# Toward Genetic Screening for Glaucoma

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*DOI:*
10.33612/diss.190720835

# CHAPTER 6

## Autonomic dysfunction and blood pressure in glaucoma patients: The Lifelines Cohort study

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This chapter was originally published in Investigative Ophthalmology and Visual Science. 2020

https://doi.org/10.1167/iovs.61.11.25

# Abstract

**Purpose**: We investigated glaucoma's relationship with autonomic dysfunction (heart rate variability [HRV]) and blood pressure (BP) related measures.

**Method**: Glaucoma was defined using a questionnaire-based algorithm in 86,841 Lifelines participants. Baseline HRV (root mean square of successive differences [RMSSD]) was calculated from resting electrocardiograms; systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP), and pulse pressure (PP) were oscillometric-based measurements. We used a generalized linear mixed model, adjusted for age, age$^2$, sex, body mass index, and familial relationships to assess the relationship of baseline HRV and BP (continuous and quartiles), hypertension and antihypertensive medication with glaucoma at follow up (median 3.8 years).

**Result**: The OR (95% confidence interval) of glaucoma was 0.95 (0.92-0.99) per unit increase in log-transformed RMSSD (in ms), indicating that autonomous dysfunction (low HRV) is associated with a higher risk of glaucoma. Per 10 mmHg increase in BP, we found ORs of 1.03 (1.01-1.05, p=0.015) for SBP, 1.01 (0.97-1.05, p=0.55) for DBP, 1.03 (1.00-1.06, p=0.083) for MAP, and 1.04 (1.01-1.07, p=0.006) for PP. The OR for the lowest vs. highest RMSSD quartile was 1.15 (1.05-1.27, p=0.003). The ORs for the highest vs. second quartile were 1.09 (0.99-1.19, p=0.091) for SBP and 1.13 (1.02-1.24, p=0.015) for PP. Glaucoma was more common among hypertensives (1.25 [1.16-1.35], p<0.001), those using ACE inhibitors (1.35 [1.18-1.55], p<0.001), or calcium-channel blockers (1.19 [1.01-1.40], p=0.039).

**Conclusion**: Low HRV, high SBP, high PP, and hypertension were associated with glaucoma. Longitudinal studies may elucidate if autonomic dysregulation and high BP also predict glaucoma incidence.


**Keywords**: Glaucoma, blood pressure, autonomic dysfunction, heart rate variability, hypertension, antihypertensive medication

# Background

Glaucoma is a group of complex ocular diseases that is accompanied by progressive damage to the optic nerve. Primary open-angle glaucoma (POAG) is the most common subtype in the western world and Africa. Mechanical,[1,2] vascular,[3,4] genetic,[5–7] and recently autonomic nervous function[8] theories have been proposed to explain the mechanisms behind glaucoma. The mechanical theory refers to axonal damage of the optic nerve that is directly related to an elevated intraocular pressure (IOP), the most important risk factor for glaucoma, or possibly to an elevated pressure difference across the lamina cribrosa (IOP versus intracranial pressure). The vascular theory proposes ischemia due to insufficient blood supply to the optic nerve head as a possible mechanism for optic nerve damage.[9] This has been linked to autonomic dysfunction, hypertension (HTN), and low blood pressure (BP). Current results are conflicting, and partially due to that, controversial.

By evaluating 24-hour BP measurements, prior studies suggest nocturnal hypotension may be a contributing factor for anterior ischemic optic neuropathy and glaucoma.[10,11] However, abnormalities in ocular blood flow occur at both high[12,13] and low[14] BP in glaucoma, yielding a J- or U-shaped[15] relationship between BP and glaucoma. This apparent controversy has led to a new hypothesis, the involvement of autonomic nervous dysfunction. Studies speculate that autonomic dysfunction affects susceptibility of the optic nerve to BP changes, and is most prominent in normal-tension glaucoma (NTG) (a glaucoma subtype where the most important risk factor, an elevated IOP, is lacking).[16,17] Autonomic dysfunction involvement in glaucoma pathogenesis is further supported by a cold provocation test, where glaucomatous individuals had greater sympathetic innervation.[17]

Compared to normal subjects, glaucoma patients exhibit blood flow abnormalities in vessels of the optic nerve head,[18] retina,[18] retrobulbar tissue,[19] and choroid.[18,20,21] With limited information regarding autonomic dysfunction involvement, several researchers proposed that ocular vessel disturbances are linked to plasma levels of endothelin-1,[22] systemic blood pressure, [23,24] and vasospasm.[25] Autonomic function reflects the effect of parasympathetic nervous system activity on the heart. Autoregulation is a related mechanism found in the nervous system, which aims to maintain a stable

blood flow despite changes in blood pressure, this also includes changes in intraocular pressure.[16,19]

There is a paucity of reports investigating the role of autonomic dysfunction in glaucoma. Dysfunctional autonomic control was reported to lead to an unstable blood supply, related to a reduced perfusion pressure in glaucomatous eyes.[17,26,27] In fact, a low heart rate variability (HRV) was associated with a faster rate of central visual field loss in glaucoma.[28] HRV is a commonly used proxy measurement for the autonomic modulation of the heart.[29]

Regarding glaucomatous damage and systemic BP, there are conflicting reports. The Rotterdam[30] and Beaver Dam[31] Eye Studies reported a higher risk of POAG with high BP, whereas the Barbados Eye Study[32] has reported the opposite. Alternatively, two US studies found a U-shaped relationship, highlighting those with either low or high BP may be at greater risk for glaucoma.[12,15]

In this study, we explored associations of HRV and BP with glaucoma in the Lifelines study, a large cohort from the Northern Netherlands, representative of the general population. We also studied the role of antihypertensive drugs in the relationship between glaucoma and BP. We hypothesized that participants with low HRV values, as well as those with high and low BP measurements, have higher odds of glaucoma.

