Long-Term Follow-Up of Normal Tension Glaucoma Patients With *TBK1* Gene Mutations in One Large Pedigree

TYLER S. QUIST, CHRIS A. JOHNSON, ALAN L. ROBIN, AND JOHN H. FINGERT

• PURPOSE: To characterize features of glaucoma associated with a TANK binding kinase 1 (*TBK1*) gene duplication, which is among the most common molecularly defined causes of normal tension glaucoma (NTG).
• DESIGN: Retrospective observational case series.
• METHODS: We conducted a retrospective case series, by reviewing medical records of 7 members of a pedigree with NTG caused by *TBK1* gene duplications. Clinical features of these patients at diagnosis, throughout management, and at latest follow-up were identified, including age, intraocular pressure (IOP), central corneal thickness (CCT), optic nerve head appearance, and mean deviation (MD) assessed with Humphrey visual field (HVF) testing protocols.
• RESULTS: At initial diagnosis, the mean age was 35 ± 7 years, IOP was 16 ± 2.1 mm Hg, cup-to-disc (C/D) ratio was 0.9 ± 0.08, and MD assessed via HVF 30-2 and/or 24-2 testing protocols was −9.0 ± 8.9 (range: −1.8 to −27) dB in the 14 study eyes. At initial diagnosis, 4 of 14 eyes (28%) had no visual field defect, 4 (28%) had early visual field defects, and 6 (43%) had severe visual field defects. Patients had a mean follow-up of 21.5 ± 9.0 years and experienced an average reduction of IOP by 28%. Four of 12 eyes (33%) had stable visual fields throughout follow-up, while 8 eyes (67%) had slow-to-moderate progression. The 30-2 and/or 24-2 HVF tests had an average change in MD of −0.53 ± 0.26 dB/year. No eyes had rapid progression with an MD > 1.0 dB/year. At final follow-up, the mean IOP was 11.5 ± 2.9, and C/D ratio was 0.94 ± 0.4. At final follow-up, 3 of 14 eyes (21%) had early visual field defects, 4 (29%) had moderate visual field defects, and 7 (50%) had severe visual field defects. Six of 14 eyes (43%) met criteria for legal blindness.
• CONCLUSIONS: We provide the first report of the clinical features and long-term clinical course in a family of NTG patients with *TBK1* gene duplications. *TBK1*-associated glaucoma exhibits classic features of NTG. Patients present with severe disease at a relatively early age and most (67%) have slow-to-moderate progression of their visual field defects. The rate of visual field change appears correlated with the magnitude of IOP, suggesting that it may be advantageous to set extremely low IOP targets for some patients with *TBK1*-associated glaucoma. (Am J Ophthalmol 2020;214:52–62. © 2020 Elsevier Inc. All rights reserved.)

GLAUCOMA IS A CHRONIC DISEASE THAT LASTS A lifetime. It is the leading cause of irreversible blindness in the world.[1] Primary open-angle glaucoma (POAG) is the most common subtype of glaucoma and is characterized by optic nerve head cupping with corresponding visual field loss. Known risk factors of POAG include advanced age, a positive family history, African or Hispanic ancestry, thin central corneal thickness (CCT), and elevated intraocular pressure (IOP).[2] Although POAG may occur at any IOP, glaucoma that occurs at lower IOP (ie, ≤21 mm Hg) may be termed normal tension glaucoma (NTG).[3]

Most cases of open-angle glaucoma are caused by a complex interaction of many environmental and genetic risk factors.[4,5] However, to date, we have found that approximately 5% of open-angle glaucoma cases seem to be caused primarily by mutations in single genes.[6–8] Myocilin (*MYOC*) mutations have been shown to cause a large proportion of juvenile-onset POAG and 3%-4% of POAG cases worldwide. Mutations in 3 glaucoma-causing genes, optineurin (*OPTN*), TANK binding kinase 1 (*TBK1*), and *MYOC*, have each been associated with approximately 1% of NTG cases.[7–12]

TBK1 encodes a serine-kinase that phosphorylates and activates many substrates that are involved in NF-kB signaling and autophagy. One target of TBK1 kinase activity is the autophagy receptor protein OPTN.[13] Autophagy is a catabolic process that cells employ to survive nutritional stress and/or to degrade defective proteins, organelles, and intracellular pathogens.[14] The recognition that

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2 NTG-causing genes, *TBK1* and *OPTN*, directly interact within the same biologic pathway suggests that dysregulation of autophagy may have a role in retinal ganglion cell death and glaucoma pathogenesis in patients with *TBK1* or *OPTN* mutations.

In 4 independent reports,[8–11] *TBK1* gene duplications or triplications were detected in 8 of 1172 (0.72%) unrelated NTG patients and in 0 of 1320 controls ($P = .0018$).[15] These reports include descriptions of mean maximum IOP, age at diagnosis, and optic disc photographs. However, the long-term clinical course of patients with *TBK1*-related NTG (or any other molecularly defined type of glaucoma) has not yet been described. In the current report, we delineate the clinical features of 7 *TBK1* glaucoma patients (14 eyes) who are all part of a large, previously described pedigree.[8] We describe features of their glaucoma at the time of diagnosis as well as their long-term follow-up (up to 31 years of examinations).

METHODS

• **STUDY DESIGN:** In this retrospective observational case series, we examined the presenting features and clinical course of glaucoma in members of a pedigree with a *TBK1* gene duplication.

• **CLINICAL ANALYSIS OF PATIENTS:** All study participants provided written informed consent and this research was conducted with the approval of the University of Iowa's Institutional Review Board. The study followed the tenets of the Declaration of Helsinki.

