Institute of Ophthalmology

Modifiable Risk Factors for Glaucoma and Related Traits

Kelsey Vernon Stuart

University College London

A thesis submitted for the degree of

Doctor of Philosophy

April 2024

Supervisors:

Professor Paul Foster

Professor Anthony Khawaja

I, Kelsey Vernon Stuart, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Glaucoma is the leading cause of irreversible blindness globally and an important public health concern. The disease has a complex multifactorial aetiology, with a combination of environmental and genetic factors contributing to disease risk. The identification and characterisation of novel modifiable risk factors may offer insights into pathogenesis, direct future therapies, and inform lifestyle advice for individuals with glaucoma.

In this thesis, I set out to quantify the burden of glaucoma in Europe, before exploring the relationship of several important modifiable factors – including dietary components, lifestyle behaviours, and systemic medication use – with glaucoma and related traits.

To address the first aim, I conduct a large multicentre meta-analysis of glaucoma prevalence data, pooling results from fourteen European population-based eye studies. I report updated prevalence estimates and calculate the number of affected individuals in Europe, demonstrating that both the current and future burden of disease may be substantially greater than previously reported.

I then utilise datasets from large population-based cohort studies and international genetic consortia, employing a variety of epidemiological techniques to assess associations of alcohol consumption, cigarette smoking, dietary salt intake, and physical activity with glaucoma. These analyses implicate alcohol and salt as potentially modifiable risk factors for glaucoma and suggest that adverse relationships may only be apparent in individuals at high underlying genetic risk.

Lastly, I report the results of two complementary association studies of systemic medication use, both of which implicate calcium channel blockers as potentially detrimental for glaucoma.

This work has important implications for public health policy and service provision and may inform dietary recommendations and targeted lifestyle advice for glaucoma patients in the future.

Impact statement

This project demonstrates how big data can be leveraged to further our understanding of complex disease through both descriptive and exploratory analyses. Previous studies of risk factors for glaucoma have often been limited by size or scope, and this thesis serves as an exemplar of how large population-based cohort studies, coupled with new epidemiological techniques and concepts, may lead to novel disease insights.

The results of this work have been disseminated widely in peer-reviewed scientific journals and conference presentations, and derived variables and code lists have facilitated further research in a variety of related projects.

The results of the glaucoma prevalence meta-analysis have been provided to working groups of the European Glaucoma Society and the Royal College of Ophthalmologists and will form the basis of proposed glaucoma screening models and workplace planning strategies. It is also envisioned that the results will contribute to future glaucoma-related epidemiological studies and public health policy.

Several novel findings have emerged, with this project implicating alcohol and dietary salt as potentially modifiable risk factors for glaucoma. While causal relationships have yet to be established, many results have already been replicated in independent cohorts, and it is hoped that this project lays the foundation for ongoing work into environmental determinants of glaucoma.

In particular, the identification of underlying gene-environment interactions may partly explain the lack of consistent findings in the past and should be a consideration in any future study of glaucoma risk factors. These results may prove to particularly relevant as we move towards population-scale genotyping and personalised

medicine. As individuals become increasingly aware of their fixed genetic susceptibility to disease, they need to be empowered with advice on behaviours that may modify their risk for progression.

Better characterisation of the relationship between common systemic medications and glaucoma – and the identification of calcium channel blockers as potentially detrimental, in particular – is especially pertinent given projections for ongoing demographic ageing and the rising prevalence of multimorbidity and polypharmacy.

Currently, intraocular pressure remains the only proven modifiable risk factor for glaucoma, yet some patients still progress despite maximal therapy. It is hoped that the work presented in this thesis informs discussions concerning potentially beneficial complementary options, and ultimately leads to dietary recommendations and lifestyle advice for individuals with glaucoma.

Publications arising from the work described in this thesis:

1. **Stuart KV**, Madjedi K, Luben RN, Chua SYL, Warwick AN, Chia M, et al. Alcohol, Intraocular Pressure, and Open-Angle Glaucoma: A Systematic Review and Meta-analysis. *Ophthalmology*. 2022;129(6):637-652.

2. **Stuart KV**, Luben RN, Warwick AN, Madjedi KM, Patel PJ, Biradar MI, et al. The Association of Alcohol Consumption with Glaucoma and Related Traits: Findings from the UK Biobank. *Ophthalmology Glaucoma*. 2023;6(4):366-379.

3. Tran JH, **Stuart KV**, de Vries V, Vergroesen JE, Cousins CC, Hysi PG, et al. Genetic Associations Between Smoking- and Glaucoma-Related Traits. *Translational Vision Science & Technology.* 2023;12(2):20.

4. **Stuart KV**, Pasquale LR, Kang JH, Foster PJ, Khawaja AP. Towards Modifying the Genetic Predisposition for Glaucoma: An Overview of the Contribution and Interaction of Genetic and Environmental Factors. *Molecular Aspects of Medicine*. 2023;93:101203.

5. Vergroesen JE*, Schuster AK*, **Stuart KV**, Asefa NG, Cougnard-Grégoire A, Delcourt C, et al. Association of Systemic Medication Use with Glaucoma and Intraocular Pressure: The European Eye Epidemiology Consortium. *Ophthalmology*. 2023;130(9):893-906.

6. Kastner A*, **Stuart KV***, Montesano G, De Moraes CG, Kang JH, Wiggs JL, et al. Calcium Channel Blocker Use and Associated Glaucoma and Related Traits among UK Biobank Participants. *JAMA Ophthalmology*. 2023;141(10):956-964.

7. Madjedi KM, **Stuart KV**, Chua SYL, Ramulu PY, Warwick A, Luben RN, et al. The Association of Physical Activity with Glaucoma and Related Traits in the UK Biobank. *Ophthalmology*. 2023;130(10):1024-1036.

8. **Stuart KV**, Madjedi KM, Luben RL, Biradar MI, Wagner SK, Warwick AN, et al. Smoking, Corneal Biomechanics, and Glaucoma: Results from Two Large Population-Based Cohorts. *Investigative Ophthalmology & Visual Science*. 2024;65(1):11.

9. **Stuart KV**, Biradar MI, Luben RL, Dhaun N, Wagner SK, Warwick AN, et al. The Association of Urinary Sodium Excretion with Glaucoma and Related Traits in a Large United Kingdom Population. *Ophthalmology Glaucoma*. 2024. (*In press*).

Manuscripts in preparation or under review arising from this thesis:

1. **Stuart KV**, de Vries VA, Schuster AK, Yu Y, van der Heide FCT, Delcourt C, et al. Prevalence of Glaucoma in Europe and Projections to 2050: Findings from the European Eye Epidemiology (E3) Consortium. *(In preparation*).

Other publications arising during the time of this thesis:

1. **Stuart KV**, Dold C, van der Westhuizen DP, de Vasconcelos S. The epidemiology of ocular trauma in the Northern Cape, South Africa. *African Vision and Eye Health*. 2022;81(1):a710.

2. Madjedi KM, **Stuart KV**, Chua SYL, Foster PJ, Strouthidis NG, Luben RN, et al. The Association of Female Reproductive Factors with Glaucoma and Related Traits: A Systematic Review. *Ophthalmology Glaucoma*. 2022;5(6):628-647.

3. **Stuart KV**, Shepherd DJ, Kruger M, Singh E. The Incidence of Retinoblastoma in South Africa: Findings from the South African National Cancer Registry (2004–2018). *Ophthalmic Epidemiology*. 2022;29(6):681-687.

4. Madjedi KM, **Stuart KV**, Chua SYL, Luben RN, Warwick A, Pasquale LR, et al. The Association between Serum Lipids and Intraocular Pressure in 2 Large United Kingdom Cohorts. *Ophthalmology*. 2022;129(9):986-996.

5. Chia MA, Taylor JR, **Stuart KV**, Khawaja AP, Foster PJ, Keane PA, et al. Prevalence of Diabetic Retinopathy in Indigenous and Non-Indigenous Australians: A Systematic Review and Meta-analysis. *Ophthalmology*. 2023;130(1):56-67.

6. Wagner SK, Cortina-Borja M, Silverstein SM, Zhou Y, Romero-Bascones D, Struyven RR, et al. Association Between Retinal Features From Multimodal Imaging and Schizophrenia. *JAMA Psychiatry*. 2023;80(5):478-487.

7. Warwick A, Curran K, Hamill B, **Stuart K**, Khawaja AP, Foster PJ, et al. UK Biobank retinal imaging grading: methodology, baseline characteristics and findings for common ocular diseases. *Eye*. 2023;37(10):2109-2116.

8. **Stuart KV**, Khawaja AP. Genomics enabling personalised glaucoma care. *British Journal of Ophthalmology*. 2024;108(1):5-9.

9. **Stuart KV**, Shepherd DJ, Lombard A, Höllhumer R, Muchengeti M. Incidence and epidemiology of conjunctival squamous cell carcinoma in relation to the

HIV epidemic in South Africa: a 25-year analysis of the National Cancer Registry (1994–2018). *British Journal of Ophthalmology*. 2024;108(2):175-180.

10. Wagner SK, Patel PJ, Huemer JC, Khalid H, **Stuart KV**, Chu CJ, et al. Periodontitis and outer retinal thickness: A cross-sectional analysis of the UK Biobank cohort. *Ophthalmology Science*. 2024;4(4):100472.

11. Sun Z, Zhang B, Smith S, Atan D, Khawaja AP, **Stuart KV**, et al. Structural Correlations between Brain Magnetic Resonance Image-Derived Phenotypes and Retinal Neuroanatomy. *European Journal of Neurology*. 2024;31(7):e16288.

12. Gouws D, van der Westhuizen DP, **Stuart KV**. Bilateral Anterior Lens Capsule Ruptures in Alport Syndrome: Case Series and Literature Review. *Digital Journal of Ophthalmology*. 2024. (*Accepted*).

13. Founti P, **Stuart KV**, Nolan W, Khawaja AP, Foster PJ. Targeted Screening Strategies to Improve Glaucoma Outcomes: Screening Strategies and Methodologies. *Journal of Glaucoma*. 2024. (*Accepted*).

14. Rajesh AE, Olvera-Barrios A, Warwick AN, Wu Y, **Stuart KV**, Biradar MI, et al. Ethnicity is not biology: retinal pigment score to evaluate biological variability from ophthalmic imaging using machine learning. *Nature Communications*. (*In revision*).

Research Paper Declaration Forms

Completed declaration forms for all publications referenced in this thesis are available in **Appendix A**.

Acknowledgements

This thesis has only been possible thanks to the support, kindness, and contribution of numerous individuals and organisations.

I am grateful to UCL for awarding me an Overseas Research Scholarship and to Fight for Sight and the Desmond Foundation for providing grant support throughout the duration of the project. I would also like to thank Dr Neville Passmore and the trustees of the Skye Foundation for affording me the privilege of pursuing a master's degree at the University of Oxford, which provided the technical groundwork upon which this thesis is built, and where the idea for this project first took seed.

It has been a great pleasure working under my two supervisors, Professors Paul Foster and Anthony Khawaja, who have been unwavering in their support and encouragement over the past three years. Together, they represent a formidable combination in the field of ophthalmic epidemiology – covering the full spectrum of fundamental to cutting edge research – and it has been a wonderful opportunity learning from them. I am especially grateful for the active role both have played in promoting and supporting my development as a researcher and clinician.

I am thankful for the many friends and colleagues I have had the pleasure of working alongside, both within the Institute of Ophthalmology and more widely, particularly those who have contributed to the work described here – Dr Robert Luben, Dr Alasdair Warwick, and Dr Kian Madjedi.

Finally, a special thank you to my wife, Nikita, and to my parents and family for their unconditional support, patience, and encouragement over the years.

Table of contents

List of figures

List of tables

List of abbreviations

ACEI	Angiotensin-converting enzyme inhibitor
ACG	Angle-closure glaucoma
ADH1B	Alcohol dehydrogenase 1B
AIC	Akaike Information Criteria
ALDH2	Aldehyde dehydrogenase 2
ARB	Angiotensin receptor blocker
ARVO	Association for Research in Vision and Ophthalmology
ASB	Anterior segment biometry
ATC	Anatomical Therapeutic Chemical
BMI	Body mass index
BWHS	Black Women's Health Study
CH	Corneal hysteresis
CCB	Calcium channel blocker
CCT	Central corneal thickness
CDR	Cup-disc ratio
CFP	Colour fundus photography
CH	Corneal hysteresis
CI	Confidence interval
CLSA	Canadian Longitudinal Study on Aging
CRF	Corneal resistance factor
CV	Coefficient of variation
DM	Diabetes mellitus
E3	European Eye Epidemiology
eGFR	Estimated glomerular filtration rate
EPIC	European Prospective Investigation into Cancer and Nutrition
FFQ	Food frequency questionnaire
GCIPL	Ganglion cell-inner plexiform layer
GHS	Gutenberg Health Study
GRADE	Grading of Recommendations Assessment, Development and Evaluation
GSCAN	GWAS & Sequencing Consortium of Alcohol and Nicotine use

GWAS	Genome-wide association study
HbA1c	Glycated haemoglobin
HPFS	Health Professionals Follow-Up Study
HR	Hazard ratio
HTG	High tension glaucoma
ICD	International Classification of Diseases
IOP	Intraocular pressure
IOPcc	Corneal-compensated intraocular pressure
IOPg	Goldmann-correlated intraocular pressure
IPAQ	International Physical Activity Questionnaire
IQR	Interquartile range
ISGEO	International Society for Geographical and Epidemiological Ophthalmology
IV	Instrumental variable
IVW	Inverse-variance weighted
LD	Linkage disequilibrium
LST	Leisure screen time
MAF	Minor allele frequency
MCV	Mean corpuscular volume
MET	Metabolic equivalent of task
MOOSE	Meta-analysis of Observational Studies in Epidemiology
MR	Mendelian randomisation
mRNFL	Macular retinal nerve fibre layer
MTAG	Multitrait analysis of GWAS
MVPA	Moderate-to-vigorous physical activity
NHS	National Health Service
NHS	Nurses' Health Study
NICOLA	Northern Ireland Cohort for the Longitudinal Study of Ageing
NTG	Normal tension glaucoma
OAG	Open-angle glaucoma
OCT	Optical coherence tomography
OHT	Ocular hypertension
ONH	Optic nerve head

OPP	Ocular perfusion pressure
OR	Odds ratio
ORA	Ocular Response Analyzer
PACG	Primary angle-closure glaucoma
PMH	Postmenopausal hormone
POAG	Primary open-angle glaucoma
PRESSO	Pleiotropy residual sum and outlier
pRNFL	Peripapillary retinal nerve fibre layer
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRS	Polygenic risk score
RAS	Renin angiotensin system
RGC	Retinal ganglion cell
RNFL	Retinal nerve fibre layer
ROBINS-E	Risk Of Bias In Non-randomised Studies of Exposures
ROBINS-I	Risk Of Bias In Non-randomised Studies of Interventions
RR	Risk ratio
SBP	Systolic blood pressure
SD	Standard deviation
SE	Spherical equivalent
SLO	Scanning laser ophthalmoscopy
SLP	Scanning laser polarimetry
SNP	Single nucleotide polymorphism
SRMA	Systematic review and meta-analysis
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TES	Thessaloniki Eye Study
TM	Trabecular meshwork
UEMS	Ural Eye and Medical Study
UNa:Cr	Urine sodium-creatinine ratio
UK	United Kingdom
US	United States
VF	Visual field

PART I

INTRODUCTION

1

Introduction

1.1 Overview

Glaucoma, a chronic progressive optic neuropathy, is the leading cause of irreversible blindness worldwide and an important public health concern. The disease has a complex multifactorial aetiology, with both environmental and genetic factors playing a role. The identification and characterisation of modifiable risk factors for glaucoma may offer insights into underlying pathogenesis, direct future therapies, and inform lifestyle advice for affected individuals.

