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METTL23 Variants and Patients With Normal-Tension Glaucoma

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IMPORTANCE This research confirms and further establishes that pathogenic variants in a fourth gene, *METTL23*, are associated with autosomal dominant normal-tension glaucoma (NTG).

OBJECTIVE To determine the frequency of glaucoma-causing pathogenic variants in the *METTL23* gene in a cohort of patients with NTG from Iowa.

DESIGN, SETTING, AND PARTICIPANTS This case-control study took place at a single tertiary care center in Iowa from January 1997 to January 2024, with analysis occurring between January 2023 and January 2024. Two groups of participants were enrolled from the University of Iowa clinics: 331 patients with NTG and 362 control individuals without glaucoma. Patients with a history of trauma; steroid use; stigmata of pigment dispersion syndrome; exfoliation syndrome; or pathogenic variants in *MYOC*, *TBK1*, or *OPTN* were also excluded.

MAIN OUTCOMES AND MEASURES Detection of an enrichment of *METTL23* pathogenic variants in individuals with NTG compared with control individuals without glaucoma.

RESULTS The study included 331 patients with NTG (mean [SD] age, 68.0 [11.7] years; 228 [68.9%] female and 103 [31.1%] male) and 362 control individuals without glaucoma (mean [SD] age, 64.5 [12.6] years; 207 [57.2%] female and 155 [42.8%] male). There were 5 detected instances of 4 unique *METTL23* pathogenic variants in patients with NTG. Three *METTL23* variants—p.Ala7Val, p.Pro22Arg, and p.Arg63Trp—were judged to be likely pathogenic and were detected in 3 patients (0.91%) with NTG. However, when all detected variants were evaluated with either mutation burden analysis or logistic regression, their frequency was not statistically higher in individuals with NTG than in control individuals without glaucoma (1.5% vs 2.5%; $P = .27$).

CONCLUSION AND RELEVANCE This investigation provides evidence that pathogenic variants in *METTL23* are associated with NTG. Within an NTG cohort at a tertiary care center, pathogenic variants were associated with approximately 1% of NTG cases, a frequency similar to that of other known normal-tension glaucoma genes, including optineurin (*OPTN*), TANK-binding kinase 1 (*TBK1*), and myocilin (*MYOC*). The findings suggest that *METTL23* pathogenic variants are likely involved in a biologic pathway that is associated with glaucoma that occurs at lower intraocular pressures.

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Supplemental content

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Primary open-angle glaucoma (POAG) is the most common form of glaucoma that is defined by characteristic optic disc damage (cupping) and corresponding patterns of visual field defects.[1] High intraocular pressure (IOP) is a strong risk factor for POAG,[2,3] although disease can occur at any IOP. Normal-tension glaucoma (NTG) is a subset of POAG that occurs with IOP of 21 mm Hg or less. In the Baltimore Eye Survey,[4] half of patients with glaucoma had a screening IOP measurement of 20 mm Hg or less. Of the patients with definite open-angle glaucoma identified in the Beaver Dam Eye Study,[5] 32% had IOP of 21 mm Hg or less. Moreover, population-based studies[6-9] of Japanese, Chinese, and Malay people have suggested that a large proportion (80% to 92%) of patients with POAG in the groups in these studies have IOP measurements of 21 mm Hg or less. NTG is a common form of glaucoma.

Most cases of POAG have a complex genetic basis and are due to the combined actions of many (perhaps hundreds) of genes.[10,11] However, approximately 5% of POAG cases are caused by pathogenic variants in single genes, including myocilin (*MYOC*),[12] optineurin (*OPTN*),[13] and TANK-binding kinase 1 (*TBK1*).[14] Pathogenic variants in each of these 3 genes have been associated with POAG that occurs with maximum IOP of 21 mm Hg or less—that is, NTG. Although *MYOC* pathogenic variants are more typically detected in patients with high IOP, 1 *MYOC* variant (Gln368Ter) was detected in 0.6% of NTG cases in cohorts of patients from Iowa and Massachusetts.[15] The most commonly detected NTG-associated *OPTN* pathogenic variant (Glu50Lys) has been detected in 1% to 2% of NTG cases.[13,16,17] Finally, *TBK1* gene duplications and triplications have been associated with 0.4% to 1.3% of NTG cases.[18]

