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## TBK1 gene duplication and normal-tension glaucoma

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### Abstract

**IMPORTANCE**—Normal-tension glaucoma (NTG) is a common cause of vision loss.

**OBJECTIVE**—To investigate the role of TANK binding kinase1(*TBK1*) gene duplications in NTG to gain insights into the causes of glaucoma that occurs at low intraocular pressure (IOP).

**DESIGN, SETTING, AND PARTICIPANTS**—In this multicenter case-control study, we investigated patients who met the criteria for NTG, including glaucomatous optic neuropathy, visual field defects, and maximum recorded untreated IOP of 21 mm Hg or less, and matched controls. Participants (N = 755) were recruited from Southampton, United Kingdom (180 patients and 178 controls), Rochester, Minnesota (65 patients and 12 controls), New York, New York (96 patients and 16 controls), and Iowa City, Iowa (208 controls).

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**MAIN OUTCOMES AND MEASURES**—Detection of *TBK1* gene duplications and comparison of the extent of the identified DNA that is duplicated with prior *TBK1* copy number variations associated with NTG.

**RESULTS**—A *TBK1* gene duplication was detected in 1 of 96 patients (1.0%) from New York and none of the controls. Analysis of duplication borders with comparative genome hybridization demonstrated that this patient has a novel duplication that has not been previously reported. No gene duplications were detected in any of the other cohorts of patients or controls.

**CONCLUSIONS AND RELEVANCE**—Duplication of the *TBK1* gene is a rare cause of NTG. The identification of another case of NTG attributed to *TBK1* gene duplication strengthens the case that this mutation causes glaucoma.

The genetic basis of primary open-angle glaucoma (POAG) is complex. Recent large population-based studies have identified numerous genetic factors related to POAG, including *CAVI/CAV2*,<sup>1, 2</sup> *CDKN2B-AS1*,<sup>3-7</sup> *ATOH7*,<sup>4, 5</sup> *SIX1/SIX6*,<sup>4</sup> *TMCO1*,<sup>3, 8</sup> *TLR4*,<sup>9</sup> *SRBD1*,<sup>10</sup> and *ELOVL5*.<sup>10</sup> These glaucoma risk factors are observed in healthy individuals; however, they are more commonly detected in patients with POAG than in healthy controls. Each of these genetic factors contributes a small risk for POAG, and although none may cause the disease on their own, in combination they may lead to the development of glaucoma.<sup>11</sup>

Studies of familial POAG have led to the identification of several genes that cause glaucoma with simple Mendelian inheritance patterns. These cases of POAG, with autosomal dominant inheritance, are caused primarily by individual genes acting alone. Mutations in *MYOC* (OMIM 601652)<sup>12</sup> or *OPTN* (OMIM 602432)<sup>13</sup> can cause POAG with minimal influence from other genes or environmental factors. Mutations in *MYOC* cause 3% to 4% of POAG cases worldwide.<sup>14</sup> Patients with *MYOC*-related glaucoma typically have markedly elevated intraocular pressure (IOP) and early-onset disease.<sup>15</sup> Mutation of *OPTN* is associated with POAG that occurs at lower IOP (ie, normal-tension glaucoma [NTG]).<sup>13</sup> *OPTN* mutations have been linked to 1% to 2% of NTG cases.<sup>16,17</sup> Overall, the known single-gene causes of POAG are responsible for approximately 5% of cases of POAG.<sup>11</sup>

More recently, a third glaucoma gene, *TBK1*, that is associated with NTG has been identified.<sup>18</sup> Prior studies<sup>18,19</sup> have found that several African American, white, and Asian patients with NTG have duplications on chromosome 12q14 that span the *TBK1* gene. *TBK1* encodes a kinase protein that directly interacts with and phosphorylates *OPTN*,<sup>20,21</sup> the protein encoded by the only other known NTG gene.<sup>13</sup> *TBK1* is the only gene encompassed by all known chromosome 12q14 duplications in NTG patients.<sup>18,19</sup> Moreover, *TBK1* is specifically expressed within the ocular tissue most affected by NTG, the retinal ganglion cell layer, and duplication of the *TBK1* gene leads to a significant increase in its transcription level.<sup>18</sup> The sum of these data strongly suggest that duplication of *TBK1* causes 0.4% to 1.3% of NTG cases.<sup>18,19</sup> However, animal and/or functional studies will be required to definitively prove that chromosome 12q14 duplications cause NTG by altering the function of *TBK1* rather than through effects on other neighboring genes.

