Mitochondrial DNA Variant Discovery in Normal-Tension Glaucoma Patients by Next-Generation Sequencing

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Purpose. Normal-tension glaucoma (NTG) is a disease of late onset, complex trait with multiple risk factors. In this study, we discovered a mitochondrial DNA variant in NTG patients using next-generation sequencing (NGS).

Methods. The DNA was extracted from the peripheral blood of NTG patients and normal control subjects. Sequencing of the entire mitochondrial DNA (mtDNA) using NGS revealed new genetic risk variants for NTG patients (discovery sample, $n = 20$). For the candidate genetic variants, we performed a disease–gene association study in the independent case–control populations (validation sample; NTG, $n = 196$ and normal control, $n = 202$) using Sanger sequencing.

Results. This study identified 148 different novel mtDNA-sequence changes. Of these, 21 sequence variants identified at a frequency greater than 15% were located in the *ND2-ND6, RNR1, RNR2, COX1, COX3, ATP6, ATP8,* and *CYTB* genes. Of the 21 candidate genetic variants, the frequencies of m.4883C>T (*ND2* gene), m.9540T>C (*COX3* gene), and m.14766C>T (*CYTB* gene) were significantly different between NTG patients and controls (28.4% vs. 15.3%, $P = 0.002$; 56.5% vs. 44.4%, $P = 0.020$; and 3.1% vs. 0.0%, $P = 0.030$, respectively). The association with m.4883C>T in the *ND2* gene resisted the Bonferroni correction for multiple tests. The NTG patients of T genotype in the m.4883C>T variant have more advanced visual field loss than those who carry the C genotype ($P = 0.009$).

Conclusions. This study reveals a spectrum of mtDNA variants in patients with NTG. Our results identified a synonymous change, m.4883C>T variant, which was more prevalent in the NTG cohorts than in the controls. This finding suggests that the identified variant may be a genetic risk factor for the development of NTG.

Keywords: normal-tension glaucoma, mitochondria, mitochondrial DNA, genetic variation

Glaucoma is a leading cause of blindness worldwide and is the second most frequent cause of nonaccidental blindness in industrialized countries.[1–3] The global prevalence of glaucoma was estimated at 67 million people in 2001.[4] Normal-tension glaucoma (NTG) is a condition involving progressive glaucomatous optic neuropathy and corresponding visual field defects with IOP in the statistically normal range. Because of the normal IOP, other factors, such as genetic predisposition, are believed to have an important role in the pathogenesis of NTG. Since up to 21% of NTG patients were reported to have a family history, it has been suggested that these patients may be genetically predisposed to developing NTG.[5] Over the last two decades, several genes have been identified that may be responsible for predisposition to NTG, but no causative gene has yet been confirmed in NTG subjects to our knowledge.

Glaucoma is recognized as a neurodegenerative disorder characterized by the accelerated loss of retinal ganglion cells and their axons. Previous studies point to the possibility that mitochondrial dysfunction in certain individuals may predispose them to glaucoma.[6–8]

The advent of next-generation sequencing (NGS) has reduced sequencing costs and has significantly increased throughput, enabling population-level surveys of human genomic variation. After the advent of NGS and the complete sequencing of a large number of individuals from different populations, the site frequency spectrum has been enriched rapidly with rare and low frequency variants.[9] Considering that rare variants may exhibit geographic or ancestry-based specificity,[10,11] population-based genetic information may provide new insights into the pathogenesis of glaucoma.

In this study, we discovered a mitochondrial DNA variant in NTG patients by using NGS technology. This study identified a synonymous change, m.4883C>T variant, which was more prevalent in the NTG cohorts than in the controls. Results of this study may improve our understanding of the pathophysiology of NTG and allow us to construct a new disease prediction model of NTG.

Methods

This study was approved by the Institutional Review Board of the Seoul National University Hospital Clinical Research Institute. Informed consent was obtained from all participants

at enrollment. All procedures were designed and conducted in accordance with the tenets of the Declaration of Helsinki.

Subjects

We included 20 NTG patients (discovery sample) in this study. Cases diagnosed as pseudoexfoliation glaucoma and those with a known myocilin mutation were excluded. The entire mitochondrial DNA (mtDNA) was sequenced using NGS for the 20 NTG patients. From these results, we revealed new genetic risk variants for NTG patients. For the candidate genetic variants, we performed a disease–gene association study, using Sanger sequencing, in the independent case–control populations (validation sample). The validation sample consisted of 196 NTG patients and 202 normal control subjects.

