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Glaucoma

Risk Score Predicting Primary Open-Angle Glaucoma Patients With Vascular Predisposition

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Received: August 20, 2024
Accepted: February 17, 2025
Published: April 7, 2025

Keywords: primary open-angle glaucoma (POAG); flammer syndrome (FS); optic nerve head (ONH) blood flow; laser speckle flowgraphy (LSFG); precision medicine

Citation: Takahashi N, Shiga Y, Kiyota N, Yasuda M, Takahashi N, Sato K, Arita R, Kikuchi A, Takayama S, Ishii T, Nakazawa T. Risk score predicting primary open-angle glaucoma patients with vascular predisposition. Transl Vis Sci Technol. 2025;14(4):9, https://doi.org/10.1167/tvst.14.4.9

Purpose: We tested the hypothesis that a questionnaire-based risk score predicts the prevalence of patients with primary open-angle glaucoma (POAG) with vascular predisposition.

Methods: The Flammer Syndrome Questionnaire (FSQ) was used to determine vascular risk scores in 823 healthy subjects and 512 patients with POAG. Next, we characterized blood flow pulsatility changes within the optic nerve head (ONH) in Flammer syndrome (FS) using laser speckle flowgraphy (LSFG) in 358 eyes of 206 patients with normal-tension glaucoma (NTG). Last, we examined the association between changes in Mean blur rate (MBR_{Ave}), an LSFG-derived ONH blood flow measurement, during cold provocation and the FSQ risk score in 56 eyes of 56 patients with NTG.

Results: Five FSQ-related symptoms were significantly associated in patients with POAG patients; cold hands/feet (odds ratio [OR] = 1.82), low blood pressure (BP; OR = 3.29), increased response to drugs (OR = 2.27), underweight (OR = 1.99), and tendency toward perfectionism (OR = 1.88). The vascular risk score showed the best discriminative accuracy in differentiating healthy subjects from patients with NTG (area under the curve [AUC] = 0.73). In the NTG eyes, ONH pulsatile blood flow in the FS group was characterized by greater pulsatility. Moreover, the negative correlation between the high FSQ risk score and the cold-induced ONH blood flow reduction was pronounced in eyes with NTG (correlation coefficient = –0.41).

Conclusions: The FSQ risk score can be a screening tool to identify patients with POAG with increased vascular stiffness and further reduced ONH blood flow during cold stress.

Translational Relevance: The vascular risk score may help tailor individual glaucoma care.

Introduction

A common framework in the pathophysiology of glaucoma is the progressive loss of retinal ganglion cells (RGCs) and their axons.[1] As the optic nerve head (ONH) is constantly exposed to fluctuating blood flow and biomechanical stress, efficient vascular perfusion is considered a critical element to meet the high metabolic demands of RGC axons.[2] Therefore, vascular dysfunction may compromise the ONH perfusion and play a pivotal role in the disease process of primary open-angle glaucoma (POAG),[3,4] notably normal-tension glaucoma (NTG). Importantly, previous randomized studies in NTG eyes have shown that whereas intraocular pressure (IOP) lowering therapy reduces disease progression, the treatment is less effective in patients with vascular components, including low systemic blood pressure (BP) and/or low ocular perfusion pressure (OPP).[5–7] Thus, developing tools to estimate individuals predisposed to vascular vulnerability may provide new insights into understanding heterogeneity in glaucoma pathogenesis and management.

Flammer syndrome (FS), also known as primary vascular dysregulation syndrome, is a phenotype characterized by complex symptoms resulting from vascular dysregulation.[8,9] Previous studies have revealed that FS is associated with several ocular diseases, including retinitis pigmentosa and NTG.[10] The Flammer Syndrome Questionnaire (FSQ), consisting of 15 symptoms commonly observed in individuals with FS, has been used to screen for this phenotype.[11–13] However, the frequency of each symptom used in the questionnaire and its effect size varies from disease to disease. Notably, the involvement of FS-related symptoms in glaucoma subtypes, such as high-tension glaucoma (HTG), remains an open question.

Vascular stiffness, a diminished capacity for vascular elasticity, has emerged as a critical component associated with neurodegeneration[14,15] and aging,[16] a major risk factor for POAG. Stiffer vessels result in reduced vascular compliance or increased vascular resistance, accelerating blood flow instability through increased transmission of pulsatile energy (greater pulsatility) in the capillaries.[17] A study examining retrobulbar blood flow using color Doppler imaging showed that elevated vascular resistance was found in subjects with FS.[18] Nonetheless, the ONH blood flow characteristics at the capillary level in patients with glaucoma with FS remain elusive.

Laser speckle flowgraphy (LSFG) imaging technology offers high-resolution in vivo assessment of deep ONH hemodynamics derived from the ciliary arteries that nourish the lamina region, a central site of glaucomatous RGC axonal damage.[19] The mean blur rate (MBR), a key parameter of LSFG, is linearly correlated with blood flow,[19,20] and continuously recorded changes in MBR allow parameterization of the pulsatile waveform.[21,22] Although there is no direct way to measure vascular resistance within the ONH yet, it has been shown that the LSFG pulsatile parameters, which are implicated with high vascular resistance to the ONH, alters with decreasing OPP[23,24] and predicts disease progression in glaucoma suspects.[25]

Furthermore, LSFG has the advantage of assessing hemodynamics within the ONH challenged by changes in OPP,[23,24,26] posture,[27] hyperoxia,[28–30] and cold temperature.[31] Cold exposure induces capillary vasoconstriction via sympathetic autonomic activation,[32] and excessive cold-induced nail capillary constriction has been reported in patients with NTG and individuals with FS.[33–35] Recently, we established a protocol using LSFG to evaluate autonomic innervated deep ONH blood flow dynamics during cold-water provocation and found that decreased ONH blood flow after cold stress in NTG eyes correlated with peripheral nail capillary constriction.[31] Thus, the longitudinal evaluation of the blood flow alterations subjected to cold provocation, as well as the analysis of ONH blood flow pulsatility using LSFG, is of great value in characterizing ONH blood flow impairment in glaucoma with vascular susceptibility.

In this study, we used the FSQ and LSFG to address the following key questions: (i) Which FS-related symptoms that comprise the FSQ are more common in patients with POAG, including NTG and HTG? (ii) Is there a characteristic ONH blood flow pulsatility in patients with NTG with FS stratified by the FSQ risk score based on the effect sizes of glaucoma-related symptoms? (iii) Is there a correlation between the FSQ risk score and ONH blood flow changes during cold stress in NTG eyes? Our results suggest that the FSQ risk score may be a promising tool in identifying POAG, notably patients with NTG with vascular susceptibility who exhibit blood flow instability within the ONH and markedly reduced ONH blood flow during cold stress.

Materials and Methods

Study Subjects

An overview of the experimental design, consisting of three-step protocols, is shown in Supplementary

Table 1. Demographic Characteristics of the Study Subjects

Variables	FSQ			LSFG Analysis	Cold Provocation
	Controls	HTG	NTG	NTG	NTG
Number of subjects	823	219	293	206	56
Subject age, y	55.0 (43.0–68.0)	63.0 (54.0–71.0)	59.0 (47.0–68.0)	56 .0 (45.0–65.3)	62.0 (53.3–70.8)
BMI, kg/m^2	23.0 (20.5–25.5)	23.0 (21.2–25.4)	20.1 (19.7–24.1)	22.1 (19.7–24.5)	22.7 (20.6–25.0)
Male/female, *n*	333/490	122/97	127/166	89/117	34/22
SBP mm Hg	130 (118–140)	134 (121–148)	124 (113–136)	122 (112–136)	128 (110–156)
DBP, mm Hg	78.0 (70.0–85.0)	78.0 (71.0–88.0)	73.0 (65.0–81.0)	73.0 (66.0–81.0)	78.0 (64.3–85.8)
Pulse rate, bpm	75.0 (69.0–83.0)	70.5 (63.0–76.3)	69.0 (62.0–76.0)	69.0 (62.0–77.0)	69.0 (60.3–73.5)
FSQ risk score	1.76 (1.28–2.56)	2.25 (1.88–2.83)	2.29 (1.68–3.27)	2.28 (1.85–2.89)	2.57 (2.04–2.91)

BMI, body mass index; DBP, diastolic blood pressure; FSQ, Flammer Syndrome Questionnaire; HTG, high tension glaucoma; LSFG, laser speckle flowgraphy; NTG, normal tension glaucoma; SBP, systolic blood pressure.

Data are expressed as the median (interquartile range).

Figure S1. The procedure in all experiments followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Tohoku Graduate School of Medicine (Protocol number: 2021-1-430). After written informed consent, all subjects were enrolled in the study.