In this thesis, I seek to: (1) quantify and characterise the burden of glaucoma in Europe, and (2) explore the relationship of several important modifiable factors – including dietary components, lifestyle behaviours, and systemic medication use – with glaucoma and related traits.

Chapter 1 serves as an introduction and provides background information on important concepts central to this thesis, while Chapter 2 details the data sources and describes the key methods used for subsequent chapters. Chapter 3 is the first of six results chapters and lays out the findings of a multicentre European glaucoma prevalence meta-analysis. Chapter 4 (alcohol), Chapter 5 (smoking), Chapter 6 (dietary salt), Chapter 7 (systemic medication), and Chapter 8 (physical activity) present the results of cross-sectional, gene-environment interaction, and Mendelian randomisation analyses conducted in several large population-based cohorts. Key findings are highlighted and discussed in Chapter 9.

1.2 Background

This section introduces glaucoma as a leading cause of global blindness and provides a brief overview of key disease concepts relevant to this report. Modifiable risk factors are discussed and the current literature on the role of these factors in glaucoma is summarised. Deficiencies and limitations in the existing knowledge base are highlighted and potential avenues for future research are introduced, providing a contextual framework for the introduction of the overall study aims. This section is partly based on review article published in *Molecular Aspects of Medicine.*[1] I was responsible for all aspects of the referenced work. The relevant declaration form for previously published material is located in **Appendix A**.

1.2.1 Glaucoma

1.2.1.1 Definition and classification

Glaucoma comprises a heterogeneous group of disorders characterised by chronic, progressive optic neuropathy and corresponding stereotypical visual field changes (**Figure 1.1**). The final common pathway for all forms of the disease is marked by retinal ganglion cell (RGC) degeneration and optic nerve fibre loss.

Despite extensive research, the precise pathophysiological mechanisms underlying glaucomatous RGC degeneration remain unclear, although numerous hypotheses have been proposed.[2] The biomechanical theory implicates intraocular pressure (IOP)-mediated mechanical stress as the primary driver of this process, while insufficiency or alteration in blood flow to the optic nerve head (ONH) is considered the major aetiological factor in the vascular theory. A third theory suggests a primary neurodegenerative component to glaucoma, especially when glaucomatous changes occur in the absence of raised IOP (normal-tension glaucoma, NTG). It is

hypothesised that certain factors, including vascular dysregulation and abnormal pressure gradients across the lamina cribrosa, may render individuals with NTG more susceptible to IOP-mediated mechanical stress, resulting in glaucomatous damage at seemingly normal pressures.[3] Ultimately, it is possible that these theories may all prove to be valid determinants of disease and that glaucomatous neurodegeneration represents varying combinations of a multifactorial process.

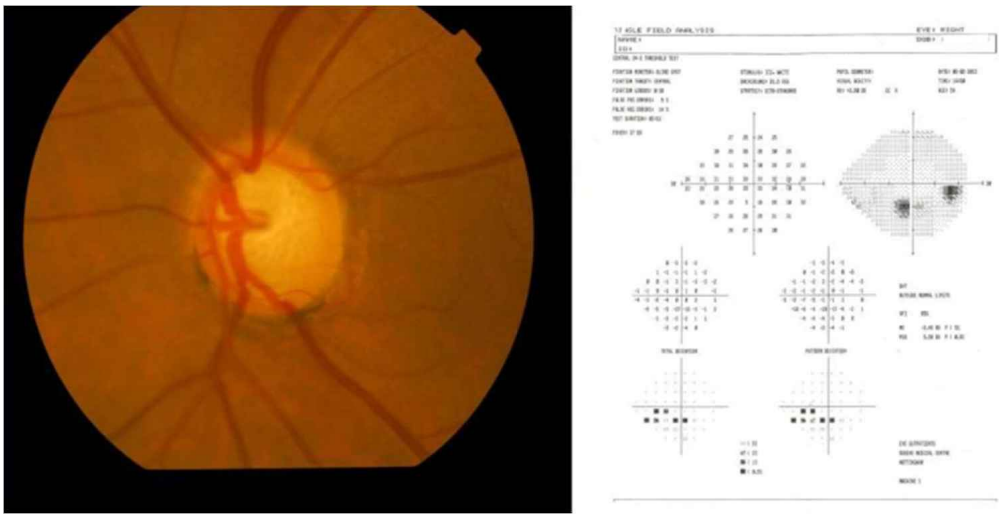

Figure 1.1 Glaucoma structure-function relationship

Example of a glaucomatous optic disc with typical structural changes (left) and an associated early visual field defect (right).

Figure from: King A, Azuara-Blanco A, Tuulonen A. Glaucoma. British Medical Journal. 2013:346. Reproduced with permission from the BMJ Publishing Group Ltd.

Glaucoma can be broadly categorised into two categories according to the configuration of the anterior chamber drainage angle (**Figure 1.2**). In open-angle glaucoma (OAG), the drainage angle has a normal anatomical configuration, while in angle-closure glaucoma (ACG), it is narrowed or closed. Chronic resistance to aqueous humour egress through the trabecular meshwork (TM), Schlemm's canal, and/or distal drainage pathways of the eye results in sustained ocular hypertension

(OHT) in most cases of OAG with elevated IOP. Consequently, the early stages of OAG tend to be asymptomatic and the disease may remain undiagnosed in up to 50% of individuals.[4,5] In contrast, the physical obstruction to the drainage angle in ACG generally results in abrupt, symptomatic, and extreme IOP elevations, leading to rapid visual loss.

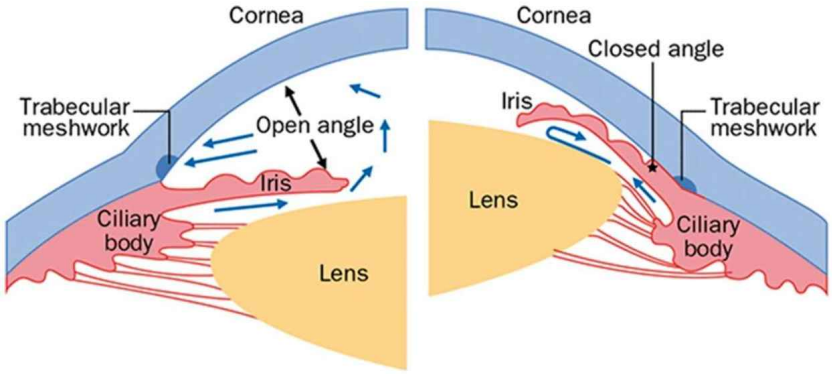

Figure 1.2 Schematic diagram of the anterior chamber drainage angle of the eye

Under normal conditions (open angle, left) the aqueous humour formed by the ciliary body flows around the lens and iris (blue arrows) and exits the eye through the trabecular meshwork, through Schlemm's canal and empties into aqueous veins and the episcleral venous system. In the closed angle (right), the iris and lens are positioned anteriorly causing an obstruction of aqueous flow through the trabecular meshwork.

Figure from: Wiggs JL, Pasquale LR. Genetics of glaucoma. Human Molecular Genetics. 2017;26(R1):R21-27. Reproduced with permission from Oxford University Press.

Both subtypes can occur as primary disease (>90% of cases) or secondary to an identifiable underlying mechanism.[6] In primary open-angle glaucoma (POAG), the commonest form of the disease, there is a normal anatomical drainage angle and no identifiable secondary cause for glaucoma (e.g., ocular pigment, pseudoexfoliative material, or inflammatory debris). Similarly, in primary angle-closure glaucoma (PACG), the anatomical drainage angle obstruction is not directly attributable to any underlying pathophysiological process (e.g., trauma or neovascularisation).

1.2.1.2 Epidemiology and public health importance

Glaucoma is the leading cause of irreversible blindness worldwide, estimated to affect more than 76 million individuals aged 40–80 years in 2020, with projections rising to 112 million by 2040.[7] There is, however, considerable regional and ethnic variation in glaucoma subtype and disease risk. POAG accounts for >80% of all glaucoma cases worldwide and global prevalence is estimated at 3.1%, with figures ranging from 2.3% in Asia to 4.2% in Africa.[7] With a global prevalence of 0.5%, PACG is far less common, but also exhibits considerable geographic variation, with estimates ranging from 0.3% in North America to 1.1% in Asia,[7] where it constitutes a significantly greater proportion of all cases relative to other world regions.[8]

Of all those affected by glaucoma, 2.1 million are blind and a further 4.2 million are visually-impaired as a result of the disease.[9] Glaucoma-related visual impairment has in turn been associated with greater inpatient and home-based healthcare service utilisation, higher total medical costs, and various non-glaucomatous health conditions, including falls and depression.[10]

The total economic burden of glaucoma is difficult to quantify but is likely to be substantial.[11] The annual medical costs of glaucoma in the United States alone have been estimated at $6.1 billion and are projected to increase to $17.3 billion by 2050.[12] Direct costs include medications, diagnostic tests, clinic visits and surgery, while indirect costs include loss of income, decreased productivity, missed work days, and the long-term costs associated with chronic visual impairment or blindness.[13] As this burden often extends to patients' families, the healthcare system and society at large, it is clear that glaucoma represents a significant public health concern.

1.2.1.3 Risk factors

Risk factors for glaucoma differ widely by disease subtype. In PACG, anatomical considerations play an important role, with factors including a shallow anterior chamber, an anteriorly positioned or displaced lens, iris morphology, and angle crowding predisposing affected individuals to appositional approximation or contact between the iris and TM.[14] Consequently, the risk of PACG is higher among women, the elderly, and the hyperopic, and the disease is more prevalent in Asia.[14] Similarly, in secondary forms of disease, an identifiable underlying cause is responsible for elevated IOP and subsequent glaucomatous damage, often through direct physical obstruction or damage of the TM and/or drainage angle.[15]

POAG, on the other hand, is a highly complex disease, with both genetic and environmental determinants, and this complexity can make elucidating the role of individual factors difficult. Well-established non-modifiable risk factors for POAG include older age, non-White ethnicity, and a family history of glaucoma[2] – with the last two almost certainly reflecting some degree of genetic influence. Similarly, elevated IOP, the only known modifiable risk factor, is a heritable trait, with considerable overlap in the underlying genetic architecture of IOP and glaucoma.[16]

Numerous landmark interventional studies have proven the clinical benefit of lowering IOP on reducing the risk for the onset or progression of disease,[17,18] and this beneficial effect has also been demonstrated for NTG,[19] in which baseline IOP is within the normative population range, even before intervention. Although all currently approved glaucoma interventions (including medication, laser, and surgery) work by lowering IOP, there is considerable interest in identifying other modifiable risk factors which may complement existing treatment strategies or guide lifestyle recommendations.

1.2.1.4 Genetic considerations

Glaucoma is one of the most heritable of all complex human diseases (estimated h^2, 0.70),[20] with first-degree relatives of individuals with glaucoma having an almost 10-fold greater lifetime risk of disease compared to the general population.[21] In the paediatric setting, monogenic mutations associated with primary congenital glaucoma are the most common cause of disease, accounting for a significant proportion of all childhood blindness.[22] Conversely, only a small proportion of POAG in adults (estimated to be <5%) is inherited in a Mendelian fashion.[2] *MYOC* (myocilin) gene sequence variations give rise to the most common form, characterized by elevated IOP; while rare missense mutations in *OPTN* (optineurin) and copy number variations involving *TBK*-1 (TANK-binding protein 1) can cause familial NTG.[23] These highly penetrant autosomal-dominant genetic mutations tend to have large biological effects, causing clinically severe, early-onset disease in affected individuals.[24]

The genetics underpinning the vast majority of adult-onset POAG, however, is far more complex. In these cases, a multitude of genetic factors, each relatively common but of small individual effect, cumulatively contribute towards the risk of disease. In the last decade, hypothesis-free genome-wide association studies (GWAS) have driven the discovery of these common genetic determinants of glaucoma, with more than 100 POAG susceptibility loci reported to date (**Figure 1.3)**.[25,26]

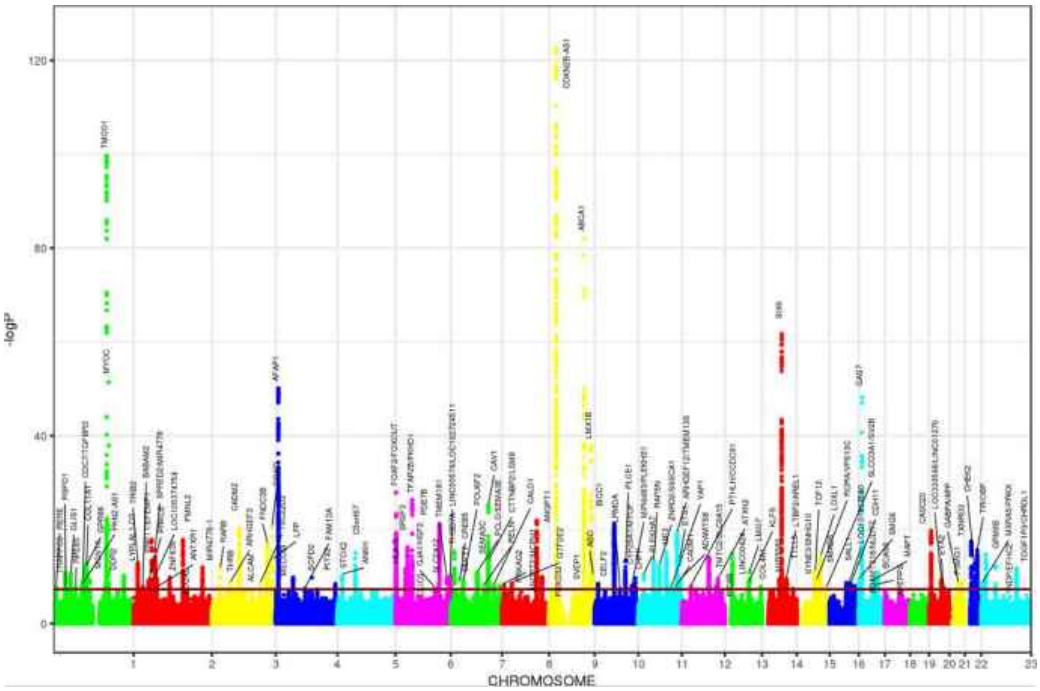

Figure 1.3 Manhattan plot of the results of a large cross-ancestry genome-wide association study meta-analysis for primary open-angle glaucoma

Each dot represents a single nucleotide polymorphism (SNP), the *x*-axis the chromosome where each SNP is located, and the *y*-axis the −log10 *P*-value of the association of each SNP with primary open-angle glaucoma in the cross-ancestry meta-analysis (34 179 cases vs. 349 321 controls). The red horizontal line shows the genome-wide significant threshold. The nearest gene to the most significant SNP in each locus has been labelled.

