JAMA Ophthalmology | Brief Report

Myocilin Mutations in Patients With Normal-Tension Glaucoma

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IMPORTANCE Mutations in the myocilin (*MYOC*) gene are the most common molecularly defined cause of primary open-angle glaucoma that typically occurs in patients with high intraocular pressures (IOP). One *MYOC* mutation, p.Gln368Ter, has been associated with as many as 1.6% of primary open-angle glaucoma cases that had a mean maximum recorded IOP of 30 mm Hg. However, to our knowledge, the role of the p.Gln368Ter mutation in patients with normal-tension glaucoma (NTG) with an IOP of 21 mm Hg or lower has not been investigated.

OBJECTIVE To evaluate the role of the p.Gln368Ter *MYOC* mutation in patients with NTG.

DESIGN, SETTING, AND PARTICIPANTS In this case-control study of the prevalence of the p.Gln368Ter mutation in patients with NTG, cohort 1 was composed of 772 patients with NTG and 2152 controls from the United States (Iowa, Minnesota, and New York) and England and cohort 2 was composed of 561 patients with NTG and 2606 controls from the Massachusetts Eye and Ear Infirmary and the NEIGHBORHOOD consortium. Genotyping was conducted using real-time polymerase chain reaction that was confirmed with Sanger sequencing, the imputation of genome-wide association study data, or an analysis of whole-exome sequence data. Data analysis occurred between April 2007 and April 2018.

MAIN OUTCOMES AND MEASURES Comparison of the frequency of the p.Gln368Ter *MYOC* mutation between NTG cases and controls with the Fisher exact test.

RESULTS Of 6091 total participants, 3346 (54.9%) were women and 5799 (95.2%) were white. We detected the p.Gln368Ter mutation in 7 of 772 patients with NTG (0.91%) and 7 of 2152 controls (0.33%) in cohort 1 ($P = .03$). In cohort 2, we detected the p.Gln368Ter mutation in 4 of 561 patients with NTG (0.71%) and 10 of 2606 controls (0.38%; $P = .15$). When the cohorts were analyzed as a group, the p.Gln368Ter mutation was associated with NTG (odds ratio, 2.3; 95% CI, 0.98-5.3; $P = .04$).

CONCLUSIONS AND RELEVANCE In cohorts 1 and 2, the p.Gln368Ter mutation in *MYOC* was found in patients with IOPs that were 21 mm Hg or lower (NTG), although at a frequency that is lower than previously detected in patients with higher IOP. These data suggest that the p.Gln368Ter mutation may be associated with glaucoma in patients with normal IOPs as well as in patients with IOPs that are greater than 21 mm Hg.

JAMA Ophthalmol. doi:10.1001/jamaophthalmol.2019.0005
Published online February 28, 2019.

Supplemental content

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Primary open-angle glaucoma (POAG) is a leading cause of visual disability and has a significant genetic basis. Three genes, myocilin (*MYOC*), optineurin (*OPTN*), and TANK binding kinase 1 (*TBK1*), are known to be associated with some cases of glaucoma with little influence from other genetic factors.[1-3] Mutations in *MYOC* are responsible for 3% to 4% of POAG cases that typically have a high intraocular pressure (IOP) (>21 mm Hg).[1,4] One *MYOC* mutation, p.Gln368Ter (also known as Gln368Stop), has been associated with 1.6% of POAG cases with high IOP (>21 mm Hg).[5] In this article, we investigate the role of *MYOC* in patients with normal-tension glaucoma (NTG) with an IOP 21 mm Hg or lower by testing cohorts of patients with NTG and controls for the most common *MYOC* mutation, p.Gln368Ter.

Key Points

Question Is the most common, known glaucoma-causing mutation, p.Gln368Ter in the myocilin gene, associated with glaucoma that occurs with intraocular pressures (IOPs) of 21 mm Hg or lower (normal-tension glaucoma) as well as glaucoma that occurs with high IOPs?