All experiments were consecutive and included 551 patients with POAG recruited at Tohoku University Hospital. All patients with POAG underwent a complete set of ophthalmologic examinations, including IOP as measured with Goldmann applanation tonometry, iridocorneal angle assessment by gonioscopy, circumpapillary retinal nerve fiber layer thickness (cpRNFLT) measurement with a spectral-domain optical coherence tomography (SD-OCT 2000; Topcon Co., Tokyo, Japan), and visual field measured with 24-2 program of the Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, CA, USA). All patients with POAG were diagnosed by glaucoma specialists and met the following diagnostic criteria: (i) glaucomatous optic disc changes, including neuroretinal rim thinning, notching, or cupping; (ii) glaucomatous visual field defects that meet Anderson-Pattela criteria[36] obtained from reliable visual testing (fixation errors <20%, false positives <33%, and false negatives <33%); (iii) normal open-angle, and (iv) no history of ocular or systemic disease causing optic nerve degeneration or secondary vascular dysregulation,[9] and potentially associated with FS other than glaucoma,[10] including high myopia (axial length >26.5 mm), optic neuritis, retinitis pigmentosa, multiple sclerosis, Raynaud's disease, breast cancer, and metastatic cancer, as well as cardiovascular events due to metabolic syndrome throughout the studies and of ocular laser or incisional surgery for experiments 2 and 3.

Eight hundred twenty-three healthy subjects were recruited from volunteers at Katta General Hospital in the same region as the Tohoku University Hospital. The inclusion criteria were normal findings on a slit-lamp and funduscopic examination. Exclusion criteria were history of ophthalmic or general disorders, ocular laser or incisional surgery in either eye, systemic or topical medication, and family history of glaucoma based on self-report. Thus, by recruiting individuals from the same region diagnosed by detailed eye examinations, we attempted to ensure an unbiased selection of POAG cases and healthy controls and minimize potential regional disparities to identify FS symptoms commonly seen in glaucoma. The demographic characteristics of the study subjects are shown in Table 1.

The Flammer Syndrome Questionnaire

To estimate the risk of vascular predisposition in patients with glaucoma, 512 patients with POAG and 823 healthy subjects completed a modified Japanese version of the FSQ developed at the University of Basel, Switzerland (Supplementary Fig. S2). As in the original version, the questionnaire consisted of 15 items, and subjects were asked to select one of the following 3 or 4 options for each question except for thirst sensation and body shape: "often (yes)," "sometimes," "never (no)," or "I don't know."[12] Similarly, for the feeling of thirst, as in the original version, subjects were asked to choose one of the following four options: "I am a little thirsty and I drink a little," "I am a little thirsty and I drink because I know I have to drink," "I am very thirsty and I drink a lot," or "Both thirst and drinking behavior are normal." However, in the cohort of our preliminary study, we found that self-assessment of body shape varied widely

among individuals. Therefore, we modified the four options presented in the original version: "very slim," "slim," "average weight," and "overweight" to describe their height and weight to calculate body mass index (BMI). After subsequent preliminary validation of the comprehensibility of the modified FSQ, we found that 5 of these 15 questions showed significant associations with the POAG group and were extracted: Q1 cold hands/feet, Q3 low BP, Q9 increased response to certain drugs, Q12 underweight, and Q13 tendency toward perfectionism. Then, an FSQ risk score for glaucoma was calculated based on their effect sizes (experiment 1.1).

Next, we examined whether the frequency as well as effect size of each questionnaire differed in the glaucoma subclass. Based on IOP values measured with Goldmann applanation tonometry at the time of untreated or diagnosis, the cases were further classified into 219 HTG, defined as peak IOP greater than or equal to 22 mm Hg, and 293 NTG, defined as peak IOP less than 22 mm Hg, and the FSQ risk score was compared for each disease type (experiment 1.2).

Laser Speckle Flowgraphy

Of the 293 patients with NTG enrolled in experiment 1.2, there were 358 eyes of 206 patients with NTG with high-quality LSFG images and no history of ocular laser or incisional surgery that could affect LSFG images that were included in the analysis (experiment 2). Subjects were classified into an FS group (50 eyes of 27 patients) and a non-FS group (308 eyes of 179 patients), using the mean of FSQ risk score obtained from healthy subjects + 2 standard deviations cutoff value. The ONH blood flow and its pulsatility were characterized using LSFG for each group.

ONH blood flow was measured with the LSFG-NAVI (Softcare, Co., Ltd., Fukutsu, Japan). The principles of LSFG have been previously described in detail.[37] Briefly, the LSFG device consists of a fundus camera equipped with a diode laser (830 nm wavelength) and an ordinary charge-coupled device sensor (750 × 360 pixels). This instrument measures the speckle contrast pattern generated by the interference of the laser scattered by blood cells moving in the blood vessels.

The MBR, a primary variable, varies temporally and spatially with the amount and rate of blood movement and correlates well with blood flow within the ONH.[19,20] MBR images of the fundus are acquired continuously at 30 frames per second over 4 seconds and then averaged to produce a composite map of ocular blood flow. The included internal analysis software automatically separates the MBR map into the large vessel and tissue (capillary) regions of the ONH to determine values specific to each. In this study, we focused on the MBR of the tissue (capillary) area because it has been reported to represent the blood flow of the deeper regions at the ONH.[19]

Furthermore, the LSFG-NAVI software synchronizes all MBR images taken with the cardiac cycle, displays the average MBR of the heartbeat as a heartbeat map, and generates many pulsatile waveform parameters. A schematic description and the formulas used to calculate these variables are described elsewhere.[22,24,38] In this study, we included the following waveform parameters, which associated consistently with changes in OPP,[23,24,38,39] as well as the mean MBR value:

Average mean blur rate (MBR_{Ave}) – MBR_{Ave}

represents a surrogate measurement for mean ONH blood flow. A low MBR_{Ave} indicates reduced blood flow.

Blowout score (BOS) – The BOS is considered a measure of blood flow maintained in one heartbeat (heart rate width) and is calculated using the difference between the maximum and minimum MBR and the mean MBR. A low BOS indicates a large pulsatile waveform of blood flow during the cardiac cycle and stiffer vessels.

Resistivity index (RI) – The RI is the range of MBR values expressed as a percentage of the maximum value, (maximum-minimum)/maximum, which has the same interpretation as BOS, but the signs are reversed (i.e. a high RI means stiffer vessels). It is similar to the widely used resistance index defined as ($Velocity_{systole}$–$Velocity_{diastole}$)/$Velocity_{systole}$,[40] which relies on vascular resistance and compliance.[41]

Fluctuation – This parameter indicates blood flow instability, proportional to the average variation in MBR divided by the average MBR of the waveform.

Flow acceleration index (FAI) – FAI is the maximum increase in MBR between two image frames (frame rate 1/30 second).

Prior to LSFG measurements, the patient's pupils were dilated with the muscarinic antagonist 0.4% tropicamide (Mydrin M; Santen Pharmaceuticals, Osaka, Japan). After administration of Mydrin M, the patient sat in a dark, quiet room for 15 minutes to allow the pupil dilation to stabilize, LSFG was taken 3 times, and the mean of the LSFG data with ensured image quality in the entire series (ONH vascular cloud $\geq$0.28) was used. BP and pulse rate were monitored in the brachial artery at the height of the heart (HBP-1300; Omron Colin Co., Ltd., Tokyo, Japan). Mean arterial BP and mean OPP were calculated as follows: mean BP

= diastolic BP + 1/3 × (systolic BP – diastolic BP), and OPP= 2/3 × mean BP – IOP.

Cold-Water Provocation Test

Last, the cold-water provocation test was used to detect the impaired vascular response to external stimuli, a primary symptom of FS. This prospective study comprised 56 eyes of 56 patients with NTG, including newly enrolled 17 eyes of 17 patients with NTG (experiment 3).

The provocation test was performed as previously described.[31] All subjects were tested in the evening, between 4:00 PM and 6:00 PM, after 15 minutes of seated rest in a dark, quiet room at a temperature of 20°C. A representative image of the testing protocol is shown in Supplementary Figure S1. The testing protocol included four phases: (1) a baseline phase, in which baseline measurements of LSFG-derived parameters, BP, pulse rate, and IOP were taken three times each; (2) a warm phase, in which subjects immersed their right hand in warm water at 40°C for 2 minutes; (3) in the cold phase, subjects immersed their right hand in cold water at 4°C for 1 minute; And (4) in the recovery phase, LSFG-derived parameters, BP, pulse rate, and IOP were measured 2 and 4 minutes after the cold-thermal induction. We measured baseline IOP with a Goldmann tonometer, whereas IOP during the cold-provocation test was measured with a portable tonometer (Icare; Tiolat Oy, Helsinki, Finland).