Complete ophthalmologic examinations including Goldmann applanation tonometry, slit-lamp examination, gonioscopy, ophthalmoscopy, and all testing were performed by 1 of the authors (A.L.R.). Visual acuity (VA) was measured using the Snellen VA eye chart and converted to logMAR. IOP was measured with Goldmann applanation. Visual fields were assessed with a Humphrey Field Analyzer (Zeiss Meditec, Dublin, California, USA). A total of 299 fields were available for analysis, including 26 (8.7%) Full-Threshold, 255 (85%) SITA-Standard, 10 (3.3%) SITA-FAST, and 8 (2.7%) FAST-PAC. We identified 243 visual fields that were obtained using either Full-Threshold or SITA-Standard protocols that met reliability standards based on the Normal Tension Glaucoma Study criteria.[16] SITA-Standard and Full-Threshold tests were included in our analysis, given data suggesting acceptable comparability.[17,18] Of the 243 visual field tests included in this study, 114 (46)% were performed in a 10-2 test pattern, 105 (42%) were performed in a 24-2 test pattern, and 24 (12%) were performed in a 30-2 test pattern. Of the 243 visual field tests, 216 (89%) were performed in SITA-Standard and 27 (11%) were performed in Full-Threshold prior to the commercial availability of the SITA protocols. Visual field tests were graded as mild, moderate, or severe using the Hodapp-Parrish-Anderson classification system.[19] Retinal nerve fiber layer (RNFL) was measured using spectral-domain optical coherence tomography (OCT) (Cirrus HD-OCT; Zeiss Meditec, Dublin, California, USA). Patients with glaucomatous cupping of the optic disc and corresponding visual field defects were diagnosed with NTG, as described in a previous report of this pedigree[8] and using standard criteria.[20] Legal blindness is defined as the best-corrected VA being ≤20/200 or a visual field defect such that the widest diameter of the visual field is no greater than 20° in the better-seeing eye with a Goldmann III4e target or equivalent Humphrey Visual Field Analyzer stimulus size III, threshold equal to or worse than 10 db defect.

• **GENETIC ANALYSIS:** DNA from each family member was tested for glaucoma-causing mutations. The proband of the pedigree, III-1, tested negative for the Glu50Lys mutation in *OPTN* and negative for a *MYOC* mutation using Sanger sequencing assays, as previously described,[8] but tested positive for a *TBK1* gene duplication using a quantitative polymerase chain reaction assay and comparative genome hybridization as part of a previous report.[8] The other 6 family members with NTG in this study also tested positive for the same *TBK1* gene duplication.

RESULTS

WE PREVIOUSLY REPORTED A LARGE AFRICAN-AMERICAN pedigree with 10 family members that were both diagnosed with NTG and had a *TBK1* gene duplication, as shown in Figure 1.[8] Long-term follow-up data from March 1985 through May 2018 were available from 7 of these family members (Figure 1: II-2, II-5, II-9, III-1, III-2, III-6, and III-7). Five of the 7 patients were female. The mean length of follow-up was 21.1 ± 10 years with a range of 5-31 years.

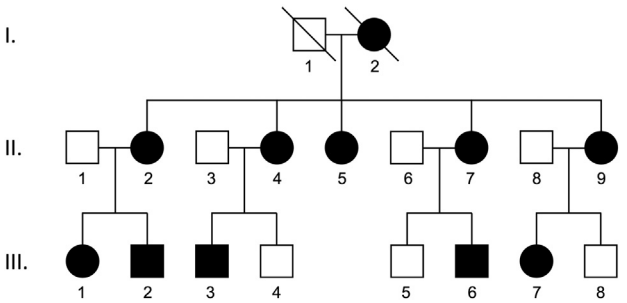

FIGURE 1. A 3-generation African-American pedigree with normal tension glaucoma (NTG) caused by a *TBK1* gene duplication. Family members diagnosed with NTG are indicated by shaded symbols. All 10 living family members with NTG carry a *TBK1* gene duplication. This family has been described in a previous report.[8]

TABLE 1. Clinical Features of Patients With a *TBK1* Gene Duplication at Initial Examination and at Last Available Examination

	II-2		II-5		II-9		III-1		III-2		III-6		III-7		Mean (SD)	
Family Member (Pedigree Symbol)	Initial Exam	Final Exam	Initial Exam	Final Exam	Initial Exam	Final Exam	Initial Exam	Final Exam	Initial Exam	Final Exam	Initial Exam	Final Exam	Initial Exam	Final Exam	Mean Initial Exam (SD)	Mean Final Exam (SD)
Age (years)	46	77	43	73	35	60	31	57	34	48	36	41	22	39	35 (8)	56 (15)
VA (logMAR)	0.0 OD	0.3 OD	0.0 OD	0.3 OD	0.0 OD	0.1 OD	0.0 OD	0.0 OD	0.0 OD	0.0 OD	0.0 OD	0.0 OD	0.0 OD	0.0 OD	0.0 (0.0)	0.13 (0.2)
	0.0 OS	0.6 OS	0.0 OS	0.4 OS	0.0 OS	0.1 OS	0.0 OS	0.0 OS	0.0 OS	0.0 OS	0.0 OS	0.0 OS	0.0 OS	0.0 OS		
IOP (mm Hg)	14.0 OD	6.0 OD	18.0 OD	16.0 OD	17.0 OD	12.0 OD	15.0 OD	10 OD	20.0 OD	14.0 OD	15.0 OD	14.0 OD	14.0 OD	11.0 OD	16.0 (2.1)	11.5 (2.9)
	14.0 OS	7.0 OS	17.0 OS	9.0 OS	16.0 OS	13.0 OS	14.5 OS	10 OS	20.0 OS	14.0 OS	15.0 OS	14.0 OS	14.0 OS	11.0 OS		
CCT (μm)[a]	532 OD	NA	533 OD	NA	487 OD	NA	496 OD	NA	490 OD	NA	524 OD	NA	474 OD	NA	506 (25)	NA
	541 OS		526 OS		504 OS		497 OS		486 OS		533 OS		463 OS			
C/D ratio	0.99 OD	0.99 OD	0.99 OD	0.99 OD	0.80 OD	0.90 OD	0.90 OD	0.99 OD	0.80 OD	0.90 OD	0.90 OD	0.90 OD	0.80 OD	0.90 OD	0.90 (0.1)	0.94 (0.04)
	0.99 OS	0.99 OS	0.99 OS	0.99 OS	0.80 OS	0.90 OS	0.90 OS	0.99 OS	0.90 OS	0.95 OS	0.90 OS	0.90 OS	0.80 OS	0.99 OS		
OCT nerve fiber layer (μm)	NA	58.0 OD	NA	61.0 OD	NA	62.0 OD	NA	54.0 OD	NA	86.0 OD	NA	88.0 OD	NA	58.0 OD	NA	66.6 (13.6)
		49.0 OS		61.0 OS		90.0 OS		62.0 OS		71.0 OS		80.0 OS		56.0 OS		