The diagnostic criteria for NTG were normal IOP, glaucomatous optic disc cupping, glaucomatous visual field defects, open anterior chamber angle, and the absence of any contributing ocular or systemic disorders. The IOP was measured using a Goldmann applanation tonometer. Normal IOP was defined as a diurnal IOP persistently below 21 mm Hg without medication. Visual fields of NTG patients were evaluated using the 30-2 program of the Humphrey Visual Field Analyzer Model 750 (Zeiss, Inc., San Leandro, CA) or Model 630 (Allergan, Inc., San Leandro, CA). Reliable fields were those with a fixation loss rate of $\leq$20%, and false-positive and false-negative error rates of $\leq$20%. A glaucomatous visual field defect was defined as a Glaucoma Hemifield Test outside normal limits on at least two fields, or a cluster of three or more nonedge points in a location typical for glaucoma, all of which are depressed on the pattern deviation plot at a $P < 5\%$ level and one of which is depressed at a $P < 1\%$ level on two consecutive fields, or a pattern standard deviation that occurs in less than 5% of normal fields. Exclusion criteria included historical or neuroimaging evidence of another possible optic neuropathic condition affecting either eye, significant visual loss in both eyes not associated with glaucoma, or refusal to participate.

Normal control subjects who had completed medical and ophthalmologic examinations at the Health Promotion Clinic served as controls. These subjects had best corrected visual acuities of 20/25 and IOPs of 21 mm Hg or less. Subjects with any suspicious findings of glaucoma in the disc and fundus (e.g., cup-to-disc ratio of more than 0.6, notch in the neural rim, vertically oval cup, zone beta peripapillary atrophy, retinal nerve fiber layer defect, choroidal sclerosis, or abnormalities of the disc vessels) were not included.

Sequence Analysis of the Mitochondrial DNA: Discovery Sample

DNA Extraction and Long-Range PCR. Genomic DNA was extracted from peripheral blood using the QIAamp DNA extraction kit (QIAGEN, Valencia, CA). The entire mitochondrial genome was amplified in two overlapping fragments with sizes of 9.3 and 7.6 kilobases (kb). The primer sets for long-range PCR were as follows: F-5′-AACCAAACCCCAAAGACACC-3′ and R-5′-GCCAATAATGACGTGAAGTCC-3′ for the 9.3 kb-sized fragment I, and F-5′-TCCCACTCCTAAACACATCC-3′ and R-5′-TTTATGGGGTGATGTGAGCC-3′ for the 7.6 kb-sized fragment II. Long-range PCR was performed using LA Taq polymerase (TAKARA, Shiga, Japan).

Library Preparation, Emulsion PCR, and Sequencing Reaction. Sequencing reactions were carried out using Roche GS sequencing technology. The library was prepared following the manufacturer's protocol. Briefly, equimolar amounts of PCR products were pooled for each patient and were sheared to yield smaller fragments of 300 to 500 base pairs (bp). Then, barcoded adaptors were ligated to each fragment with DNA ligase. Adaptor-modified fragments were amplified clonally by emulsion PCR. The clonal amplicons were sequenced on the GS Jr platform.

Bioinformatics Analysis. NextGENe software (Softgenetics, State College, PA) was used to analyze the reads and align against the revised Cambridge reference sequence of human mtDNA.[12] Variant calls were filtered using the criteria of quality score of $\geq$20 and minimum coverage $\geq \times 10$.

Disease–Gene Association Study: Validation Sample

From the results of the discovery sample, we selected new genetic risk variants for NTG patients. The mtDNA sequence variants, which were identified at a frequency of greater than 15% in the discovery sample, were considered as possible candidate genetic variants for NTG. We performed an allele association study on a new case–control population that is highly discordant for clinical phenotypes. A subset of 196 cases and 202 controls meeting the eligibility criteria were selected as a validation sample for genotyping. All candidate variants were confirmed by Sanger sequencing using original DNA from an independent biological sample and not from the whole genome amplified sample used in the discovery stage. Predicting effect of nonsynonymous variants on protein function was done using Polyphen-2 software.[13] The haplogroup determination was based on the phylogenetic network for European mtDNA, as described by Finnila et al.[14] and the literature by Accetturo et al.[15]

Statistical Analysis

The frequencies of the genotypes and alleles of the polymorphisms were compared between NTG patients and controls. The significance of association was determined by contingency table analysis using Fisher's exact test. The results were considered statistically significant when the P value was <0.05. For the Fisher's exact test, double-sided P values were calculated using the sum of small P values.[14] For genotype-phenotype analysis, we used independent t-tests to compare group means of those carrying the sequence variant being tested against the group means of those carrying the normal, reference sequence. The statistical analyses were performed using SPSS for Windows version 19.0 (SPSS, Inc., Chicago, IL).