As our previous study showed that MBR_{Ave} fell the most 4 minutes after the cold provocation,[31] the percentage changes of MBR_{Ave} at 4 minutes relative to baseline was calculated and analyzed for correlation with the FSQ risk score.

Statistical Analysis

Univariable correlation analysis was performed prior to multivariable analysis throughout all experimental protocols.

In experiment 1, a logistic regression analysis was performed to determine the effect size of each FSQ item among patients with POAG and healthy subjects as an exploratory data analysis. Following a previous study,[12] for each item, the most positive response, "often (yes)," or "I am little thirsty and I drink a little" was set to 1, whereas the other response categories ("sometimes," "never (no)," and "I do not know") were set to 0. For body shape, BMI was calculated from the weight and height reported in the questionnaire. Based on the World Health Organization (WHO) reference,[42] a BMI of less than 18.5 kg/m^2 was defined as underweight and set to 1; otherwise, it set to 0. For this analysis, the female patients were set to 0 and the male patients to 1. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated for each item. Principle component analysis (PCA) was implemented to extract items that contribute primarily to the first principal component. The ability to discriminate between healthy and POAG, NTG, or HTG eyes was analyzed by the area under the curve (AUC) of receiver operating characteristic (ROC) curve analysis adjusted for covariates by age and sex, using risk scores calculated from the effect size of items that showed a significant association with POAG. Z-test was used for the AUC comparison between NTG and HTG eyes. Fisher's exact test was used to compare the frequency of FS with high FSQ risk scores in the control and POAG groups.

In experiment 2, we conducted a regression analysis using a linear mixed-effects model, with each clinical parameter as the dependent variable and the distinction between the FS and non-FS groups as the independent variable. A nested random-effects structure was applied, with subjects modeled as a random effect with random intercepts, and both eyes treated as repeated measures to account for within-subject correlation. Multivariable linear mixed-effects model regression analysis was used to analyze LSFG pulsatile waveform parameters to identify factors contributing to FS. LSFG pulsatile waveform parameters, age, sex, pulse rate, mean OPP, axial length, and cpRNFLT were explanatory variables in the model.

In experiment 3, a 1-way analysis of variance (ANOVA) test was used for the changes in MBR_{Ave}, BP, pulse rate, IOP, and mean OPP during the cold-water provocation test. Pearson correlation coefficient was used to correlate the percentage changes of MBR_{Ave} with the FSQ risk score.

All statistical analyses were performed using R software (version 3.2.5). Because the FSQ risk score calculated from POAG-related questions with P values less than 0.05 showed the highest R-squared value (0.14) and discriminative power (AUC = 0.69, 95% CI = 0.66–0.72) in the present POAG cohort, we set the significance threshold at $P < 0.05$ for all experimental protocols.

Results

Experiment 1.1. Development of Vascular Risk Score for POAG

First, we examined correlations between the 15 questions from FSQ and age and sex. Univariable

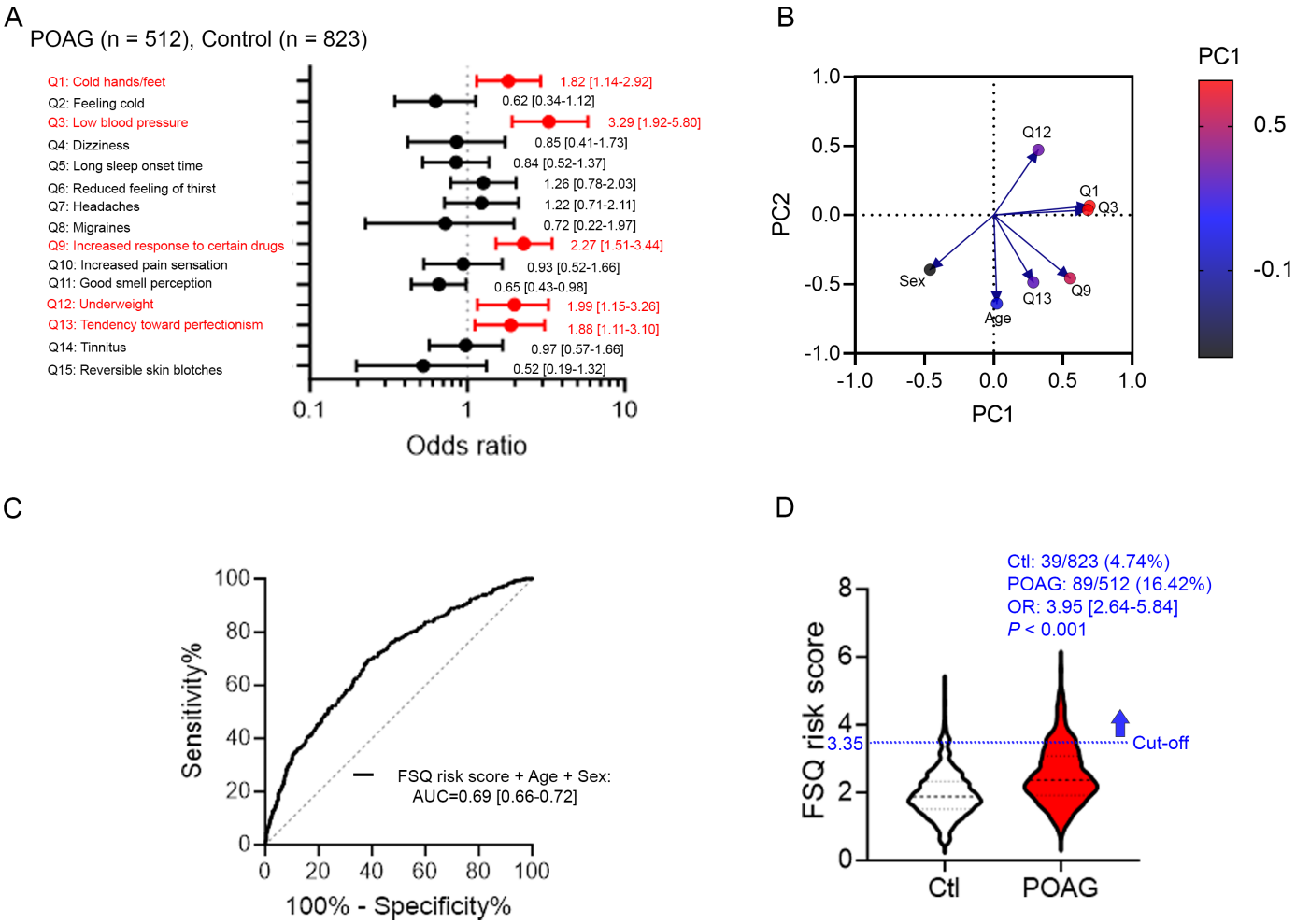

Figure 1. (**A**) Forest plot showing ORs and 95% CIs for each FSQ item in patients with POAG. *Red fonts* indicate items that showed significant associations in the POAG. (**B**) PCA results of FSQ items significantly associated with POAG, age, and gender. (**C**) Discrimination ability between healthy subjects and patients with POAG in calculated FSQ risk score showing AUC and 95% CI. (**D**) Violin plot showing the distribution of FSQ risk scores in healthy subjects and patients with POAG. The *horizontal blue dotted line* indicates the cutoff value of the FSQ risk score. The *dotted lines* in each plot indicate upper and lower quartiles and medians (Fisher's exact test; ***$P < 0.001$).

correlation analysis revealed that those questions have weak positive and negative correlations with age and sex (Supplementary Fig. S3). Thus, we conducted a logistic regression analysis adjusted by age and sex in the 512 healthy controls and 823 patients with POAG. The results showed that 5 of the 15 questions were significantly associated in patients with POAG: Q1 cold hands/feet (OR = 1.82, 95% CI = 1.14–2.92, $P = 0.01$), Q3 low BP (OR = 3.29, 95% CI = 1.92–5.80, $P < 0.001$), Q9 increased response to certain drugs (OR = 2.27, 95% CI = 1.51–3.44, $P < 0.001$), Q12 underweight (OR = 1.99, 95% CI = 1.15–3.26, $P = 0.01$), and Q13 tendency toward perfectionism (OR = 1.88, 95% CI = 1.11–3.10, $P = 0.01$; Fig. 1A). Additionally, PCA revealed that the contribution of cold hands/feet (Q1), low BP (Q3), and increased response to certain drugs (Q9) to PC1 was significant (contribution variables to PC1: 0.30, 0.26, and 0.13, respectively), with Q1 and Q3 being elements with similar characteristics (Fig. 1B).

