TBK1* had been solely associated with autosomal dominant inheritance of NTG.[26] The discovery of a mutation in a *glaucoma-causing* gene in a patient with XFG is a novel finding. Previous studies of XFG have primarily investigated a complex genetic basis of disease in which many genetic and environmental "risk factors" can each increase the likelihood of disease but are unable to cause disease on their own. Common genetic mutations in the lysyl oxidase–like 1 (*LOXL1*) gene[87] and the calcium channel, voltage dependent, P/Q type, alpha-1A subunit (*CACNA1A*) gene[88] are important contributors to the development of XFS. More recently, environmental risk factors have also been implicated in the pathogenesis of XFS. There is an association between latitude and potentially between sun exposure and the risk for developing XFS.[89,90] Other potentially important dietary influences for XFS, such as caffeine and folate consumption, have also been reported.[91,92] While the preponderance of the available data suggests a complex genetic basis for XFS and XFG, there is some evidence that rare cases may have a simple genetic (single gene) basis. A few rare pedigrees with familial/autosomal dominant inheritance of XFS have been reported.[93-95] These large XFS families suggest that a subset of the disease might be caused by a single gene as a Mendelian trait, such as by a *TBK1* CNV mutation.

The detection of a *TBK1* gene duplication in one (0.44%) of 225 XFG patients (patient GGR-623-1) suggests that mutations that alter *TBK1* gene dosage (ie, a gene duplication) might have a role in rare cases of XFS and XFG. Alternatively, it is also possible that patient GGR-623-1 had independent factors predisposing NTG and XFG (ie, NTG caused by a *TBK1* gene duplication and XFG due to different genetic and environmental factors). Such concurrent conditions would likely result in a single diagnosis of XFG and is the most plausible explanation for this patient's disease. In either case, the results of this study suggest that one might consider *TBK1* CNVs as a possible mechanism of disease in patients diagnosed with XFG. Studies of additional cohorts of patients with XFG will be necessary to establish a role for the *TBK1* gene in this condition.

MODEL OF *TBK1*-RELATED GLAUCOMA

Most cases of glaucoma associated with *TBK1* CNVs occur with lower IOP. Consequently, we have developed a model that focuses on the pathophysiology of NTG described below. The possible role of the *TBK1* gene in XFG and glaucoma that occurs at higher IOP remains unclear and will require further study and more data.

Two genes known to cause Mendelian cases of NTG (*TBK1* and *OPTN*) and one gene associated with complex genetic forms of NTG (*TLR4*) also have important roles in autophagy. The interaction of these three genes to activate autophagy has been best described with studies of the innate immune system's response to intracellular pathogens.[64,96] Intracellular bacteria (eg, *Salmonella enterica*) may be recognized and cleared via a form of autophagy known as xenophagy. Cells respond to bacteria in the cytosol by coating them with the protein ubiquitin. A cascade of events involving TLR4, *TBK1*, and OPTN leads to formation of an autophagosome around the ubiquitin-coated bacterium and ultimately to fusion of the autophagosome with a lysosome, in which it will be degraded. When exposed to lipopolysaccharide from *Salmonella*, TLR4 stimulates *TBK1* to phosphorylate OPTN. Via its multiple binding domains, phosphorylated OPTN then brings together key components of the autophagosome, including the microtubule-associated protein LC3, and facilitates envelopment of ubiquitin-coated *Salmonella* within the autophagosome. Finally, the autophagosome fuses with the lysosome and its cargo *Salmonella* is digested. Importantly, OPTN's function in autophagy requires phosphorylation of its LC3 binding domain by *TBK1*.[64,96] These data are significant because they define a mechanistic link between known glaucoma genes, autophagy, and NTG pathogenesis.

Although it is remarkable that three NTG genes participate in xenophagy, there is little evidence to suggest an infectious component to the pathophysiology of glaucoma. Instead, we hypothesize that the same key molecules that activate xenophagy (TLR4, *TBK1*, and OPTN) interact in a similar fashion to stimulate autophagy in retinal ganglion cells of patients with NTG. We have begun

to investigate the mechanism by which *TBK1* gene duplications and triplications cause NTG using cell culture approaches. Early studies using fibroblast cell lines obtained from NTG patients in pedigree GGO-441 with a *TBK1* gene duplication have shown that the *TBK1* gene duplication results in increased transcription of *TBK1* mRNA.[44] This data suggests that the gene duplication may result in increased *TBK1* kinase function and ultimately in increased activation of autophagy.