# Methodology

## Ethical approval

The Lifelines data collection was approved by the medical ethics committee of the University Medical Center Groningen. Written informed consent was obtained from all participants and the data collection was conducted in accordance with the tenets of the Declaration of Helsinki.

## Study design and sample

We used phenotypic data from the Lifelines cohort study and Biobank, a multi-disciplinary prospective population-based cohort study of the Northern Netherlands. This cohort employs a broad range of investigative procedures in assessing the socio-demographic, biomedical, physical, behavioral and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics. Lifelines participants were asked to invite their family members (i.e., partners, parents, and children), that realized the formation of a three-generation family study. In the current study, 17,379 families, with an average family size of 3.32, and 29,082 singletons (i.e., no family members included) were included. A questionnaire on diagnosis and treatment of eye conditions, also including the National Eye Institute Visual Function Questionnaire (NEI-VFQ-25),[33] was administered to all participants (n=110,759) aged 18 and older during the first follow-up visit between 2014-2017, after a median period of 3.8 years after baseline. Further details on design and data collection approaches used by Lifelines are described elsewhere.[34,35]

## Measurement and definition of glaucoma

We used a previously described algorithm for defining glaucoma in Lifelines,[36] which was based on self-report of glaucoma diagnosis and treatment in combination with the NEI-VFQ-25.[33] For details see Neustaeter, et al (2020).[36] In short, this algorithm classifies participants as definite, probable, or possible glaucoma cases, or as healthy. Definite glaucoma cases were those who reported incisional surgery for glaucoma. These cases were also used to define a glaucoma-specific complaints pattern within the NEI-VFQ-25. Probable cases were those who self-reported glaucoma (including the use of IOP-lowering medication and a history of glaucoma laser treatment)

together with glaucoma specific complaints above a certain threshold. Possible cases were those who either self-reported glaucoma or had glaucoma-specific complaints. As such, the algorithm includes both participants who were aware (definite, probable, and possible glaucoma by self-report) and unaware (possible glaucoma by complaint) of their disease status. The algorithm was applied to participants in the first follow-up visit with available eye questionnaire data. In this study, unless specified otherwise, the term 'glaucoma' refers to the definite, probable and possible cases combined. Aiming for primary glaucoma, the proxy excluded participants with self-reported macular degeneration, or (laser)surgery for diabetes or retinal detachment. The questionnaire did not allow for discrimination between open-angle and narrow-angle glaucoma. Based on the prevalence ratio of open-angle and narrow-angle glaucoma in the western world, however, the majority of cases will have open-angle glaucoma.[37,38]

## Predictor variables and covariates

Predictor variables were measured during the baseline visit from Lifelines. HRV was represented as the log of the root mean square of successive differences (RMSSD) in ms, which quantifies normal beat-to-beat variance in heart rate, and is used to estimate the vagally-mediated modulation of the heart.[39] RMSSD was calculated with a ten second resting electrocardiogram (ECG) reading (see Tegegne et al[40] and Munoz et al[41] for details). We previously demonstrated the validity of RMSSD based on an ultra-short (10-second) ECG recording as a measure of HRV by comparing it to the current gold standard recording of 4-5 min.[41,42] For BP, ten consecutive BP measurements were obtained in a supine position using an automated oscillometric method (Dinamap, PRO 100V2); the last three measurements were averaged to yield SBP, DBP, and MAP values. MAP was provided by the device; PP was calculated as: PP = SBP-DBP.[43]

High BP was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg, and HTN as high BP and/or use of antihypertensive medication. The generic names and ATC-Codes (Anatomical Therapeutic Chemical) of all common antihypertensive medications were obtained; angiotensin-converting enzyme (ACE) inhibitors are assigned in ATC code C09A, calcium-channel blockers in ATC code C08, diuretics in ATC code C03, and beta-antagonists in ATC code C07. Effects of (i) any antihypertensive medication use, (ii) number of antihypertensive medications, and (iii) different antihypertensive medication classes were investigated.

Based on evidence of associations with glaucoma, data analyses were adjusted for the effects of age, age$^2$, sex, and BMI. [37,44–46] BMI was calculated as weight in kilo-

grams divided by the square of height in meter (kg/m$^2$); from baseline assessment.

## Data analysis

Our analyses estimated the association of HRV and BP-related measurements at baseline with glaucoma at follow-up, with a median (IQR) interval of 3.8 (3.1-4.5) years. We separately investigated the association of HTN and antihypertensive medication use with glaucoma. To test associations, we applied generalized linear mixed models (GLMM), in order to adjust for family membership.