Results

Subject Characteristics

Clinical features of discovery and validation samples are depicted in Table 1. In this study, all subjects were Korean. For the validation sample, no significant differences were found between the two groups in terms of age, sex ratio, and IOP. The frequency of family history of glaucoma was significantly higher in the NTG patients (14.3%) than in the controls (0%, $P < 0.001$). In the case–control sample, no subject was identified with inherited mitochondrial disorders.

Sequence Analysis of the Mitochondrial DNA

For the discovery sample, the entire mtDNA was sequenced using NGS. This study identified 148 different novel mtDNA-sequence changes (see Supplementary Table S1 for a list of mtDNA-sequence changes). Of these, 21 sequence variants identified at a frequency greater than 15% were located in the

Table 1. Characteristics of Study Subjects

	Discovery Sample	Validation Sample		
	NTG Patients, $n = 20$	NTG Patients, $n = 196$	Normal Controls, $n = 202$	*P* Value
Age, y	58.5 ± 9.3	57.0 ± 13.4	59.0 ± 15.3	0.184*
Sex ratio, male-to-female	5:15	89:107	98:104	0.603†
Baseline IOP, mm Hg	15.3 ± 2.4	15.3 ± 2.7	15.0 ± 2.8	0.286*
Family history of glaucoma	5 (25.0%)	28 (14.3%)	0 (0.0%)	<0.001†
Social history				
Smoking	3 (15.0%)	19 (9.7%)	22 (10.9%)	0.820†
Consumption of alcohol	6 (30.0%)	71 (36.2%)	78 (38.6%)	0.697†
Medical history				
Diabetes	2 (10.0%)	24 (12.2%)	21 (10.4%)	0.672†
Hypertension	1 (5.0%)	64 (32.7%)	55 (27.2%)	0.284†
Humphrey C30-2 visual field				
MD, dB	−6.88 ± 4.78	−7.99 ± 8.75		
PSD, dB	8.65 ± 5.24	6.52 ± 4.53		

MD, mean deviation; PSD, pattern standard deviation.
* *P* values by independent *t*-test.
† *P* value by χ^2 test.

ND2-ND6, RNR1, RNR2, COX1, COX3, ATP6, ATP8, and *CYTB* genes.

From the 148 mtDNA-sequence changes, 20 nonsynonymous mtDNA changes were detected in 20 patients with NTG, and these were not considered to be Leber's hereditary optic neuropathy (LHON) mutations (Table 2). These 20 mtDNA changes spanned the mitochondrial coding region; 10 (50.0%) in complex I, 3 (15.0%) in complex III, 2 (10.0%) in complex IV, and 5 (25.0%) in ATP synthase. Six of these changes were predicted to be damaging to the corresponding protein structure and/or function.

Seven nonsynonymous mtDNA changes were present in a heteroplasmic status. The heteroplasmy levels were 13% for m.3745G>A, 6% for m.6102T>C, 8% for m.6114A>G, 11% for m.8894A>T, 5% for m.10854T>C, 6% for m.12005T>C, and 11% for m.12436C>A.

Disease-Gene Association Study

Of the 148 mtDNA abnormalities, the 21 sequence variants identified at a frequency greater than 15% were selected for the disease-gene association study. Of the 21 candidate genetic variants, the frequencies of m.4883C>T (*ND2* gene), m.9540T>C (*COX3* gene), and m.14766C>T (*CYTB* gene) were significantly different between NTG patients and controls (28.4% vs. 15.3%, $P = 0.002$; 56.5% vs. 44.4%, $P = 0.020$; 3.1% vs. 0.0%, $P = 0.030$, respectively). Additionally, the association with m.4883C>T in *ND2* gene resisted the Bonferroni