Recently, Pan and colleagues[19] identified a missense pathogenic variant, c.A83G p.Glu28Gly, in the methyltransferaselike 23 (*METTL23*) gene in an NTG pedigree. This variant results in aberrant splicing of *METTL23* messenger RNA, haploinsufficiency, and abnormal cellular localization of the encoded METLL23 protein, a histone arginine methyltransferase. Moreover, pathogenic variants in *METTL23* recapitulate glaucoma phenotypes in genetically engineered mice. Finally, METTL23 is abundantly produced in retinal ganglion cells, which are a primary site of NTG pathology. Together, these data have established *METTL23* as another gene associated with NTG.

METTL23 encodes a protein that regulates transcription via its interactions with GA-binding protein transcription factor α subunit (*GABPA*).[20] Previous reports have demonstrated that *METTL23* variants may be associated with cognitive impairment.[21,22] The mechanism by which *METTL23* variants may cause NTG are unknown. However, loss of *METTL23* function was shown to promote aberrant histone methylation (H3R17) and dysregulation of NF-kB signaling, which may contribute to retinal ganglion cell death and NTG pathogenesis.[19,23]

The prevalence of *METTL23* pathogenic variants among patients with NTG is unknown, and only 1 NTG pedigree has been reported with a *METTL23* variant. We sought to confirm the association between *METTL23* variants and NTG as well as estimate the prevalence of potential disease-causing variants in a cohort of patients with NTG.

Key Points

Question What is the prevalence of *METTL23* pathogenic variants in patients with normal-tension glaucoma (NTG) within 1 tertiary care center?

Findings In this case-control study, 3 of 331 patients (0.91%) with NTG had *METTL23* pathogenic variants judged likely to be pathogenic based on analysis with mutation algorithms, protein modeling, and sequence conservation, while no such pathogenic variants were detected in in matched control individuals without glaucoma (n = 362).

Meaning *METTL23* pathogenic variants at this tertiary care center were associated with NTG and had a prevalence among patients with NTG (approximately 1%) similar to pathogenic variants in other known glaucoma-causing genes, including optineurin (*OPTN*), TANK-binding kinase 1 (*TBK1*), and myocilin (*MYOC*).

Methods

Study Population

Approval for this study was provided by the University of Iowa institutional review board, and all patients or their parents provided written informed consent for participation. Participants received no stipends or other incentives to participate. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline was followed.

Patient Cohort

A total of 331 patients with NTG seen at the University of Iowa were examined by board certified ophthalmologists at the University of Iowa with glaucoma fellowship training and were judged to have NTG based on standard criteria as previously described.[14,15,24,25] Optic nerve criteria for glaucoma are cup-disc ratios greater than 0.7, thinning of the neural rim, asymmetry of the optic nerve cup-to-disc ratio of 0.2 or greater, or progressive increase in cupping. Visual field evidence of glaucoma was based on the Collaborative Normal-Tension Glaucoma Treatment Trial criteria.[26] Patients tested with manual kinetic perimetry were required to exhibit arcuate defects or nasal steps. Maximum recorded IOPs of 21 mm Hg or less were required for a diagnosis of NTG. Patients with a history of trauma, steroid use, or stigmata of pigment dispersion syndrome or exfoliation syndrome were excluded. Patients with pathogenic variants in *MYOC*, *TBK1*, or *OPTN* were also excluded.

Glaucoma-Free Control Cohort

A total of 362 control individuals without glaucoma seen at the University of Iowa were examined by board certified ophthalmologists and were judged not to have glaucoma or ocular hypertension. Patients with a history of ocular hypertension (IOP >21 mm Hg) or stigmata of pigment dispersion syndrome or exfoliation syndrome were excluded.