CCT = central corneal thickness; C/D = cup-to-disc; IOP = intraocular pressure; NA = not available; OCT = optical coherence tomography; VA = visual acuity.

Age, VA, IOP, CCT, C/D ratio, and average retinal nerve fiber layer thickness from OCT was measured in 14 eyes of 7 patients from a single African-American pedigree with normal tension glaucoma caused by a *TBK1* gene duplication. Patients are indicated by their pedigree symbol identification number from Figure 1.

[a]A single measurement of corneal thickness was made at some point during follow-up and is listed under "Initial Exam" column.

TABLE 2. Humphrey Visual Field Test Parameters of Patients With a *TBK1* Gene Duplication at Initial and Last Available Examination

Family Member (Pedigree Symbol)	II-2				II-5				II-9		III-1				III-2			III-6		III-7	
HVF Protocol	24-2 (dB)		10-2 (dB)		30-2 or 24-2 (dB)		10-2 (dB)		24-2 (dB)		30-2 or 24-2 (dB)		10-2 (dB)		24-2 (dB)		10-2 (dB)	24-2 (dB)		24-2 (dB)	
Eye	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OS	OD	OS	OD	OS
First available HVF	−24.3	−27.0	−20.5	−21.9	−18.7	−17.3	−13.1	−23.6	−2.7	−2.96	−9.6	−8.4	−11.7	−5.2	−1.8	−3.0	−3.9	−1.8	−3.5	−2.9	−2.9
Last available HVF	−25.1	−28.7	−28.2	−27.1	−23.1	−28.3	−23.8	−32.0	−3.6	−3.0	−29.0	−27.5	−25.6	−15.1	−2.1	−8.53	−4.6	−1.2	−2.6	−2.1	−2.7
Change in HVF	−0.8	−1.7	−7.7	−5.2	−4.4	−11.0	−10.7	−8.4	−0.9	0.04	−19.4	−19.1	−13.9	−9.9	−0.3	−5.53	−0.7	0.6	0.9	0.8	0.2
Years of follow-up	2	2	19	19	15	15	20	20	20	20	24	24	16	16	14	14	2	5	5	15	15
Rate of change in HVF (dB/year)																					
First and last test only	−0.40	−0.85	−0.41	−0.27	−0.29	−0.73	−0.54	−0.42	−0.05	0.00	−0.81	−0.80	−0.87	−0.62	−0.02	−0.40	−0.35	0.12	0.18	0.05	0.01
Best linear fit (all data)	NA	NA	−0.30	−0.22	−0.44	−0.77	−0.57	−0.48	0.00	−0.03	−0.80	−0.84	−0.74	−0.52	−0.23	−0.51	NA	−0.04	0.06	0.02	−0.04
Rate of change category	NA	NA	Slow	Slow	Slow	Mod	Mod	Slow	NC	NC	Mod	Mod	Mod	Mod	Slow	Mod	NA	NA	NA	NC	NC

HVF = Humphrey visual field; Mod = moderate; NA = not available owing to limited data; NC = no significant change.

The first available HVF, last available HVF, change in HVF, and rate of change in HVF were recorded in 14 eyes of 7 patients from a single African-American pedigree with normal tension glaucoma caused by a *TBK1* gene duplication. Rate of change was calculated by compared first and last available HVF tests and by plotting the best linear fit to all of the available HVF data. The rate of change in mean deviation was categorized as "slow" if the rate was less than −0.5 dB/year and "moderate" if the rate was between −0.5 dB/year and −1.0 dB/year. The rate of change in mean deviation was categorized as no change or "NC" if the rate was not statistically different from 0 dB/year.

The mean number of clinic visits was 59 ± 44 with a range of 3-137 visits per patient. We evaluated ophthalmic features of glaucoma (Table 1) and corresponding visual fields (Table 2) at the first and last available examination. For 3 of 7 patients, we were not able to obtain visual field tests at the time of glaucoma diagnosis as the patients were referred for the question of the etiology of the nerve and visual field changes. Visual fields were assessed with the 24-2 or 30-2 testing protocols initially; however, 3 of 7 family members were later tested using the 10-2 test protocol. The earliest and latest optic disc photographs and Humphrey visual field (HVF) tests available are shown for each eye in Figure 2.

• **OPHTHALMIC FEATURES AT DIAGNOSIS/INITIAL EXAMINATION:** *Ocular examination*. The mean age at diagnosis for these 7 patients was 35 ± 8 years with a range of 22-46 years. All eyes presented with a Snellen VA of 20/20 (logMAR = 0). All eyes had an IOP ≤21 mm Hg at presentation and throughout follow-up, with a mean initial IOP of 16.0 ± 2.1 mm Hg and a range of 14-20 mm Hg. CCT was thin in some patients and average in others, with a mean thickness of 506.1 ± 25.1 μm and range of 463-541 μm, which is thinner than the reported mean CCT for African Americans of ~530 μm.[21,22] Gonioscopy revealed wide-open angles without visible pathology in all cases. At initial examination, glaucomatous optic nerve damage was detected in all cases with ophthalmoscopy and optic disc photographs. All 14 eyes had extensive cupping, with a mean cup-to-disc (C/D) ratio of 0.90 ± 0.08 and range of 0.80 to 0.99, as determined by physician assessment. At initial examination, 1 eye (7%) met criteria for legal blindness.