Findings In this case-control study of 1333 patients and 4758 controls, the p.Gln368Ter mutation was detected at a significantly higher frequency in patients with normal-tension glaucoma than in control participants.

Meaning These findings suggest that myocilin mutations are associated with glaucoma that occurs with a broader range of IOP than previously recognized, including individuals with pressures that are 21 mm Hg or lower.

Methods

Study Participants

Participants gave informed consent and the University of Iowa institutional review board approved the research. The criteria for NTG were glaucomatous optic nerve damage and visual field loss with a maximum recorded IOP of 21 mm Hg or lower.[3,4] Patients with secondary glaucoma were excluded. Patients with mutations in *TBK1* or *OPTN* were excluded. The criteria for healthy control participants included being older than 50 years, an IOP of 21 mm Hg or lower, failure to meet the criteria for glaucoma, and an examination by an ophthalmologist. Another cohort from the Retina Clinic at the University of Iowa with no known history of glaucoma (n = 1434) was used as a population control. Cohort 1 was composed of 406 patients with NTG, 528 healthy controls, and 1434 population control participants from Iowa; 93 patients with NTG and 37 healthy controls from Minnesota; 125 patients with NTG from New York; and 148 patients with NTG and 153 healthy controls from England.

The POAG cases in the NEIGHBORHOOD Consortium or from the Massachusetts Eye and Ear Infirmary were classified as NTG if the highest known IOP at or before enrollment was less than 22 mm Hg. Patients with glaucoma with an IOP of less than 22 mm Hg who were receiving IOP-lowering treatment at enrollment were also classified as having NTG if there were no recorded IOPs that were greater than 22 mm Hg before treatment. Pretreatment IOP was available for 376 (67%) of the NEIGHBORHOOD cases overall and was available for all of the cases classified as NTG. Control participants had an IOP of 21 mm Hg or lower without glaucoma features.[6] Cohort 2 was composed of 166 patients with NTG and 344 healthy controls from Massachusetts Eye and Ear Infirmary and 395 patients with NTG and 2262 healthy controls from the NEIGHBORHOOD consortium.

Genotyping

Whole-exome sequencing results in cohort 1 had been obtained from 134 patients with NTG, 362 healthy controls, and 1434 population controls as previously described[7] and the presence of the p.Gln368Ter mutation was assessed by inspection. The remainder of cohort 1 (638 patients with NTG and 356 healthy controls) was tested for the p.Gln368Ter mutation using a real-time polymerase chain reaction TaqMan assay (Applied Biosystems) following the manufacturer's protocol. All instances of p.Gln368Ter were confirmed with Sanger sequencing as previously described.[5]

The presence or absence of the p.Gln368Ter mutation in *MYOC* among cohort 2 members was inferred from the posterior probabilities imputed from genome-wide association study genotypes as previously described,[8] removing minor allele frequency filters. IMPUTE2[9] assigned a heterozygous p.Gln368Ter genotype (rs74315329) based on flanking marker genotypes using the 1000 Genomes Project as a reference panel. A posterior probability of 0.7 or more was used as a threshold to assign p.Gln368Ter. DNA was not available for Sanger sequencing to confirm imputed results. However, a similar approach reported 100% sensitivity, 99.9% specificity, and 95.7% positive predictive value for identifying p.Gln368Ter with imputation.[8] The frequency of p.Gln368Ter mutation was compared between NTG cases and controls using the Fisher exact test with a threshold for significance of $P < .05$.

Results

Genetic Analyses

We evaluated the role of the p.Gln368Ter mutation in *MYOC* in NTG by assessing its frequency in 2 cohorts of patients with NTG and control participants. We used real-time polymerase chain reaction, Sanger sequencing, whole-exome sequencing, and the imputation of genome-wide association study genotypes to test for the p.Gln368Ter mutation.