Molecular Genetic Testing

Whole-Exome Analysis

Whole-exome data were collected using standard methods. Eighty patients with NTG had whole-exome sequencing done

Table 1. Demographic Features of the Normal-Tension Glaucoma (NTG) and Glaucoma-Free Control Cohorts[a]

Characteristic	NTG (n = 331)	Control (n = 362)	*P* value
Age, mean (SD), y	68.0 (11.7)	64.5 (12.6)	<.001
Sex, No. (%)			
Female	228 (68.9)	207 (57.2)	.002
Male	103 (31.1)	155 (42.8)	

[a] Ages were compared between patients with NTG and control individuals without glaucoma using an unpaired Wilcoxon test and sex was compared with χ^2 analyses. All patients with NTG and control individuals self-reported non-Hispanic European ancestry. Ancestry data were collected because it is need to ensure proper matching of control cohorts and because of known differences in the genetics of glaucoma between populations with disparate ancestry.

in collaboration with the Yale Center for Mendelian Diseases Genomics as previously described,[27] while an additional 55 patients with NTG had whole-exome sequencing done at the University of Iowa as previously reported.[28] Whole-exome data including the coding sequence of the *METTL23* gene was obtained from our cohort of control individuals without glaucoma as part of a previously reported study of pigmentary glaucoma.[29] Variants detected with whole-exome analysis were confirmed with Sanger sequencing. RefSeq transcript NM_001378349.1 was used to interpret the coding consequence of the variants.

Sanger Sequencing Analysis

An additional 196 patients with NTG from Iowa were tested for pathogenic variants in the coding sequences of *METTL23* with Sanger sequencing as previously reported.[30,31] DNA spanning each of the transcribed exons of *METTL23* was polymerase chain reaction–amplified using standard reactions and analyzed with Sanger sequencing on a 3730 automated capillary sequencer (Applied BioSystems).

Variant Analysis

Variants present at 1% or greater in the European cohorts in the Genome Aggregation Database (gnomAD) were excluded from analysis. Identified sequence changes were evaluated for potential pathogenicity using mutation analysis algorithms Polymorphism Phenotyping version 2 (PolyPhen-2),[32] sorting intolerant from tolerant (SIFT),[33] MutationTaster,[34] combined annotation dependent depletion (CADD),[35] Blocks Substitution Matrix 62 (BLOSUM62),[36] and AlphaMissense.[37] We also examined conservation of the *METTL23* amino acids that are altered by detected variants using the University of California, Santa Cruz, genome browser. Finally, we investigated the effects of detected variations on *METTL23* protein structure by developing a model of wild-type *METTL23* protein structure using AlphaFold[38] followed by our physics-based protocol as described previously.[39] Then we extended our analysis by adding detected sequence variations to the refined structure, followed by repacking of nearby residues using a rotamer optimization algorithm[40,41] and a potential energy function defined by the atomic multipole optimized energetics for biomolecular applications (AMOEBA) force field[42] in the program Force Field X[43] as previously described.[44]

Fibroblast Cell Culture

We obtained 4-mm skin biopsies from non–sun-exposed skin on the forearm. We then isolated skin fibroblast cells from the biopsy using methods previously described.[14]

Real-Time Polymerase Chain Reaction

Complementary DNA was produced from fibroblast RNA using standard techniques.[14] Primers were designed to amplify full-length *METTL23* complementary DNA–spanning introns flanking the exon containing detected pathogenic variants. Polymerase chain reaction products were assessed for size with agarose gel electrophoresis and visualized with ethidium bromide staining.

Statistical Analysis

Age at enrollment was compared between patients with NTG and control individuals without glaucoma using a *t* test. Sex was compared between groups using a χ^2 test. Variant frequencies in the NTG and glaucoma-free control cohorts were compared with age and sex as covariates using Firth logistic regression for rare variants and optimized sequence kernel association test (SKAT-O) as a variant burden analysis.[45]

Results

A cohort of 331 patients with NTG (mean [SD] age, 68.0 [11.7] years; 228 [68.9%] female and 103 [31.1%] male) and 362 control individuals without glaucoma (mean [SD] age, 64.5 [12.6] years; 207 [57.2%] female and 155 [42.8%] male) were assembled to study the role of *METTL23* variants in NTG (**Table 1**). The mean age at enrollment of patients with NTG was 3.5 years older than the mean age at enrollment of control individuals ($P < .001$). The proportion of female participants in the NTG group was higher than in the control group ($P = .002$).