Figure from: Gharahkhani P, Jorgenson E, Hysi P, Khawaja AP, Pendergrass S, Han X, et al. Genome-wide meta-analysis identifies 127 open-angle glaucoma loci with consistent effect across ancestries. Nature Communications. 2021;12:1258. Reproduced under a Creative Commons CC-BY 4.0 license.

This line of research has been greatly accelerated by the emergence of large-scale biobank-based cohorts and collaborative genetic consortia, the widespread availability of GWAS summary statistics to the scientific community, and advances in post-GWAS genetic analyses.[25] Despite this rapid progress, current knowledge of genome-wide significant ($P < 5 \times 10^{-8}$) single nucleotide polymorphisms (SNPs) explains less than 10% of the genetic contribution to POAG susceptibility, suggesting that additional variants are yet to be discovered.[27]

1.2.1.5 Environmental considerations

While genetic susceptibility undoubtedly contributes a substantial proportion to individual risk, environmental determinants also play a role. Some factors, such as playing high-resistance wind instruments, ingesting caffeine, certain yoga positions, wearing tight neckties, and lifting weights are known to increase IOP; while others, including general physical activity and consuming alcohol, lower IOP.[28] However, it is unclear whether these short-term changes are sufficient to meaningfully impact glaucoma risk, and the overall effect of habitual behaviours are less clear.

Certain factors may also influence glaucoma risk through IOP-independent mechanisms by affecting the rate of RGC apoptosis[29] – various dietary factors, including antioxidants and essential fatty acids, have been implicated as potentially neuroprotective in glaucoma,[30,31] while others, notably alcohol, are known to be neurotoxic.[32] A further consideration is the potential for “environmental antagonistic pleiotropism” – in which an environmental exposure may simultaneously generate biological responses that offset one another.[28] However, despite extensive research and numerous reported associations, no single environmental factor has been proven as an interventional target for glaucoma in clinical trials.

1.2.1.6 Diagnostics

Various diagnostic tests may be used in the assessment of glaucoma, including perimetry (visual field testing), gonioscopy (assessment of the anterior chamber drainage angle), IOP measurement, central corneal thickness (CCT) measurement, and evaluation of the ONH and retinal nerve fibre layer (RNFL) through fundus photography and optical coherence tomography (OCT).[2]

In general, the diagnosis of glaucoma relies on the demonstration of compatible structural changes with corresponding functional deficits, the exclusion of alternative underlying pathology which may better account for the observed changes, and should be independent of measured IOP. There are, however, no universally accepted diagnostic criteria and individualised diagnosis may be difficult or subjective.[33] Although rapid advances in imaging and technology have improved diagnostic capabilities in recent decades, this lack of standardisation and consensus can often prove a major limitation to glaucoma-related research.

On a population level, the International Society for Geographical and Epidemiological Ophthalmology (ISGEO) criteria proposed by Foster, et al. in 2002, based largely on ONH and visual field parameters, are the most well-established diagnostic guidelines used in prevalence studies, and have greatly aided the interpretation and comparability of epidemiological studies of glaucoma (**Table 1.1**).[34]

Table 1.1 The diagnosis of glaucoma in cross-sectional prevalence surveys

Category	Description	Criteria
1	Structural and functional evidence.	Eyes with a CDR or CDR asymmetry ≥97.5th percentile for the normal population, or a neuroretinal rim width reduced to ≤0.1 CDR (between 11 to 1 o'clock or 5 to 7 o'clock) that also showed a definite visual field defect consistent with glaucoma.
2	Advanced structural damage with unproven field loss.	If the subject could not satisfactorily complete visual field testing but had a CDR or CDR asymmetry ≥99.5th percentile for the normal population, glaucoma was diagnosed solely on the structural evidence. In diagnosing category 1 or 2 glaucoma, there should be no alternative explanation for CDR findings (dysplastic disc or marked anisometropia) or the visual field defect (retinal vascular disease, macular degeneration, or cerebrovascular disease).
3	Optic disc not seen. Field test impossible.	If it is not possible to examine the optic disc, glaucoma is diagnosed if: **(A)** The visual acuity <3/60 and the IOP >99.5th percentile, *or* **(B)** The visual acuity <3/60 and the eye shows evidence of glaucoma filtering surgery, or medical records were available confirming glaucomatous visual morbidity.

Adapted from: Foster PJ, Buhrmann R, Quigley HA, Johnson GJ. The definition and classification of glaucoma in prevalence surveys. British Journal of Ophthalmology. 2002;86(2):238–242.

CDR, cup-disc ratio; IOP, intraocular pressure.

1.2.2 Glaucoma-related traits

1.2.2.1 Introduction

Given the diagnostic difficulties described above, population-based glaucoma studies are often prone to a significant risk of misclassification bias. This, coupled with the decreased statistical power associated with a binary outcome, has resulted in considerable interest in the use of glaucoma-related traits, or endophenotypes, as alternative outcome measures. These continuous, objective, often structural measures, act as glaucoma biomarkers and afford greater statistical power and minimise the risk of misclassification bias.[35] The availability of multiple outcomes additionally allows for an assessment of the consistency of any observed associations and may offer insights into underlying pathophysiology. This multitrait approach has also greatly aided genetic discovery and enabled improved polygenic risk prediction in glaucoma.[27]

1.2.2.2 Intraocular pressure

Although a diagnosis of glaucomatous optic neuropathy should be independent of IOP, the two are inextricably linked, and given its widespread availability and relative ease of measurement, IOP represents the most commonly investigated glaucoma-related trait. While interventional studies have proven elevated IOP to be a major causal risk factor for glaucoma,[17,18] observational studies have consistently demonstrated a strong positive association between higher IOP and prevalence of glaucoma on a population level (**Figure 1.4**). Both cross-sectional and longitudinal studies suggest a 10–15% increase in the risk for the onset and progression of glaucoma for every 1mmHg increase in IOP.[36] It has also been shown that there is

considerable overlap in the underlying genetic architecture of IOP and glaucoma,[16] further highlighting the importance of this endophenotype.

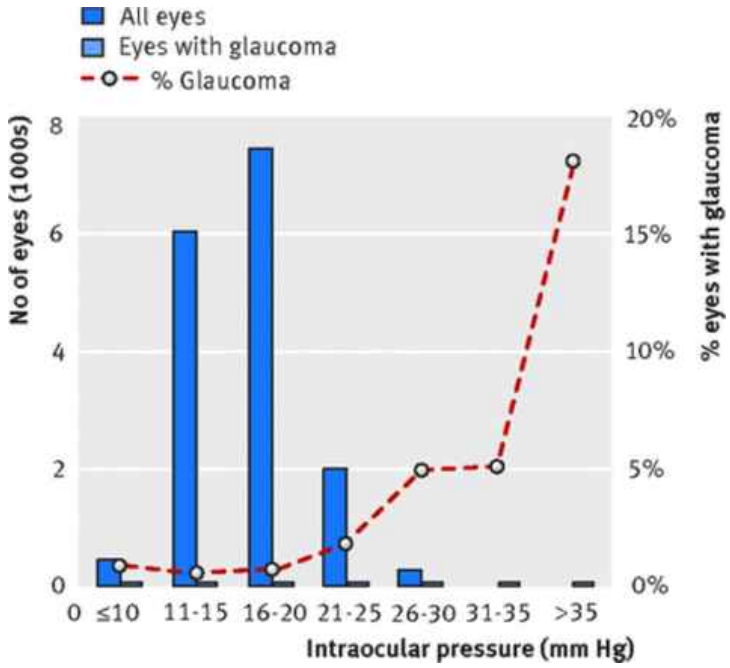

Figure 1.4 Relationship between IOP and glaucoma in the EPIC-Norfolk Eye Study

Figure from: Chan MPY, Broadway DC, Khawaja AP, Yip JLY, Garway-Heath DF, Burr JM, et al. Glaucoma and intraocular pressure in EPIC-Norfolk Eye Study: cross sectional study. British Medical Journal. 2017:358. Reproduced under a Creative Commons CC-BY-NC 4.0 license.

1.2.2.3 Optic nerve head parameters

Numerous ONH characteristics and parameters have proven useful in the assessment and evaluation of glaucoma.[37] One of the most common is the vertical cup-disc ratio (CDR), a simple but crude measure of neuroretinal tissue loss at the ONH (**Figure 1.5**). Although the relationship between CDR and glaucoma is complex (CDR is related both physiologically to disc size and pathologically to glaucomatous damage),[38] it has proven useful as a population-based glaucoma biomarker, aiding diagnosis, risk stratification, and genetic discovery.[27,34,36]

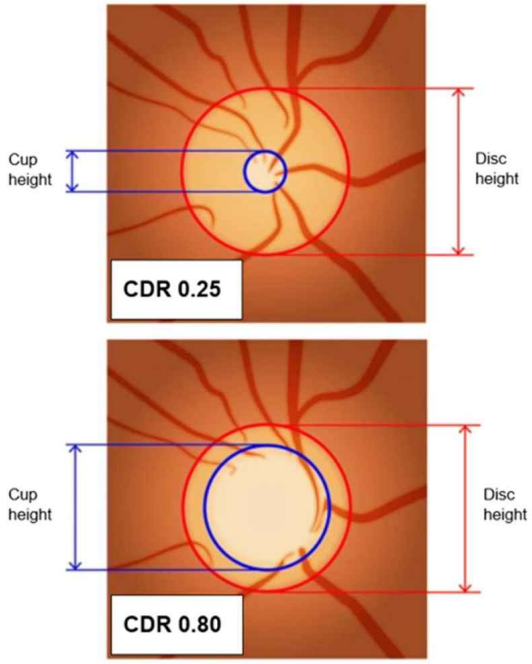

Figure 1.5 Schematic representation of the vertical cup-disc ratio

The vertical cup-disc ratio (CDR) is calculated as the ratio of the optic disc cup height to the optic disc height. Compared to normal eyes (top), the ratio increases as glaucomatous damage progresses (bottom).

Figure adapted from: Barros DMS, Moura JCC, Freire CF, Taleb AC, Valentim RAC, Morais PSG. Machine learning applied to retinal image processing for glaucoma detection: review and perspective. BioMedical Engineering OnLine. 2020;19:20. Reproduced with adaptions under a Creative Commons CC-BY 4.0 license.

1.2.2.4 Inner retinal parameters

Recent advances in diagnostic imaging, most notably OCT, have allowed for incredibly detailed delineation of the anatomical layers of the human retina. In addition to assessment of the peripapillary RNFL (pRNFL), various macular inner retinal parameters, particularly the macular retinal nerve fibre layer (mRNFL) and macular ganglion-cell inner plexiform layer (GCIPL), have been shown to be useful glaucoma-related biomarkers (**Figure 1.6**). Although these measures are non-specific, they have proven utility in both the diagnosis and management of

glaucoma,[39,40] and are increasingly available in large population-based epidemiological studies.

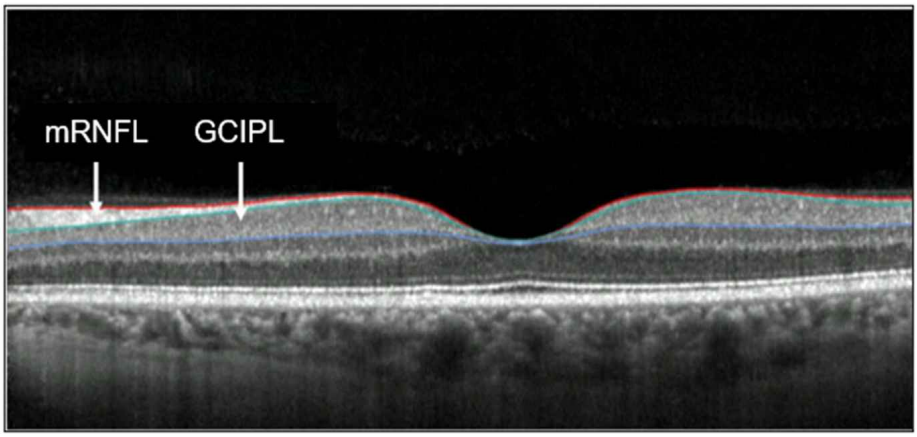

Figure 1.6 Ocular coherence tomography scan demonstrating the macular retinal nerve fibre layer (mRNFL) and the ganglion cell-inner plexiform layer (GCIPL)

1.2.2.5 Genetic discovery

To aid POAG genetic discovery, the definitive case-control GWAS approach has been supplemented by the examination of these endophenotypes. This approach is not reliant only on data from disease cases but can instead leverage data from a healthy population by assessing the variation of an endophenotype across a spectrum of health and disease. In this way, population cohorts can contribute to analyses, greatly increasing sample size and power to detect small associations. Statistical power is also increased by analysing continuous traits rather than binary outcomes. Using this approach, more than 100 genetic loci associated with both IOP[16,41] and CDR[42] have been identified. Results from these analyses have shed light on the underlying pathophysiology of glaucoma,[25] while meta-analysis of this genetic data, using a multitrait approach, has further enabled POAG genetic discovery.[27]

1.2.3 Modifiable risk factors

1.2.3.1 Introduction

The identification of underlying risk factors is an important undertaking for any disease process and may lead to pathophysiological insights or aid screening, diagnosis, and management options. Potentially modifiable risk factors are of particular importance as intervening on these factors may be effective and inexpensive approaches to improving health outcomes on both an individual and a population level. Broadly speaking, risk factors can be classified as environmental and occupational risks, behavioural risks, and metabolic risks; and collectively these factors have been shown to be substantial contributors to global morbidity and mortality.[43]

In particular, a number of these risk factors, especially those associated with an unhealthy lifestyle, are intricately related and contribute to multiple diseases and adverse health outcomes. These include alcohol consumption, tobacco smoking, salt intake, low physical activity, and dyslipidaemia, which have all been implicated in the rising prevalence and burden of non-communicable diseases worldwide.[43,44]

Another important consideration, and potentially modifiable factor, is the use of systemic medication. Several medication classes are known or suspected to modulate glaucoma risk and, with ongoing population ageing, polypharmacy has become increasingly prevalent, especially in older individuals.[45,46] Given that glaucoma is a strongly age-related condition, investigating the potential role that systemic medication may play in glaucoma is a particular research priority.