More recently, we examined the activation of autophagy in cell cultures from NTG patients with *TBK1* gene duplications. Fibroblast cells from these patients were reprogrammed to become induced pluripotent stem cells (iPSCs) and then differentiated into retinal ganglion cell–like neurons.[97] Both fibroblast cell cultures and retinal ganglion cell–like neurons were assessed for activation of autophagy. During the formation of the autophagosome, LC3 becomes lipidated (termed LC3-II). Increasing levels of LC3-II is a reliable marker of activation of autophagy.[98] Elevated levels of LC3-II were detected in retinal ganglion cell–like neurons from an NTG patient with a *TBK1* gene duplication when compared to controls.[97] These data provide additional evidence that duplication of *TBK1* may activate autophagy in cell types most relevant to NTG.

Thus, several observations suggest that *TBK1* gene dosage mutations (duplications and triplications) might cause NTG by activating autophagy. One of *TBK1*'s functions is to stimulate autophagy. Moreover, extra copies of the *TBK1* gene in NTG patients have been shown to result in increased transcription of *TBK1* mRNA in fibroblasts and increased production of the key marker of autophagy, LC3-II, in iPSC-derived retinal ganglion cell–like neurons. Together, these data suggest that mutations that increase *TBK1* gene dosage (ie, *TBK1* gene duplications or triplications) cause optic nerve damage and glaucoma by increasing autophagy in retinal ganglion cells. This discovery has important implications in the study of NTG pathogenesis and the potential development of new glaucoma therapies that target autophagy in the eye.

FUTURE DIRECTIONS

Transgenic Mouse Studies of *TBK1*-Related Normal-Tension Glaucoma

Compelling evidence suggests that mutations that alter *TBK1* gene dosage (ie, *TBK1* gene duplications or triplications) cause NTG. *TBK1* CNVs have been detected in NTG patients from the United States,[44,58] Japan,[57] and Australia,[59] but have never been detected in control subjects with no glaucoma or in public databases. Moreover, *TBK1* gene duplications have been shown to cause increased *TBK1* gene activity in cultured fibroblast cells, indicating that these mutations have functional consequences on gene transcription (at least in this cell culture system).[44] Moreover, *TBK1* is known to be specifically expressed in retinal ganglion cells of human retina, a key site of NTG pathology.[44] Finally, *TBK1* is known to interact with the only other gene (*OPTN*) known to cause autosomal dominant, Mendelian forms of NTG. Together, these data strongly suggest that *TBK1* CNVs cause NTG. However, the most definitive proof that CNVs spanning *TBK1* cause NTG would be to generate transgenic mice that carry extra doses of the *TBK1* gene and to see if they develop signs of glaucoma. Such mice have been engineered and are currently being evaluated for signs that *TBK1* CNVs in mice recapitulate human glaucoma. Moreover, ocular tissue from transgenic *TBK1* mice might also be studied for signs of activation of autophagy (ie, increased production of LC3-II and other markers of autophagy) to solidify the role of this process in *TBK1*-related glaucoma.

Cell Culture Studies of *TBK1*-Related Normal-Tension Glaucoma

Preliminary studies of iPSC-derived retinal ganglion cell–like neurons have suggested that *TBK1* gene duplications activate autophagy. However, more rigorous studies to investigate autophagy in these cells are necessary, including confirmatory studies with cell lines from more NTG patients with *TBK1* CNVs. Moreover, these iPSC-derived cells provide an ideal system to test our hypothesis that *TBK1* CNVs increase signaling via TBK1 and OPTN to activate autophagy and lead to retinal ganglion cell death. The same iPSC-derived cells will also be a powerful tool for testing new therapies directed at targets in the autophagy pathway.