First, we combined the three glaucoma classes. Next, we performed the analyses after excluding possible glaucoma cases, as there is less certainty in this category. Finally, we performed the analyses separately for those who were aware of having glaucoma and for those who were not aware (for definitions, see subsection Measurement and definition of glaucoma). The rationale behind this separation is that clinical glaucoma cases (aware) differ from glaucoma cases captured during glaucoma screening (unaware); in the latter group NTG dominates,[47,48] and as such, unawareness may serve as a surrogate for NTG in the current study. We used GLMM to test the association of HRV, BP-related measurements, HTN, and antihypertensive medications with glaucoma. A separate model was built for each predictor, except for the classes of antihypertensive medications, which were put together in one model. Measurements were analysed as continuous traits and as quartiles, to accommodate potential non-linear effects. In all models, age, age$^2$, sex, and BMI were used as covariates. In models investigating effects of BP variables, we additionally investigated interaction by antihypertensive medication status followed by a stratified analysis. In models investigating effects of the various antihypertensive medication classes, we additionally adjusted for the different BP measurements.

In the mixed models, we assumed individuals as level-1 observation units clustered by family membership (level-2). Fitting the logit link function, the odds of glaucoma for individual i, (i=1,2,3,...n) in a family j (j=1,2,3,...m) was estimated as follows:

$$log(p_{ij}/1\text{-}p_{ij}) = \mu + \beta_i X_i + a_j + e_{ij}, \text{ with } a_j \sim N(0,\sigma_j^2) \text{ and } e_{ij} \sim N(0,\sigma_e^2)$$

Where, $p_{ij}$ = probability of glaucoma status, μ = overall mean, $\beta i$ = fixed effects, $X_i$= prediction variables and covariates (age, age$^2$, sex, BMI), $a$ = random effect due to the family relationship, and $e$= random error.

Data reorganization and cleaning, and descriptive analyses were performed in R package version 3.5.3 and SPSS version 23. GLMM analyses were executed in ASReml, version 4.1. Statistical significance was set at a P-value of 0.05 or less.

# Results

Of the 110,759 participants invited for the follow-up assessment, 88,584 (80%) had eye-related data. After excluding 1,743 participants who underwent laser treatment or surgery for diabetes or retinal detachment, or who had AMD (see Methods section), the total number of study participants included in the analyses was 86,841. The number of definite, probable and possible glaucoma cases were 102 (0.11%), 348 (0.40%), and 3,388 (3.90%), respectively. Of the possible cases, 1,585 (1.82%) were possible by self-report and 1,803 (2.07%) possible by complaint. Table 1 shows the characteristics of the study population and the distribution of HRV and BP data between cases and controls.

**Table 1: Population characteristics and distribution of HRV and BP data stratified by glaucoma status**

| Variables | All cases (n=3,838) | *Possible* cases excluded (n=450) | Controls (n=83,003) |
|---|---|---|---|
| Age in years, mean (SD) | 53.4 (12.7) | 58.4 (12.1) | 46.1 (12.6) |
| Sex (females), n (%) | 2,461 (64.1) | 268 (59.6) | 48,921 (58.9) |
| BMI (kg/m$^2$), median (IQR) | 26.2 (23.8-29.1) | 26.1 (23.9-29.3) | 25.4 (23.1-28.1) |
| HRV (lnRMSSD, milliseconds), median (IQR) | 3.1 (2.6-3.5) | 3.0 (2.6-3.5) | 3.3 (2.8-3.7) |
| SBP (mmHg), median (IQR) | 127(117-140) | 130 (120-142) | 124 (115-135) |
| DBP (mmHg), median (IQR) | 74 (68-81) | 74 (68-82) | 73 (67-80) |
| MAP (mmHg), median (IQR) | 94 (88-102) | 95 (89-103) | 92 (87-99) |
| PP (mmHg), median (IQR) | 53 (45-62) | 54 (46-64.3) | 50 (44-58) |
| High BP, n (%) | 1,018 (26.6) | 135 (30) | 15,410 (18.6) |
| HTN, n (%) | 1,568 (40.9) | 215 (47.8) | 21,609 (26.0) |

BP: blood pressure; SD: standard deviation; IQR: inter-quartile range; BMI: body mass index; HRV: heart rate variability; lnRMSSD: logarithm of root mean square of successive differences between normal-to-normal intervals; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; HTN: hypertension. High BP was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg and HTN as high BP and/or use of antihypertensive medication.

We confirmed the well-known relationship between glaucoma and age, i.e., compared to individuals 55 years, we found a significant positive relationship between glaucoma and age across all age groups (Supplementary Table S1).

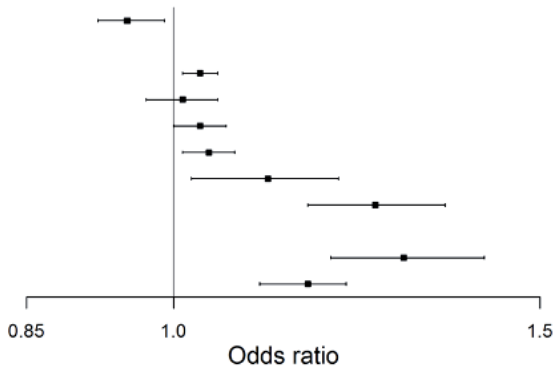
## All glaucoma cases combined

We found a negative relationship between glaucoma and baseline HRV, represented by RMSSD: the OR (95% confidence interval [CI]) of glaucoma was 0.95 (0.92-0.99), p=0.005, per unit increase in log-transformed RMSSD (with RMSSD in ms), which means that autonomous dysfunction (low HRV) is associated with higher risk of glaucoma; Figure 1A). Alternatively, most BP related measurements, including SBP,

PP, high BP, HTN, and the use of antihypertensive medication, showed a significant positive association with glaucoma, see Figure 1A for the results of the continuous trait analysis. After stratifying by type of medication, only ACE inhibitors and calcium-channel blockers were statistically significant (Table 2), showing a harmful effect. These medication effects did not change when adjusting for BP measurements (Supplementary Table S2).