Despite their importance, the role of these factors in glaucoma remains unclear. Previous studies in the field have often been limited by size, design, or methodology,

and are often at a high risk of bias. While interventional studies have been used to examine short-term exposures to these factors, their role in assessing the long-term effects of habitual or chronic exposure are generally limited by practical, ethical, and financial constraints. Small participant numbers, residual confounding, reverse causality, and misclassification bias are particular concerns in observational studies of these relationships. A brief summary of the relevant literature follows below.

1.2.3.2 Alcohol

The short-term effects of alcohol ingestion include a transient, dose-dependent reduction in IOP[47–54] and an increase in ONH blood flow,[53,55] theoretically playing a protective role in the development of glaucoma. The effects of habitual alcohol consumption on IOP and glaucoma, however, are less clear, with several population-based studies reporting an adverse association between alcohol use and IOP,[56–61] although this is not always a consistent finding.[62,63]

Very few studies have been designed specifically to assess the relationship between alcohol consumption and glaucoma, and while adverse associations have been reported,[61,64] most observational studies have yielded null results.[56,65–73] Alcohol intake does, however, appear to be consistently associated with a thinner inner retina[61,74–77] – a structural characteristic of glaucoma.[39,40]

1.2.3.3 Smoking

Exposure to harmful compounds found in tobacco smoke has been postulated to be a risk factor for glaucoma through ischaemic or oxidative mechanisms.[78] Conversely, nicotine has been hypothesised to be a protective factor through nitric oxide-induced vasodilatory properties.[79] While acute exposure has been shown to have detrimental

effects on the ocular surface and tear function,[80] there appears to be little short-term effect on IOP or ONH perfusion.[81]

Despite these experimental results, multiple population-based studies have reported higher IOP in smokers compared to non-smokers,[82–84] with findings from the UK Biobank suggesting that this may be related to altered corneal biomechanical properties rather than a true ocular hypertensive effect.[85] The evidence for the role of smoking in glaucoma is conflicting and inconclusive. Most studies have reported null[64,67,86–92] or adverse[70,93–96] associations, especially in current or heavy smokers,[78] but there is also evidence suggesting a potentially protective association,[97–99] despite an uncertain explanatory mechanism.

1.2.3.4 Diet

There is considerable interest in the role that diet may play in modulating glaucoma risk and various individual dietary components have been studied in relation to the disease.[28,31] Studies suggest that oxidative stress may play a role in glaucoma,[30] and many dietary factors are hypothesised to be neuroprotective through antioxidative mechanisms. These include *Ginkgo biloba* extract (which may also increase ocular blood flow and be of particular importance in NTG),[100–106] flavonoids (a polyphenol compound commonly found in green tea, red wine, and cocoa),[107,108] fruits and vegetables (nitrate-rich green leafy vegetables, in particular, are further hypothesised to play a role through nitric oxide signalling).[109–111] Despite these findings, the use of antioxidant supplementation has not consistently shown a beneficial association with glaucoma.[112–114] There is also evidence that dietary niacin (vitamin B3) may be protective in glaucoma, potentially through favourable effects on neural tissue and mitochondrial function.[115,116]

Dietary factors implicated as potentially harmful in glaucoma include essential fatty acids (specifically an omega-3:omega-6 imbalance),[117–119] and excessive sodium intake.[120] Although low-carbohydrate dietary patterns, theorised to enhance mitochondrial function and have antioxidant effects,[121] were not consistently associated with glaucoma in three large US prospective studies,[122] a combined Mediterranean and DASH (Dietary Approaches to Stop Hypertension) diet, which incorporates various individual dietary components discussed above, was recently associated with a lower risk for incident glaucoma in the Rotterdam Study.[123]

1.2.3.5 Physical activity

Bouts of physical activity are well documented to cause a transient reduction in IOP in both healthy individuals[124–134] and glaucoma patients,[129,134] as well as an increase in ocular blood flow and perfusion of the ONH and retina.[133,135–137] Fewer studies have assessed the association of habitual physical activity with IOP[138,139] and glaucoma.[140–143] While protective associations have been reported for both greater levels of physical activity and greater cardiovascular fitness,[138,139,141,142] this is not always a consistent finding in epidemiological studies.[140,143]

1.2.3.6 Systemic medication

Several classes of medication are known or suspected to modulate the risk of glaucoma, either through an effect on IOP or via IOP-independent mechanisms.[45] Corticosteroid-induced OHT is a well-established cause of secondary OAG in susceptible individuals,[144,145] while systemic beta-blockers are protective through their ocular hypotensive effect.[146,147] Certain medications – including statins, metformin, selective serotonin reuptake inhibitors, and postmenopausal hormones – have been implicated as potentially protective in glaucoma, although the evidence

for these agents is less consistent.[45] Contradictory findings have been reported in relation to the role of calcium channel blockers (CCB) – a drug class commonly prescribed for various cardiovascular conditions, particularly hypertension – and the risk of glaucoma. While some studies have suggested that CCBs may increase ONH perfusion and retard visual field deterioration in patients with OAG,[148–150] multiple epidemiological studies have reported an adverse association between CCB use and glaucoma.[147,151–154]

1.2.4 Epidemiological considerations

1.2.4.1 Large-scale cohort studies and biobanks

In recent decades, multiple large-scale, population-based, prospective cohort studies have been established or launched. More than 100 studies internationally now include more than 100 000 participants, with numbers often in excess of one million.[155] These studies have revolutionised epidemiological research and offer exciting new research opportunities, as well as the ability to revisit and refine research questions which may have been inadequately addressed by older studies.

A prominent example is the UK Biobank, a prospective cohort study of half-a-million UK adults.[156] This unparalleled resource boasts a wealth of phenotypic and genotypic information, a comprehensive eye and vision assessment, national health-related record-linkage, regular follow-up, and multiple repeat assessments.[157,158]

1.2.4.2 Polygenic risk scores

Although each SNP identified through GWAS explains only a small proportion of glaucoma heritability and is generally insufficient to cause disease, the additive effects of multiple common variants across the genome can confer a genetic risk

equivalent to that seen in monogenic disease.[159] This cumulative genetic burden can be distilled into a single probabilistic value – a polygenic risk score (PRS) – that represents a quantitative summary of an individual's genetic susceptibility to a specific trait or disease.[160]

At its most basic, an unweighted PRS is a simple sum of the number of risk variants carried by an individual. More commonly, however, the variants are weighted by their magnitude of effect (based on the GWAS results), allowing for better risk prediction by accounting for both the total number of variants and the individual variant effect sizes.[161]

In a clinical context, the utility of a PRS is not as a diagnostic tool, but rather as a means of disease risk stratification, allowing for categorisation of individuals according to their level of underlying genetic risk. Those identified to be at high risk of disease (with a PRS in the top 20% of the normal population or study cohort, for example) may then benefit from modified screening approaches or targeted interventions (**Figure 1.7**).[160] The clinical utility of PRS has already been reported in a host of complex non-communicable diseases, including cardiovascular disease, diabetes, and cancer.[162]

Given the high heritability of POAG, as well as the clinical effectiveness of early interventions in preventing otherwise irreversible vision loss, the application of PRS to glaucoma risk stratification has been a research focus in recent years.[160] Early studies, generally based on a restricted set of genetic variants and applied to relatively small cohorts, were only able to demonstrate modest discriminatory powers, with limited clinical potential.[163–165]

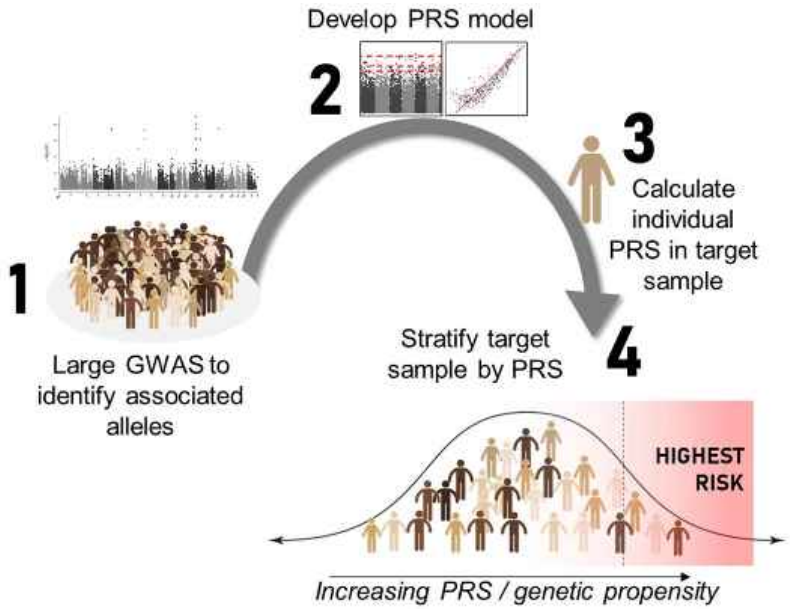

Figure 1.7 Process of polygenic risk score calculation to identify individuals at high genetic risk for disease

1) Disorder-specific genome-wide association study (GWAS) conducted on largest possible sample to identify associated alleles. **2)** Polygenic risk score (PRS) model derived from the GWAS data, incorporating associated SNPs weighted by effect size. **3)** The polygenic risk model is applied to individuals in a target sample (independent of GWAS sample) to calculate a single PRS that reflects genetic propensity to the phenotype. **4)** Identify highest risk individuals based on genetic propensity alone, or in combination with other factors.

Figure from: Kennedy HL, Dinkler L, Kennedy MA, Bulik CM, Jordan J. How genetic analysis may contribute to the understanding of avoidant/restrictive food intake disorder (ARFID). Journal of Eating Disorders. 2020;19:20. Reproduced under a Creative Commons CC-BY 4.0 license.

Backed by larger GWAS, however, recent work has been able to demonstrate risk stratification and predictive ability with clear potential for translational benefit. For example, a glaucoma PRS based on 146 IOP-associated SNPs was found to be associated with higher IOP, younger age of glaucoma diagnosis, more family members affected, and higher treatment intensity in an independent cohort.[166] More recently, a comprehensive POAG PRS, based on 2 673 uncorrelated genetic variants identified using a multitrait approach, demonstrated even greater risk

stratification in an independent cohort, with those in the top decile of the PRS distribution having an almost 15-fold greater risk for glaucoma relative to those in the bottom decile.[27] The same PRS was also found to predict disease progression in early manifest glaucoma cases and surgical intervention in advanced disease.[27,167]

Although not intended as diagnostic tools, regression-based POAG risk prediction models based on recent PRS can now achieve an area under the receiver operating characteristic curve of 0.76,[16,27] considered an "acceptable" level of discriminatory power for a diagnostic test.[168]

Given recent advances in glaucoma PRS development, it may soon be possible to identify individuals at high risk of glaucoma before they exhibit any signs of disease, making the identification of environmental factors that could potentially modify genetic risk a particular priority.

1.2.4.3 Gene-environment interaction

While both genetic and environmental factors can independently influence glaucoma risk, a further aetiological consideration is the interplay between the two. Studies of gene-environment interaction aim to describe how genetic and environmental factors jointly influence disease risk.[169] Importantly, the combined effect of gene and environment may confer a risk that reflects a departure from the simple additive effect of the two (**Figure 1.8**). For example, an environmental exposure may only cause an effect or be associated with a disease in the presence of a certain genetic variant (e.g., the alcohol-induced flushing response seen in individuals with low-activity polymorphisms in the *ALDH2* (aldehyde dehydrogenase 2) gene).[170] Alternatively stated, the risk of disease associated with a particular genotype may be modified by changing the level of exposure to an environmental risk factor (e.g., the

risk of developing emphysema in individuals with alpha-1 antitrypsin deficiency caused by *SERPINA1* (serpin family A1) mutations can be modified by altering exposure to cigarette smoke).[171]

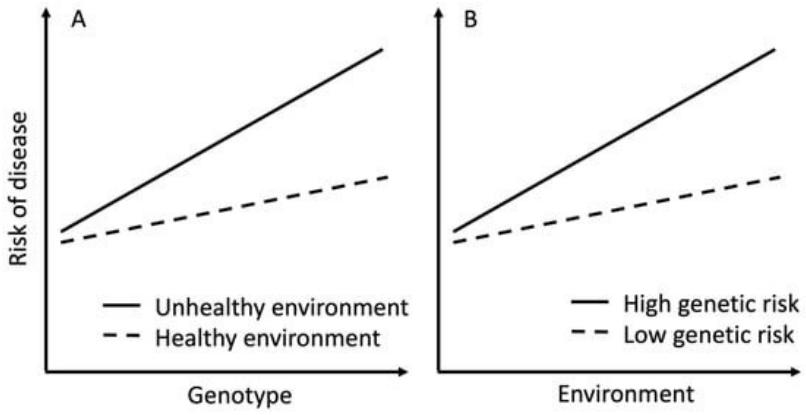

Figure 1.8 Graphical representation of a simple gene-environment interaction

a) The risk for a certain disease (y-axis) increases as genetic susceptibility to the disease (x-axis) increases. However, when compared to the relationship observed in a healthy environment, a stronger genotype-disease association (steeper slope) is noted in the presence of an unhealthy environment. **b)** Similarly, the association between a particular environmental risk factor and disease is modified by underlying genetic risk, with a stronger adverse effect observed in those at higher genetic risk for the disease. In both scenarios, the risk of disease associated with a particular (fixed) genotype can be reduced by modifying exposure to a certain environmental risk factor.