Visual field and optic nerve image analyses. Humphrey Visual Field Analyzer perimetry data (24-2 and 30-2 protocol) were available from 10 of the 14 eyes (71%) at or within 1 year of initial examination and from 4 of the 14 eyes (29%) within 5-8 years of initial examination. Reduced global sensitivity was detected, with a mean MD of −9.0 ± 8.9 (range: −1.8 to −27) dB. Using the Hodapp-Parrish-Anderson classification system,[19] 4 of the 14 eyes (29%) had no glaucomatous visual field defects, 4 (29%) had early visual field defects, and 6 (43%) had severe defects. Visual field defects typical for glaucoma (ie, paracentral scotomas, arcuate defects, and nasal steps[23]) were detected in patients with *TBK1*-associated glaucoma (Figure 2). Analysis of the 4 eyes with early defects on presentation indicates that 2 eyes had arcuate defects and 2 eyes had nasal step defects. OCT analyses were not available at initial examination.

• **CLINICAL COURSE DURING LONG-TERM FOLLOW-UP EXAMINATIONS:** The mean IOP of the 7 members of this pedigree with glaucoma caused by a *TBK1* gene duplication was lowered by topical medications, laser trabeculoplasty, and/or trabeculectomy from 16.0 ± 2.1 mm Hg to 11.5 ± 2.9 mm Hg, for a mean reduction of 4.5 ± 2.4 mm Hg, a decrease in IOP of 28% ± 15%. Eleven of the 14 eyes (79%) were managed only with topical antihypertensives and/or argon laser trabeculoplasty, while 3 (21%) underwent glaucoma surgery (trabeculectomy) at some point during follow-up. Several patients also underwent cataract extraction when indicated to restore vision. No optic disc hemorrhages were detected during any of the optic disc examinations or identified retrospectively in disc photographs from any patients.

• **VISUAL FIELD ANALYSIS:** We analyzed visual fields and determined the rate of change in mean deviation (MD) for each glaucoma patient as a measure of overall change in severity of visual field damage (Table 2).

30-2 and 24-2 Humphrey visual field tests. Of the 14 eyes in this study, all had 30-2 and or 24-2 HVF tests. These eyes had a total of 129 Humphrey 30-2 or 24-2 visual field tests, with a mean number of 9.2 ± 5.1 visual field tests per eye. Ten of 14 eyes (71%) had more than 5 30-2 and or 24-2 visual field tests that were suitable for assessing progression rate. The rate of change in MD for these 10 eyes ranged from −0.84 to 0.017 dB/year and the average rate of change was −0.38 ± 0.36 dB/year. Four eyes had no statistically significant change in MD during follow-up. The average rate of change in MD increased to −0.62 ± 0.23 dB/year if these 4 stable eyes were excluded. For each of these 10 eyes, we also compared the mean of IOP measurements during follow-up with the rate of change in MD and found a correlation of $r = 0.71$ and $r^2 = 0.51$ ($P = .019$). IOP and rate of change of MD in Humphrey 24-2 visual fields in this family were moderately correlated.

10-2 Humphrey visual field tests. Of the 14 eyes in this study, 7 had 10-2 HVF tests. These eyes had a total of 114 Humphrey 10-2 visual field tests, with a mean number of 14.3 ± 9.5 visual field tests per eye. Six of the 14 eyes (42%) had more than 5 10-2 visual field tests that were suitable for assessing progression rate. The rate of change in MD for these 6 eyes ranged from −0.21 dB/year to −0.57 dB/year, with a mean rate of change of −0.47 ± 0.19 dB/year. The rate of change in MD of 10-2 visual field tests was highly correlated to mean IOP, with $r = 0.81$ and $r^2 = 0.66$ ($P = .05$).

None of the family members' eyes had progression >1 dB/year with HVF tests (24-2 or 10-2), which is a frequently used threshold for rapid progression.[24]

We placed the 12 eyes with more than 5 visual field tests into 3 groups: (1) stable MD, (2) slowly progressive MD (<0.5 dB/year), and (3) moderately progressive MD (0.5 to 1.0 dB/year).

Group 1—Stable mean deviation

Four of 12 eyes (II-9 OU and III-7 OU) (33%) had visual fields with stable MD throughout follow-up. For each of