Table 2. Nonsynonymous mtDNA Nucleotide Changes

Nucleotide Substitution	Gene	Amino Acid Substitution	Location	Heteroplasmy Level, %	PolyPhen-2 Prediction	Base Substitution
m.3745G>A	*ND1*	p.Ala147Thr	Complex I	13	Benign	Transition
m.4129A>G	*ND1*	p.Thr275Ala	Complex I	NA	Benign	Transition
m.4824A>G	*ND2*	p.Thr119Ala	Complex I	NA	Possibly damaging	Transition
m.5178C>A	*ND2*	p.Leu237Met	Complex I	NA	Probably damaging	Transversion
m.5442T>C	*ND2*	p.Phe325Leu	Complex I	NA	Benign	Transition
m.6102T>C	*COX1*	p.Phe67Leu	Complex IV	6	Benign	Transition
m.6114A>G	*COX1*	p.Met71Val	Complex IV	8	Possibly damaging	Transition
m.8414C>T	*ATP8*	p.Leu17Phe	ATP synthase	NA	Probably damaging	Transition
m.8701A>G	*ATP6*	p.Thr59Ala	ATP synthase	NA	Benign	Transition
m.8794C>T	*ATP6*	p.His90Tyr	ATP synthase	NA	Benign	Transition
m.8860A>G	*ATP6*	p.Thr112Ala	ATP synthase	NA	Benign	Transition
m.8894A>T	*ATP6*	p.Asn123Ile	ATP synthase	11	Benign	Transversion
m.10398A>G	*ND3*	p.Thr114Ala	Complex I	NA	Benign	Transition
m.10854T>C	*ND4*	p.Leu32Pro	Complex I	5	Probably damaging	Transition
m.12005T>C	*ND4*	p.Trp416Arg	Complex I	6	Benign	Transition
m.12358A>G	*ND5*	p.Thr8Ala	Complex I	NA	Unknown	Transition
m.12436C>A	*ND5*	p.His34Asn	Complex I	11	Probably damaging	Transversion
m.14766C>T	*CYTB*	p.Thr7Ile	Complex III	NA	Benign	Transition
m.15071T>C	*CYTB*	p.Tyr109His	Complex III	NA	Benign	Transition
m.15326A>G	*CYTB*	p.Thr194Ala	Complex III	NA	Benign	Transition

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Table 3. Genotype Frequencies of mtDNA Sequence Changes in NTG Patients and Normal Control Subjects

mtDNA Sequence Changes	Haplogroup	Associated Disease	Location	Amino Acid Substitution	Genotype	Patients	Controls	*P* Value*
m.663A>G	A		*RNR1* gene		A	169	178	1.000
					G	20	20	
m.709G>A			*RNR1* gene		G	155	155	0.525
					A	35	42	
m.4883C>T	D		*ND2* gene		C	139	171	0.002
					T	55	31	
m.5178C>A	D	Longevity, blood iron metabolism	*ND2* gene	p.Leu237Met	C	138	140	0.825
					A	55	60	
m.5231G>A	L0a		*ND2* gene		G	186	193	0.797
					A	8	7	
m.5417G>A	N9		*ND2* gene		G	185	186	0.392
					A	9	14	
m.8414C>T	D4	Longevity	*ATP8* gene	p.Leu17Phe	C	144	145	0.641
					T	46	53	
m.8701A>G	L/M		*ATP6* gene	p.Thr59Ala	A	89	84	0.478
					G	105	115	
m.8794C>T	A	Exercise endurance, coronary atherosclerosis risk	*ATP6* gene	p.His90Tyr	C	174	179	1.000
					T	20	20	
m.9540T>C	L/M		*COX3* gene		T	83	110	0.020
					C	108	88	
m.10398A>G	M	PD protective factor, longevity, altered cell PH, metabolic syndrome, breast cancer risk	*ND3* gene	p.Thr114Ala	A	77	65	0.143
					G	117	135	
m.10400C>T	M		*ND3* gene		C	85	82	0.611
					T	109	118	
m.10873T>C	L/M		*ND4* gene		T	86	81	0.478
					C	110	121	
m.12358A>G	N9a		*ND5* gene	p.Thr8Ala	A	186	189	1.000
					G	9	10	
m.12372G>A	U	Altered brain pH	*ND5* gene		G	180	190	0.211
					A	15	9	
m.12705C>T	N/M/L		*ND5* gene		C	57	49	0.310
					T	139	151	
m.14668C>T	D4	Major depressive disorder	*ND6* gene		C	147	145	0.564
					T	47	54	
m.14766C>T	HV		*CYTB* gene	p.Thr7Ile	C	6	0	0.030
					T	189	193	
m.14783T>C	M		*CYTB* gene		T	86	79	0.539
					C	109	114	
m.15043G>A	M	Major depressive disorder	*CYTB* gene		G	86	81	0.476
					A	108	119	
m.15301G>A	L/M		*CYTB* gene		G	87	81	0.417
					A	108	119	