The standard regression coefficients for the five questions that were significant in patients with glaucoma were then used to calculate the FSQ risk score for POAG (R-squared value = 0.14) as follows:

$$\begin{aligned}\text{FSQ risk score} = {} & 0.02562 \times \text{Age [years]} + 0.6025 \\ & \times \text{Sex [Female : 0, Male : 1]} \\ & + 0.5991 \times \text{Q1} + 1.192 \times \text{Q3} + 0.8214 \\ & \times \text{Q9} + 0.6590 \times \text{Q12} + 0.6120 \times \text{Q13}\end{aligned}$$

ROC curve analysis demonstrated that the AUC value of the FSQ risk score was 0.69 (95% CI = 0.66–0.72; Fig. 1C). The probability of a higher FSQ risk

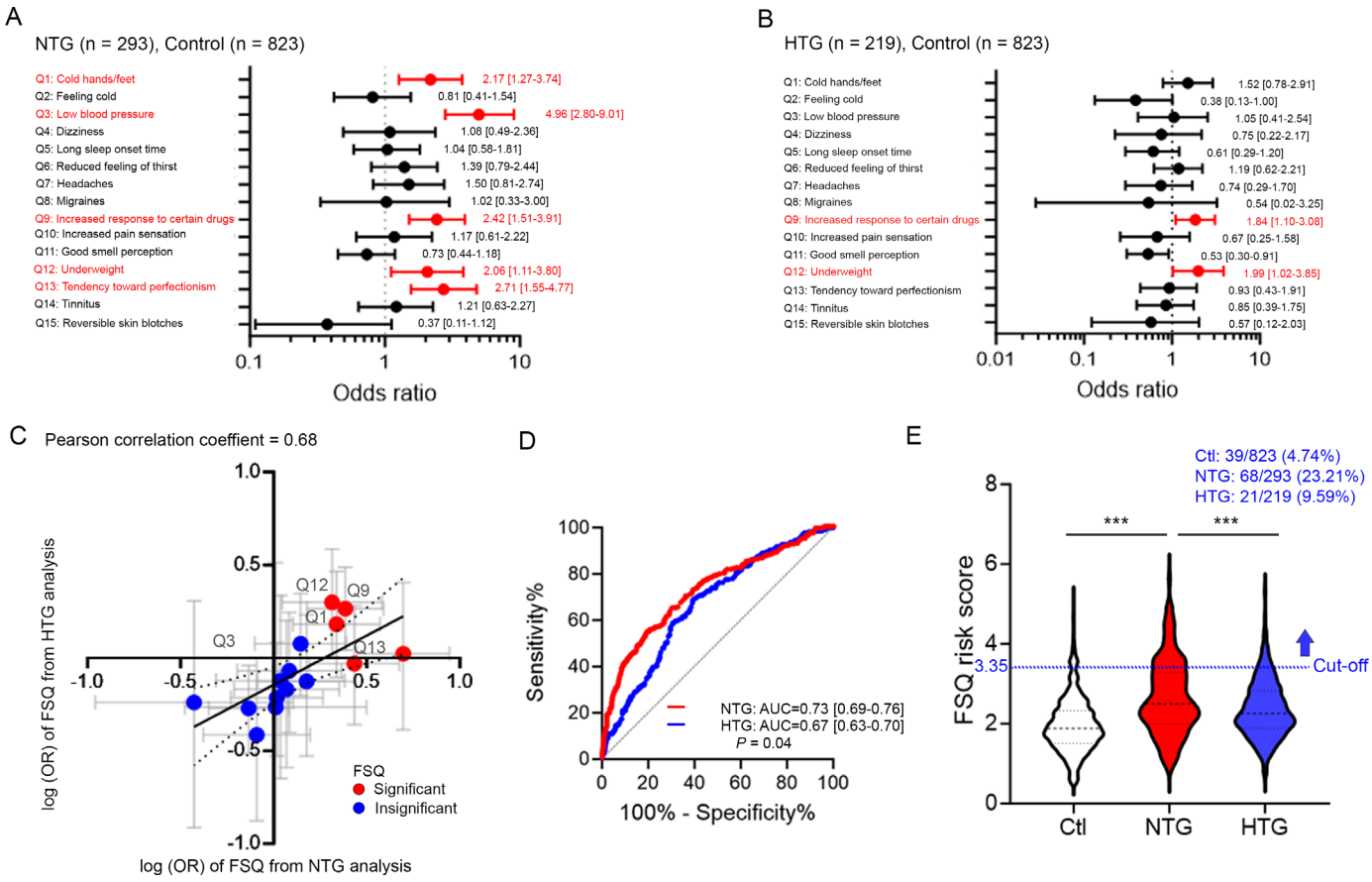

Figure 2. (**A**) Forest plot showing ORs and 95% CIs for each FSQ item in patients with NTG. *Red fonts* indicate items that showed significant associations in the NTG. (**B**) Forest plot showing ORs and 95% CIs for each FSQ item in patients with HTG. *Red fonts* indicate items that showed significant associations in the HTG. (**C**) The plotted figure shows the effect estimates for each FSQ item obtained from the NTG and HTG analyses on a log (OR) scale. *Red dots* indicate items that showed significant associations in the POAG analysis, and *blue dots* represent items that displayed insignificance. The *horizontal gray bar* on each dot represents the 95% CIs of the effect estimate in NTG. The *vertical gray bar* on each dot represents the 95% CIs of the effect estimate in HTG. The *line* is the linear regression line that best fits the data. The *dotted area* indicates the 95% CI of the regression line. (**D**) Comparison of discrimination ability between healthy subjects and patients with NTG (*red*) and between healthy subjects and patients with HTG (*blue*) in calculated FSQ risk score showing AUCs, 95% CIs, and *P* value (Z-test). (**E**) Violin plots showing the distribution of FSQ risk scores in healthy subjects, patients with NTG, and patients with HTG. The *horizontal blue dotted line* indicates the cutoff value of the FSQ risk score. The *dotted lines* in each plot indicate upper and lower quartiles and medians (Fisher's exact test; ***$P < 0.001$).

score in the patients with POAG group was significantly greater: 89 of 512 (16.42%) patients with POAG had a score above the cutoff value (3.35; mean FSQ risk score from healthy subjects + 2 standard deviations), compared to only 39 of 823 (4.74%) healthy controls (OR = 3.95, 95% CI = 2.64–5.84, $P < 0.001$; Fig. 1D).

Experiment 1.2. Vascular Risk Score Analysis in POAG Subtypes

To further assess the clinical relevance of POAG-associated FSQ risk score, we conducted the subtype-stratified analyses using 293 cases and 823 controls for NTG and 219 cases and 823 controls for HTG. All five questions that were significantly associated in the overall POAG analysis also showed significant associations in the NTG group: Q1 cold hands/feet (OR = 2.17, 95% CI = 1.27–3.74, $P < 0.01$), Q3 low BP (OR = 4.96, 95% CI = 2.80–9.01, $P < 0.001$), Q9 increased response to certain drugs (OR = 2.42, 95% CI = 1.51–3.91, $P < 0.001$), Q12 underweight (OR = 2.06, 95% CI = 1.11–3.80, $P = 0.02$), and Q13 tendency toward perfectionism (OR = 2.71, 95% CI = 1.55–4.77, $P < 0.001$; Fig. 2A). In contrast, 2 questionnaires that include Q9 increased response to certain drugs (OR = 1.84, 95% CI = 1.10–3.80, $P = 0.01$) and Q12 underweight (OR = 1.99, 95% CI = 1.02–3.85, $P = 0.04$) were associated with HTG, however, the other

Table 2. Clinical Characteristics of the FS and Non-FS Groups in NTG Eyes

Variables	Non-FS Group	FS Group	*P* Value
Number of patients, *n*	179	27	–
Number of eyes, *n*	308	50	–
Age, y	56.0 (46.0 to 65.0)	60.0 (44.5 to 65.5)	0.58
Male : female, *n*	78 : 101	11 : 16	0.78
Axial length, mm	25.7 (24.6 to 26.7)	25.7 (24.2 to 26.8)	0.82
Intraocular pressure, mm Hg	13.0 (12.0 to 15.0)	12.0 (11.0 to 14.0)	0.15
Ocular perfusion pressure, mm Hg	47.4 (41.8 to 53.1)	43.3 (38.8 to 55.4)	0.26
Mean blood pressure, mm Hg	90.5 (84.8 to 98.1)	83.0 (77.2 to 102.3)	0.15
Pulse rate, bpm	69.0 (62.0 to 77.0)	74.0 (61.0 to 82.8)	0.30
cpRNFLT, µm	75.1 (63.1 to 87.3)	75.3 (63.5 to 85.2)	0.80
Mean deviation, dB	−5.59 (−9.71 to −2.11)	−5.10 (−8.65 to −1.00)	0.52
FSQ risk score	2.17 (1.76 to 2.54)	3.64 (3.51 to 4.03)	<0.01*

cpRNFLT, circumpapillary retinal nerve fiber layer thickness; FSQ, Flammer syndrome questionnaire.

Data are expressed as the median (interquartile range).