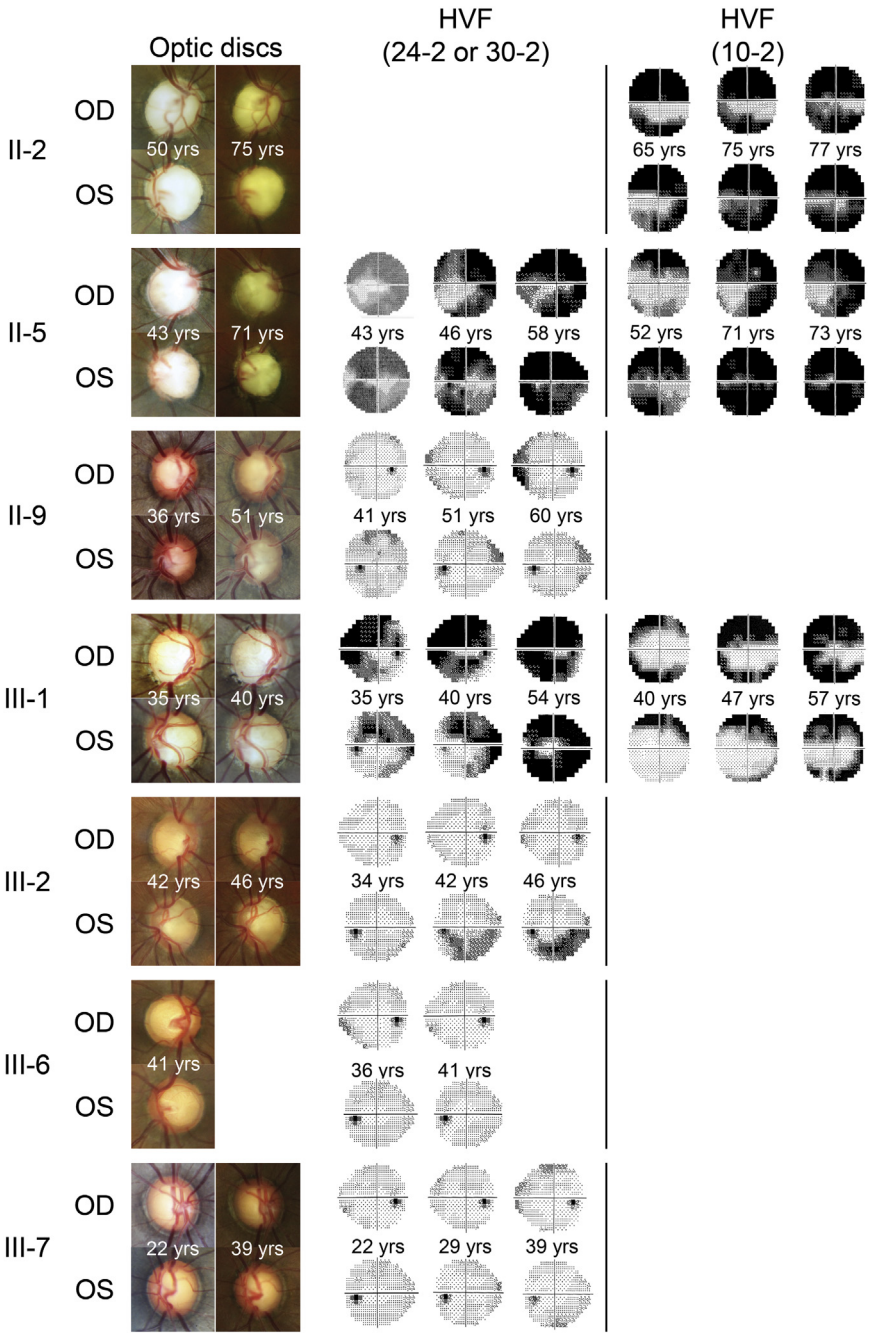

FIGURE 2. First and last available disc photographs and Humphrey visual field (HVF) tests. Long-term clinical follow-up data were available from 7 family members with normal tension glaucoma (NTG) caused by a *TBK1* gene duplication. First and last available disc photographs demonstrate progressive optic disc cupping. First and last available HVF tests (30-2, 24-2, and/or 10-2 protocols) demonstrate progressive glaucomatous visual field loss. Visual field tests that were obtained at the same time as optic disc photographs are also presented. Patients are indicated by their pedigree symbol identification number from Figure 1. Only 1 set of disc photographs was available from Patient III-6.

these eyes, the rate of change in MD was not statistically different from 0 dB/year (no change) and ranged from −0.04 to 0.017 dB/year (mean = −0.02 ± 0.03 dB/year). Two of the 4 eyes (II-9 OD and III-7 OS) had no glaucomatous visual field loss at initial examination, but both developed moderate visual field defects at final examination. Two of the 4 eyes (II-9 OS and III-7 OD) had an early defect on visual field testing at initial examination

and 1 developed a moderate visual field defect at final examination. Thus, all 4 eyes with stable MD did in fact have progression of glaucomatous changes in their visual fields when measured with other criteria (ie, Hodapp-Parrish-Anderson classification system). The Collaborative Normal Tension Glaucoma Study set a target for a 30% reduction in IOP for their normal tension glaucoma study patients.[25] The mean baseline IOP for these 4 patients was 15.5 ± 1.5 mm Hg (range, 14-17 mm Hg) and 30% reduction from baseline IOP was reached at 38% of follow-up visits. The mean IOP of these eyes during follow-up was 11.4 mm Hg (range, 5-16 mm Hg), with a mean decrease in IOP by 22.7% ± 4.6%. The MD of visual field tests and IOP measurements of Patient III-7, right and left eye, are depicted in Figure 3, A and B, as an example of the stable visual fields in this patient group. Additional diagrams for the rest of study patients are shown in the Supplemental Figure (Supplemental Material available at AJO.com).

Group 2—Slow progression of mean deviation

Three of 12 eyes (II-2 OU and III-2 OD) (25%) had worsening Humphrey visual fields (30-2, 24-2, and/or 10-2) with relatively slow rates of change in MD (<0.5 dB/year). On initial examination, 1 of these eyes (III-2 OD) had an essentially normal 24-2 visual field that developed damage at a rate of −0.23 dB/year with IOPs that were mostly between 10 and 15 mm Hg (Figure 3, C). Two other eyes (II-2 OD and II-2 OS) had severe 10-2 visual field defects on first available visual field, with MDs of −20.53 dB and 21.90 dB that worsened at a rate of −0.29 dB/year and −0.21 dB/year, respectively. Notably, these 2 eyes