In conclusion, although we found a *TBK1* gene duplication in only one patient with XFG, our sample size was not large enough to conclude that *TBK1* gene duplication is more common in NTG than in secondary open-angle glaucomas. Because NTG is generally considered to be on a continuum with POAG, we felt that it would be interesting to determine whether *TBK1* mutations are present in patients with POAG and other forms of open-angle glaucoma (JOAG, PG, XFG, and SIG). This had been the case with *MYOC* mutations, which were initially discovered in patients with JOAG and were later found to cause 3% to 4% of POAG cases[42,45] as well as rare instances of other forms of glaucoma.[99] In this report, we did not detect *TBK1* mutations in JOAG, PG, or SIG patients. Prior reports similarly did not detect *TBK1* mutations in POAG patients.[44,59] We did, however, detect a *TBK1* gene duplication in a single XFG patient with markedly high IOP, which suggests that defects in *TBK1* may also be involved in rare cases of this type of glaucoma. To date, *TBK1* mutations have been primarily found in NTG patients. This subset of patients may have a primary destruction of their retinal ganglion cells mediated through autophagy. Further investigations of autophagy, therefore, may suggest new ideas for the treatment of NTG and other glaucomas.

ACKNOWLEDGMENTS

Funding/Support: This study was supported in part by a grant (R01 EY023512) from the National Institutes of Health/National Eye Institute, Research to Prevent Blindness, and the Marlene S. and Leonard A. Hadley Glaucoma Research Fund.

Financial Disclosures: None.

Author Contributions: Design of study (J.H.F.); conduct of study (J.H.F., A.L.R., T.E.S., Y.H.K., J.M.L, R.R., W.L.M.A.); data analysis (J.H.F., T.E.S.); manuscript preparation (J.H.F.).