PD, Parkinson disease.
* *P* values by Fisher's exact test.

correction for multiple tests (Table 3). None of the other single nucleotide polymorphisms was associated with differences in baseline IOP, age at diagnosis, or mean deviation of the visual field.

Genotype-Phenotype Analysis

When we compared the clinical features of NTG patients with and without m.4883C>T mitochondrial DNA sequence changes, there was a significant difference in the mean deviation values of the visual field. The NTG patients of T genotype in the m.4883C>T variant had worse mean deviation values of the visual field compared to those who carry the C genotype ($P = 0.009$, Table 4).

Discussion

Glaucoma comprises a heterogeneous group of ocular disorders with a hereditable basis. The majority of these cases may be explained by a confluence of complex genotype and environmental risk factors.[16] The IOP is the only well recognized factor responsible for optic nerve damage in glaucoma. However, evidence from several studies suggests that mitochondrial dysfunction or altered mitochondrial

Table 4. Comparison of the Clinical Features of NTG Patients With and Without m.4883C>T mtDNA Sequence Changes

mtDNA Sequence Changes	Clinical Features	Genotype C, $n = 139$	Genotype T, $n = 55$	*P* Value
m.4883C>T	Age at diagnosis, y	50.5 ± 12.6	53.0 ± 15.7	0.316*
	Sex ratio, male-to-female	61:78	27:28	0.620†
	Baseline IOP, mm Hg	15.3 ± 2.6	15.1 ± 2.8	0.598*
	MD value of visual field, dB	−7.15 ± 5.48	−10.03 ± 8.26	0.009*

* *P* values by independent *t*-test.
† *P* value by χ^2 test.

signaling pathways are involved in glaucoma pathogenesis.[17,18] In this study, we performed a genetic analysis of the mitochondrial gene and revealed the genetic risk variants in NTG patients.

Previous studies suggest that mitochondria have a crucial role in the pathophysiology of optic neuropathies, such as Leber's optic neuropathy,[19,20] and neurodegenerative diseases, such as Parkinson's disease.[21] In this study, we found that m.4883C>T in *ND2* gene showed a significant association with NTG. The ND2 protein is one of the 46 subunits that constitute the OXPHOS complex I, which is a key enzyme in the respiratory chain. Our results are in line with previous reports that mutations in the ND subunits of complex I have an important role in oxidative phosphorylation diseases.[22] Complex I is the first site of the respiratory chain, produced by the assembly of 35 to 37 nuclear DNA and 7 mtDNA-encoded subunits. Since mtDNA is particularly vulnerable to oxidative stress, accumulation of mtDNA mutations leads to further impairment in oxidative phosphorylation and increases oxidative stress within the cell.[23–25]

In genotype–phenotype analysis, we found that NTG patients bearing a T genotype in the m.4883C>T variant exhibit worse mean deviation values of the visual field when compared to those of the C genotype. Identification of genetic variants that are associated with glaucoma or with glaucoma severity can contribute to better understanding of the disease mechanisms.[26] Since glaucoma is a late-onset disease, it can be difficult to know when the disease actually was developed. However, the determination of genotype–phenotype correlation is important because earlier onset of the disease could result in increased visual disability later in life.[27] Our results suggested that the m.4883C>T variant accounts for the more severe form of NTG, which may require intensive treatment.