Genetic Testing

We tested patients with NTG and control individuals without glaucoma from Iowa for *METTL23* gene variants. First, we searched whole-exome sequences available from our cohort of 135 patients with NTG (cohort 1) and 362 control individuals without glaucoma from Iowa for potential glaucoma-causing variants in the *METTL23* gene. All of the NTG exomes were obtained with at least 30× coverage across the coding se-

Table 2. Allele Frequencies *METTL23* Pathogenic Variants in a Cohort of Patients With Normal-Tension Glaucoma (NTG) and Control Individuals Without Glaucoma From Iowa[a]

METTL23 variant	No. (%)					Variant analysis algorithms						
	NTG cohort				gnomAD database							
	Cohort 1: n = 135 (270 alleles)	Cohort 2: n = 196 (392 alleles)	Cohorts 1 and 2: n = 331 (662 alleles)	Control cohort: n = 362 (724 alleles)	Non-Finnish European individuals: >1 100 000 alleles	Finnish European individuals: >63 000 alleles	PolyPhen-2	SIFT	BLOSUM62	MutationTaster	CADD	AlphaMissense	Protein modeling
p.Ala7Val c.20C>T rs201999820	0	1 (0.26)	1 (0.15)	0	0.041	0	0.93 (Probably damaging)	0 (Deleterious)	−3 (Pathogenic)	Disease-causing	27.3	0.9237 (Pathogenic)	Conservative changes
p.Pro22Arg c.65C>G rs368889510	0	1 (0.26)	1 (0.15)	0	0.0019	0	0.003 (Benign)	0.61 (Tolerated)	−2 (Pathogenic)	Polymorphism	9.82	0.0747 (Benign)	Altered structure
p.Arg63Trp c.187C>T rs370752836	1 (0.37)	0	1 (0.15)	0	0.0019	0	0.024 (Benign)	0.02 (Deleterious)	−3 (Pathogenic)	Disease-causing	22.8	0.0878 (Benign)	Altered structure
p.Asp166Asn c.496G>A rs138247613	0	2 (0.51)	2 (0.30)	9 (1.24)	0.90	0.091	0 (Benign)	0.29 (Tolerated)	1 (Benign)	Disease-causing	21.0	0.0695 (Benign)	Conservative changes

Abbreviations: BLOSUM62, Blocks Substitution Matrix 62; CADD, combined annotation dependent depletion; gnomAD, Genome Aggregation Database; PolyPhen-2, Polymorphism Phenotyping version 2; SIFT, sorting intolerant from tolerant.

[a] The allele frequency of each pathogenic variant was also determined in the gnomAD database of human exomes and genomes, and the pathogenicity of the variants was assessed with 6 different algorithms.

quence of *METTL23*, and 99.4% (360/362) of the glaucoma-free control samples were sequenced to 30× depth across the coding sequence of *METTL23*. We detected a rare, heterozygous variant—c.187C>T, p.Arg63Trp (rs370752836)—in 1 of 135 patients with NTG (0.74%) (**Table 2**). This variant was not identified in the control individuals without glaucoma from Iowa and was rarely detected in the gnomAD database, the publicly available genetic database of whole-exome and whole-genome sequences from more than 800 000 people of different ancestries,[46] with an overall allele frequency of 0.0019% among non-Finnish European individuals (Table 2). Three additional variants were detected in the control individuals without glaucoma from Iowa that were absent from the NTG cohort 1 (eTable in Supplement 1): p.Ala31Ala (rs377529542), p.Asp166Asn (rs138247613), and p.Leu190Pro (rs147321492). Two of these variants, p.Ala31Ala and p.Leu190Pro, were detected at a frequency of more than 1% of populations in the gnomAD database or were synonymous coding sequence variants and met exclusion criteria for analysis.