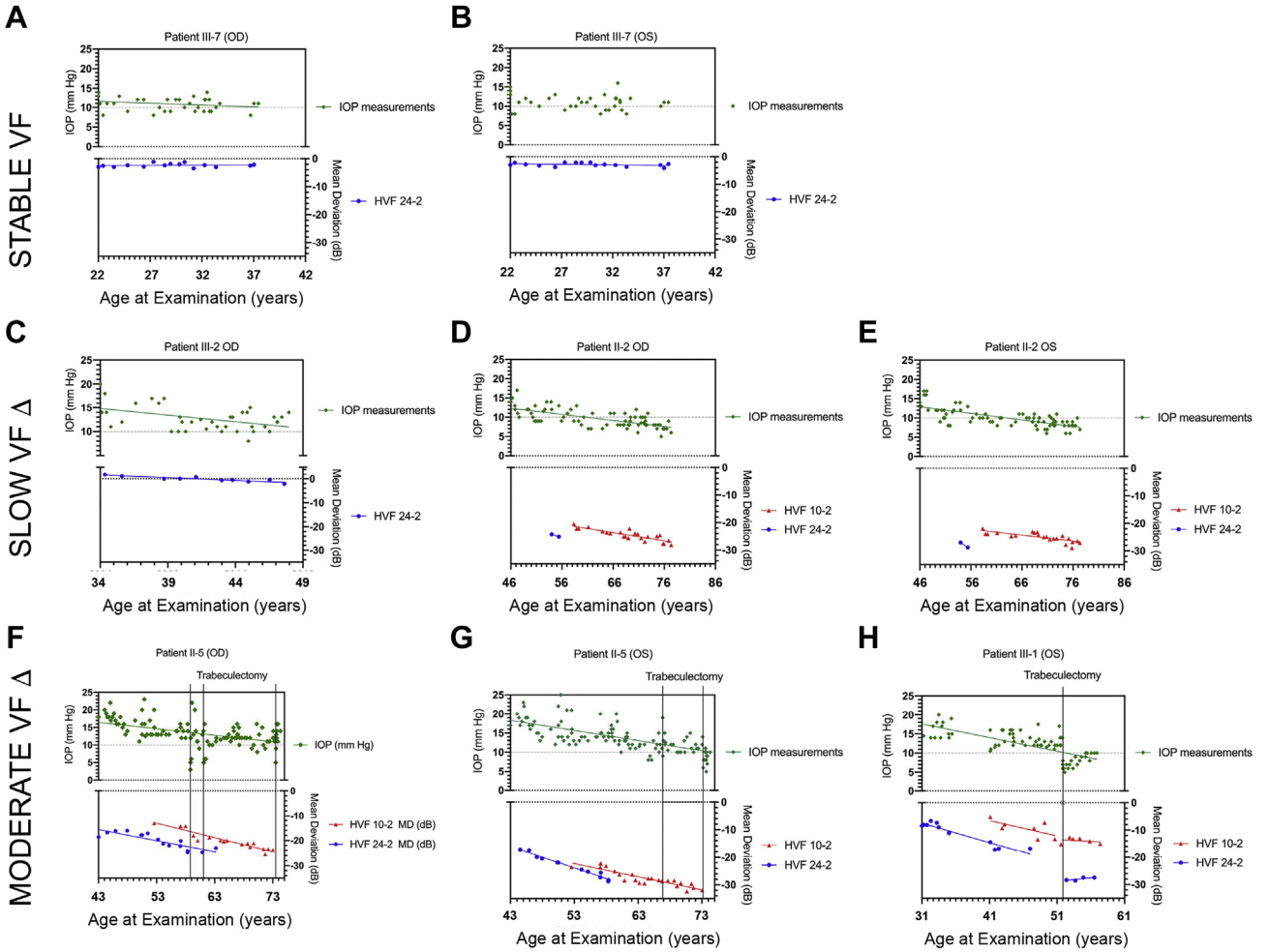

FIGURE 3. Longitudinal measures of intraocular pressure (IOP) and Humphrey visual field (HVF) tests. Panels show longitudinal plots of IOP and mean deviation (MD) from HVF tests for 8 eyes of patients with normal tension glaucoma caused by a *TBK1* gene duplication. A, B. Linear fits show plots for Patient III-7 OD and OS that have stable HVF tests throughout follow-up. C-E. Plots for Patient III-2 OD and Patient II-2 OD and OS that have HVF tests with slow progression with a change in MD < 0.5 dB/year. F-H. Plots for Patient III-5 OD and OS and Patient III-1 OS that have HVF tests with moderate progression with a change in MD of 0.5-1.0 dB/year. The time at which a trabeculectomy surgery was performed is indicated by a vertical line in these patients.

consistently worsened despite successful lowering of IOP to levels frequently <10 mm Hg (Figure 3, D and E). The 3 eyes in this group had a mean baseline IOP of 16.0 ± 3.5 mm Hg (range, 14-20 mm Hg), and a 30% reduction of IOP from baseline was reached at 62% of follow-up visits, while the mean IOP of these eyes during follow-up was 10.8 mm Hg, with a mean decrease in IOP by 45% ± 14.1%.

Group 3—Moderate progression of mean deviation

Five of 12 eyes (III-1 OU, III-2 OS, and II-5 OU) (42%) had more rapidly worsening visual fields (0.5 to 1.0 dB/year) with either 10-2, 24-2, and/or 30-2 protocols. These 5 eyes had 30-2 or 24-2 visual field defects on initial examination that ranged from mild (MD = −3.0 dB) to severe defects (MD = −18.65 dB). Humphrey visual field 30-2 and 24-2 tests in these 5 eyes worsened at an average rate of −0.70 ± 0.15 dB/year. Additionally, 10-2 visual field tests were obtained from 4 of these eyes and these visual field tests worsened at a rate of −0.75 ± 0.11 dB/year. The mean baseline IOP for these 5 eyes was 17.0 ± 2.6 mm Hg (range, 14.5-20 mm Hg) and a 30% reduction from baseline IOP was reached at 39% of follow-up visits, while the mean IOP of these eyes during follow-up was 12.8 mm Hg (range, 9-20 mm Hg), with a mean decrease in IOP by 30.5% ± 12.8%. Three of these 5 more rapidly progressing eyes (III-1 OS, II-5 OU) were treated surgically with trabeculectomy, with a mean of 2 trabeculectomies per eye (range, 1-3).