REFERENCES

1. Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology* 2014;121(11):2081-2090.
2. Leske MC. The epidemiology of open-angle glaucoma: a review. *Am J Epidemiol* 1983;118(2):166-191.
3. Kwon YH, Fingert JH, Kuehn MH, Alward WLM. Primary open-angle glaucoma. *N Engl J Med* 2009;360(11):1113-1124.
4. Gordon MO, Beiser JA, Brandt JD, et al. The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. *Arch Ophthalmol* 2002;120(6):714-720.
5. Sommer A. Ocular hypertension and normal-tension glaucoma: time for banishment and burial. *Arch Ophthalmol* 2011;129(6):785-787.
6. Benedict TWG. *Abhaundlungen zus dem Gebiete der Augenheilkunde*. Breslau: L Freunde; 1842.
7. von Arlt F. *Die Krankeiten des Auges für praktische Ärzte*. Prague: FA Credner & Kleinbub; 1853.
8. Teikari JM, Airaksinen PJ, Kaprio J, Koskenvuo M. Primary open-angle glaucoma in 2 monozygotic twin pairs. *Acta Ophthalmol* 1987;65(5):607-611.
9. Kurten H, Halle S. Ein 81 jahriges, eineiiges zwillings-bruderpaar. *Arch Rassen Gessell Biol* 1934;28:38-49.
10. Westerland KE. *Clinical and Genetic Studies on the Primary Glaucoma Diseases*. Copenhagen: Busck; 1947.
11. Courtney RH, Hill EH. Hereditary juvenile glaucoma simplex. *JAMA* 1931;97:1602-1609.
12. Crombie AL, Cullen JF. Hereditary glaucoma occurrence in five generations of an Edinburgh family. *Br J Ophthalmol* 1964;48:143-147.
13. Harris D. The inheritance of glaucoma: a pedigree of familial glaucoma. *Am J Ophthalmol* 1965;60:90-95.
14. Stokes WH. Hereditary primary glaucoma: a pedigree with five generations. *Arch Ophthalmol* 1940;24:885-909.
15. Beiguelman B, Prado D. Recessive juvenile glaucoma. *J Genet Hum* 1963;12:53-54.
16. Kellerman L, Posner A. The value of heredity in the detection and study of glaucoma. *Am J Ophthalmol* 1955;40(5 Part 1):681-685.
17. François J. Genetics and primary open-angle glaucoma. *Am J Ophthalmol* 1966;61(4):652-665.
18. Jay B, Paterson G. The genetics of simple glaucoma. *Trans Ophthalmol Soc U K* 1970;90:161-171.
19. Becker B, Kolker AE, Roth FD. Glaucoma family study. *Am J Ophthalmol* 1960;50:557-567.
20. Teikari JM. Genetic factors in open-angle (simple and capsular) glaucoma. A population-based twin study. *Acta Ophthalmol* 1987;65(6):715-720.
21. Armaly MF. The genetic determination of ocular pressure in the normal eye. *Arch Ophthalmol* 1967;78(2):187-192.
22. Klein BEK, Klein R, Lee KE. Heritability of risk factors for primary open-angle glaucoma: the Beaver Dam Eye Study. *Invest Ophthalmol Vis Sci* 2004;45(1):59-62.
23. Chang TC, Congdon NG, Wojciechowski R, et al. Determinants and heritability of intraocular pressure and cup-to-disc ratio in a defined older population. *Ophthalmology* 2005;112(7):1186-1191.