In this study, 148 mitochondrial DNA sequence variants were observed in 20 patients with NTG. Of these, 20 nonsynonymous mtDNA changes were detected, which were not considered to be LHON mutations. Results of the present study corresponded with those of an earlier study that reported 27 different novel nonsynonymous mtDNA changes in primary open-angle glaucoma (POAG) patients, and most of these mtDNA sequence alterations in patients with POAG were transversions, meaning sequence changes that alter the purine/pyrimidine orientation and imply oxidative stress.[7] In this study, however, only 3 (15%) of the 20 nonsynonymous mtDNA changes were transversions. Furthermore, the nonsynonymous mtDNA changes from the earlier study were not observed in our results, suggesting that mitochondrial abnormalities may have a different role in the pathogenesis of NTG and POAG. However, the discovery sample set in this study is relatively small. This may limit the final recovery of functionally relevant variants associated with NTG.

Of the 148 mtDNA abnormalities, we selected 21 candidate variants according to their frequency from the discovery sample. Some variants were known to be associated with certain complex diseases, like Parkinson disease or major depressive disorders.[28–32] Given that glaucoma has some typical features that are similar to neurodegenerative diseases,[33] we speculated that mtDNA mutations could create a genetic susceptibility for the development of glaucoma. Moreover, the mtDNA mutations tend to accumulate with age, possibly affecting the mitochondrial function, and providing a link between aging and glaucoma.[34–36]

Several mtDNA haplogroups were found to be associated with longevity, carcinogenesis, and other metabolic and degenerative diseases. Haplogroup D is one of these mtDNA haplogroups, which is defined by the specific variations of m.5178C>A and m.4883C>T in *ND2* gene. The m.5178C>A variant is reported to be associated with longevity and also a variety of human disorders.[37,38] There are a few studies suggesting the association of some types of cancers (endometrial cancer, esophageal cancer, and lung cancer) with m.4883C>T.[39–41] However, this association is based on a background of haplogroup D, not with m.4883C>T. Therefore, to the best of our knowledge, our study is the first to demonstrate the association of this variant with a phenotype. However, the m.4883C>T variant, which was significantly associated with NTG in our study, is a synonymous one; therefore, it is questionable that this variant will have some direct effect on protein function or structure. Further investigation is necessary for the linking of the discovered variants to mitochondrial dysfunction and NTG phenotype.

The condition of NTG is highly prevalent in Korea and Japan; therefore, the distinction between the NTG and the control groups may be confounded with population ancestry and not necessarily with disease risk. This is because the mtDNA position 4883 is a characteristic mutation of haplogroup D, which is found in Japan. However, population-based comparisons confirmed that present-day Japanese have closest genetic affinity to Northern Asian populations, especially to Koreans.[42] Moreover, the incidence of haplogroup D in Korea is similar to that in Japan.[42,43] Therefore, examination of the ancestry of mtDNA was not required in our study.

Mammalian mtDNA is present at high copy numbers in all cells of the body, which gives rise to characteristic features of mitochondrial genetics: homoplasmy and heteroplasmy. Occasionally, a subpopulation of mtDNA molecules carries pathogenic mutations. When this heteroplasmic mtDNA is present during embryogenesis, it can lead to a variety of clinical symptoms predominantly affecting muscle and nerve, along with other tissues, albeit on a lower scale.[44] In the presence of heteroplasmy, there is a threshold level of mutation that is important for the clinical expression of the disease and for biochemical defects.[45] Many disease-causing mtDNA variants are heteroplasmic and their clinical manifestation depends on the relative proportion of mutant versus normal mitochondrial genomes.[45] In our study, the heteroplasmy level ranged from 5% to 13%. However, low heteroplasmy level does not exclude pathogenic potential for the variant concerned.

Mitochondrial dysfunction has been suggested in the pathophysiology of a number of neurodegenerative diseases.

The high concentration of mitochondria at the optic nerve head may reflect a high requirement of ATP as the primary site of glaucomatous axonal injury.[6] The extent of mtDNA damage accumulating in various tissues correlates with those tissues most prone to age-related dysfunction. Mitochondrial dysfunction may have a role in the pathogenesis of glaucoma through direct involvement in a number of cellular processes, including aging, oxidative damage, and excitotoxicity.[33] Considering that glaucoma has a disproportionate prevalence among specific ethnic groups,[46] it is necessary to evaluate data from a broader ethnic distribution to better understand the spectrum of mitochondrial abnormalities in glaucoma patients.

In conclusion, this study revealed a spectrum of mtDNA variants in patients with NTG. Our results identified a synonymous change, m.4883C>T variant, which was more prevalent in the NTG cohorts than in the controls. This finding suggested that the identified variant may be a genetic risk factor for the development of NTG.

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