Linear mixed models were used to analyze the data, focusing on the relationship between the variable and the presence or absence of FS. Differences were considered significant at $P < 0.05$.

*$P < 0.01$.

3 POAG-associated questionnaires, including Q1 cold hands/feet (OR = 1.52, 95% CI = 0.78–2.91, $P = 0.20$), Q3 low BP (OR = 1.05, 95% CI = 0.41–2.54, $P = 0.91$), and Q13 tendency toward perfectionism (OR = 0.93, 95% CI = 0.43–1.91, $P = 0.84$) showed insignificant positive association (Fig. 2B).

Although each FSQ-related symptom had the same direction of effect for HTG (Pearson correlation coefficient = 0.68; Fig. 2C), the AUC values between NTG and HTG significantly differed (0.73, 95% CI = 0.69–0.76, and 0.67, 95% CI = 0.63–0.70, $P = 0.04$, respectively; Fig. 2D), and the effect was more prominent for NTG than HTG. Similarly, the NTG group had a higher proportion of the outliers in the FSQ score: 68 of 293 (23.21%) patients with NTG had a score above the cutoff value, compared to 39 of 823 (4.74%) in the healthy control group (OR = 6.07, 95% CI = 3.98–9.17, $P < 0.001$) and 21 of 219 (9.59%) patients in the HTG group (OR = 2.85, 95% CI = 1.68–4.83, $P < 0.001$; Fig. 2E).

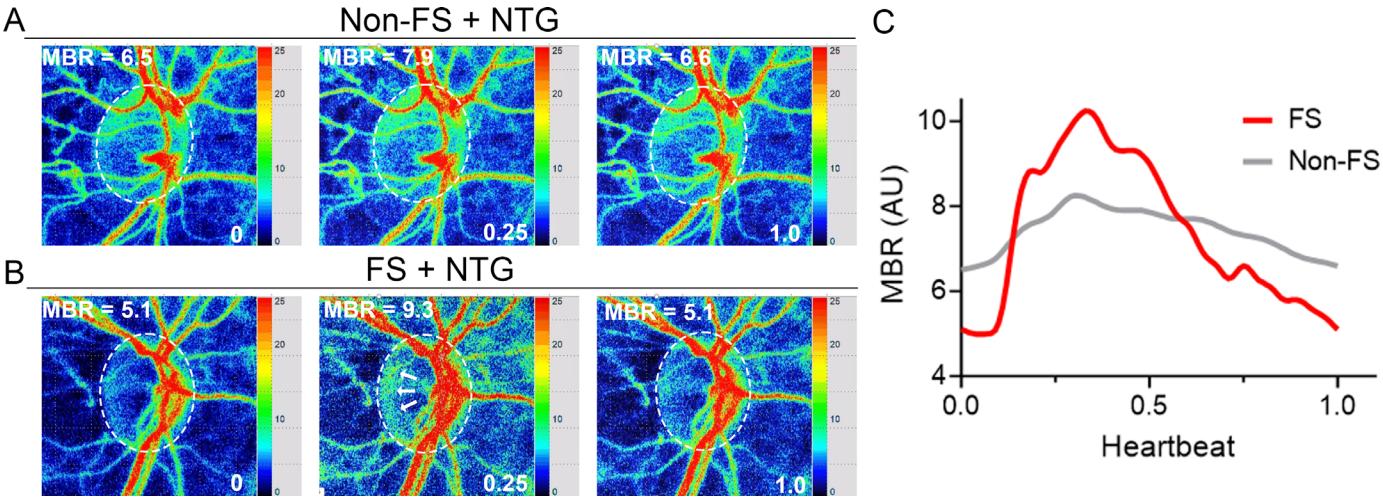

Figure 3. (**A, B**) Representative LSFG heartbeat map of ONH blood flow in NTG eyes without FS (**A**) and with FS (**B**). On each image, the MBR of the ONH region of interest (*dotted area*) is shown at each measured time point. *Arrows* highlight the increased change in MBR within ONH in a single heartbeat in the FS group. (**C**) Representative LSFG waveforms of ONH blood flow in NTG eyes without FS (*gray*) and with FS (*red*) show greater pulsatility in the FS group compared with the non-FS group.

These results indicate that the FSQ risk score can identify individuals with high vascular risk across POAG subtypes and is highly implicated in NTG.

Experiment 2. Characterizing ONH Blood Flow Pulsatility in NTG Eyes With FS

Next, we used LSFG to examine the difference in blood flow pulsatility in the ONH in patients with NTG with and without FS, because the FSQ risk score was more relevant in the NTG group than in the HTG group. Using the FSQ risk score cutoff values described above, the 358 eyes of 206 patients with NTG were stratified into FS and non-FS groups, with 50 eyes of 27 patients classified into the FS group and 308 eyes of 179 patients into the non-FS group. The clinical characteristics of each group are shown in Table 2. There were no significant differences in age, gender distribution, axial length, IOP, pulse rate, or disease severity (mean deviation [MD] values for visual field impairment and optical coherence tomography (OCT)-derived cpRNFLT measurements) in the two groups ($P = 0.15$ and $P = 0.82$, respectively). In addition, mean OPP and BP tended to be lower in the FL group, but did not reach significance. ($P = 0.15$ and $P = 0.26$, respectively).

Capillary blood flow pulsatility was mild in the ONH of patients with NTG without FS (Fig. 3A). In contrast, a marked increase in pulsatility was observed in the eyes of patients with NTG with FS (Figs. 3B, 3C). In the analysis using a linear mixed model corrected for age, sex, pulse rate, mean OPP, axial length, and cpRNFLT, we confirmed that LSFG parameters; BOS, RI, Fluctuation and FAI were also determined as significant contributors to NTG eyes with FS ($\beta = -0.13$, 95% CI = –0.24 to –0.02], $P = 0.02$; $\beta = 0.13$, 95% CI = 0.01 to 0.24], $P = 0.02$, $\beta = 0.14$, 95% CI = 0.02 to 0.26, $P = 0.01$; and $\beta = 0.20$, 95% CI = 0.07 to 0.32, $P < 0.01$, respectively; Table 3).

Collectively, these data suggest increased vascular stiffness in patients with NTG with FS.

Table 3. Linear Mixed Model Analysis of LSFG Parameters Contributing to FS

Response Variables	β (95% CI)	*P* Value
Blowout Score	−0.13 (−0.24 to −0.02)	0.02*
Resistivity Index	0.13 (0.01 to 0.24)	0.02*
Fluctuation	0.14 (0.02 to 0.26)	0.01*
Flow Acceleration Index	0.20 (0.07 to 0.32)	<0.01**

β indicates the standardized beta; 95% CI = 95% confident intervals.

Age, sex, axial length, circumpapillary retinal nerve fiber layer thickness, ocular perfusion pressure, and pulse rate were adjusted in generalized linear mixed models. Differences were considered significant at $P < 0.05$.

*$P < 0.05$.

**$P < 0.01$.

Experiment 3. Association Between Cold-Induced ONH Blood Flow Changes and Vascular Risk Score

Individuals with FS are hypersensitive to the increased sympathetic activity response to cold stimuli, which induces vasoconstriction and reduced blood flow in ocular circulation and peripheral capillaries.[8] There-

Table 4. Changes in Clinical Variables During Cold Provocation Test

	NTG, $n = 56$			
Variables	Baseline	2 min	4 min	*P* Value
MBR_{Ave}, AU	9.83 (8.40 to 11.9)	9.70 (7.90 to 11.4)	9.50 (7.90 to 11.8)	0.77
$\%MBR_{Ave}$, %	0.00 (0.00 to 0.00)	−1.52 (−8.78 to 3.76)	−2.22 (−9.83 to 7.80)	0.72
SBP, mm Hg	128.0 (110.0 to 155.8)	136.0 (115.5 to 156.0)	134.0 (115.5 to 150.8)	0.63
DBP, mm Hg	78.0 (64.3 to 85.8)	75.0 (65.3 to 86.5)	77.5 (65.3 to 88.0)	0.99
IOP, mm Hg	10.5 (9.0 to 14.0)	10.0 (9.0 to 12.8)	10.0 (9.0 to 14.8)	0.73
Pulse rate, bpm	69.0 (60.3 to 73.5)	68.0 (61.0 to 74.0)	68.0 (60.5 to 74.0)	0.89
MBP, mm Hg	93.2 (79.6 to 107.8)	96.3 (81.5 to 109.0)	95.0 (80.8 to 108.9)	0.89
OPP, mm Hg	54.5 (41.8 to 61.0)	52.9 (41.8 to 61.3)	54.1 (42.7 to 63.0)	0.95

DBP, diastolic blood pressure; IOP, intraocular pressure; MBP, mean blood pressure; MBR_{Ave}, tissue-area average mean blur rate; OPP, ocular perfusion pressure; SBP, systolic blood pressure.