24. Alsbirk PH. Corneal thickness. II. Environmental and genetic factors. *Acta Ophthalmol* 1978;56(1):105-113.
25. Toh T. Central corneal thickness is highly heritable: the Twin Eye Studies. *Invest Ophthalmol Vis Sci* 2005;46(10):3718-3722.
26. Fingert JH. Primary open-angle glaucoma genes. *Eye* 2011;25(5):587-595.
27. Thorleifsson G, Walters GB, Hewitt AW, et al. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma. *Nat Genet* 2010;42(10):906-909.
28. Kuehn MH, Wang K, Roos B, et al. Chromosome 7q31 POAG locus: ocular expression of caveolins and lack of association with POAG in a US cohort. *Mol Vis* 2011;17:430-435.
29. Wiggs JL, Kang JH, Yaspan BL, et al. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma in Caucasians from the USA. *Hum Mol Genet* 2011;20(23):4707-4713.
30. Burdon KP, MacGregor S, Hewitt AW, et al. Genome-wide association study identifies susceptibility loci for open angle glaucoma at TMCO1 and CDKN2B-AS1. *Nat Genet* 2011;43(6):574-578.
31. Ramdas WD, van Koolwijk LME, Lemij HG, et al. Common genetic variants associated with open-angle glaucoma. *Hum Mol Genet* 2011;20(12):2464-2471.
32. Burdon KP, Crawford A, Casson RJ, et al. Glaucoma risk alleles at CDKN2B-AS1 are associated with lower intraocular pressure, normal-tension glaucoma, and advanced glaucoma. *Ophthalmology* 2012;119(8):1539-1545.
33. Nakano M, Ikeda Y, Tokuda Y, et al. Common variants in CDKN2B-AS1 associated with optic-nerve vulnerability of glaucoma identified by genome-wide association studies in Japanese. *PLoS ONE* 2012;7(3):e33389.
34. Wiggs JL, Yaspan BL, Hauser MA, et al. Common variants at 9p21 and 8q22 are associated with increased susceptibility to optic nerve degeneration in glaucoma. *PLoS Genet* 2012;8(4):e1002654.
35. Gibson J, Griffiths H, De Salvo G, et al. Genome-wide association study of primary open angle glaucoma risk and quantitative traits. *Mol Vis* 2012;18:1083-1092.
36. Dimasi DP, Burdon KP, Hewitt AW, et al. Genetic investigation into the endophenotypic status of central corneal thickness and optic disc parameters in relation to open-angle glaucoma. *Am J Ophthalmol* 2012;154(5):833-842.e2.
37. Fan BJ, Wang DY, Pasquale LR, Haines JL, Wiggs JL. Genetic variants associated with optic nerve vertical cup-to-disc ratio are risk factors for primary open angle glaucoma in a US Caucasian population. *Invest Ophthalmol Vis Sci* 2011;52(3):1788-1792.
38. Osman W, Low S-K, Takahashi A, Kubo M, Nakamura Y. A genome-wide association study in the Japanese population confirms 9p21 and 14q23 as susceptibility loci for primary open angle glaucoma. *Hum Mol Genet* 2012;21(12):2836-2842.