**A**

| Risk factors | Odds ratio (95%CI) | p-value |
|---|---|---|
| **HRV** | | |
| lnRMSSD | 0.95 (0.92-0.99) | p=0.005 |
| **BP measures** | | |
| SBP per 10 mmHg | 1.03 (1.01-1.05) | p=0.015 |
| DBP per 10 mmHg | 1.01 (0.97-1.05) | p=0.550 |
| MAP per 10 mmHg | 1.03 (1.00-1.06) | p=0.083 |
| PP per 10 mmHg | 1.04 (1.01-1.07) | p=0.006 |
| High BP | 1.11 (1.02-1.20) | p=0.013 |
| HTN | 1.25 (1.16-1.35) | p<0.001 |
| **Medication use** | | |
| Any anti-HTN medication use | 1.29 (1.19-1.41) | p<0.001 |
| Number of anti-HTN medicines | 1.16 (1.10-1.21) | p<0.001 |




**B**

| | | |
|---|---|---|
| **HRV** | | |
| lnRMSSD | 1.01 (0.91-1.11) | p=0.882 |
| **BP measures** | | |
| SBP per 10 mmHg | 1.02 (0.96-1.08) | p=0.458 |
| DBP per 10 mmHg | 1.02 (0.92-1.13) | p=0.710 |
| MAP per 10 mmHg | 1.03 (0.95-1.13) | p=0.470 |
| PP per 10 mmHg | 1.03 (0.95-1.11) | p=0.484 |
| High BP | 1.00 (0.81-1.24) | p=0.979 |
| HTN | 1.18 (0.96-1.45) | p=0.116 |
| **Medication use** | | |
| Any anti-HTN medication use | 1.20 (0.95-1.51) | p=0.108 |
| Number of anti-HTN medicines | 1.13 (1.01-1.27) | p=0.046 |

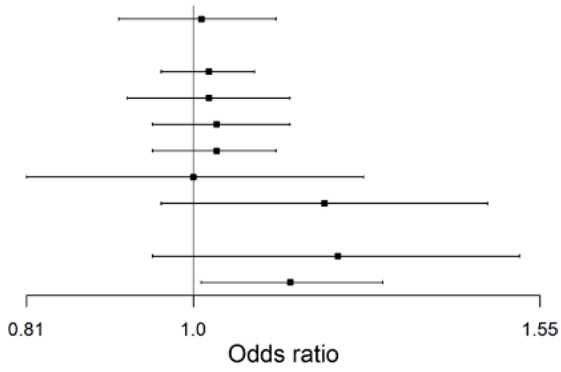



**Figure 1** Association of glaucoma with HRV and BP-related measurements before (A) and after (B) excluding *possible* glaucoma cases

CI: confidence interval; HRV: heart rate variability; BP: blood pressure; lnRMSSD: logarithm of root mean square of successive differences between normal-to-normal intervals; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; HTN: hypertension. High BP was defined as SBP ≥140 mmHg and/or DBP ≥ 90 mmHg and HTN as high BP and/or use of antihypertensive medication. Generalized linear mixed model (GLMM), adjusted for age, age2, sex, and body mass index (BMI). Number of anti-HTN medications (combinations) was modeled as a continuous variable.

**Table 2: Association of glaucoma with antihypertensive medication use**

| | All cases | | | *Possible* cases excluded | | |
|---|---|---|---|---|---|---|
| Antihypertensive medication class | Glaucoma cases/ controls | OR (95%CI) | p-value | Glaucoma cases/ controls | OR (95%CI) | p-value |
| Beta-antagonists | 403/4,613 | 1.06 (0.94-1.19) | 0.319 | 60/4,613 | 1.06 (0.78-1.42) | 0.695 |
| Diuretics | 292/2,858 | 1.09 (0.95-1.25) | 0.229 | 41/2,858 | 0.99 (0.69-1.42) | 0.982 |
| ACE inhibitors | 299/2,908 | 1.35 (1.18-1.55) | <0.001 | 50/2908 | 1.48 (1.07-2.06) | 0.017 |
| Angiotensin II blockers | 187/1,943 | 1.16 (0.98-1.37) | 0.080 | 22/1,943 | 0.86 (0.54-1.35) | 0.515 |
| Calcium-channel blockers | 191/1734 | 1.19 (1.01-1.40) | 0.039 | 30/1734 | 1.16 (0.78-1.73) | 0.443 |
| Other medication use | 25/220 | 1.29 (0.84-2.00) | 0.243 | 5/220 | 1.97 (0.79-4.92) | 0.142 |

*OR: odds ratio; CI: confidence interval; ACE: angiotensin converting enzyme. Models were adjusted for age, age$^2$, sex and body mass index (BMI). Other medications: renin inhibitors, alpha-beta blockers, alpha blockers, alpha agonists, antiadrenergics, serotonin antagonists, potassium channel enhancers, and smooth muscle relaxants.*

When analyzed in quartiles (Table 3), participants in the lowest quartile of log(RMSSD) demonstrated a higher odds ratio for glaucoma (OR=1.15, p=0.003). For BP-related measurements, PP was significantly associated with glaucoma; participants in the highest quartile of PP were 1.13 times more likely to have glaucoma (p=0.015), compared to those in the second quartile.