Data are expressed as the median (interquartile range).

$\%MBR_{Ave}$ indicates the percentage changes of MBR_{Ave} relative to baseline. Differences between groups were assessed with ANOVA test and considered significant at $P < 0.05$.

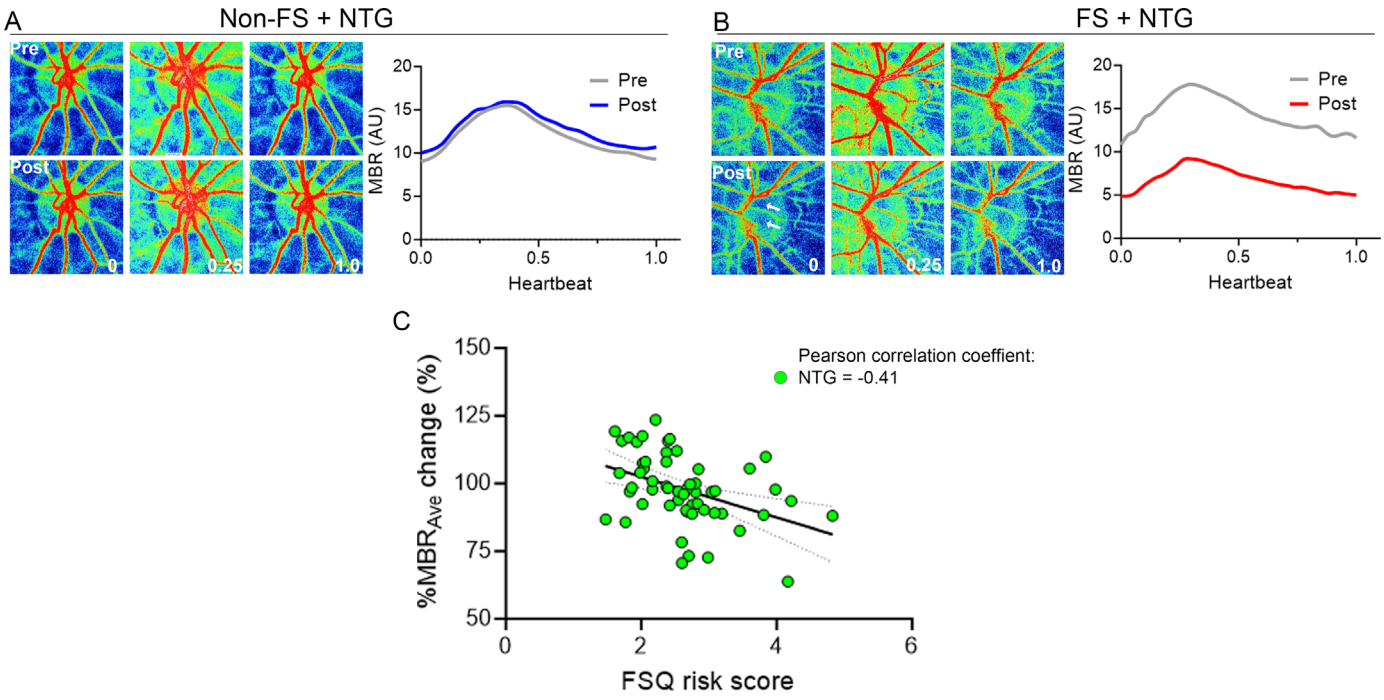

Figure 4. (**A, B**) Representative LSFG heartbeat maps and waveforms of ONH blood flow changes before and after cold provocation testing in NTG eyes without FS (**A**) and with FS (**B**). *Arrows* represent the marked decrease in capillary blood flow within the ONH after cold stress in eyes with FS. (**C**) The plotted figure shows the correlation between FSQ risk score and ONH blood flow (MBR_{Ave}) changes during cold stress in NTG eyes. The line is the linear regression line that best fits the data. The *dotted area* indicates the 95% CI of the regression line.

fore, we sought to investigate the relationship between the FSQ risk score and changes in microcirculation within the ONH during the cold-water provocation test in 56 eyes of 56 patients with NTG (mean MD = –8.95 ± 7.60 decibels [dB]).

Table 4 shows the changes in clinical variables during cold stress: ocular and systemic parameters, including IOP and OPP, did not change significantly ($P = 0.63$–0.95).

Nonetheless, the higher the FSQ risk score, the more likely MBR_{Ave} decreased after cold provocation in the eyes with NTG (Figs. 4A, 4B). Indeed, the FSQ risk score and the rate of MBR_{Ave} change showed a significant negative correlation in the NTG group (Pearson correlation coefficient = –0.41; Fig. 4C), indicating that cold stimuli can trigger a further reduction of ONH microcirculation in eyes with high FSQ risk score in patients with NTG.

Discussion

For decades, the involvement of blood flow dysregulation in the pathogenesis of glaucoma has been suggested, especially in individuals with FS.[8,43] Nevertheless, due to the lack of gold standards for diagnosis of FS, it needs to be clarified how to conveniently stratify patients with glaucoma with FS and the hemodynamic characteristics of the ONH in these patients. The FSQ-based risk score developed in this study, combined with LSFG assessment, may help identify FS with ONH blood flow instability in patients with POAG, notably NTG.

First, we used the FSQ to develop a vascular predisposition risk score for patients with POAG by examining symptoms commonly observed in the disease. We found that five items were significantly associated with POAG: cold hands and feet, low BP, increased sensitivity to certain drugs, underweight, and tendency toward perfectionism (of note, low BP and increased drug sensitivity remained associated with POAG after Bonferroni correction for the number of tests [$P < 0.05/15$]). Among them, PCA in this study revealed that low BP, cold extremities in hands and feet, and hypersensitivity to certain drugs were the main characteristics of FS associated with POAG, correlating with low BP and cold hands and feet.

Remarkably, low systemic BP showed the most significant effect size associated with patients with POAG in this study. Previous studies indicate that adequate BP levels play a pivotal role in effective

ONH blood flow autoregulation, the mechanisms that maintain blood flow despite OPP Fluctuations.[44,45] In line with this, many population studies have confirmed that low BP or low OPP (due to hypotension and/or elevated IOP) are critical risk factors for the development and progression of glaucoma.[46–52] Moreover, accumulating evidence suggests that in glaucomatous eyes, nocturnal BP dip is also associated with the worsening of the disease.[53,54] Thus, the attenuation of blood flow autoregulation due to lower BP, coupled with Fluctuations in OPP, may cause ONH ischemia-reperfusion or overperfusion injury when below the lower limit of autoregulatory capacity, accelerating the vulnerability of RGCs.

In agreement with previous findings,[55] the present study showed associations with cold hypersensitivity in hands/feet and low BP. Although the detailed mechanisms in this crosstalk are poorly understood, cold hands and feet may be associated with reduced blood flow to the peripheral tissues due to reduced mechanical stress in blood flow induced by low BP.[56] Additionally, perfectionism observed more frequently in the patients with POAG has been shown to be associated with a variety of psychiatric disorders, including anxiety.[57] This disposition may increase susceptibility to psychological stress. Collectively, increased sensitivity to cold and psychological stress may cause vascular vulnerability, especially in NTG eyes with low BP.

On the other hand, the items increased sensitivity to certain drugs and underweight were significantly associated with both NTG and HTG, whereas the greater involvement of FS in NTG was consistent with previous studies.[9] A potential mechanism for increased drug sensitivity in patients with FS has been reported to involve energy-dependent ATP-binding cassette (ABC) transporter proteins,[58] which play an essential role in drug transport out of the cell.[59] For example, the MDR1 (P-glycoprotein), also known as ABCB1, is abundantly expressed in human trabecular meshwork cells, and metabolic stress induces its dysfunction,[60] supporting the possible involvement of ABC transporters in glaucoma over a wide range of IOP levels. In addition, several population surveys have reported that being underweight increases cardiovascular risk.[61] This finding may be explained by a general metabolic decline, such as reduced protein and lipid synthesis that consist blood vessels and their associated enzyme activity.[62] Taken together, increased sensitivity to drugs and underweight may drive the induction of vascular dysregulation across POAG subtypes.