Figure from: D'Urso S, Hwang L-D. New Insights into Polygenic Score-Lifestyle Interactions for Cardiometabolic Risk Factors from Genome-Wide Interaction Analyses. Nutrients. 2023;15(22):4815. Reproduced under a Creative Commons CC-BY 4.0 license.

Better characterisation of gene-environment interactions has several possible benefits, including offering insights into underlying biological pathways, allowing for improved public health policy through targeted population screening, and filling the missing heritability gap for complex traits.[169] However, despite considerable interest, studies of gene-environment interaction have historically been limited by a lack of adequately powered studies with the necessary genetic and environmental data to

perform these analyses.[172] This challenge has been partially overcome by the advent of large-scale, population-based, prospective cohort studies, such as the UK Biobank,[156,157] which have revolutionised epidemiological research in recent decades.[155] The increasing availability of large cohorts with detailed ophthalmic, genetic, and environmental data has allowed for greater consideration to be given to gene-environment interactions in glaucoma.

An early research focus was the role of the *NOS3* (nitric oxide synthase 3) gene in mediating glaucoma risk. The NOS3 enzyme catalyses the production of nitric oxide, which in turn influences luminal smooth muscle tone.[173] This isoform is present in the human outflow pathway and the endothelial cells of the RGC vasculature,[174,175] making it of interest in glaucoma. In a nested case-control study of the Nurses' Health Study and Health Professionals Follow-up Study, while no *NOS3* polymorphism was associated with POAG overall, a significant interaction was observed between various *NOS3* SNPs and postmenopausal hormone (PMH) use in women.[176] Although PMH use has previously been implicated as a protective factor in glaucoma,[177] these findings suggest that sex-based biology and reproductive hormones may play a role in POAG pathogenesis and offer insights into potential underlying disease mechanisms.

In a similar analysis, the associations of hypertension and cigarette smoking with POAG risk were also found to depend on *NOS3* genetic polymorphisms,[178] again suggesting that nitric oxide signalling may play an important role in mediating the effect of environmental risk factors on glaucoma risk.

While these studies examined environmental interaction with a single genetic locus, recent advances in PRS development have now made it possible to assess the interaction between an environmental factor and the cumulative effect of multiple

genetic variants. For example, it has been shown that for women in the highest decile of non-modifiable risk for breast cancer (based in part on a 92-SNP breast cancer PRS), their absolute lifetime risk could be reduced to an average level by modifying body mass index, PMH use, alcohol intake and smoking.[179] Although this approach may not yield specific insights into underlying biological pathways, it may have important implications for targeted population screening and personalised recommendations for primary preventative measures.

Recently, the same approach has been applied to the study of glaucoma-related gene-environment interactions. In a study of more than 100 000 UK Biobank participants, caffeine consumption was found to be associated with both higher IOP and glaucoma prevalence, but only in those at the highest genetic susceptibility to higher IOP (based on a 111-SNP IOP PRS).[16,180] Specifically, among those with a PRS in the top 25% of the study population, consuming >480mg per day versus <80mg per day of caffeine was associated with a 0.35mmHg higher IOP.[180] Although this population-level difference may appear small, it is equivalent in magnitude to the effect of *TMCO1* rs10918274, the gene variant with the strongest effect on both higher IOP and POAG risk.[16]

1.3 Aims and objectives

The overarching aims of this thesis are to leverage large-scale datasets from epidemiological studies and international genetics consortia to:

1) Provide updated estimates and detailed characterisation of the prevalence of glaucoma in Europe;
2) Explore the role that several common modifiable risk factors may play in mediating glaucoma risk.

Specific objectives include:

- To conduct a large, multicentre meta-analysis of glaucoma prevalence data, pooling results from population-based eye studies within the European Eye Epidemiology (E3) consortium.
- To utilise a variety of epidemiological techniques and study designs to assess the relationship of:
    - (i) alcohol consumption,
    - (ii) cigarette smoking,
    - (iii) dietary salt intake,
    - (iv) physical activity, and
    - (v) systemic medication

  with glaucoma and related traits, predominantly within the UK Biobank cohort, and with additional or supplementary analyses in smaller population-based cohort studies.

1.4 Summary

Glaucoma represents a significant public health concern, responsible for substantial visual morbidity and economic costs worldwide, and this disease burden is only projected to grow further in future. Despite extensive research, IOP remains the only established modifiable risk factor for glaucoma.

Several common, modifiable risk factors are implicated in a multitude of chronic diseases and adverse health outcomes, and together are responsible for significant global morbidity and mortality. Additionally, polypharmacy has become increasingly prevalent, especially in older individuals at the highest risk of disease. The role that these factors may play in glaucoma, however, is unclear.

Given the widespread prevalence of these various factors and glaucoma, a better understanding of any underlying associations may have important clinical and public health implications. The identification of novel risk factors may also provide new insights into disease pathogenesis, guide lifestyle recommendations, and may prove to be effective, yet inexpensive, preventative or therapeutic targets.

Overall, the current evidence for the associations of these factors with glaucoma is inconsistent, inconclusive, and often marked by a high risk of bias. The availability of large-scale cohort data, as well as rapid advances in imaging, genetics, and bioinformatics, however, have culminated in the opportunity to overcome past limitations and better define the role that these modifiable risk factors may play in glaucoma.

PART II

METHODS

2

Methods

2.1 Data sources

Several datasets were utilised during the course of this project. Broadly, these fall into one of two categories: (i) population-based cohort studies, which enabled the observational analyses described in subsequent chapters, and (ii) publicly available genome-wide association study (GWAS) summary statistics, which facilitated the Mendelian randomisation (MR) analyses. This section provides an overview and description of these data sources. Supplementary material for this section can be found in **Appendix B**.

2.1.1 Population-based cohort studies

This thesis leveraged data from multiple epidemiological cohort studies. Most analyses, including those for alcohol (section 4.2), smoking (section 5.1), salt intake (section 6.1), systemic medication (section 7.2), and physical activity (section 8.1) were performed in the UK Biobank cohort. Analyses of glaucoma prevalence (section 3.1) and systemic medication (section 7.1) were conducted in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study, with subsequent meta-analysis of results using data from the European Eye Epidemiology (E3) consortium. The Canadian Longitudinal Study on Aging (CLSA) was used as a replication cohort for the analyses of smoking (section 5.1)

2.1.1.1 UK Biobank

The UK Biobank is a large, population-based cohort study and data resource of approximately 500 000 individuals aged 37–73 years at recruitment (2006–2010). Participants were recruited through National Health Service registers and invited to

attend one of 22 assessment centres across the United Kingdom where extensive phenotypic information and biological samples were collected.[156,157]

After providing electronic informed consent, participants completed an in-depth touchscreen questionnaire – detailing sociodemographic information, life-course exposures, and medical history – and an array of physical and cognitive measurements. Blood, urine, and saliva specimens were also collected and used to generate a wealth of genetic, proteomic, and metabolomic data.[181]

Multiple repeat and supplementary assessments, including an eye and vision sub-study (2009–2010), have been conducted in participant subsets to augment the baseline data.[158] Additional health-related outcomes are available through linkage with nationwide medical records and registries. Detailed descriptions, including the overall study protocol and individual test procedures, are available online (https://www.ukbiobank.ac.uk).

The UKB was approved by the National Health Service North West Multicentre Research Ethics Committee (06/MRE08/65) and the National Information Governance Board for Health and Social Care. Research described in this thesis was conducted under UKB application number 36741.

2.1.1.2 EPIC-Norfolk

EPIC is an international prospective cohort study, including more than 20 collaborating centres across 10 European countries, designed to investigate the relationship of diet, nutrition, and lifestyle with the risk of cancer.[182] EPIC-Norfolk, one of the UK study sites, comprises a population-based cohort of 25 639 participants aged 40–79 years at enrolment.[183]

Participants were recruited from 35 participating general practices across Norfolk and baseline assessments were conducted from 1993–1997. Further study details are available online (https://www.epic-norfolk.org.uk). During the third health assessment (2004–2011), a comprehensive ophthalmic examination was performed in a subset of 8 623 participants aged 48–92 years.[184] The Epic-Norfolk Eye Study forms the cohort utilised in this thesis.

Extensive socioeconomic, medical, dietary, biological, and lifestyle data have been collected at multiple timepoints through in-person assessments, postal questionnaires, and record linkage. All participants provided written informed consent and the study was approved by the Norwich Local Research Ethics Committee (05/Q0101/191) and the East Norfolk & Waveney NHS Research Governance Committee (2005EC07L). The research described in this thesis was conducted under a Data Sharing Agreement between University College London and the University of Cambridge (7086164).

2.1.1.3 Canadian Longitudinal Study on Aging

The CLSA is a national longitudinal research platform, including approximately 50 000 participants from all 10 Canadian provinces, designed to support a wide variety of ageing-related research questions.[185] Participants aged 45–85 years were recruited through random household sampling and were invited to join one of two complementary cohorts (2010–2015).

After providing written informed consent, a subset of approximately 30 000 (the Comprehensive cohort) completed a detailed in-person home interview and attended one of 11 data collection sites, where additional questionnaires, tests, physical measurements, and biological specimens (blood and urine) were collected. Active

follow up occurs every three years and record linkage with existing healthcare administrative databases is planned for approximately 90% of the cohort. Further study details, including protocols and test procedures, are available online (https://www.clsa-elcv.ca).

Ethical approval for CLSA was granted individually for each data collection site.[185] The research described in this thesis was conducted under CLSA application number 2109012.

2.1.1.4 European Eye Epidemiology consortium

The E3 consortium is a collaborative initiative of over 50 European eye studies, including more than 180 000 participants, with the aim of promoting and facilitating epidemiological research into common eye diseases.[186] More information on the E3 consortium can be found online (https://www.e3consortium.eu/). For this project, all available population-based studies were invited to contribute raw data or summary statistics towards the analyses described in Chapter 3.

Contributing studies included: the EPIC-Norfolk Eye Study (see details above),[184] the Northern Ireland Cohort for the Longitudinal Study of Ageing (NICOLA),[187] the Ural Eye and Medical Study (UEMS),[188] the Thessaloniki Eye Study (TES),[189] the Coimbra Eye Study,[190] the Rotterdam Study,[191] the ALIENOR (Antioxydants, lipides essentiels, nutrition et maladies oculaires) Study,[192] the Gutenberg Health Study,[193] the MONTRACHET (Maculopathy, optic nerve, nutrition, neurovascular and heart diseases) Study,[194] the Maastricht Study,[195] and the AugUR (Age-related diseases: understanding genetic and non-genetic influences – a study at the University of Regensburg) Study.[196]

All studies adhered to the tenets of the Declaration of Helsinki, and relevant local ethical committee approvals with specific study consent were obtained.

2.1.2 Publicly available summary statistics

In addition to the epidemiological data described above, I also utilised publicly available GWAS summary statistics for the MR analyses of alcohol (section 4.3), smoking (section 5.2), and physical activity (section 8.2), in relation to various glaucoma-related outcome measures. Full details of these data are available in the relevant sections of this thesis and are summarised in **Table 2.1**.

Table 2.1 Details of summary-level data used for Mendelian randomisation analyses

Trait	Source	Sample size	Participant ethnicity	Reference
Exposure				
Alcohol consumption	GSCAN	941 280	European	Liu, et al. (2019)[197]
Smoking initiation	GSCAN	1 232 091	European	Liu, et al. (2019)[197]
Smoking intensity	GSCAN	337 334	European	Liu, et al. (2019)[197]
Coffee consumption	CCGC	91 462	European	Cornelis, et al. (2015)[198]
Leisure screen time	Multiple consortia	606 280	European	Wang, et al. (2022)[199]
Moderate-to-vigorous physical activity	Multiple consortia	526 725	European	Wang, et al. (2022)[199]
Outcome				
Intraocular pressure	UK Biobank, EPIC-Norfolk, IGGC	139 555	European	Khawaja, et al. (2018)[16]
Macular RNFL thickness	UK Biobank	31 434	European	Currant, et al. (2021)[200]
GCIPL thickness	UK Biobank	31 434	European	Currant, et al. (2021)[200]
Vertical cup-disc ratio	IGGC	23 899	European	Springelkamp, et al. (2017)[201]
AI-derived vertical cup-disc ratio	UK Biobank, CLSA, IGGC	111 724	European	Han, et al. (2021)[42]
Primary open-angle glaucoma	IGGC	216 257	European	Gharahkhani, et al. (2021)[26]
Corneal hysteresis	UK Biobank	106 041	European	Simcoe, et al. (2020)[202]
Corneal resistance factor	UK Biobank	106 030	European	Simcoe, et al. (2020)[202]

AI, artificial intelligence; CCGC, Coffee and Caffeine Genetics Consortium; CLSA, Canadian Longitudinal Study on Aging; EPIC, European Prospective Investigation into Cancer and Nutrition; GCIPL, ganglion cell-inner plexiform layer; GSCAN, GWAS (genome-wide association study) and Sequencing Consortium of Alcohol and Nicotine Use; IGGC, International Glaucoma Genetics Consortium; RNFL, retinal nerve fibre layer.

2.2 Data description and derivation

This project utilised numerous variables from the epidemiological studies described above. This section provides a description of these data, including details of derived variables created for use in specific analyses.