We compared the mean IOPs of eyes with stable MD (n = 4), slowly progressive MD (n = 3), and moderately progressive MD (n = 5). There was no significant difference in mean IOP between these groups ($P = .0386$). There was only a slight difference in mean age between these groups ($P = .609$), with the group that did not have progression of visual field loss being slightly younger.

The eyes that were treated with trabeculectomy (III-1 OS, II-5 OU) had a mean baseline IOP of 16.5 ± 1.8 mm Hg and severe visual field defects at the time of diagnosis. These eyes had a statistically significant change in HVF 24-2 MD throughout follow-up, with a mean change in MD of −0.73 ± 0.14 dB/year, and a statistically significant change in HVF 10-2 MD throughout follow-up, with a mean change in MD of −0.53 ± 0.26 dB/year. One family member (Patient II-5) had 3 trabeculectomies in the right eye and 2 trabeculectomies in the left eye. During the course of follow-up this patient had a mean IOP of 13 ± 1.8 mm Hg in the right eye and a mean IOP of 13.5 ± 3.6 mm Hg in the left eye, with a peak IOP of up to 23 mm Hg in both eyes. Despite achieving IOP lowering, her visual fields continued to worsen (Figure 3, F and G). Another family member (Patient III-1) underwent 1 trabeculectomy in the left eye. In this patient, IOP was ultimately maintained below 10 mm Hg and there was stabilization her visual field (Figure 3, H).

• **OPHTHALMIC FEATURES AT LAST EXAMINATION:** *Ocular examination*. The mean age at final follow-up was 56 ± 15 years with a range of 39-77 years and the mean VA at final examination was a logMAR of 0.13 ± 0.2, equivalent to 20/25 Snellen. A decline in VA was detected in 6 of 14 eyes (42%). Reduced VA was likely attributable to glaucomatous visual field damage within 5 degrees of fixation in 4 of the 6 eyes and likely attributable to cataract in 2 eyes. The mean IOP at final examination was 11.5 ± 2.9 mm Hg (range, 6-16 mm Hg), which was a 28% ± 15.5% mean decrease in IOP compared with first examination or a 4.5 ± 2.4 mm Hg mean decrease in IOP. Progressive glaucomatous optic nerve damage was detected in 8 of 14 eyes (57%) with ophthalmoscopy and evaluation of optic disc photographs. Of the 6 eyes without progressive glaucomatous optic nerve damage, 4 had initial C/D ratios of 0.99. At final examination, the mean C/D ratio was 0.94 ± 0.4 with a range of 0.90 to 0.99.

Visual field and optic nerve image analyses. At final examination 3 of 14 eyes (21%) had early defects, 4 (29%) had moderate defects, and 7 (50%) had severe defects, using the Hodapp-Parrish-Anderson classification system. Half of the eyes with early visual field defects at last examination had only 5 years or less follow-up, which is much shorter than the average follow-up in this study. At final examination, 6 of 14 eyes (43%) met criteria for legal blindness based on visual field testing.

All eyes had thin RNFL when assessed using spectral-domain OCT (Cirrus HD-OCT) at last examination. Unfortunately, longitudinal OCT measures were not available for analysis owing to the date of introduction of this testing modality. The average RNFL thickness was available for 14 of 14 eyes (100%) at last examination and ranged from 49 to 90 μm, with a mean of 66.0 ± 13.6 μm. In most cases, inferior thinning of the RNFL was greater than superior thinning of the RNFL.

• **OTHER SYSTEMIC FEATURES OF PATIENTS:** Systemic hypertension treated with antihypertensive medications was present in 2 of 7 patients; however, no patient had a known history of associated nocturnal hypotension. No patients had a known history of vasospastic disease such as migraine headache or Raynaud phenomenon, autoimmune disease, or obstructive sleep apnea. A history of iron deficiency anemia associated with heavy menstruation and/or the need for 1 or more blood transfusions during childbirth was also present in 3 of 5 female patients.

DISCUSSION

APPROXIMATELY 1% OF NTG CASES MAY BE DUE TO *TBK1* mutations.[15] Duplications and triplications of *TBK1* have

been associated with glaucoma that occurs at low IOP in several reports.[8–11,26] The current report, however, is the first to describe the clinical features of *TBK1*-associated NTG at the time of diagnosis as well as during long-term (up to 31 years) follow-up. Patients were young (ranging from 22 to 46 years old) and had low, untreated IOPs (ranging from 14 to 20 mm Hg) at the time of first examination. All of these patients had extensive optic nerve damage, with C/D ratios ranging from 0.8 to 0.9, and almost half (43%) had severe visual field damage at first examination using the Hodapp-Parrish-Anderson classification.

TBK1 duplications and triplications have been reported in 10 pedigrees that include 27 patients with glaucoma.[8–11,26] These patients were diagnosed with NTG by glaucoma specialists prior to the identification of their *TBK1* mutations. However, the longitudinal features of *TBK1*-associated glaucoma have not yet been delineated. Our research finds that NTG patients with a *TBK1* mutation have classic features of NTG: progressive cupping and progressive visual field damage that occur at low IOP. We chose to primarily analyze progression of visual field defects by following change in MD. Although MD does not take the pattern of visual field loss into account, its global assessment facilitates longitudinal comparisons and simple calculation of overall rates of change.[24,27,28]

Analysis of hundreds of visual field tests from our NTG pedigree showed that 33% of patients had stable visual fields and that 67% had progressive worsening during their follow-up examinations (mean of 21.5 years). The rate at which 24-2 visual fields worsened in these patients (average change in MD = −0.57 ± 0.31 dB/year) was similar to the rate of worsening exhibited by patients in the Collaborative Normal Tension Glaucoma Study (average MD = −0.40 to −0.50 dB/year)[3] and no family members had rapidly progressing visual fields (change in MD >1.0 dB/year). The rate of visual field loss was strongly correlated with the magnitude of IOP (r = 0.66 for 24-2 and r = 0.90 for 10-2), which provides evidence that IOP influences the rate of worsening of visual fields in *TBK1*-related glaucoma. Moreover, the rate of worsening was slowed by IOP reduction in at least 1 patient. This patient, III-1, had progressive visual field loss in the left eye until her IOP became <10 mm Hg following a trabeculectomy. With lower IOP, the visual field stabilized (Figure 3, G). These data indicate that patients with *TBK1*-related glaucoma may have similar responses to lowering IOP as other open-angle glaucoma patients.[24,29,30]