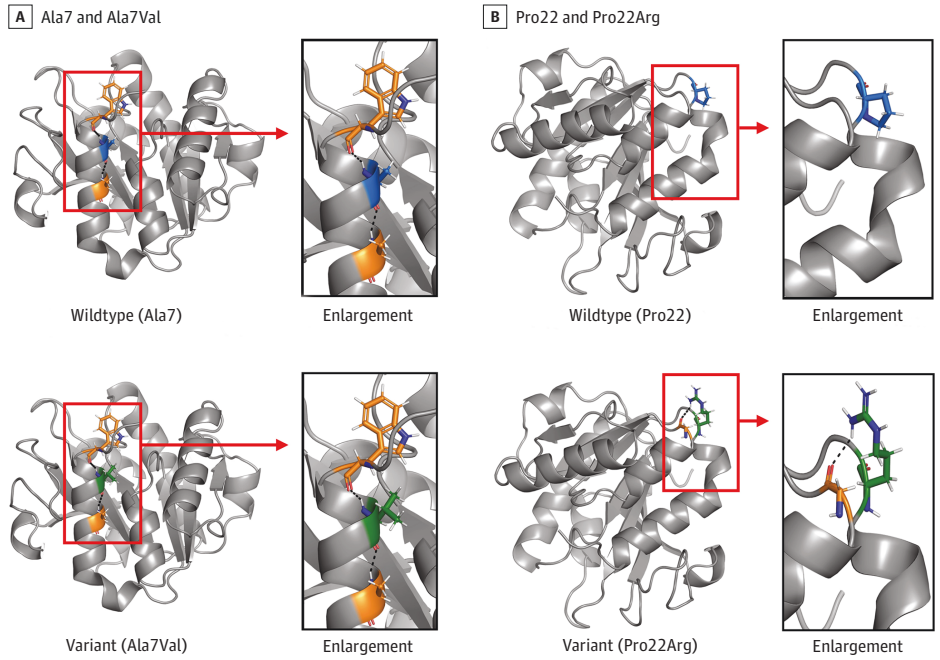
We tested an additional 196 patients with NTG from Iowa (cohort 2) for *METTL23* variants with automated Sanger sequencing. A total of 4 instances of 3 unique *METTL23* heterozygous missense variants were detected, including 1 instance of c.20C>T, p.Ala7Val (rs201999820); 1 instance of c.65C>G, p.Pro22Arg (rs368889510); and 3), and 2 instances of c.496G>A, p.Asp166Asn.

Variant Analyses

The *METTL23* gene encodes a histone arginine methyl transferase that demethylates histone proteins and may activate transcription via its effects on chromatin.[20,22] The methyltransferase domain of *METTL23* is located within amino acids 26 through 120,[47] and it encompasses 1 of the detected variants, p.Arg63Trp.

Several approaches were used to assess the pathogenicity of the detected *METTL23* variants. First, the frequencies of variants were compared between patients with NTG and control individuals without glaucoma. One heterozygous variant, p.Asp166Asn, was present in 2.5% of control individuals (9 of 363) and 0.6% of patients with NTG (2 of 331). The other 3 *METTL23* variants were absent from the glaucoma-free control cohort and were observed at extremely low frequency in a large public database, gnomAD (Table 2). When cohort 1 and 2 were combined—these 3 rare missense variants, p.Ala7Val, p.Pro22Arg, and p.Arg63Trp—were detected in a total of 3 (0.91%) of 331 patients with NTG and in none of the 362 control individuals. However, when analyzed as a group, the frequencies of all 4 detected *METTL23* variants in patients with NTG were not significantly higher than in control individuals using either logistic regression or the mutation burden test SKAT-O (1.5% vs 2.5%; $P = .27$). Second, we analyzed each of the 4 *METTL23* variants with 6 different algorithms (PolyPhen2, SIFT, Blosum62, MutationTaster, CADD, and AlphaMissense) to estimate their likely pathogenicity (Table 2). All 6 variant analysis algorithms suggested that p.Ala7Val is likely pathogenic, and 4 of 6 algorithms suggested p.Arg63Trp is pathogenic. Conversely, most algorithms suggested the other 2 variants (p.Pro22Arg and p.Asp166Asn) are likely nonpatho-