2.2.1 Glaucoma and related traits

2.2.1.1 UK Biobank

IOP: In 2009–2010, IOP measurements in both eyes of approximately 115 000 participants were taken using an Ocular Response Analyzer (ORA) non-contact pneumotonometer (Reichert Corp., Philadelphia, PA, USA).[158] Participants reporting an eye infection or eye surgery within the previous four weeks did not undergo IOP assessment. Individual-level IOP values were calculated as the mean of available right and left eye values and extreme IOP values in the top and bottom 0.5 percentiles were excluded. For most analyses, I used corneal-compensated IOP (IOPcc), a measure derived from a linear combination of inward and outward applanation tensions which is least influenced by corneal biomechanical properties.[203] Individual-level IOP values were calculated as the mean of available right and left eye values. I excluded participants with a history of glaucoma surgery or laser therapy, corneal graft or refractive surgery, or visually significant ocular trauma (these participants were not excluded from the analyses of OCT parameters or glaucoma status). I imputed pre-treatment IOP values for participants using ocular hypotensive agents by dividing the measured IOP by 0.7, as previously described.[16]

OCT: In 2009–2010, macular spectral domain OCT imaging using a Topcon 3D OCT-1000 Mark II (Topcon Corp., Tokyo, Japan) was performed in both eyes of

approximately 65 000 participants.[158] The image handling, segmentation and quality control protocols have been described previously.[74] For all analyses, I used macular retinal nerve fibre layer (mRNFL) thickness and macular ganglion cell inner plexiform layer (GCIPL) thickness, as these measures have been shown to be useful glaucoma-related biomarkers.[39,40] I calculated individual level OCT values as the mean of all available right and left eye measurements. As I aimed to explore associations in the general population, I did not exclude individuals with retinal (or other) pathology from the OCT analyses.

Glaucoma status: From 2006–2010, the touchscreen questionnaire administered to approximately 175 000 participants included a question on physician-diagnosed eye disorders. Participants were considered cases if they reported a diagnosis of glaucoma, or previous surgical or laser treatment for glaucoma, in either eye. I also included any participant carrying an International Classification of Diseases (ICD) code for glaucoma (ICD 9th revision: 365.* (excluding 365.0); ICD 10th revision: H40.* (excluding H40.0 and H42.*)) in their linked hospital records at any point prior to, and up to 1 year after, the baseline assessment. I excluded cases who were diagnosed prior to 30 years of age, and controls who reported using ocular hypotensive medication or carrying an ICD code for glaucoma suspect (ICD 9th revision: 365.0; ICD 10th revision: H40.0).

2.2.1.2 EPIC-Norfolk

IOP: All participants of the EPIC-Norfolk Eye Study underwent measurement of IOP through non-contact tonometry. The first 443 sequential participants were measured with an AT555 device (Reichert Corp., Philadelphia, PA, USA), with the ORA used for all remaining participants.[204] Three ORA readings were taken per eye and the best

signal value for each eye was used (based on the best quality pressure waveform as assessed by the ORA software). Individual-level IOP values were calculated as the mean of available right and left eye values. Participants with a history of glaucoma laser therapy or surgery were excluded, and imputation of pre-treatment IOP for participants using topical hypotensive agents was performed using the same method described above.

Glaucoma status: Case ascertainment was determined from a detailed ophthalmic examination that included visual acuity, tonometry, assessment of the ONH and pRNFL with scanning laser ophthalmoscopy and polarimetry, and visual field assessment through automated perimetry.[184] Those participants with abnormal findings, based on predefined criteria, were referred for a definitive eye examination by a consultant ophthalmologist with a special interest in glaucoma. Full details of glaucoma case ascertainment in the EPIC-Norfolk Eye Study have been described previously.[184,204]

2.2.1.3 Canadian Longitudinal Study on Aging

IOP: All approximately 30 000 Comprehensive cohort CLSA participants underwent a detailed ophthalmic examination as part of the baseline assessment, including measurement of IOP using the ORA. Individual-level IOPcc was calculated as the mean of available right and left eye values, and extreme values in the top and bottom 0.5 percentiles of the distribution were excluded. I excluded participants using ocular hypotensive medication or those reporting recent eye surgery.

Glaucoma status: Case ascertainment in CLSA was based on a self-reported history of glaucoma at the time of the baseline assessment. A detailed eye examination was not performed, and medical record linkage was not available.

2.2.2 Modifiable risk factors

2.2.2.1 Alcohol

See section 4.2.3.2 for a detailed description of the assessment and quantification of alcohol intake in the UK Biobank.

2.2.2.2 Smoking

See section 5.1.3.3 for details of the derivation of the smoking-related exposure measures used in the UK Biobank and CLSA.

2.2.2.3 Salt intake

See section 6.1.3.2 for a description of the assessment and quantification of dietary salt intake and urinary sodium excretion in the UK Biobank.

2.2.2.4 Systemic medication

See section 7.1.2.2 and section 7.2.3.3 for details of the determination of systemic medication use in the UK Biobank and EPIC-Norfolk, respectively.

2.2.2.5 Physical activity

See section 8.1.2.2 for details of physical activity measures used in the UK Biobank.

2.2.3 Genotyping and polygenic risk scores

Genetic data from approximately 490 000 UK Biobank participants were generated using two closely related genotyping platforms. The Affymetrix UK BiLEVE Axiom Array returned genotypes at 807 411 markers for approximately 50 000 participants, while the Affymetrix UK Biobank Axiom Array provided genotypes at 825 925 markers for the remaining approximately 440 000 participants.[205] Quality control and imputation were performed jointly for these two platforms, as previously described.[157] Imputation (genotypic determination based on inference and not by direct typing) was based on the UK10K and Haplotype Reference Consortium reference panels.

I constructed a polygenic risk score (PRS) based on 2 673 independent single nucleotide polymorphisms (SNPs) associated with glaucoma (at $P \leq 0.001$) from a recent multi-trait analysis of GWAS (MTAG) which included UK Biobank data.[27] Glaucoma is a complex polygenic disease and I considered the MTAG PRS to be a better representation of genetic variation in glaucoma than any individual or limited set of variants. I used the effect estimates from the original MTAG study to generate a glaucoma PRS for each participant using a standard weighted sum of individual SNPs:

$$\sum_{i=1}^{2\,673} \hat{\beta}_{(i)} * SNP_{(i)}$$

where $\hat{\beta}_{(i)}$ is the estimated effect size of $SNP_{(i)}$ on glaucoma. The PRS was normalised with a mean of 0 and a standard deviation (SD) of 1 for analyses. This glaucoma MTAG PRS has been found to be predictive of earlier age at glaucoma diagnosis, glaucoma progression, and need for surgical intervention in an independent cohort.[27]

2.2.4 Covariables

A notable strength of the epidemiological studies included in this project, and the UK Biobank in particular, is the extensive participant phenotyping and wealth of additional data available for analysis. Detailed descriptions of these variables, including individual test procedures, protocols, and quality control, are available online for the UK Biobank (https://biobank.ndph.ox.ac.uk/showcase/), EPIC-Norfolk (https://www.epic-norfolk.org.uk/for-researchers/data-dictionary/), and CLSA (https://datapreview.clsa-elcv.ca/datasets). Additional covariable information, including details of cleaning, categorisation, and derivation, can be found in the methods sections of Chapters 3–8. A summary of UK Biobank covariables used in this project are available in **Table B1**.

2.2.5 Other

In addition to the variables described above, I also derived several other UK Biobank variables over the course of this project, many of which have been utilised for analyses conducted by both departmental and external research groups. Notably, together with Dr Alasdair Warwick, I curated a comprehensive glaucoma code list (comprising diagnostic, procedural, and prescription codes) from linked primary care and hospital admission records of UK Biobank participants (**Table B2**).

2.3 Analytical approaches

Multiple analytical approaches were utilised in the course of this project. This section provides an introduction and brief overview of the various study designs employed. Specific details and comprehensive descriptions can be found in the relevant methods sections of Chapters 3–8.

2.3.1 Systematic review

A systematic literature review is a secondary research approach characterised by a detailed and comprehensive search strategy and analysis plan developed a priori, with the goal of reducing bias by identifying, appraising, and synthesising all relevant studies on a particular topic.[206,207] This is in contrast to traditional narrative reviews which tend to be mainly descriptive and are often focused on a subset of studies based on availability or author selection. Systematic reviews aim to minimise this potential selection bias and can be particularly informative if similar studies have conflicting results or divergent conclusions.[206]

A systematic review typically follows several well-defined steps, which often include, (i) formulation of a clear and logical research question; (ii) preliminary research and idea validation; (iii) development and pre-registration of study protocol; (iv) comprehensive search strategy for identification of relevant studies; (v) screening and selection of studies meeting pre-defined inclusion criteria; (vi) data extraction; (vii) assessment of study quality; (viii) synthesis, analysis, and interpretation of results; and (ix) dissemination of study findings. To minimise individual error and bias, several steps are performed independently by at least two researchers. Systematic reviews may also include a quantitative meta-analysis of study results (see section 2.3.2 below).

This research methodology was applied in Chapter 4 (alcohol) of this thesis and was conducted in accordance with the Meta-analysis of Observational Studies in Epidemiology (MOOSE) and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[208,209] Further details of specific systematic review methods used in this thesis are available in section 4.1.

2.3.2 Meta-analysis

Meta-analysis is a quantitative research method involving the statistical combination of results from multiple (two or more) separate studies addressing a similar research question. This approach is often applied to the results of studies identified through a systematic review but can also be used to pool findings from multicentre studies or research consortia. Potential benefits include the ability to answer questions not posed by individual studies, increased statistical power, and the opportunity to settle controversies arising from conflicting study results.[210]

Most meta-analytical methods are variations on a weighted average of the effect estimates from individual studies. A variety of statistical techniques have been developed to enable pooling of results commonly reported in biomedical literature, including mean differences, odds ratios, prevalence estimates, and measures of relative risk.[210] When performing meta-analysis, it is critically important to assess differences in study design, within-study biases, variation across studies, and reporting biases, as these have the potential to lead to misleading results if not appropriately considered.[210]

Meta-analysis was employed in Chapter 3 (glaucoma prevalence) and Chapter 4 (alcohol) of this thesis in accordance with Cochrane Collaboration guidelines.[210] Further details of meta-analytical methods used in this thesis are available in sections 3.1 and 4.1.

2.3.3 Cross-sectional analyses

A cross-sectional study is an observational study design involving the analysis of data from a population at a single point in time.[211] In comparison to case-control studies (where participants are selected based on the outcome status and exposures

are assessed retrospectively) and cohort studies (where participants are selected based on the exposure status and outcomes are assessed prospectively), cross-sectional studies measure exposures and outcomes in study participants at the same time.

Cross-sectional studies are often used to describe the features of a population or to measure the prevalence of health outcomes (descriptive studies), or to assess associations between exposures and outcomes in order to understand determinants of health (analytical studies). While this study design offers a relatively quick and inexpensive approach to studying multiple exposures and outcomes, it is unable to investigate temporal relationships and is susceptible to various biases which affect the ability to make causal inferences.[211] In analytical cross-sectional studies, a variety of methodological and statistics techniques can be utilised in an attempt to minimise potential biases.[212]

Although a longitudinal study design (in which exposures and outcomes are measured at different timepoints and associations with incident outcomes are assessed) is often preferred when making causal inferences from observational data, this approach, although possible, was not considered appropriate for this thesis for several reasons highlighted below.

Glaucoma is a chronic, progressive disease largely managed on an outpatient basis.[2] Early stages of the disease are often asymptomatic and a substantial proportion of cases in the community remain undiagnosed.[5] In the UK, opportunistic case finding occurs largely at the level of community-based optometrists,[213] and case detection in the general population may be influenced by several socioeconomic factors.[214] In the UK Biobank (and other epidemiological studies utilised in this thesis), incident disease outcomes are based on record-linkage with

hospital inpatient data and, for any particular hospital episode, all disease codes (both responsible for and coincidental to the reason for admission) are recorded.

This means that the longitudinal glaucoma data available in the cohorts utilised in this thesis, (i) are likely to be a significant underestimation of true glaucoma prevalence, (ii) may not be an accurate reflection of true glaucoma status, (iii) do not reflect the onset of the disease process, (iv) are subject to various potential selection, identification, classification, and reporting biases, and (v) do not include relevant glaucoma-related measures, such as IOP or structural OCT biomarkers.

A cross-sectional study design was employed in Chapters 3–8 of this thesis, with findings reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[215] For most analyses described in this thesis, I employed descriptive summary metrics and statistical tests to characterise the population under study and constructed multivariable regression models to assess exposure-outcome relationships. Full details of the methods and techniques employed in this thesis are available in sections 3.1, 4.2, 5.1, 6.1, 7.1, 7.2, and 8.1.

2.3.4 Gene-environment interaction

A gene-environment interaction occurs when the relationship between an exposure and an outcome differs in individuals with different genotypes (or when a particular genotype has a different effect in individuals with different environmental exposures).[216] To assess whether observed exposure-outcome associations were modified by genetic factors, I included the glaucoma MTAG PRS in final regression models and tested the significance of a multiplicative interaction term between the relevant exposure and the PRS. This statistical approach was employed in Chapter 4

(alcohol), Chapter 6 (salt intake), and Chapter 8 (physical activity) of this thesis. Further details of these analyses are available in sections 4.2, 6.1, and 8.1.

2.3.5 Mendelian randomisation

Mendelian randomisation (MR) is an analytical method that uses genetic variants as instrumental variables (or proxies) for modifiable risk factors that affect population health.[217] A population is first stratified according to genetic makeup before measurable traits or disease outcomes are compared across strata.

For example, certain variants in the C-reactive protein (CRP) gene are known to specifically influence mean CRP concentration (but not protein structure) on a population level.[218] On average, individuals carrying these genetic variants have higher lifetime CRP levels than those who do not. Due to the random allocation of alleles at conception, stratifying a population on these variants is analogous to a naturally occurring randomised controlled trial, with individuals differing only by their lifetime exposure to circulating CRP levels and no other factors. Any difference between the two study groups (incident heart disease, for example) can therefore be causally attributed to the effect of CRP.

Compared to traditional observational techniques, MR is less likely to be affected by confounding and reverse causation, but requires that certain assumptions be satisfied in order to make valid causal inferences.[217] The key assumptions for a valid MR study are: (i) the genetic variants must be associated with the risk factor of interest (the relevance assumption), (ii) there are no unmeasured confounders of the gene-outcome association (the independence assumption), and (iii) the genetic variants affect the outcome only through their effect on the risk factor of interest (the exclusion restriction assumption) (**Figure 2.1**).