Second, we used LSFG to characterize ONH blood flow pulsatile waveforms in NTG eyes with FS stratified by FSQ risk score. The results showed that despite similar disease severity and mean ONH blood flow in the FS and non-FS groups, ONH hemodynamics in the FS group was characterized by greater pulsatility (lower BOS, higher RI, Fluctuation, and FAI). Recent studies in healthy human volunteers evaluated LSFG-derived pulsatile parameter changes in ONH capillaries after acute elevation of IOP using tonometry.[23,24] The results showed that a decrease in OPP due to acute IOP elevation causes higher resistance to ONH blood flow, as indicated by changes in pulsatile parameters such as decreased BOS and increased RI and FAI. In contrast, studies that followed changes in pulsatile parameters of ONH blood flow in glaucomatous eyes after trabeculectomy over 3 to 6 months showed an increase in BOS and a decrease in RI and Fluctuation after surgery, along with an increase in OPP due to decreased IOP.[38,39] Although there are differences in the degree to which IOP or BP-dependent reduction in OPP affects ONH blood flow,[45] the present study clearly demonstrates that ONH blood flow pulsatile parameters that are sensitive to changes in OPP – lower BOS, higher RI, Fluctuation, and FAI – are signature features of NTG eyes with FS, allowing the quantification of increased vascular stiffness and blood flow instability at the ONH capillaries as a central pathogenesis of the FS.

Last, we examined the relationship between FSQ risk score and ONH blood flow changes during sympathetic-dependent capillary constriction induced by cold provocation. The results showed that the higher the FSQ risk score, the lower the mean ONH blood flow during cold stress, and this negative correlation was pronounced in the NTG eyes. There are several potential mechanisms for the relationship between FSQ risk scores in NTG eyes and reduced ONH capillary blood flow during cold stimulus induction: (i) impaired ONH blood flow autoregulation under glaucomatous conditions,[63] (ii) increased vascular resistance and pre-existing capillary diameter constriction due to tissue remodeling,[64] and (iii) hyperexcitability in the autonomic nervous system due to autonomic dysfunction,[65] which may cause capillary hypercontraction in response to external cold stress.

There are several limitations in this study. First, central corneal thickness, diurnal IOP Fluctuations, and the number of IOP measurements may affect IOP readings. This may lead to misdiagnosis of NTG in some patients. Second, the present study lacks a replication cohort, although the FSQ risk score in this study was generated using case-control under strict criteria and corroborates the ONH circulation characteristics of NTG eyes with FS using LSFG, which ensures the reliability of the FSQ risk score. A meta-analysis with larger sample size is expected further to improve the accuracy of the FSQ risk score to identify

glaucomatous eyes with FS. Third, longitudinal and prospective studies are needed to examine the relationship between treatment response to IOP reduction and disease progression in glaucomatous eyes with FS stratified using the FSQ risk score. Despite these caveats, the vascular risk score developed in this study may be potentially useful, for example, in determining the target population for glaucoma with a predisposition to vascular vulnerability in clinical trials.

In conclusion, the FSQ risk score helps detect patients with POAG with vascular predisposition, especially patients with NTG, and the LSFG can be a valuable tool to quantify the increased vascular stiffness and instability of ONH blood flow in these patients and the marked decrease in ONH blood flow in response to cold stimuli.

Acknowledgments

The authors thank Noriko Kakubari and other staff in the Department of Ophthalmology, Katta general hospital for technical support.

Supported by JSPS KAKENHI Grants-in-Aid for Scientific Research (B) (26293372) for Scientific Research (C) (23K09055) and for Exploratory Research (26670751) and JST COI (grant number JPMJCE1303).

Author Contributions: Conceptualization: Y.S., N.T., and T.N.; Investigation/experimentation: N.T. and Y.S.; Data analyses/interpretation: N.T., Y.S., N.K., M.Y., N.T., and K.S.; Questionnaire translation: R.A., A.K., S.T., and T.I.; Writing-original draft: Y.S., N.T., and T.N.; Writing-review and editing: Y.S., N.T., N.K., M.Y., N.T., K.S., R.A., K.A., S.T., T.I., and T.N.; Visualization: Y.S. and N.T.; Supervision: Y.S. and T.N.; Funding acquisition: T.N.

Disclosure: **N. Takahashi**, None; **Y. Shiga**, None; **N. Kiyota**, None; **M. Yasuda**, None; **N. Takahashi**, None; **K. Sato**, None; **R. Arita**, None; **A. Kikuchi**, None; **S. Takayama**, None; **T. Ishii**, None; **T. Nakazawa**, None

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References

1. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. *JAMA*. 2014;311:1901–1911.
2. Burgoyne CF. A biomechanical paradigm for axonal insult within the optic nerve head in aging and glaucoma. *Exp Eye Res*. 2011;93:120–132.
3. Flammer J, Orgul S, Costa VP, et al. The impact of ocular blood flow in glaucoma. *Prog Retin Eye Res*. 2002;21:359–393.
4. Resch H, Garhofer G, Fuchsjager-Mayrl G, Hommer A, Schmetterer L. Endothelial dysfunction in glaucoma. *Acta Ophthalmol*. 2009;87:4–12.
5. Anderson DR, Normal Tension Glaucoma Study. Collaborative normal tension glaucoma study. *Curr Opin Ophthalmol*. 2003;14:86–90.
6. De Moraes CG, Liebmann JM, Greenfield DS, et al. Risk factors for visual field progression in the low-pressure glaucoma treatment study. *Am J Ophthalmol*. 2012;154:702–711.
7. Furlanetto RL, De Moraes CG, Teng CC, et al. Risk factors for optic disc hemorrhage in the low-pressure glaucoma treatment study. *Am J Ophthalmol*. 2014;157:945–952.
8. Flammer J, Konieczka K, Flammer AJ. The primary vascular dysregulation syndrome: implications for eye diseases. *EPMA J*. 2013;4:14.
9. Konieczka K, Ritch R, Traverso CE, et al. Flammer syndrome. *EPMA J*. 2014;5:11.
10. Konieczka K, Erb C. Diseases potentially related to Flammer syndrome. *EPMA J*. 2017;8:327–332.
11. Konieczka K, Koch S, Schoetzau A, Todorova MG. Increased prevalence of Flammer syndrome in patients with retinitis pigmentosa. *Klin Monbl Augenheilkd*. 2016;233:448–452.
12. Konieczka K, Choi HJ, Koch S, Fankhauser F, Schoetzau A, Kim DM. Relationship between normal tension glaucoma and Flammer syndrome. *EPMA J*. 2017;8:111–117.
13. Uzunkopru C, Beckmann Y. Flammer syndrome in multiple sclerosis: diagnostics, prediction, and personalization of treatments. *EPMA J*. 2019;10:437–444.
14. Nation DA, Edmonds EC, Bangen KJ, et al. Pulse pressure in relation to tau-mediated neurodegeneration, cerebral amyloidosis, and progression to dementia in very old adults. *JAMA Neurol*. 2015;72:546–553.
15. Moore EE, Liu D, Li J, et al. Association of aortic stiffness with biomarkers of neuroinflammation, synaptic dysfunction, and neurodegeneration. *Neurology*. 2021;97:e329–e340.
16. Lacolley P, Regnault V, Segers P, Laurent S. Vascular smooth muscle cells and arterial stiffening: relevance in development, aging, and disease. *Physiol Rev*. 2017;97:1555–1617.
17. Hughes TM, Craft S, Lopez OL. Review of 'the potential role of arterial stiffness in the patho-