In cohort 1 (**Table 1**), we detected the p.Gln368Ter mutation in 7 of 772 patients with NTG (0.91%) and 7 of 2152 controls (0.33%) (odds ratio [OR], 3.2; 95% CI, 0.96-10.8; $P = .03$). In cohort 2 (Table 1), we detected the p.Gln368Ter mutation in 4 of 561 patients with NTG (0.71%) and 10 of 2606 controls (0.38%) (OR, 2.3; 95% CI, 0.52-7.9; $P = .15$). When cohorts 1 and 2 were pooled, p.Gln368Ter was observed in 11 of 1333 patients with NTG (0.83%) and 17 of 4758 control participants (0.36%) (OR, 2.3; 95% CI, 0.98-5.3; $P = .04$).

Table 1. Patients With NGT With p.Gln368Ter Mutations

	Cohort 1							Cohort 2					Cohorts 1 and 2		
	No. (%)							No. (%)					No. (%)		
Participants	Iowa	Minnesota	New York	England	Total	Odds Ratio (95% CI)	*P* Value	Massachusetts Eye and Ear Infirmary	NEIGHBORHOOD Consortium	Total	Odds Ratio (95% CI)	*P* Value	Total	Odds Ratio (95% CI)	*P* Value
Patients with NTG	4/406 (1.0)	2/93 (2.2)	0/125	1/148 (0.7)	7/772 (0.9)	3.2 (0.96-10.8)	.03	0/166 (0)	4/395 (1.0)	4/561 (0.7)	2.3 (0.52-7.9)	.15	11/1333 (0.8)	2.3 (0.98-5.3)	.04
Control participants	5/1962 (0.3)	1/37 (2.7)	NA	1/153 (0.7)	7/2152 (0.3)			1/344 (0.3)	9/2262 (0.4)	10/2606 (0.4)			17/4758 (0.4)		

Abbreviations: NA, not applicable; NTG, normal-tension glaucoma.

Table 2. Clinical Features of 7 Patients With NTG With p.Gln368Ter Mutations

	Cohort 1								Cohort 2				
Characteristic	Iowa				Minnesota		England	Mean (SD)	NEIGHBORHOOD Consortium				Mean (SD)
Patient ID	2087-1	2393-1	2332-1	1132-1	1234602412	1305706063	92		NA1125	NA1375	NF7503	NI01430	
Max IOP OD, mm Hg	20	20	15	20	20	20	21	20.0 (1.4)	17	20	14	13	15.8 (2.8)
Max IOP OS, mm Hg	21	21	17	20	20	NA[a]	20		18	20	13	13	
Diurnal IOP measured	No	Yes	No	Yes	No	No	No	NAp	No	No	No	No	NAp
Central corneal thickness OD, microns	566	585	483	NA	547	552	NA	548 (42.3)	550	572	620	NA	573 (30.9)
Central corneal thickness OS, microns	566	607	469	NA	559	546	NA		549	546	600	NA	
History of disc hemorrhage	Yes	Not noted	Not noted	Not noted	Not noted	Not noted	Not noted	NAp	Not noted	Not noted	Not noted	Not noted	NAp
Family history of glaucoma (first-degree relative)	Yes	Yes	Yes	Yes	Yes	No	Yes	NAp	No	No	No	NA	NAp

Abbreviations: IOP, intraocular pressure; NA, not available; NAp, not applicable; NTG, normal-tension glaucoma.

[a] Patient 1305706063 had a central retinal vein occlusion and developed neovascular glaucoma in the left eye after receiving a diagnosis of NTG. The IOP measurements before the vein occlusion were not available.

Clinical Features of Patients With NTG With p.Gln368Ter Mutations

We examined the clinical features of the 11 patients with NTG with p.Gln368Ter mutations (**Table 2**). The mean (SD) maximum IOP in patients with NTG with the p.Gln368Ter mutation from cohort 1 was 20.0 (1.4) mm Hg (range, 17-21 mm Hg). Disc photographs and visual field test results from a representative case are shown in the eFigure in the Supplement. The mean (SD) central corneal thickness of patients with p.Gln368Ter mutations from both cohorts was 557 (39.3) µm (range, 469-620 microns). A disc hemorrhage was observed in 1 patient, while 6 patients had a family history of glaucoma in a first-degree relative.