39. Writing Committee for the Normal Tension Glaucoma Genetic Study Group of Japan Glaucoma Society, Meguro A, Inoko H, Ota M, Mizuki N, Bahram S. Genome-wide association study of normal tension glaucoma: common variants in SRBD1 and ELOVL5 contribute to disease susceptibility. *Ophthalmology* 2010;117(7):1331-1338.
40. Shibuya E, Meguro A, Ota M, et al. Association of Toll-like receptor 4 gene polymorphisms with normal tension glaucoma. *Invest Ophthalmol Vis Sci* 2008;49(10):4453-4457.
41. Takano Y, Shi D, Shimizu A, et al. Association of Toll-like receptor 4 gene polymorphisms in Japanese subjects with primary open-angle, normal-tension, and exfoliation glaucoma. *Am J Ophthalmol* 2012;154(5):825-832.e1.
42. Stone EM, Fingert JH, Alward WLM, et al. Identification of a gene that causes primary open angle glaucoma. *Science* 1997;275(5300):668-670.
43. Rezaie T, Child A, Hitchings R, et al. Adult-onset primary open-angle glaucoma caused by mutations in optineurin. *Science* 2002;295(5557):1077-1079.
44. Fingert JH, Robin AL, Ben R Roos, et al. Copy number variations on chromosome 12q14 in patients with normal tension glaucoma. *Hum Mol Genet* 2011;20(12):2482-2494.
45. Fingert JH, Héon E, Liebmann JM, et al. Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. *Hum Mol Genet* 1999;8(5):899-905.
46. Zode GS, Bugge KE, Mohan K, et al. Topical ocular sodium 4-phenylbutyrate rescues glaucoma in a myocilin mouse model of primary open-angle glaucoma. *Invest Ophthalmol Vis Sci* 2012;53(3):1557-1565.
47. Zode GS, Kuehn MH, Nishimura DY, et al. Reduction of ER stress via a chemical chaperone prevents disease phenotypes in a mouse model of primary open angle glaucoma. *J Clin Invest* 2011;121(9):3542-3553.
48. Alward WLM, Kwon YH, Kawase K, et al. Evaluation of optineurin sequence variations in 1,048 patients with open-angle glaucoma. *Am J Ophthalmol* 2003;136(5):904-910.
49. Monemi S, Spaeth G, DaSilva A, et al. Identification of a novel adult-onset primary open-angle glaucoma (POAG) gene on 5q22.1. *Hum Mol Genet* 2005;14(6):725-733.
50. Pasutto F, Keller KE, Weisschuh N, et al. Variants in ASB10 are associated with open-angle glaucoma. *Hum Mol Genet* 2012;21(6):1336-1349.
51. Hewitt AW, Dimasi DP, Mackey DA, Craig JE. A glaucoma case-control study of the WDR36 gene D658G sequence variant. *Am J Ophthalmol* 2006;142(2):324-325.
52. Fingert JH, Alward WLM, Kwon YH, et al. No association between variations in the WDR36 gene and primary open-angle glaucoma. *Arch Ophthalmol* 2007;125(3):434-436.
53. Hauser MA, Allingham RR, Linkroum K, et al. Distribution of WDR36 DNA sequence variants in patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci* 2006;47(6):2542-2546.
54. Pasutto F, Matsumoto T, Mardin CY, et al. Heterozygous NTF4 mutations impairing neurotrophin-4 signaling in patients with primary open-angle glaucoma. *Am J Hum Genet* 2009;85(4):447-456.
55. Liu Y, Liu W, Crooks K, Schmidt S, Allingham RR, Hauser MA. No evidence of association of heterozygous NTF4 mutations in patients with primary open-angle glaucoma. *Am J Hum Genet* 2010;86(3):498-499.
56. Fingert JH, Roos BR, Solivan-Timpe F, et al. Analysis of ASB10 variants in open angle glaucoma. *Hum Mol Genet* 2012;21(20):4543-4548.
57. Kawase K, Allingham RR, Meguro A, et al. Confirmation of TBK1 duplication in normal tension glaucoma. *Exp Eye Res* 2012;96(1):178-180.
58. Ritch R, Darbro B, menon G, et al. TBK1 Gene duplication and normal-tension glaucoma. *JAMA Ophthalmo*l 2014;132(5):544-548.
59. Awadalla MS, Fingert JH, Roos BE, et al. Copy number variations of TBK1 in Australian patients with primary open-angle glaucoma. *Am J Ophthalmol* 2015;159(1):124-130.
60. Bennett SR, Alward WL, Folberg R. An autosomal dominant form of low-tension glaucoma. *Am J Ophthalmol* 1989;108(3):238-244.
61. Tu D, Zhu Z, Zhou AY, et al. Structure and ubiquitination-dependent activation of TANK-binding kinase 1. *Cell Rep* 2013;3(3):747-758.
62. Ma X, Helgason E, Phung QT, et al. Molecular basis of Tank-binding kinase 1 activation by transautophosphorylation. *Proc Natl Acad Sci* 2012;109(24):9378-9383.
63. Pomerantz JL, Baltimore D. NF-kappaB activation by a signaling complex containing TRAF2, TANK and TBK1, a novel IKK-related kinase. *EMBO J* 1999;18(23):6694-6704.
64. Wild P, Farhan H, McEwan DG, et al. Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. *Science* 2011;333(6039):228-233.
65. Helgason E, Phung QT, Dueber EC. Recent insights into the complexity of Tank-binding kinase 1 signaling networks: the emerging role of cellular localization in the activation and substrate specificity of TBK1. *FEBS Lett* 2013;587(8):1230-1237.
66. Sharma S, tenOever BR, Grandvaux N, Zhou GP, Lin R, Hiscott J. Triggering the interferon antiviral response through an IKK-related pathway. *Science* 2003;300(5622):1148-1151.
67. Pilli M, Arko-Mensah J, Ponpuak M, et al. TBK-1 promotes autophagy-mediated antimicrobial defense by controlling autophagosome maturation. *Immunity* 2012;37(2):223-234.