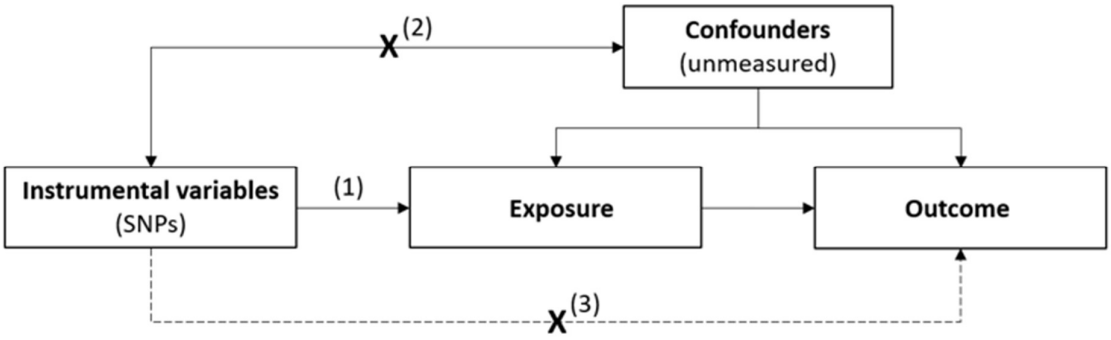

Figure 2.1 Directed acyclic graph illustrating the principals and assumptions of Mendelian randomisation

SNP, single nucleotide polymorphism. Instrumental variable (IV) assumptions: (1) IV is associated with the exposure of interest, (2) IV is not associated with confounders of the exposure-outcome association, (3) IV only affects the outcome via the exposure and not through alternative pathways.

The simplest MR studies use a single genetic variant as an instrumental variable for the risk factor of interest. This approach can be particularly persuasive when using variants in a gene with a well understood function, as the core MR assumptions can be supported by biological knowledge.[217] However, for many complex traits, single genetic variants generally explain only a small proportion of the variation in a phenotype. More complex MR techniques can utilise multiple genetic variants as instrumental variables through aggregate approaches or regression-based methods.

A particular concern with this approach, especially when genetic variants are chosen on a statistical rather than a biological basis, is the potential for horizontal pleiotropy. This occurs when genetic variants affect the outcome through pathways other than through the risk factor of interest (a violation of the exclusion restriction assumption).

Several MR methods have been developed that allow for genetic pleiotropy and consistency of results across multiple techniques strengthens causal inferences.[219–221]

MR analyses can be performed in a one-sample (using a single dataset to yield the causal estimate of the risk factor on the outcome) or two-sample framework.[217] In the two-sample approach, different study populations are used to estimate the instrument-risk factor and instrument-outcome associations. This is particularly useful when the exposure or outcome are difficult or expensive to measure, and additionally allows for substantially increased statistical power, by incorporating data from multiple sources, including large genetic consortia.

Two-sample MR analyses were conducted in Chapter 4 (alcohol), Chapter 5 (smoking), and Chapter 8 (physical activity) of this thesis. These were reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomisation (STROBE-MR) guidelines.[222] Full details of these analyses are provided in sections 4.3, 5.2, and 8.1.

PART III

RESULTS

3

Glaucoma prevalence

3.1 Glaucoma prevalence meta-analysis

This section describes the results of a multicentre glaucoma prevalence meta-analysis currently in preparation for submission to the *British Medical Journal*. I am grateful to the numerous researchers of the E3 consortium who contributed study-level raw data or summary statistics for use in this project. A full list of collaborators and the relevant declaration form is located in **Appendix A**. I was responsible for all other aspects of the work described here. Supplementary material for this section can be found in **Appendix C**.

3.1.1 Abstract

Objective: To provide updated estimates of European glaucoma prevalence and future disease burden.

Design: Quantitative meta-analysis of individual-level prevalence data.

Setting: Fourteen population-based European eye studies of the E3 consortium.

Participants: A total of 56 611 participants (53.9% women), aged 36–106 years, from seven countries were included.

Main outcome measures: Age-standardised prevalence (2013 European Standard Population) of glaucoma for individuals aged ≥40 years. Annual projections of total European glaucoma burden from 2024 to 2050.

Results: A total of 2 021 participants were diagnosed with glaucoma, with an overall age-standardised prevalence of 2.99% (95% CI, 2.86–3.12). Prevalence increased with older age, reaching 10.74% (95% CI, 8.86–12.76) in those aged 85+ years, and was higher in men (3.32%; 95% CI, 3.12–3.53), Eastern Europe (5.42%, 95% CI, 4.74–6.10), and in studies with case ascertainment based on specialist opinion

(3.38%, 95% CI, 3.12–3.63). More than half (56.4%) of all cases were newly diagnosed, with a higher proportion of undiagnosed disease in younger participants. Prevalence of POAG, PACG, and secondary glaucoma was 2.51% (95% CI, 2.29–2.73, 79.9% of cases), 0.21% (95% CI, 0.16–0.27, 9.1% of cases), and 0.29% (95% CI, 0.22–0.36, 11.0% of cases), respectively. There are currently an estimated 12.26 million glaucoma cases across Europe, a figure projected to increase to 13.52 million by 2050.

Conclusions: This study provides detailed estimates of glaucoma prevalence in Europe and updated projections of the future burden of disease. It corroborates many previous findings, but also offers novel insights into the burden of undiagnosed disease, as well as highlighting regional and diagnostic differences in glaucoma prevalence estimates.

3.1.2 Introduction

Glaucoma is the leading cause of irreversible blindness globally and the second most common cause in Europe, where it accounts for 14% of total blindness.[223,224] The disease is strongly age related – while rare before the age of 40 years, prevalence approaches 10% in those older than 80 years.[225] Considering projections for ongoing European demographic ageing, the substantial health and economic burden posed by glaucoma is likely to increase in coming decades.[11,226]

Previous meta-analyses of glaucoma prevalence data have often relied on statistical modelling or broad subgroup analyses to account for interstudy differences in age structure, since individual-level data are generally not available.[7,225,227,228] Differences in modelling approaches and assumptions, however, may lead to widely disparate projections of glaucoma burden.[7,225,228] Detailed stratum-specific

prevalence estimates are essential for accurate projections of future glaucoma prevalence used to inform public health policy.

Despite the progressive, irreparable nature of glaucoma, early stages of the disease are asymptomatic, with up to two-thirds of those affected in Europe undiagnosed, or at least unaware of their diagnosis.[204,229] Quantification of the burden of undiagnosed disease therefore represents a valuable undertaking and an important consideration when planning the allocation of limited healthcare resources or designing screening strategies. Other relevant factors include how disease prevalence varies over time and according to sex, ethnicity, geographic region, diagnostic criteria used, and glaucoma subtype.

In this study, I investigate the prevalence of both total and previously undiagnosed glaucoma across Europe, using pooled data from population-based studies of the E3 consortium. I provide updated age-standardised prevalence estimates, assess for temporal trends, and investigate heterogeneity across clinically relevant subgroups. Lastly, I apply these new estimates to European population projections to predict the number of individuals with glaucoma through to 2050.

3.1.3 Methods

3.1.3.1 Study population

See section 2.1.1.4 for a detailed description of the E3 consortium.

For this analysis, I considered only population-based studies with glaucoma prevalence data based on direct participant examination. Studies with estimates based on self-report, record-linkage, or other methods were excluded, as these studies will systematically underestimate glaucoma prevalence due to the high proportion of undiagnosed glaucoma in the general population. Ultimately, 55 415

participants aged 40 years and older from fourteen E3 studies were included (**Figure 3.1**). Thirteen studies (n = 48 698) had data on previously undiagnosed glaucoma and three studies (n = 14 559) had detailed and comparable glaucoma subtype data.

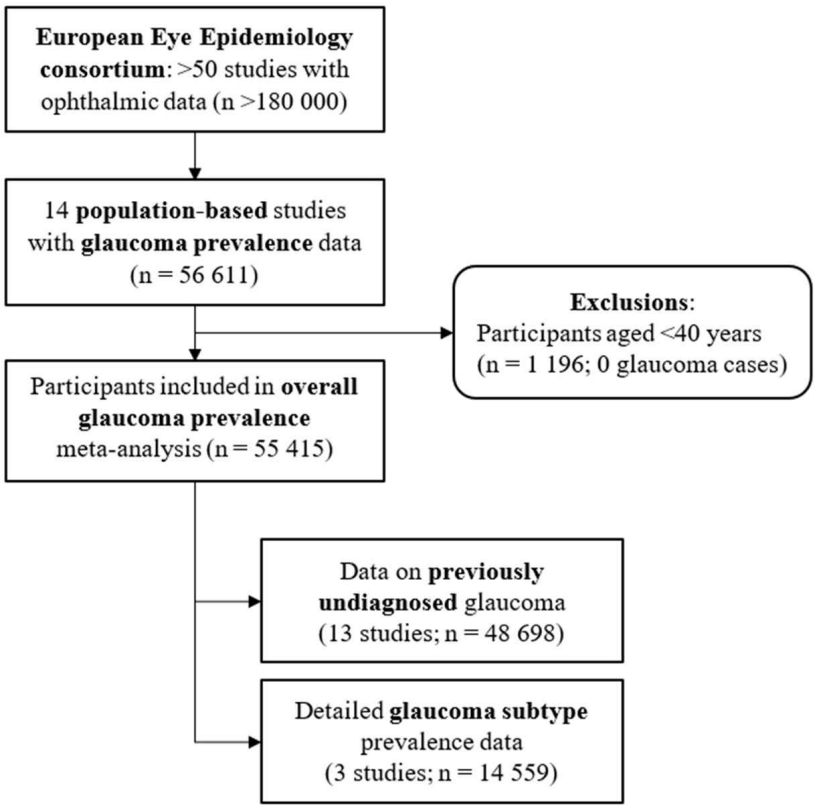

Figure 3.1 Flow diagram of study and participant selection in the glaucoma prevalence meta-analysis

Participants were recruited between 1991 and 2020 from seven European countries. Across all studies, mean age was 63.8 years (range, 32–106 years), with a slight predominance of women (53.9%) and minimal ethnic diversity (where available, 99.3% were of European descent). Further details of each contributing study are available in **Table 3.1**, **Appendix C**, and previous publications.[65,189,204,229–232]

Table 3.1 Description of the fourteen studies included in the glaucoma prevalence meta-analysis

Study	Years	Country	Participants (n)	Age (mean, range)	Sex (% women)	Ethnicity (% European)	Glaucoma cases (n)	Crude prevalence (%)
Northern Europe								
EPIC-Norfolk	2006–2011	United Kingdom	8 623	68.7 (48–92)	55.2	99.7	363	4.21
NICOLA	2014–2018	United Kingdom	3 386	63.6 (32–96)	52.1	N/A	99	2.92
Eastern Europe								
UEMS	2015–2017	Russia	5 545	59.1 (37–91)	56.8	N/A	247	4.45
Southern Europe								
TES	1995–2005	Greece	2 554	71.5 (60–95)	47.1	100.0	154	6.03
Coimbra	2015–2017	Portugal	1 603	72.5 (60–91)	57.0	99.6	142	8.86
Western Europe								
Rotterdam-I	1991–1993	Netherlands	6 717	69.4 (55–106)	59.6	99.0	354	5.27
Rotterdam-II	2000–2001	Netherlands	2 240	64.3 (55–97)	54.8	97.9	106	4.73
ALIENOR	2006–2008	France	936	80.1 (73–94)	61.8	N/A	45	4.81
Rotterdam-III	2006–2008	Netherlands	3 434	56.9 (45–90)	56.4	96.9	73	2.13
GHS	2007–2012	Germany	12 089	54.9 (35–74)	49.7	100.0	128	1.06
MONTRACHET	2009–2011	France	1 153	82.3 (76–96)	62.7	N/A	100	8.67
Maastricht	2010–2020	Netherlands	6 018	59.8 (40–79)	50.2	98.6%	87	1.45
AugUR-I	2013–2015	Germany	1 043	77.5 (70–95)	45.0	100.0	49	4.70
AugUR-II	2017–2019	Germany	1 270	79.0 (70–95)	57.9	100.0	74	5.83
TOTAL								
14 studies	**1991–2020**	**7 countries**	**56 611**	**63.8 (32–106)**	**53.9**	**99.3**	**2 021**	**3.65**

ALIENOR, Antioxydants, lipides essentiels, nutrition et maladies oculaires; AugUR, Age-related diseases: understanding genetic and non-genetic influences - a study at the University of Regensburg; EPIC, European Prospective Investigation into Cancer; GHS, Gutenberg Health Study; MONTRACHET, Maculopathy, optic nerve, nutrition, neurovascular and heart diseases; N/A, not available; NICOLA, Northern Ireland Cohort for the Longitudinal Study of Ageing; TES, Thessaloniki Eye Study; UEMS, Ural Eye and Medical Study.

Ethnicity proportions are reported only for participants with known ethnicity data.

3.1.3.2 Glaucoma case ascertainment

Both eyes of included participants were examined according to study-specific protocols and guidelines. These examinations, summarised in **Table 3.2,** consisted of varying combinations of structural and functional ONH and anterior drainage angle assessments. Despite significant advances in glaucoma diagnostic technology (and hence, availability) over the 30-year period spanned by this meta-analysis, most studies performed both ONH-centred colour fundus photography and visual field assessment through automated perimetry.

Similarly, there are no universal diagnostic criteria for glaucoma and case definitions have evolved in parallel with advances in diagnostic technology. On a population level, the ISGEO criteria proposed in 2002, based largely on ONH and visual field parameters, are the most well-established diagnostic guidelines used in prevalence studies.[34] Glaucoma classification was performed according to study-specific criteria and participants were considered cases if at least one eye was classified as having 'probable' or 'definite' glaucoma, according to local definitions. In most studies, glaucoma diagnosis was based either on the opinion of a glaucoma specialist or on ISGEO criteria (or modifications thereof). Further details of glaucoma case ascertainment are available in **Table 3.2** and **Appendix C**.