The discovery that *TBK1* is a glaucoma gene suggests biological pathways that may be important in the pathogenesis of NTG. Both known NTG genes, *TBK1* and *OPTN*, function in nuclear factor- $\kappa$ B (NF- $\kappa$ B) signaling pathways,<sup>22,23</sup> which have been previously connected with apoptosis and cell death. *TBK1* and *OPTN* also have essential functions in autophagy, a pathway for eliminating damaged or accumulating intracellular materials. Prior studies<sup>20,24</sup> have also indicated that *TBK1* colocalizes with *OPTN* and that *TBK1* phosphorylates *OPTN* as part of a cascade of events that ultimately leads to activation of autophagy. Autophagy can protect cells in times of nutrient deprivation or serve as a means to degrade accumulating intracellular proteins, dysfunctional organelles (eg, damaged mitochondria), or intracellular pathogens. Excessive autophagy may also lead to cell death in retinal ganglion cells.<sup>25,26</sup> Previous investigations demonstrated that *TBK1* gene duplications in NTG patients lead to increased transcription of *TBK1* messenger RNA,<sup>18</sup> which may lead to retinal ganglion cell death by activation of autophagy or altering NF- $\kappa$ B signaling. In this report, we investigated the role of *TBK1* gene duplication in 3 additional NTG patient populations to further explore the role of the *TBK1* gene in NTG.

## METHODS

All participants provided written informed consent, and research was conducted with the approval of the institutional review board of the University of Iowa. All participants were examined by a fellowship-trained glaucoma specialist. Criteria for diagnosis of NTG included typical glaucomatous optic nerve damage and visual field loss with a maximum recorded IOP of 21 mm Hg or less, as previously described.<sup>15,18,19</sup> Three cohorts of patients and controls were enrolled from Southampton, United Kingdom (180 patients and 178 controls), Rochester, Minnesota (65 patients and 12 controls), and New York, New York (96 patients and 16 controls). An additional 208 controls from Iowa were also enrolled. None of the patients or controls in the current report were included in previous studies of *TBK1*.

DNA from NTG patients and controls was examined for *TBK1* gene duplications using a quantitative polymerase chain reaction assay (TaqMan Number Assay; Applied Biosystems) as previously described.<sup>18,19</sup> Positive quantitative polymerase chain reaction results were confirmed, and duplication borders were defined with comparative genome hybridization (CGH) using whole genome microarrays (NimbleGen 720 000 microarray; Roche NimbleGen) following the manufacturer's protocol. The borders and extent of detected *TBK1* gene duplications were compared with previously reported *TBK1* gene duplications in other NTG patients using the current build of the human genome (hg19).<sup>18,19</sup>

## RESULTS

A total of 755 participants from 3 populations (Southampton, United Kingdom; Rochester, Minnesota; and New York, New York) were tested for duplication of the *TBK1* gene using a quantitative polymerase chain reaction assay. A *TBK1* gene duplication was detected in 1 (patient GGR-590-1) of 96 patients (1.0%) from New York. No gene duplication was detected in any of the controls or in the other NTG cohorts. The extent of the chromosome 12q14 duplication in patient GGR-590-1 was determined by examination with a CGH

microarray. The duplication encompasses 370 kilobase pairs (kbp), extends from 64 563 to 64 933 kbp, and spans the *TBK1* gene and part of the *XPOT* gene (Figure 1).

### Case Report

Patient GGR-590-1 is a 65-year-old white woman who was diagnosed as having NTG at 47 years of age with maximum recorded IOP of 16 mm Hg in both eyes, progressive visual field damage (left eye greater than right eye), and glaucomatous cup-to-disc ratios. As part of her evaluation, magnetic resonance imaging ruled out nonglaucomatous optic neuropathy. She had no known family history of glaucoma. Argon laser trabeculoplasty was performed in both eyes at 48 years of age.