genesis of Alzheimer's disease'. *Neurodegener Dis Manag*. 2015;5:121–135.
18. Kaiser HJ, Schoetzau A, Stumpfig D, Flammer J. Blood-flow velocities of the extraocular vessels in patients with high-tension and normal-tension primary open-angle glaucoma. *Am J Ophthalmol*. 1997;123:320–327.
19. Wang L, Cull GA, Piper C, Burgoyne CF, Fortune B. Anterior and posterior optic nerve head blood flow in nonhuman primate experimental glaucoma model measured by laser speckle imaging technique and microsphere method. *Invest Ophthalmol Vis Sci*. 2012;53:8303–8309.
20. Aizawa N, Nitta F, Kunikata H, et al. Laser speckle and hydrogen gas clearance measurements of optic nerve circulation in albino and pigmented rabbits with or without optic disc atrophy. *Invest Ophthalmol Vis Sci*. 2014;55:7991–7996.
21. Luft N, Wozniak PA, Aschinger GC, et al. Ocular blood flow measurements in healthy white subjects using laser speckle flowgraphy. *PLoS One*. 2016;11:e0168190.
22. Enomoto N, Anraku A, Tomita G, et al. Characterization of laser speckle flowgraphy pulse waveform parameters for the evaluation of the optic nerve head and retinal circulation. *Sci Rep*. 2021;11:6847.
23. Kiyota N, Shiga Y, Ichinohasama K, et al. The Impact of intraocular pressure elevation on optic nerve head and choroidal blood flow. *Invest Ophthalmol Vis Sci*. 2018;59:3488–3496.
24. Iwase C, Iwase T, Tomita R, et al. Changes in pulse waveforms in response to intraocular pressure elevation determined by laser speckle flowgraphy in healthy subjects. *BMC Ophthalmol*. 2021;21:303.
25. Gardiner SK, Cull G, Fortune B. Retinal vessel pulsatile characteristics associated with vascular stiffness can predict the rate of functional progression in glaucoma suspects. *Invest Ophthalmol Vis Sci*. 2023;64:30.
26. Witkowska KJ, Bata AM, Calzetti G, et al. Optic nerve head and retinal blood flow regulation during isometric exercise as assessed with laser speckle flowgraphy. *PLoS One*. 2017;12:e0184772.
27. Shiga Y, Shimura M, Asano T, et al. The influence of posture change on ocular blood flow in normal subjects, measured by laser speckle flowgraphy. *Curr Eye Res*. 2013;38:691–698.
28. Shiga Y, Sato M, Maruyama K, et al. Assessment of short-term changes in optic nerve head hemodynamics in hyperoxic conditions with laser speckle flowgraphy. *Curr Eye Res*. 2015;40:1055–1062.
29. Kiyota N, Shiga Y, Suzuki S, et al. The effect of systemic hyperoxia on optic nerve head blood flow in primary open-angle glaucoma patients. *Invest Ophthalmol Vis Sci*. 2017;58:3181–3188.
30. Kiyota N, Shiga Y, Yasuda M, et al. The optic nerve head vasoreactive response to systemic hyperoxia and visual field defect progression in open-angle glaucoma, a pilot study. *Acta Ophthalmol*. 2020;98:e747–e753.
31. Takahashi N, Kiyota N, Kunikata H, et al. Vasoreactivity of the optic nerve head, nailfold, and facial skin in response to cold provocation in normal-tension glaucoma patients. *BMC Ophthalmol*. 2023;23:316.
32. Victor RG, Leimbach WN, Jr., Seals DR, Wallin BG, Mark AL. Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension*. 1987;9:429–436.
33. Drance SM, Douglas GR, Wijsman K, Schulzer M, Britton RJ. Response of blood flow to warm and cold in normal and low-tension glaucoma patients. *Am J Ophthalmol*. 1988;105:35–39.
34. Orgul S, Kaiser HJ, Flammer J, Gasser P. Systemic blood pressure and capillary blood-cell velocity in glaucoma patients: a preliminary study. *Eur J Ophthalmol*. 1995;5:88–91.
35. Gherghel D, Hosking SL, Cunliffe IA. Abnormal systemic and ocular vascular response to temperature provocation in primary open-angle glaucoma patients: a case for autonomic failure? *Invest Ophthalmol Vis Sci*. 2004;45:3546–3554.
36. Anderson DR, Patella VM. *Automated static perimetry*. 2nd ed. St. Louis, MO: Mosby; 1999:xiv, 363 p.
37. Sugiyama T, Araie M, Riva CE, Schmetterer L, Orgul S. Use of laser speckle flowgraphy in ocular blood flow research. *Acta Ophthalmol*. 2010;88:723–729.
38. Masai S, Ishida K, Anraku A, Takumi T, Tomita G. Pulse waveform analysis of the ocular blood flow using laser speckle flowgraphy before and after glaucoma treatment. *J Ophthalmol*. 2019;2019:1980493.
39. Takeshima S, Higashide T, Kimura M, et al. Effects of trabeculectomy on waveform changes of laser speckle flowgraphy in open angle glaucoma. *Invest Ophthalmol Vis Sci*. 2019;60:677–684.
40. Pourcelot L. [Indications of Doppler's ultrasonography in the study of peripheral vessels [Article in French]. *Rev Prat*. 1975;25:4671–4680.
41. Bude RO, Rubin JM. Relationship between the resistive index and vascular compliance and resistance. *Radiology*. 1999;211:411–417.
42. Weir CB, Jan A. *BMI classification percentile and cut off points*. Treasure Island, FL: StatPearls; 2025.

43. Alarcon-Martinez L, Shiga Y, Villafranca-Baughman D, et al. Neurovascular dysfunction in glaucoma. *Prog Retin Eye Res*. 2023;97:101217.
44. Liang Y, Downs JC, Fortune B, Cull G, Cioffi GA, Wang L. Impact of systemic blood pressure on the relationship between intraocular pressure and blood flow in the optic nerve head of nonhuman primates. *Invest Ophthalmol Vis Sci*. 2009;50:2154–2160.
45. Wang L, Cull GA, Fortune B. Optic nerve head blood flow response to reduced ocular perfusion pressure by alteration of either the blood pressure or intraocular pressure. *Curr Eye Res*. 2015;40:359–367.
46. Tielsch JM, Katz J, Sommer A, Quigley HA, Javitt JC. Hypertension, perfusion pressure, and primary open-angle glaucoma. A population-based assessment. *Arch Ophthalmol*. 1995;113:216–221.
47. Bonomi L, Marchini G, Marraffa M, Bernardi P, Morbio R, Varotto A. Vascular risk factors for primary open angle glaucoma: the Egna-Neumarkt study. *Ophthalmology*. 2000;107:1287–1293.
48. Memarzadeh F, Ying-Lai M, Chung J, Azen SP, Varma R, Los Angeles Latino Eye Study Group. Blood pressure, perfusion pressure, and open-angle glaucoma: the Los Angeles Latino Eye Study. *Invest Ophthalmol Vis Sci*. 2010;51:2872–2877.
49. Zheng Y, Wong TY, Mitchell P, Friedman DS, He M, Aung T. Distribution of ocular perfusion pressure and its relationship with open-angle glaucoma: the Singapore Malay Eye Study. *Invest Ophthalmol Vis Sci*. 2010;51:3399–3404.
50. Kim H, Choi B. Nonlinear relationship between blood pressure and glaucoma in US adults. *Am J Hypertens*. 2019;32:308–316.
51. Lee K, Yang H, Kim JY, Seong GJ, Kim CY, Bae HW. Risk factors associated with structural progression in normal-tension glaucoma: intraocular pressure, systemic blood pressure, and myopia. *Invest Ophthalmol Vis Sci*. 2020;61:35.
52. Jammal AA, Berchuck SI, Mariottoni EB, Tanna AP, Costa VP, Medeiros FA. Blood pressure and glaucomatous progression in a large clinical population. *Ophthalmology*. 2022;129:161–170.
53. Graham SL, Drance SM, Wijsman K, Douglas GR, Mikelberg FS. Ambulatory blood pressure monitoring in glaucoma. The nocturnal dip. *Ophthalmology*. 1995;102:61–69.
54. Kwon J, Jo YH, Jeong D, Shon K, Kook MS. Baseline systolic versus diastolic blood pressure dip and subsequent visual field progression in normal-tension glaucoma. *Ophthalmology*. 2019;126:967–979.
55. Bae KH, Lee Y, Go HY, Kim SJ, Lee SW. The relationship between cold hypersensitivity in the hands and feet and health-related quality of life in Koreans: a nationwide population survey. *Evid Based Complement Alternat Med*. 2019;2019:6217036.
56. Bevan JA, Hwa JJ. Myogenic tone and cerebral vascular autoregulation: the role of a stretch-dependent mechanism. *Ann Biomed Eng*. 1985;13:281–286.
57. Wright A, Fisher PL, Baker N, O'Rourke L, Cherry MG. Perfectionism, depression and anxiety in chronic fatigue syndrome: a systematic review. *J Psychosom Res*. 2021;140:110322.
58. Wunderlich K, Zimmerman C, Gutmann H, Teuchner B, Flammer J, Drewe J. Vasospastic persons exhibit differential expression of ABC-transport proteins. *Mol Vis*. 2003;9:756–761.
59. Flammer J, Konieczka K. The discovery of the Flammer syndrome: a historical and personal perspective. *EPMA J*. 2017;8:75–97.
60. Grybauskas A, Koga T, Kuprys PV, et al. ABCB1 transporter and Toll-like receptor 4 in trabecular meshwork cells. *Mol Vis*. 2015;21:201–212.
61. Lin DS, Lo HY, Yu AL, Lee JK, Chien KL. Mortality risk in patients with underweight or obesity with peripheral artery disease: a meta-analysis including 5,735,578 individuals. *Int J Obes (Lond)*. 2022;46:1425–1434.
62. Wells JC, Sawaya AL, Wibaek R, et al. The double burden of malnutrition: aetiological pathways and consequences for health. *Lancet*. 2020;395:75–88.
63. Leung DYL, Tham CC. Normal-tension glaucoma: current concepts and approaches - a review. *Clin Exp Ophthalmol*. 2022;50:247–259.
64. Trivli A, Koliarakis I, Terzidou C, et al. Normal-tension glaucoma: pathogenesis and genetics. *Exp Ther Med*. 2019;17:563–574.
65. Pasquale LR. Vascular and autonomic dysregulation in primary open-angle glaucoma. *Curr Opin Ophthalmol*. 2016;27:94–101.