Discussion

MYOC was discovered to be associated with glaucoma via genetic studies of pedigrees with juvenile-onset primary open-angle glaucoma (JOAG). The patients with JOAG in these studies were found to harbor a set of *MYOC* mutations (GLY364VAL, THR377MET, TYR437HIS, and ILE477ASN) that were associated with mean maximum IOPs of 36, 31, 44, and 40 mm Hg, respectively.[4] The p.Gln368Ter mutation has been associated with adult-onset POAG with high IOP (mean maximum IOP of 26-30 mm Hg).[4,10,11] As a result, *MYOC*-associated glaucoma has been considered synonymous with glaucoma that occurs with high IOP.

Isolated instances of *MYOC* mutations have been reported in patients with NTG. Alward et al[12] reported a TRP286ARG mutation in 1 patient with NTG. This mutation had been reported in 3 other patients with POAG with high IOP and in 1 case of JOAG with high IOP.[5,13,14] One occurrence of a p.Gln368Ter mutation was detected in a patient with NTG from Germany.[15] These observations, coupled with the 11 cases of patients with NTG with the p.Gln368Ter mutation in this article, provide evidence that the *MYOC* gene is associated with glaucoma across a broader range of IOP than was previously recognized.

Limitations

This study had limitations. Pretreatment IOPs were not available for all patients with NTG. Also, variation in central corneal thickness influences IOP measurement and could potentially have led to a misdiagnosis of NTG for some patients. Similarly, some patients who received a diagnosis of NTG might have had an unrecognized IOP of more than 21 mm Hg because of diurnal variation or infrequent testing. This study should be evaluated in the context of these limitations.

Conclusions

One interpretation of these data is that the p.Gln368Ter mutation may be associated with glaucoma with peak IOP less than an arbitrary threshold of 21 mm Hg (NTG) or greater than this threshold (POAG). Rather than separate the glaucoma associated with the p.Gln368Ter mutation into these categories of NTG or high-tension glaucoma, it may be more useful to note that *MYOC*-associated glaucoma occurs over a range of IOPs with a prevalence that increases with maximum IOP. However, ophthalmologists tend to describe open-angle glaucoma as NTG if the maximum IOP is 21 mm Hg or lower. With such definitions, the p.Gln368Ter mutation in *MYOC* may be responsible for up to 0.83% of NTG cases, making it the third most common, known cause of NTG behind *OPTN* and *TBK1*.[2,3]

ARTICLE INFORMATION

Accepted for Publication: December 22, 2018.

Published Online: February 28, 2019. doi:10.1001/jamaophthalmol.2019.0005

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Obtained funding: Fingert.
Administrative, technical, or material support: Alward, Roos, Sivaprasad, Lotery, Stone, Scheetz, Kwon, Fingert.
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Conflict of Interest Disclosures: Dr Pasquale reports receiving grants support for the National Eye Institute and personal fees from Eyenovia and Basuch+Lomb Inc. Dr Ritch reports receiving personal fees from Aeon Astron, ISonic Medical, and Santen Pharmaceuticals and consulting fees from Diopsys Inc, GLIA LLC, Guardion Health Sciences, Intelon Optics, Axim Biotechnologies Inc, Miotech, and Glauconix Inc. Dr Sivaprasad reports receiving grants and personal fees from Boehringer Inglehein, Roche, Novartis, Allergan, and Bayer and personal fees from Heidelberg Engineering. No other disclosures are reported.

Funding/Support: The research in this report was supported in part by National Institutes of Health grants R01EY023512, R01EY022305, R01EY015473, and P30EY014104 and Donald Heineking and the Hadley-Carver Chair in Glaucoma.

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; the collection, management, analysis, and interpretation of the data; the preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

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