She was first seen by one of us (R.R.) at 53 years of age. At that time her medications consisted of topical timolol, dorzolamide hydrochloride, brimonidine tartrate, and travoprost for both eyes. She denied a history of migraine, Raynaud phenomenon, or cardiac arrhythmia but stated that she had chronic low blood pressure. A complete eye examination revealed 20/20 visual acuity in both eyes, IOPs of 10 mm Hg in both eyes, and thin corneas. Gonioscopy revealed grade IV open angles with trace pigmentation. Her cup-to-disc ratios were 0.8 OD and 0.9 OS in 2002. Optic nerve head cupping progressively worsened in both eyes (Figure 2A), and the cup-to-disc ratio was 0.99 OU by 2011. Automated perimetry (24-2 Swedish Interactive Thresholding Algorithm Standard) in 2002 revealed superior and inferior arcuate scotomas with a superior paracentral defect in both eyes. The loss was greater in the left eye (Figure 2B). Visual field loss also progressed from 2002 to 2012 (Figure 2, C–E) despite maintaining an IOP of 12 mm Hg or less in both eyes.

Subsequent 24-hour blood pressure monitoring revealed persistent nocturnal dipping between 12:00 and 4:00 AM. The lowest readings reported were 98 mm Hg systolic, 44 mm Hg diastolic, and 62 mm Hg mean arterial pressure. The nocturnal mean arterial pressure was 30% to 40% lower than the diurnal mean arterial pressure. Findings of magnetic resonance imaging of the brain with and without contrast were unremarkable except for optic nerve thinning.

### Analysis of *TBK1* Gene Duplications

Three different chromosome 12q14 duplications spanning the *TBK1* gene were previously detected in 1 African American NTG pedigree and 2 white NTG pedigrees (Figure 1).<sup>18</sup> A fourth *TBK1* gene duplication was later detected in a Japanese NTG pedigree, GGJ-414,<sup>19</sup> but the borders of this duplication were not reported. Using CGH, we found that the borders of the *TBK1* gene duplication in this Japanese NTG pedigree span 267 kbp of DNA on chromosome 12q14, from 64 830 to 65 096 kbp. These data indicate that NTG patients in pedigree GGJ-414 have a novel *TBK1* mutation (Figure 1).

Each of the 5 known *TBK1* gene duplications was detected in unrelated NTG pedigrees. These duplications are novel and appear to have arisen independently. No evidence of a founder effect or common ancestry was detected for those carrying these copy number variations. Moreover, no repetitive DNA sequences were identified in the region that might predispose patients to relatively frequent development of copy number variations.

## DISCUSSION

*TBK1* gene duplications were recently reported to be associated with 0.4% to 1.3% of NTG cases in white and Asian populations.<sup>18,19</sup> We report identification of an additional case of *TBK1* gene duplication in 1 of 96 NTG patients (1.0%) from New York. These data further confirm the role of *TBK1* gene dosage in the pathogenesis of NTG and are consistent with prior reports that suggest approximately 1 in 100 NTG patients may carry a *TBK1* gene duplication. When the data from all of the populations studied in the prior 2 reports of *TBK1* and NTG<sup>18,19</sup> are combined with the data from the current report, 5 of 803 NTG patients (0.62%) were found to carry *TBK1* gene duplications, whereas no such mutations were identified in 1116 controls. *TBK1* duplications have been detected in African American, white, and Asian NTG patients, suggesting that although these mutations are rare, they may be found worldwide. Moreover, each of the 5 duplications differ from each other in the extent of duplicated DNA, which does not support a founder effect and suggests that each mutation arose independently.

Patients with NTG that is associated with *TBK1* gene duplications have some characteristic clinical features. Patient GGR-590-1 and other previously described patients with *TBK1*-associated NTG had early onset of severe disease.<sup>18,19</sup> Many patients have a strong family history of NTG<sup>18,19</sup>; however, patient GGR-590-1 reported no history of NTG in her family. Similarly, many but not all NTG patients have thin central corneas.<sup>18,19</sup> Finally, 2 NTG patients were evaluated for low blood pressure, and nocturnal hypotension was detected in 1 patient (patient GGR-590-1). Unfortunately, nocturnal blood pressure data were not available from the other patients with *TBK1*-associated NTG. Low blood pressure has been studied as a contributor to the pathophysiology of NTG<sup>27,28</sup>; however, its role in *TBK1*-related disease is unclear.