Figure 1. Protein Structure of *METTL23* Part 1



A, In the top subpanel, the wildtype *METTL23* protein contains an alanine (blue) at position 7 that has 2 hydrogen bonds (black dashed lines) to neighboring amino acids (orange) in an α helix. Enlargement of the boxed region shows the alanine and its hydrogen bonds. In the bottom subpanel, the Ala7Val variant introduces a valine residue (green). Enlargement of the boxed region shows that the variant valine (green) does not change the hydrogen bonding pattern relative to the native *METTL23* structure and likely does not alter the stability of the protein structure. B, In the top subpanel, the wildtype *METTL23* protein contains a proline (blue) at position 22 that is surface exposed and has no hydrogen bonds. Enlargement of the boxed region shows the surface exposed proline amino acid. In the bottom subpanel, the Pro22Arg variant introduces an arginine residue (green). Enlargement of the boxed region shows that the variant arginine (green) adds a hydrogen bond to a neighboring amino acid (orange), altering the stability of the protein at that site.

genic. Third, we analyzed the conservation of the amino acids affected by the 4 variants across 10 different species of vertebrate animals (eFigure 1 in Supplement 1). The amino acids altered by the p.Ala7Val and p.Pro22Arg variants were highly conserved among vertebrate species, suggesting they may be vital for *METTL23* function. The amino acids altered by p.Arg63Trp and p.Asp166Asn were also conserved among vertebrate species, but to a lesser degree. Notably, the amino acid at position 63 in the protein encoded by rat *METTL23* is a tryptophan that matches the variant allele in the human gene.

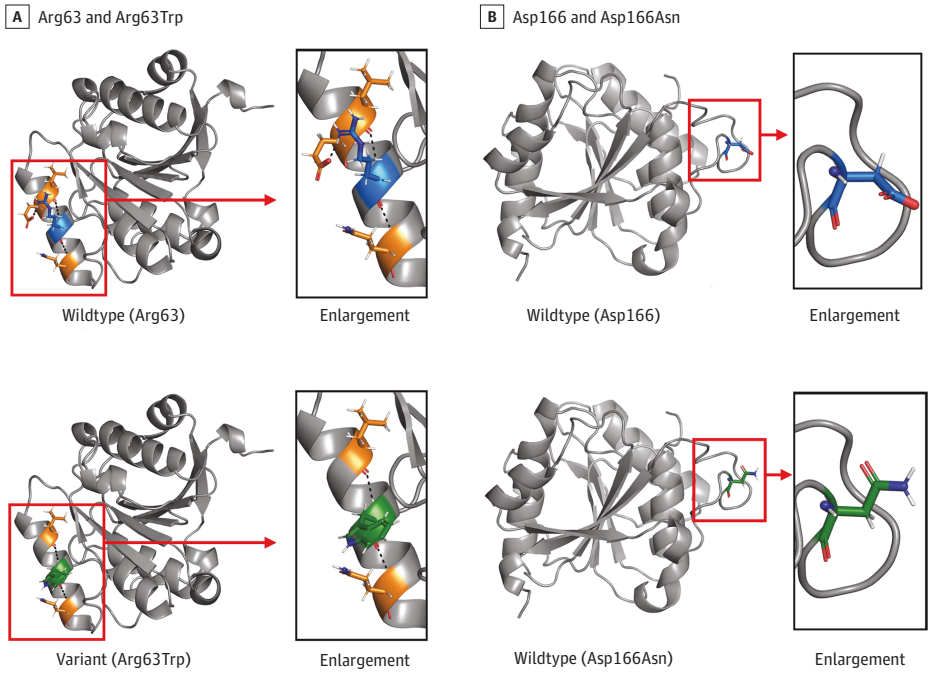
We also investigated the potential pathogenicity of variants by modeling their effects on the molecular structure of *METTL23* protein. The p.Ala7Val variant does not alter any local interactions in the *METTL23* structure (**Figure 1**A). Conversely, the p.Pro22Arg variant adds a hydrogen bond in place of the proline backbone bond, which might cause a change in protein stability (Figure 1B). The p.Arg63Trp variant alters hydrogen bonding with neighboring amino acids and introduces a tryptophan at the surface of *METTL23*, which increases its hydrophobicity[48] and may be associated with an increased propensity for protein aggregation[49-51] (**Figure 2**A). The p.Asp166Asn variant does not appear to alter protein folding and structure (Figure 2B). The molecular modeling of *METTL23* provides some additional support for the pathogenicity of 2 variants, namely, p.Pro22Arg and p.Arg63Trp.