68. Morton S, Peggie M, Cohen P. Enhanced binding of TBK1 by an optineurin mutant that causes a familial form of primary open angle glaucoma. *FEBS Lett* 2008;582(6):997-1002.
69. Mankouri J, Fragkoudis R, Richards KH, et al. Optineurin negatively regulates the induction of IFNbeta in response to RNA virus infection. *PLoS Pathog* 2010;6(2):e1000778.
70. Minegishi Y, Iejima D, Kobayashi H, et al. Enhanced optineurin E50K-TBK1 interaction evokes protein insolubility and initiates familial primary open-angle glaucoma. *Hum Mol Genet* 2013;22(17):3559-3567.
71. Suh W, Kim S, Ki CS, Kee C. Toll-like receptor 4 gene polymorphisms do not associate with normal tension glaucoma in a Korean population. *Mol Vis* 2011;17:2343-2348.
72. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell* 2008;132(1):27-42.
73. Levine B. Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. *Cell* 2005;120(2):159-162.
74. Nassif M, Hetz C. Targeting autophagy in ALS: a complex mission. *Autophagy* 2011;7(4):450-453.
75. Nixon RA. The role of autophagy in neurodegenerative disease. *Nat Med* 2013;19(8):983-997.
76. Park HYL, Kim JH, Park CK. Activation of autophagy induces retinal ganglion cell death in a chronic hypertensive glaucoma model. *Cell Death Dis* 2012;3:e290.
77. Piras A, Gianetto D, Conte D, Bosone A, Vercelli A. Activation of autophagy in a rat model of retinal ischemia following high intraocular pressure. *PLoS ONE* 2011;6(7):e22514.
78. Rodríguez-Muela N, Germain F, Mariño G, Fitze PS, Boya P. Autophagy promotes survival of retinal ganglion cells after optic nerve axotomy in mice. *Cell Death Differ* 2012;19(1):162-169.
79. Wong E, Cuervo AM. Autophagy gone awry in neurodegenerative diseases. *Nat Neurosci* 2010;13(7):805-811.
80. Rodríguez-Muela N. Axonal damage, autophagy and neuronal survival. *Autophagy* 2012;8(2):286-288.
81. Lazarou M, Sliter DA, Kane LA, et al. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature* 2015;524(7565):309-314.
82. Heo JM, Ordureau A, Paulo JA, Rinehart J, Harper JW. The PINK1-PARKIN mitochondrial ubiquitylation pathway drives a program of OPTN/NDP52 recruitment and TBK1 activation to promote mitophagy. *Mol Cell* 2015;60(1):7-20.
83. Liu Y, Garrett ME, Yaspan BL, et al. DNA copy number variants of known glaucoma genes in relation to primary open-angle glaucoma. *Invest Ophthalmol Vis Sci* 2014;55(12):8251-8258.
84. Alward WL, Fingert JH, Coote MA, et al. Clinical features associated with mutations in the chromosome 1 open-angle glaucoma gene (GLC1A). *N Engl J Med* 1998;338(15):1022-1027.
85. Buffone GJ, Darlington GJ. Isolation of DNA from biological specimens without extraction with phenol. *Clin Chem* 1985;31(1):164-165.
86. Stone EM, Aldave AJ, Drack AV, et al. Recommendations for genetic testing of inherited eye diseases: report of the American Academy of Ophthalmology task force on genetic testing. *Ophthalmology* 2012;119(11):2408-2410.
87. Thorleifsson G, Magnusson KP, Sulem P, et al. Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma. *Science* 2007;317(5843):1397-1400.
88. Aung T, Ozaki M, Mizoguchi T, et al. A common variant mapping to CACNA1A is associated with susceptibility to exfoliation syndrome. *Nat Genet* 2015;47(4):387-392.
89. Stein JD, Pasquale LR, Talwar N, et al. Geographic and climatic factors associated with exfoliation syndrome. *Arch Ophthalmol* 2011;129(8):1053-1060.
90. Pasquale LR, Jiwani AZ, Zehavi-Dorin T, et al. Solar exposure and residential geographic history in relation to exfoliation syndrome in the United States and Israel. *JAMA Ophthalmol* 2014;132(12):1439-1445.
91. Pasquale LR, Wiggs JL, Willett WC, Kang JH. The relationship between caffeine and coffee consumption and exfoliation glaucoma or glaucoma suspect: a prospective study in two cohorts. *Invest Ophthalmol Vis Sci* 2012;53(10):6427-6433.
92. Kang JH, Loomis SJ, Wiggs JL, Willett WC, Pasquale LR. A Prospective study of folate, vitamin B6, and vitamin B12 intake in relation to exfoliation glaucoma or suspected exfoliation glaucoma. *JAMA Ophthalmol* 2014;132(5):549-559.
93. Orr AC, Robitaille JM, Price PA, et al. Exfoliation syndrome: clinical and genetic features. *Ophthalmic Genet* 2001;22(3):171-185.
94. Hardie JG, Mercieca F, Fenech T, Cuschieri A. Familial pseudoexfoliation in Gozo. *Eye (Lond)* 2005;19(12):1280-1285.
95. Forsman E, Cantor RM, Lu A, et al. Exfoliation syndrome: prevalence and inheritance in a subisolate of the Finnish population. *Acta Ophthalmol Scand* 2007;85(5):500-507.
96. Galluzzi L, Kepp O, Kroemer G. Autophagy and innate immunity ally against bacterial invasion. *EMBO J* 2011;30(16):3213-3214.
97. Tucker BA, Solivan-Timpe F, Roos BR, et al. Duplication of TBK1 stimulates autophagy in iPSC-derived retinal cells from a patient with normal tension glaucoma. *J Stem Cell Res Ther* 2014;3(5):161.
98. Klionsky DJ, Abdalla FC, Abeliovich H, et al. Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy* 2012;8(4):445-544.
99. Alward WLM, Kwon YH, Khanna CL, et al. Variations in the myocilin gene in patients with open-angle glaucoma. *Arch Ophthalmol* 2002;120(9):1189-1197.