The specific mechanism by which *TBK1* gene duplications lead to NTG is not known. However, the known functions of *TBK1* and other NTG genes (*OPTN* and *TLR4*) suggest that mutations of *TBK1* may cause NTG through abnormal activation of autophagy. *TBK1*, *OPTN*, and *TLR4* each encode proteins that interact to activate autophagy. On the basis of these data, we hypothesize that mutations (such as a *TBK1* gene duplication) may abnormally activate autophagy and lead to retinal ganglion cell death and glaucoma. *TBK1* also regulates the immune response and inflammation (ie, noncanonical NF- $\kappa$ B signaling). Consequently, the role of NF- $\kappa$ B signaling and other downstream pathways cannot be ruled out as an alternative for causing *TBK1*-associated NTG. Interestingly, *TBK1* may also mediate crosstalk between the autophagic and NF- $\kappa$ B pathways.<sup>29–31</sup> This type of crosstalk has been found with other innate immunity signaling pathways, such as activation of NOD2 by intracellular pathogens, that also result in activation of both autophagy and NF- $\kappa$ B.<sup>32</sup> These findings suggest a high degree of coordination between these pathways. They also suggest that pathogenesis of NTG caused by defects in one particular branch of the pathway could be mediated by other pathways connected by crosstalk.

## CONCLUSIONS

This report provides additional evidence that defects in the *TBK1* gene (ie, gene duplication) may cause 0.4% to 1.3% of NTG cases in numerous populations. Future studies of *TBK1* function using a range of approaches and resources, including human donor eyes, transgenic mice, and cell culture, may clarify the role of autophagy and/or NF- $\kappa$ B signaling in the pathogenesis of NTG. These investigations may provide new insights into the mechanisms by which *TBK1*, *OPTN*, and *TLR4* gene defects lead to glaucoma and may suggest new methods of intervention.