Family members were available for study from the patient with a p.Ala7Val *METTL23* variant, who had a sibling with NTG who also had the p.Ala7Val variant. Overall, a range of different variant analyses provide support for the pathogenicity of the p.Ala7Val and p.Arg63Trp variants and mixed results for the p.Pro22Arg variant. The control population frequency and variant analyses suggest that the p.Asp166Asn variant is likely benign. Using American College of Medical Genetics and Genomics and the Association for Molecular Pathology criteria,[52] 3 of the *METTL23* variants may be classified as a variant of unknown significance, p.Ala7Val (PM2, PP1, and PP3), p.Pro22Arg (PM2 and BP4), p.Arg63Trp (PM2 and PP3), while 1 variant is likely benign, namely, p.Asp166Asn (BS1 and BP4).

Splicing Analyses

A previously reported *METTL23* variant, p.Glu28Gly, is known to alter splicing and cause NTG. Consequently, we sought to determine if the variants we detected might also lead to alternative splicing. Fibroblast cells had been previously collected via a skin biopsy from each patient with NTG with a *METTL23* pathogenic variant as well as from age- and ethnicity-matched control individuals without glaucoma. RNA was isolated from the fibroblast cells and real-time polymerase chain reaction experiments detected no *METTL23* splicing variants (data not shown).

Figure 2. Protein Structure of *METTL23* Part 2



A, In the top subpanel, the wildtype *METTL23* protein contains an arginine (blue) at position 63, which hydrogen bonds (black dashed lines) to neighboring amino acids (orange). Enlargement of the boxed region shows 3 hydrogen bonds between the native arginine and neighboring leucine, glutamic acid, and glutamine residues. In the bottom subpanel, the Arg63Trp variant introduces a tryptophan residue (green). Enlargement of the boxed region shows that the variant tryptophan (green) disrupts the original arginine-glutamic acid hydrogen bond. It also increases hydrophobicity at the protein surface, which may promote hydrophobic aggregation. B, In the top subpanel, the wildtype *METTL23* protein contains an aspartic acid (blue) at position 166 that is solvent exposed. Enlargement of the boxed region shows the surface exposed aspartic acid has no hydrogen bonds to neighboring amino acids in the native structure. In the bottom subpanel, the Asp166Asn variant introduces an asparagine residue (green). Enlargement of the boxed region shows that the variant asparagine (green) remains solvent exposed and introduces no hydrogen bonds or significant changes to the protein structure.

Clinical Features of Patients With NTG and *METTL23* Variants

The clinical features of the NTG associated with these *METTL23* pathogenic variants are described in the eAppendix in Supplement 1. All 4 patients had asymmetric glaucoma, with the right side greater than the left.

Discussion

This case-control study was conducted because, even though glaucoma is known to have a major genetic component,[53,54] the specific genetic factors involved in most cases of NTG are unknown. Several risk factor genes that contribute to cases of polygenic forms of NTG have been discovered, including *CDKN2B-AS1*,[55-57] *TLR4*,[58] *ELOVL5*, *SRBD1*,[59] and a chromosome 9q21 locus.[56,60,61] Single pathogenic variants in any of 4 genes may independently cause NTG. Missense variants in *OPTN* (p.Gln50Lys)[13] and *MYOC* (p.Gln368Ter)[15] and *TBK1* gene duplication or gene triplications[24] have each been shown to cause approximately 1% of NTG cases. More recently, Pan and colleagues[19] showed that a missense variant in *METTL23* (p.Glu28Gly) caused NTG in a 3-generation glaucoma pedigree with 9 affected family members. This cohort study from a tertiary care center in Iowa provides confirmation that *METTL23* pathogenic variants are associated with NTG. This report of 3 disease-associated *METTL23* pathogenic variants—p.Ala7Val, p.Pro22Arg, and p.Arg63Trp—strengthens the association between this gene and glaucoma pathogenesis. These additional variants were present in patients with NTG, absent from control individuals without glaucoma, and only rarely detected in large public exome databases. Multiple mutation analyses also suggested their pathogenicity. These analyses suggest that as many as 1 in 100 cases of NTG may be caused by *METTL23* pathogenic variants. The prevalence of *METTL23* pathogenic variants in patients with NTG from Iowa was similar to that of *OPTN, MYOC*, and *TBK1* pathogenic variants, suggesting that variants in the *METTL23* gene may be 1 of the 4 most common causes of NTG. More studies with larger and more diverse patient populations are needed to determine if these findings in Iowa cohorts are generalizable.