Neither continuous nor categorical BP analyses showed significant interactions by antihypertensive medication status (data not shown), but when stratified by antihypertensive medication status, more clearly positive associations between BP and glaucoma in those not taking antihypertensive medication was observed (Supplementary Table S3).

Table 4 shows the effect of the various risk factors for participants who were aware of their glaucoma status (definite, probable, and possible by self-report; n=2035

cases) versus those who were not (possible by complaint; n=1803; a NTG surrogate as described in the Methods section). The risk of high BP was observed only among participants who were aware of their glaucoma status. In contrast, HRV was significantly associated with glaucoma in both groups, as was the effect of antihypertensive medication. A similar pattern was seen in the corresponding quartile analysis (Table 5).

## After exclusion of possible glaucoma cases

We repeated association testing between HRV, BP, high BP, HTN, and medication use with glaucoma status after excluding the possible cases. Although the direction of the effects remained unchanged, excluding 88% (n=3,388 possible cases) of the 3,838 total glaucoma cases resulted in low power to detect significant differences between cases and controls (Figure 1B, Table 3).

**Table 3:** Odds ratios of glaucoma at different categories of HRV and BP-related measurements (quartiles)

| Predictor | | All cases | | | *Possible* cases excluded | | |
|---|---|---|---|---|---|---|---|
| | | Glaucoma cases/ controls | OR (95%CI) | p-value | Glaucoma cases/ controls | OR (95%CI) | p-value |
| HRV | ≤2.85 | 1,272/19,905 | 1.15 (1.05-1.27) | 0.003 | 159/19,905 | 1.07 (0.82-1.41) | 0.610 |
| (lnRMS-SD) | 2.86-3.30 | 946/19,968 | 1.05 (0.95-1.16) | 0.312 | 113/19,968 | 1.09 (0.82-1.45) | 0.561 |
| | 3.31-3.77 | 796/20,124 | 1.03 (0.93-1.14) | 0.603 | 91/20,124 | 1.14 (0.85-1.54) | 0.384 |
| | ≥3.78 | 645/20,267 | Reference | - | 62/20,267 | Reference | -- |
| SBP | ≤115 | 805/21,907 | 0.99 (0.89-1.09) | 0.818 | 70/21907 | 0.84 (0.61-1.15) | 0.284 |
| (mmHg) | 116-124 | 826/20,347 | Reference | -- | 94/20,347 | Reference | -- |
| | 125-135 | 965/21,019 | 1.02 (0.93-1.12) | 0.689 | 131/21,019 | 1.12 (0.85-1.46) | 0.417 |
| | ≥136 | 1,238/19,696 | 1.09 (0.99-1.19) | 0.091 | 155/19,696 | 0.91 (0.70-1.20) | 0.515 |
| DBP | ≤67 | 879/21,456 | 0.98 (0.89-1.08) | 0.450 | 96/21,456 | 0.93 (0.71-1.21) | 0.575 |
| (mmHg) | 68-73 | 1027/22,166 | Reference | -- | 128/22,166 | Reference | -- |
| | 74-80 | 916/20,337 | 0.91 (0.83-1.00) | 0.048 | 97/20,337 | 0.74 (0.57-0.97) | 0.03 |
| | ≥81 | 1012/19,010 | 1.01 (0.92-1.11) | 0.810 | 129/19,010 | 0.95 (0.74-1.22) | 0.681 |
| MAP | ≤87 | 719/21,191 | 0.97 (0.88-1.07) | 0.660 | 55/21,191 | 0.68 (0.50-0.91) | 0.008 |
| (mmHg) | 88-92 | 774/21,863 | Reference | -- | 92/21,863 | Reference | -- |
| | 93-99 | 949/20,023 | 0.97 (0.88-1.07) | 0.528 | 119/20,023 | 0.81 (0.62-1.06) | 0.118 |
| | ≥100 | 1,391/19,885 | 1.01 (0.92-1.11) | 0.849 | 184/19,885 | 0.78 (0.61-1.00) | 0.05 |
| PP | ≤44 | 842/22,795 | 1.00 (0.91-1.11) | 0.968 | 84/22,795 | 0.90 (0.66-1.21) | 0.483 |
| (mmHg) | 45-50 | 757/18,987 | Reference | -- | 88/18,987 | Reference | -- |
| | 51-58 | 890/20,619 | 1.00 (0.91-1.11) | 0.936 | 108/20,619 | 0.97 (0.73-1.29) | 0.841 |
| | ≥59 | 1,315/20,568 | 1.13 (1.02-1.24) | 0.015 | 170/20,568 | 0.94 (0.72-1.24) | 0.674 |

*OR: odds ratio; CI: confidence interval; HRV: heart rate variability; BP: blood pressure; lnRMSSD: logarithm of root mean square of successive differences between normal-to-normal intervals; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure. Generalized linear mixed model (GLMM), adjusted for age, age$^2$, sex, and body mass index (BMI).*