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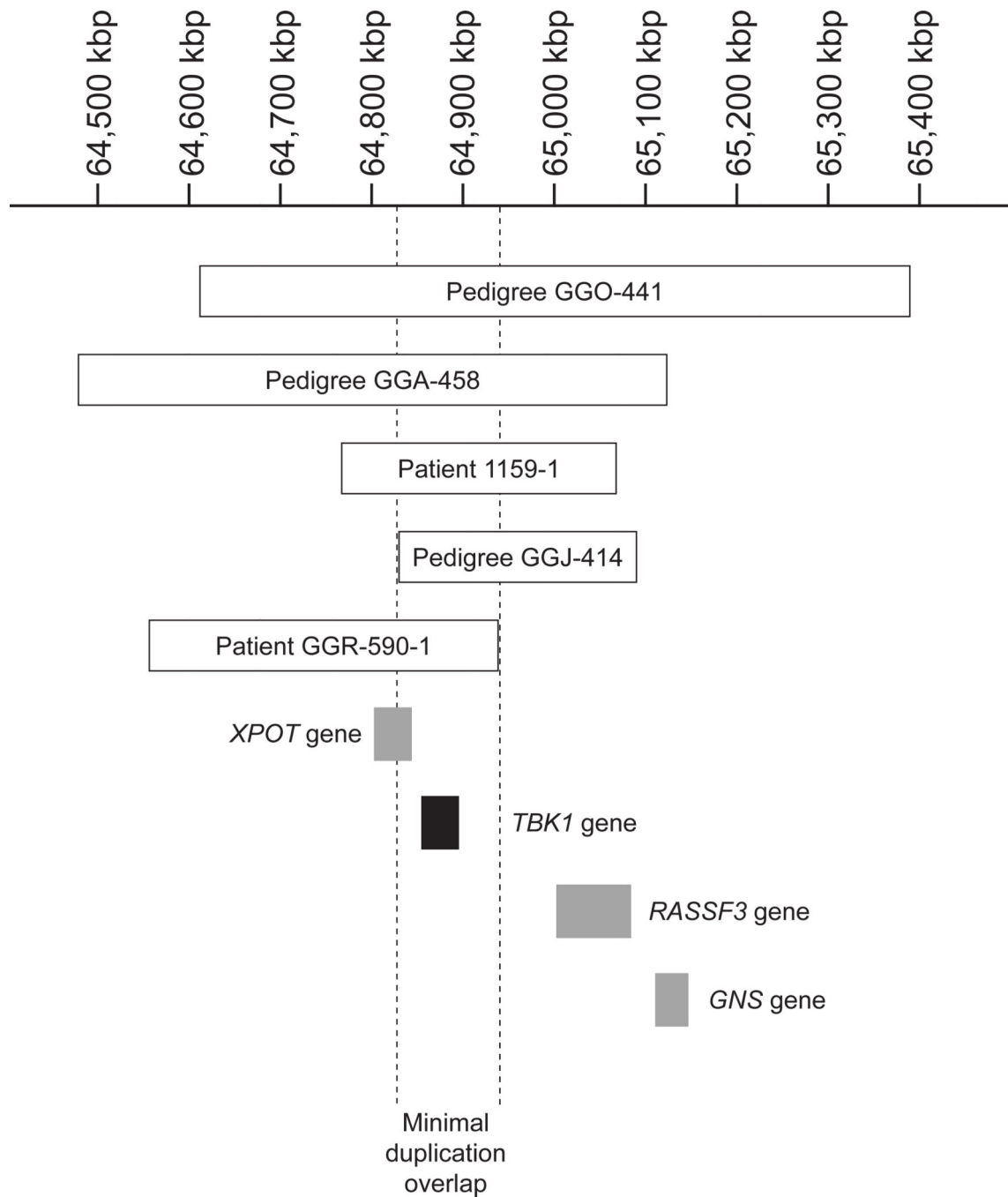
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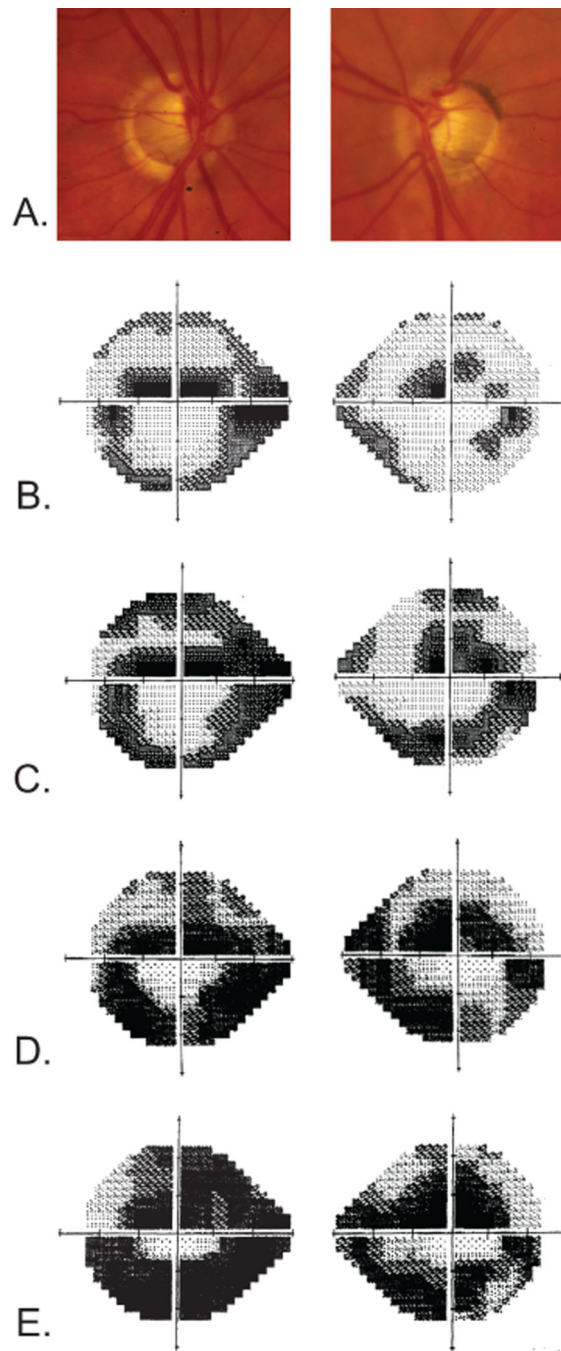
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**Figure 1. *TBK1* gene duplications**

The position and extent of each of the detected *TBK1* gene duplications and their relationship to flanking genes are shown using the current human genome build (hg19).<sup>18,19</sup> Duplications in normal-tension glaucoma pedigrees GGO-441, GGA-416, GGA-1159, and GGJ-414 were previously reported (using the hg18 genome build). kbp indicates kilobase pair.





**Figure 2. Patient GGR-590-1 clinical data**

A, Disc photographs at 56 years of age that demonstrate significant cupping. Humphrey visual field tests (24-2 Swedish Interactive Thresholding Algorithm Standard) performed at 53 years of age (B), 56 years of age (C), 59 years of age (D), and 64 years of age (E) demonstrate progressive glaucomatous visual field loss despite maximum intraocular pressure of 12 mm Hg in both eyes.