All 4 patients with plausible NTG-causing variants had asymmetric disease that was worse in the right eye than the left eye (eFigure 2 in Supplement 1). Asymmetry is a common feature in exfoliation glaucoma,[62,63] and prior studies have also suggested the presence of some asymmetry in POAG,[64] perhaps related in part to asymmetric intraocular pressure.[65-67] Some reports suggested POAG may have some asymmetry that is worse in the left eye, while others did not.[68] The potential causes of asymmetry in POAG are unknown, although some have suggested that differences in vascular anatomy might

have a role in promoting asymmetric disease, ie, worse in the left eye.[69] It is possible that the asymmetry of disease observed in our report (worse in the right eye) is a random occurrence related to the small number of patients evaluated (n = 4). In the first report of *METTL23* related glaucoma, Pan et al[19] made no note of asymmetric disease, and the severe visual field defects presented from 1 patient with NTG with a *METTL23* pathogenic variant were symmetric. Larger studies of patients with *METTL23*-related NTG are needed to determine if worse disease in the right eye is a feature of this type of glaucoma.

Several homozygous, truncating *METTL23* variants have previously been associated with autosomal recessive inheritance of developmental delay and intellectual disability.[20,22] Pan et al[19] identified a heterozygous *METTL23* missense variant, p.Glu28Gly, that is linked with autosomal dominant inheritance of NTG in a large Japanese pedigree. We confirm this finding by identifying 3 novel heterozygous *METTL23* missense pathogenic variants in patients with NTG from Iowa. While truncating *METTL23* variants are associated with central nervous system abnormalities, some missense variants are associated with NTG. The p.Glu28Gly pathogenic variant was shown to cause *METTL23* splicing abnormalities, which resulted in loss of *METTL23* protein production and dysregulation of its transcriptional function.[19] The mechanism by which the novel *METTL23* pathogenic variants in the current report may cause NTG is unclear. However, our preliminary studies suggest that *METTL23* function is not dysregulated through splicing abnormalities in the patients in this cohort. Further studies of these novel pathogenic variants with animal models and cell culture models of disease are warranted to confirm their pathogenicity, to investigate the mechanisms by which they may cause disease, and to explore new potential variant-specific therapies.

Limitations

This study has limitations. First, this analysis involved individuals of non-Hispanic, European ancestry and the results may not be generalizable to populations of patients of other races and ethnicities. Similarly, this analysis was made with patients from a tertiary care center and may not represent what would be observed in other patient populations. Second, the relatively small size of the NTG cohort may have biased the detected frequency of *METTL23* pathogenic variants. Moreover, the small cohort size limited the power of this study to detect a statistically significant enrichment of pathogenic variants among patients with NTG. Third, the cohort of patients with NTG was slightly older and had a greater proportion of female participants than the cohort of control individuals. These differences might be a source of bias in the variant analyses. Fourth, the analyses in this report support the pathogenicity of several *METTL23* variants, but functional studies or transgenic animal studies are needed to provide the strongest evidence for their role in glaucoma.

Conclusions

This case-control study provides more evidence that *METTL23* variants are associated with NTG. Moreover, the study estimated a prevalence of pathogenic *METTL23* variants to be approximately 1% in patients with NTG from a tertiary care center in Iowa. Replication and functional studies to confirm and extend these results are warranted.

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Concept and design: Roos, Fingert.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Tollefson, Fingert.
Critical review of the manuscript for important intellectual content: Scheetz, Roos, Boese, Pouw, Stone, Schnieders, Fingert.
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Data Sharing Statement: See Supplement 2.

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