**Table 4:** Association of glaucoma with HRV, BP-related measurements and antihypertensive medication use for cases who were *aware* of their glaucoma status versus *unaware*

| Variables | Glaucoma cases aware of their glaucoma status/controls (2,035/83,003) | | Glaucoma cases unaware of their glaucoma status/controls (1,803/83,003) | |
|---|---|---|---|---|
| | **OR (95%CI)** | **P-value** | **OR (95%CI)** | **P-value** |
| **HRV** | | | | |
| lnRMSSD | 0.95 (0.91-0.99 | 0.045 | 0.95 (0.90-1.00) | 0.044 |
| **BP measurements** | | | | |
| SBP per 10 mm Hg | 1.08 (1.04-1.11) | <0.001 | 0.98 (0.95-1.01) | 0.173 |
| DBP per 10 mm Hg | 1.07 (1.02-1.12) | 0.008 | 0.95 (0.90-1.01) | 0.078 |
| MAP per 10 mm Hg | 1.10 (1.05-1.14) | <0.001 | 0.96 (0.92-1.01) | 0.107 |
| PP per 10 mm Hg | 1.09 (1.05-1.13) | <0.001 | 0.99 (0.95-1.03) | 0.681 |
| High BP | 1.21 (1.09-1.35) | <0.001 | 0.97 (0.86-1.10) | 0.659 |
| HTN | 1.33 (1.20-1.47) | <0.001 | 1.16 (1.04-1.29) | 0.008 |
| **Medication Use** | | | | |
| Any anti-HTN use | 1.25 (1.11-1.40) | <0.001 | 1.35 (1.19-1.53) | <0.001 |
| Number of anti-HTN medications | 1.13 (1.06-1.21) | <0.001 | 1.18 (1.1-1.26) | <0.001 |

*OR: odds ratio; CI: confidence interval; HRV: heart rate variability; BP: blood pressure; lnRMSSD: logarithm of root mean square of successive differences between normal-to-normal intervals; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; HTN: Hypertension. Generalized linear mixed model (GLMM), adjusted for age, age2, sex, and body mass index (BMI). Number of anti-HTN medications (combinations) was modeled as a continuous variable.*

**Table 5:** Association of glaucoma with HRV and BP-related measurements for cases who were aware of their glaucoma status versus unaware (quartiles)

| | | Glaucoma cases aware of their glaucoma status/controls (2,035/83,003) | | Glaucoma cases unaware of their glaucoma status/controls (1,803/83,003) | |
|---|---|---|---|---|---|
| **Variables (quartile)** | | **OR (95% CI)** | **P-value** | **OR (95% CI)** | **P-value** |
| **HRV** | | | | | |
| LnRMSSD (ms) | ≤2.85 | 1.14 (1.00-1.29) | 0.043 | 1.16 (1.01-1.33) | 0.031 |
| | 2.86-3.30 | 1.02 (0.89-1.17) | 0.749 | 1.09 (0.94-1.25) | 0.250 |
| | 3.31-3.77 | 1.02 (0.88-1.17) | 0.787 | 1.04 (0.90-1.20) | 0.610 |
| | ≥3.78 | Reference | -- | Reference | -- |
| **BP** | | | | | |
| SBP (mmHg) | ≤115 | 0.92 (0.79-1.06) | 0.262 | 1.05 (0.91-1.20) | 0.515 |
| | 116-124 | Reference | -- | Reference | -- |
| | 125-135 | 1.08 (0.94-1.23) | 0.258 | 0.96 (0.84-1.11) | 0.596 |
| | ≥136 | 1.21 (1.06-1.38) | 0.003 | 0.94 (0.82-1.08) | 0.412 |
| DBP (mmHg) | ≤67 | 0.92 (0.80-1.05) | 0.2 | 1.04 (0.91-1.19) | 0.548 |
| | 68-73 | Reference | -- | Reference | -- |
| | 74-80 | 0.91 (0.80-1.03) | 0.149 | 0.91 (0.80-1.04) | 0.167 |
| | ≥81 | 1.07 (0.94-1.20) | 0.3 | 0.93 (0.82-1.07) | 0.327 |
| MAP (mmHg) | ≤87 | 0.86 (0.75-0.99) | 0.043 | 1.07 (0.93-1.22) | 0.342 |
| | 88-92 | Reference | -- | Reference | -- |
| | 93-99 | 0.93 (0.81-1.06) | 0.271 | 1.02 (0.89-1.17) | 0.779 |
| | ≥100 | 1.11 (0.98-1.26) | 0.093 | 0.87 (0.76-1.00) | 0.058 |
| PP (mmHg) | ≤44 | 0.90 (0.78-1.04) | 0.149 | 1.11 (0.97-1.28) | 0.138 |
| | 45-50 | Reference | -- | Reference | -- |
| | 51-58 | 1.03 (0.89-1.18) | 0.667 | 0.98 (0.85-1.14) | 0.810 |
| | ≥59 | 1.18 (1.03-1.34) | 0.011 | 1.07 (0.93-1.24) | 0.317 |

OR: odds ratio; CI: confidence interval; HRV: heart rate variability; BP: blood pressure; lnRMSSD: logarithm of root mean square of successive differences between normal-to-normal intervals; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure. Generalized linear mixed model (GLMM), adjusted for age, age$^2$, sex, and body mass index (BMI).