Although disc hemorrhages have been more frequently detected in patients with NTG than in other forms of glaucoma,[31,32] none were reported, nor did we observe any disc hemorrhages in these *TBK1*-associated NTG patients despite extensive review of all clinical notes and optic disc photographs. A disc hemorrhage was documented, however, in a recent report of another NTG pedigree with glaucoma due to a *TBK1* gene duplication.[33] Although the follow-up period for this pedigree was long, the sample size may have been too small to detect optic disc hemorrhages. However, these data and observations suggest that disc hemorrhages in this specific pedigree may occur with *TBK1*-associated NTG, at reduced frequency. Also, a reduced rate of disc hemorrhages has been reported in African-American glaucoma patients when compared with white patients.[34,35] Thus, the African ancestry of our *TBK1* glaucoma pedigree may have contributed to the absence of disc hemorrhages in our study. *TBK1* duplications and triplications have been also been detected in white and Asian NTG patients[8–11,26] and rate of optic disc hemorrhages in these patients has not yet been investigated.

Several types of data demonstrate that *TBK1* mutations are the primary cause of disease in a subset of NTG cases. The pathogenicity of *TBK1* gene duplications has been confirmed by statistical analyses[15] and by recapitulation of disease in transgenic animals with analogous *TBK1* mutations.[36] *TBK1*-associated glaucoma also has autosomal dominant inheritance, which indicates a single-gene cause of disease.[8] Nonetheless, additional coexisting genetic and environmental risk factors may have contributed to disease in members of the pedigree described in this report. First, members of this pedigree have African ancestry, which may increase overall risk for glaucoma and severity of disease. Second, the majority of patients in this study had a thin or very thin CCT, with only 1 patient having an average CCT when compared with African-American normative data. Third, 3 patients had significant iron deficiency anemia, related to either heavy menses or postpartum hemorrhage, that required blood transfusions. Unfortunately, the amount of blood loss, hemoglobin level, and these patients' resulting blood pressures were not documented. Also, several patients in this NTG pedigree were taking oral medication for systemic hypertension. None of these patients was known to have nocturnal hypotension as a result of these medications. It is possible that reduced ocular perfusion owing to these hemodynamic influences may have contributed to glaucoma in these patients with *TBK1* mutations.

Our study had additional limitations. Although our NTG pedigree is one of the largest known families with glaucoma caused by a *TBK1* gene duplication, longitudinal clinical data were available from only 7 family members. This relatively small sample size from a single pedigree may have biased our results. However, *TBK1* mutations were only recently discovered and individuals with this abnormality are just beginning to be recognized. Additionally, this family's clinical course may not be representative for patients with *TBK1*-associated glaucoma who are of different racial/ethnic ancestry. The vast majority of the visual field tests in our study were obtained using the SITA-Standard 24-2 protocol. However, we also included 24-2 Full-Threshold, 30-2 SITA-Standard, and 30-2 Full-Threshold visual field tests in our analysis. Data from each of these visual field test protocols are not

completely interchangeable owing to differences in algorithms used to find threshold sensitivities as well as differences in patient fatigue and intertest variability related to differences in test times.[37] However, prior analyses have demonstrated that the test results from 24-2 and 30-2 protocols have minimal differences.[18] Moreover, global indices, like MD, are determined by comparison with normative databases that are specific to each testing strategy (ie, SITA-Standard and Full-Threshold). Consequently MDs calculated from different testing strategies are very similar, with little to no statistical difference,[38] and we focused our analyses on this robust metric.

This study demonstrates that one must be more cautious and observant in younger patients with glaucoma. Although the mean MD loss per year was not that great, when following a relatively young patient for decades, this small loss/year becomes clinically significant.

In conclusion, this is the first paper to report the natural history of NTG caused by *TBK1* gene duplication. These data demonstrated that *TBK1* mutations are associated with progressive optic nerve and visual field damage that are typical for NTG. Standard therapies achieved an average IOP reduction of 28% during the course of follow-up and overall family members experienced slow-to-moderate progressive worsening of Humphrey 24-2 visual fields (rate of MD change was −0.35 dB/year). Although 25% of family members had stable MD throughout follow-up, even these individuals experienced some objective worsening of visual fields with Hodapp-Parrish-Anderson classification. None of the family members had rapidly worsening visual fields (rate of MD change >1.0 dB/year); however, substantial visual disability commonly occurred in members of this family, perhaps owing in part to the early onset of disease. Finally, the rate of visual field worsening in these patients was correlated with IOP and, in at least 1 patient, reduction of IOP to <10 mm Hg served to stabilize disease, which indicates that lowering IOP may slow progression of *TBK1*-associated glaucoma. Such mutation-specific data may provide physicians with better information to counsel patients with *TBK1*-associated glaucoma about the likely course of their disease and may also assist with setting treatment goals (ie, target IOP). Finally, documenting the natural history of NTG caused by a *TBK1* mutation is important to clinicians to help them counsel and treat their patients and will also be necessary for designing future gene-targeted glaucoma therapies and evaluating their effectiveness. Similar studies in other forms of NTG and POAG will better help clinicians manage their patients.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

TYLER S. QUIST: METHODOLOGY, FORMAL ANALYSIS, INVESTIGATION, Writing - original draft, Writing - review & editing, Visualization. **Chris A. Johnson:** Methodology, Writing - review & editing. **Alan L. Robin:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing. **John H. Fingert:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition.

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