# Discussion

Our findings show that HRV has a negative relationship with glaucoma, whereas BP-related measurements, including HTN, high BP, and antihypertensive medication use (especially ACE inhibitors and calcium-channel blockers), have a positive association with glaucoma prevalence.

Our findings support previous work where lower HRV was associated with higher prevalence and faster rates of central visual field loss in NTG.[28,49] Our findings regarding HRV also agree with reports proposing that autonomic dysfunction contributes to the pathophysiology of glaucoma. For example, choroidal parasympathetic innervation increases choroidal blood flow, while sympathetic innervation has the opposite effect. Given the choroid contributes to the prelaminar blood supply of the optic nerve head,[50] and predominant sympathetic innervation over-constricts microvessels nourishing the optic nerve head, the inability to maintain a constant blood supply may promote the occurrence of the disease.[27,51–53] The results of our NTG surrogate subanalysis also strengthen previous evidence that autonomic dysregulation plays a role in glaucoma.[28,49] HRV was at least as significant in the unaware group than it was in the aware group at similar sample size.

Our results showed an increasing trend of glaucoma at high BP. These findings support previous population-based cross-sectional studies where increases in BP are associated with increased odds of glaucoma. This was found in the Rotterdam Eye study,[54] the Beaver Dam Eye study,[31] the Blue Mountain Eye study,[13] and the Egna-Neumarkt Eye study.[55] Our findings also agree with the Los Angeles Latino Eye study with respect to SBP and MAP, but not for DBP (see next paragraph). Our findings contradict the Barbados Eye study in terms of SBP; they reported an OR for glaucoma of 0.91 (0.84-1.0) per 10 mmHg increase in SBP.[32] Our findings are in line with a general practitioner study from the UK, where glaucoma was more common among hypertensive individuals (OR 1.29 [1.23-1.36], $p<0.001$).[56] Furthermore, a meta-analysis of 27 observational studies reported an overall relative risk (RR) of 1.16 (1.05-1.28, $p<0.05$) of glaucoma among hypertensive individuals.[57]

In the current study, we did not find a J- or U-shaped relationship (higher risk at both extremes) between glaucoma and BP. In the NHANES cross-sectional study, Kim et al reported a U-shaped relationship between SBP and glaucoma among individ-

uals without antihypertensive medications, but not among those taking medication.[15] However, this finding contradicts the notion that overtreatment of HTN (excessive BP lowering due to antihypertensive medication) may reduce ocular blood flow, with ischemia as a consequence.[9] Similarly, in the Los Angeles Latino Eye Study (LALES), the U-shaped relationship came from high SBP (SBP >170 mmHg, OR 2.1 [1.1-4.0]) and low DBP (DBP ≤ 60, OR 1.9 [1.1-3.0]).[12] Possible explanations for the apparent discrepancy between NHANES and LALES and our study are differences in ethnicity, model building, and glaucoma definition. The reported proportion of Caucasian ethnicity was 76% and 0% in NHANES[15] and LALES,[12] respectively, vs. 98.3% in our study. Furthermore, the final statistical model in LALES[12] was corrected for the effects of IOP, a history of high BP, and use of antihypertensive medication, whereas the model used in NHANES was adjusted for antihypertensive medication but not for IOP. We did not adjust for IOP (data not available), and blood pressure related variables were studied one at a time. Stratification of our BP analyses by antihypertensive medication status clearly confirmed the positive association between BP and glaucoma, in those not taking antihypertensive medication, but there was no indication of a J- or U-shaped relationship. Glaucoma was defined based on fundus photographs only (NHANES) and a combination of fundus photographs and a visual field test (LALES), whereas our glaucoma definition was based on a questionnaire-based algorithm. Cases detected with fundus photography and visual field testing in a population-based setting tend to have a normal to near normal IOP, that is, NTG is frequently found. The role of a low blood pressure has previously been linked to NTG.[58] Self-reported glaucoma is one of the features of the glaucoma classification algorithm in Lifelines, that is, glaucoma with elevated pressures might be more frequent in this sample. This may explain the non-significance of hypotension in our primary analysis. Interestingly, the subgroup of participants who were unaware of their disease status, our NTG surrogate, did not show a positive relationship with any of the BP-related traits but did show an association with antihypertensive medication, where overtreatment may play a role. The fact that high BP was only significant in the aware group suggests that either high BP is mainly associated with glaucoma with elevated pressures or that ascertainment bias plays a role (those with high BP are more likely to have their glaucoma detected). A possible reason why high BP relates to glaucoma could be the association between blood pressure and IOP.[59]

Suggested mechanisms that explain how high BP affects the optic nerve head, with ischemia as a consequence, include blood vessel size that determines blood flow velocity to the optic nerve head.[9,60] The significant relationship between high PP and

glaucoma may reflect the role of vascular aging; thickening and deposition of collagen in the arterial walls alters normal blood flow to vital organs, including the eye.[61]

There are controversies among studies investigating the effects of antihypertensive medication on glaucoma. Our study demonstrated that glaucoma is more prevalent in hypertensive individuals, and the use of each additional antihypertensive medication was associated with a 16% increase in the odds of glaucoma. However, when we stratified by class of antihypertensive medications, only ACE inhibitors and calcium-channel blockers showed statistically significant relationships. These effects were independent of BP, per se, as shown by additionally adjusting for BP measurements. Our findings agree with previous population-based work in the UK, where ACE inhibitors and calcium-channel blocker medication users were more often diagnosed with glaucoma (ORs 1.16 [1.09-1.24] and 1.34 [1.24-1.44], respectively).[56] Similarly, a 6.5 year follow-up study (Rotterdam Study, median age 71 years, and predominantly Caucasian descent)[62] showed an increased glaucoma incidence (a 1.8-fold [1.04–3.2], p=0.037) among individuals taking calcium-channel blockers. They did not find any effect (neither protective nor harmful) for ACE inhibitors. ACE inhibitors are effective in controlling BP and are typically used in patients with renal hypertension.[63] Thus, the strong association of ACE inhibitors with glaucoma as observed by us may indicate a role for the kidney in glaucoma, which is worth exploring in future studies. In the Barbados Eye Studies[32] (mean age 56.9 years, and > 90% African descent) with 9 year follow-up, antihypertensive medication did not show a relationship with glaucoma risk. However, the effect of separate antihypertensive medication classes was not investigated, i.e., the effect of antihypertensive medication was simply modeled as 'yes' or 'no'. In a recent clinical study by Zheng et al.,[64] calcium-channel blocker usage was associated with a 26% increased risk of glaucoma ($p<0.05$), although no dose-response relationship was observed. In the Thessaloniki Eye study, all classes of antihypertensive medications were associated with more glaucomatous damage among subjects with DBP < 90 mmHg.[65] In the same cohort, when compared to untreated normal DBP (< 90 mmHg), a significantly larger cup area and cup-to-disc ratio were observed in those with the same DBP secondary to antihypertensive medication.[66] One explanation could be that excessive BP lowering in response to antihypertensive medication use, in eyes with compromised vessels due to chronic high BP, may result in lower perfusion of blood to retinal ganglion cells with ischemia as a consequence.[67,68] In contrast, a large prescription-based study from Denmark[69] reported that, except for vasodilators, all classes of antihypertensive medications were significantly associated with a later onset of glaucoma. Because the vascular component is a suggested mechanism

involved in glaucoma development and progression, it seems logical to assume that all antihypertensive medications could also prevent the occurrence of glaucoma. The authors defined glaucoma by the use of any glaucoma medication; given that glaucoma medications are also prescribed for ocular hypertension, their outcome measure could be a mixture of glaucoma and ocular hypertension. This suspicion is supported by the overall prevalence estimate of glaucoma in the Danish[69] paper (1996 to 2012) of 4.3% in people aged 40-95 years, higher than the registry-based, i.e., ICD-10-based definition of glaucoma (ranging from 1.4 to 1.9%)[70] and also higher than the European prevalence estimate of 2.93% [1.85-4.40%] from 2013.[37] An alternative explanation could be that, in the Danish health care system, a high BP is detected and treated relatively early, resulting in less damage to the blood vessels (see below). Similar to the Danish study, a recent prospective cohort study of glaucoma patients and glaucoma suspects, the Groningen Longitudinal Glaucoma Study, reported that the use of ACE inhibitors and angiotensin II receptor blockers was associated with a lower conversion rate of glaucoma suspects.[71]

Clearly, the complex association between glaucoma, high BP, and antihypertensive drugs is not yet fully understood. By merging the above mentioned findings, a tentative hypothesis could be that some antihypertensive drug classes possess neuroprotective properties, but that these effects are only visible if prescribed timely;[69,71] a high BP itself promotes glaucoma, and if treated late and/or suboptimally, antihypertensive drugs may simply reflect a history of high BP, which may, in the past, have increased risk of glaucoma development.

Our study has a number of strengths, but also some limitations. Our findings are based on a much larger sample size than most previous studies, rendering more power to precisely estimate effect sizes; however, we need to be aware of the predictive limitation of the cross-sectional design in establishing exposure-outcome relationships. Furthermore, we used GLMM to properly adjust for familial relationships that otherwise may have inflated the significance of associations of HRV and BP with glaucoma. We used a systematic approach for defining glaucoma, but it is likely that not all possible glaucoma cases are truly affected, which may result in misclassification errors. Almost all study participants (98.3%) were White Caucasians; direct comparison with other multi-ethnic study results may not be possible. Therefore, the results of this study should be interpreted with these limitations taken into account.

# Conclusion

In summary, using a large population-based cohort, we examined the association of HRV, BP, high BP, HTN, and antihypertensive medication use with glaucoma. Except for HRV, which showed a negative relationship, all other variables (BP, high BP, HTN, and antihypertensive medication use) showed a positive association with glaucoma. Our results indicate that in addition to the traditional risk factors (elevated eye pressure, family history, age, and ethnicity), low HRV and high BP may play a role in glaucoma pathophysiology.

Further population-based prospective studies may help to better understand the effect of BP and the role of autonomic dysregulation in predicting glaucoma. Given that the relationship of antihypertensive medication with glaucoma remains controversial even in prospective studies,[32,62] other designs such as randomized clinical trials or Mendelian randomization studies are needed to investigate which classes of antihypertensive medications truly have a causal relationship with glaucoma.[72]

## Acknowledgement

We gratefully acknowledge for the funding received from European Union's Horizon 2020. The authors also thank Lifelines data management staff who arranged the required software in the Lifelines working space.

## Funding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 661883. Additional funding has been provided by the Rotterdamse Stichting Blindenbelangen under grant number B20150036. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Conflict of interest:

None.

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