Table 3.2 Glaucoma case ascertainment in the fourteen studies included in the glaucoma prevalence meta-analysis

Study	Glaucoma assessment			Diagnostic criteria	Glaucoma classification		Previously undiagnosed glaucoma	
	Structural	Functional	Angle		Subtypes available	Meta-analysis	Definition used	Meta-analysis
Rotterdam-I	CFP	VF	SL, Hx	Other	Overall*	–	N/A	–
TES	SL	VF	Gonio	Specialist	Overall, POAG, PACG, 2°	✓	Self-report	✓
Rotterdam-II	CFP	VF	Hx	Other	Overall*	–	Self-report	✓
ALIENOR	CFP, SL, SLO	VF	Gonio	ISGEO	Overall, OAG	–	Self-report	✓
Rotterdam-III	CFP	VF	Hx	Other	Overall*	–	Self-report	✓
EPIC-Norfolk	CFP, SL, SLO, SLP	VF	Gonio	Specialist	Overall, POAG, PACG, 2°	✓	Self-report, medical records	✓
GHS	CFP, SL	VF	SL	ISGEO	Overall, OAG	–	Self-report	✓
MONTRACHET	CFP, OCT	VF	Gonio	ISGEO	Overall*, OAG	–	Self-report	✓
Maastricht	CFP	VF	–	ISGEO	Overall	–	Self-report	✓
AugUR-I	CFP	–	–	M-ISGEO	Overall	–	Self-report	✓
NICOLA	CFP, OCT, SL	VF	Gonio	ISGEO	Overall, POAG, PACG, 2°	✓	Self-report	✓
Coimbra	CFP, OCT	–	–	Specialist	Overall	–	Self-report	✓
UEMS	CFP, SL, OCT	VF	ASB	ISGEO	Overall, OAG, ACG	–	Self-report	✓
AugUR-II	CFP	–	–	M-ISGEO	Overall	–	Self-report	✓

2°, secondary; ACG, angle-closure glaucoma; ALIENOR, Antioxydants, lipides essentiels, nutrition et maladies oculaires; ASB, anterior segment biometry; AugUR, Age-related diseases: understanding genetic and non-genetic influences - a study at the University of Regensburg; CFP, colour fundus photography; EPIC, European Prospective Investigation into Cancer; GHS, Gutenberg Health Study; Gonio, gonioscopy; Hx, medical history; ISGEO, International Society of Geographical and Epidemiological Ophthalmology; M-ISGEO, modified ISGEO; MONTRACHET, Maculopathy, optic nerve, nutrition, neurovascular and heart diseases; N/A, not available; NICOLA, Northern Ireland Cohort for the Longitudinal Study of Ageing; OAG, open-angle glaucoma; OCT, optical coherence tomography; PACG, primary angle-closure glaucoma; POAG, primary open-angle glaucoma; SL, slit lamp biomicroscopy; SLO, scanning laser ophthalmoscopy; SLP, scanning laser polarimetry; TES, Thessaloniki Eye Study; UEMS, Ural Eye and Medical Study; VF, visual field.

* Excludes cases of angle-closure glaucoma (not quantified in Rotterdam studies, *n* = 2 in MONTRACHET). Studies are listed in chronological order according to the median year of assessment.

Thirteen studies included data on whether glaucoma cases were newly diagnosed by the study or had been previously diagnosed. Participants were considered previously diagnosed cases if they reported a history of glaucoma diagnosis or therapy in either eye at the time of the study assessment. Studies were included in the glaucoma subtype analysis if they included: (1) diagnosis based on both structural and functional ONH assessment, (2) gonioscopic assessment of the anterior drainage angle, (3) slit lamp biomicroscopy to assess for secondary causes of disease, and (4) prevalence data for all of POAG, PACG, and secondary glaucoma. Further details of glaucoma ascertainment in all studies are available in **Table 3.2**.

3.1.3.3 Demographic and study variables

Participants were stratified by 5-year age group, sex, and, where available, ethnicity (European, non-European). For the purposes of this study, participants were considered European if they self-identified as being of White ethnicity or were classified as being of European descent, based on principal component genetic analysis. There were no glaucoma cases in participants younger than 40 years of age (*n* = 1 196) and subsequent analyses were restricted to individuals aged 40 years and older. Due to small participant numbers in older age groups, participants aged 85 years and older were collapsed into a single age category. European geographic regions were defined according to the United Nations Geoscheme (M49) classification system.[233]

3.1.3.4 Statistical analysis

Within each study, I calculated crude glaucoma prevalence for each age group separately for men and women. To address variance instability and normality assumptions, I applied a Freeman-Tukey double-arcsine transformation to crude

estimates, before pooling results using random-effects meta-analysis, stratified by age and weighted by sample size.[234] A random-effects model was chosen to allow for expected heterogeneity between studies as a result of varying study design. This enabled calculation of a pooled prevalence estimate for each 5-year age group, with a corresponding 95% CI based on score procedures.[235] This approach was repeated for the analysis of undiagnosed glaucoma and for each glaucoma subtype. Proportions of undiagnosed glaucoma and glaucoma subtypes were calculated for each 5-year age group, using only studies with available data as the denominator, and compared using two-sample *z* tests of proportion.

Age-standardised prevalence estimates were then calculated with demographic distribution adjustments to age-specific estimates according to the European Standard Population (2013 revision).[236] This resulted in glaucoma prevalence estimates representative of the European population, with appropriate weighting to the age demographic distribution of Europe. The primary outcome measure used in this study was the age-standardised prevalence for individuals 40 years and older.

Subsequent random-effects meta-analyses were performed with stratification by age and each of: sex, geographic region, and glaucoma diagnostic criteria used. In these subgroup analyses, heterogeneity was assessed with Cochran's *Q* statistic and quantified using the I^2 statistic.[237] To avoid biased estimates, substrata with fewer than 50 participants were excluded. Age-standardised prevalence estimates for each subgroup were then calculated in the manner described above. Due to minimal ethnic variation in studies where these data were available, I was unable to perform a meaningful subgroup analysis for ethnicity, but performed a sensitivity analysis in which I restricted analyses to participants of European ethnicity only.

To assess for study-level determinants of glaucoma prevalence and to investigate temporal trends, I performed random-effects meta-regression. Meta-regression is an extension to standard meta-analysis that investigates the extent to which statistical heterogeneity between results of multiple studies can be related to one or more characteristics of the studies.[238] Random-effects meta-regression fits a linear model allowing for residual heterogeneity (between-study variance not explained by the covariates). I used a multilevel meta-regression framework to model the logit of glaucoma prevalence, using data from 186 age- and sex-specific strata from across the 14 included studies, with incorporation of random effects at the study level. Models were adjusted for age, sex, and the median year of the study assessment period. Geographic region and diagnostic criteria used were not concurrently added to the model due to strong collinearity.

3.1.3.5 Glaucoma projections

The projected number of glaucoma cases was calculated using the latest United Nations World Population Prospects for Europe, assuming constant fertility and mortality.[239] Age- and sex-specific glaucoma prevalence estimates (plus their respective 95% CIs) derived from the meta-analysis were applied to the corresponding population projections for the years 2024 to 2050. The predicted number of affected individuals was then summed across all groups to arrive at an estimate of total glaucoma burden. Based on the results of the meta-analysis, glaucoma prevalence estimates were assumed to remain constant over the next 26 years for these calculations. For the glaucoma subtype projections, age-specific proportions of POAG, PACG, and secondary glaucoma were applied to the corresponding estimates of the total number of affected individuals in each age

group, before summing across all age groups. Projections were then compared to figures from previous glaucoma prevalence meta-analyses.[7,225,228,240]

Statistical analysis was performed in Stata/MP (StataCorp. 2023. *Stata Statistical Software: Release 18*. College Station, TX: StataCorp LLC), including the *meta* and *metaprop* packages.[241]

3.1.4 Results

3.1.4.1 Overall glaucoma prevalence

Of the 55 415 participants included in the main analysis, 2 021 were diagnosed with glaucoma (crude prevalence, 3.65%), with an age-standardised European prevalence of 2.99% (95% CI, 2.86–3.12) for individuals aged 40 years and older. Age-specific prevalence increased from 0.22% (95% CI, 0.03–0.51) in those aged 40–44 years to 10.74% (95% CI, 8.86–12.76) in those aged 85+ years (**Figure 3.2** and **Table 3.3**).

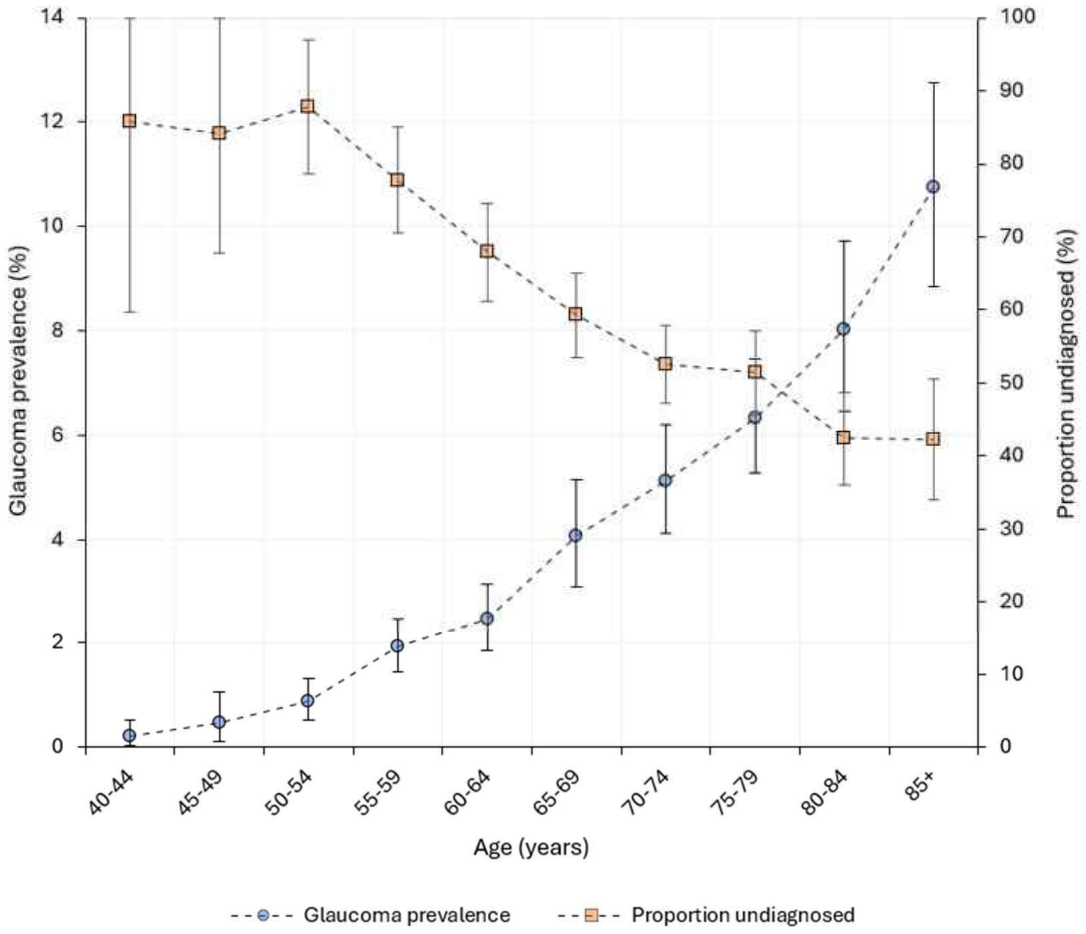

Figure 3.2 Age-stratified glaucoma prevalence and proportion of previously undiagnosed cases in participants aged 40 years and older

Table 3.3 Age-specific and age-standardised European glaucoma prevalence estimates, overall and by sex

Age (years)	Women			Men			Overall			Proportion undiagnosed (%)
	N	Prevalence (%)	95% CI	*N*	Prevalence (%)	95% CI	*N*	Prevalence (%)	95% CI	
Age-specific prevalence										
40–44	1 280	0.17	(0.00, 0.55)	991	0.30	(0.01, 0.83)	2 271	0.22	(0.03, 0.51)	85.7
45–49	1 797	0.48	(0.07, 1.16)	1676	0.50	(0.00, 1.77)	3 473	0.47	(0.10, 1.05)	84.2
50–54	2 758	0.85	(0.30, 1.61)	2322	0.90	(0.44, 1.48)	5 080	0.88	(0.52, 1.33)	87.8
55–59	4 379	1.93	(1.31, 2.66)	3614	1.94	(1.17, 2.89)	7 993	1.93	(1.44, 2.48)	77.8
60–64	5 423	2.00	(1.29, 2.86)	4483	3.01	(2.08, 4.09)	9 906	2.47	(1.87, 3.14)	67.9
65–69	4 564	3.61	(2.34, 5.12)	4301	4.58	(3.13, 6.26)	8 865	4.07	(3.10, 5.15)	59.3
70–74	3 940	4.54	(3.54, 5.66)	3811	5.90	(4.10, 7.97)	7 751	5.12	(4.13, 6.21)	52.5
75–79	2 869	6.19	(4.85, 7.69)	2378	6.49	(4.84, 8.34)	5 247	6.33	(5.28, 7.47)	51.4
80–84	1 864	7.65	(5.70, 9.83)	1342	8.48	(5.97, 11.36)	3 206	8.02	(6.47, 9.71)	42.4
85+	1 003	9.93	(7.80, 12.27)	620	12.70	(8.46, 17.62)	1 623	10.74	(8.86, 12.76)	42.3
Age-standardised prevalence										
Overall	29 897	1.46	(1.37, 1.55)	25 538	1.76	(1.65, 1.87)	55 415	1.59	(1.52, 1.65)	56.4
≥40	29 897	2.76	(2.59, 2.93)	25 538	3.32	(3.12, 3.53)	55 415	2.99	(2.86, 3.12)	56.4
≥50	26 800	3.63	(3.41, 3.86)	22 871	4.37	(4.10, 4.65)	49 671	3.94	(3.77, 4.11)	55.9
≥60	19 663	4.83	(4.52, 5.15)	16 935	5.95	(5.56, 6.34)	36 598	5.30	(5.05, 5.54)	53.0
≥70	9 676	6.53	(6.02, 7.04)	8 151	7.74	(7.11, 8.38)	17 827	6.99	(6.59, 7.38)	48.4
≥80	2 867	8.79	(7.72, 9.86)	1 962	10.59	(9.12, 12.06)	4 829	9.38	(8.52, 10.24)	42.4

CI, confidence interval; *N*, sample size.

Age-standardised prevalences are based on the European Standard population (2013 revision).

3.1.4.2 Subgroup analyses

Men had a higher overall glaucoma prevalence (3.32%; 95% CI, 3.12–3.53) than women (2.76%; 95% CI, 2.59–2.93), with this sex-specific difference apparent at all ages (**Table 3.3**). There was significant heterogeneity by geographic region, with the lowest prevalence observed in Western Europe (2.55%; 95% CI, 2.39–2.71) and the highest in Eastern Europe (5.42%, 95% CI, 4.74–6.10). Studies based on specialist opinion yielded the highest glaucoma prevalence estimate (3.38%, 95% CI, 3.12–3.63) and those on modified ISGEO criteria the lowest (2.17%, 95% CI, 1.79–2.56). Further details of subgroup analyses are presented in **Figure 3.3** and **Figure 3.4**.

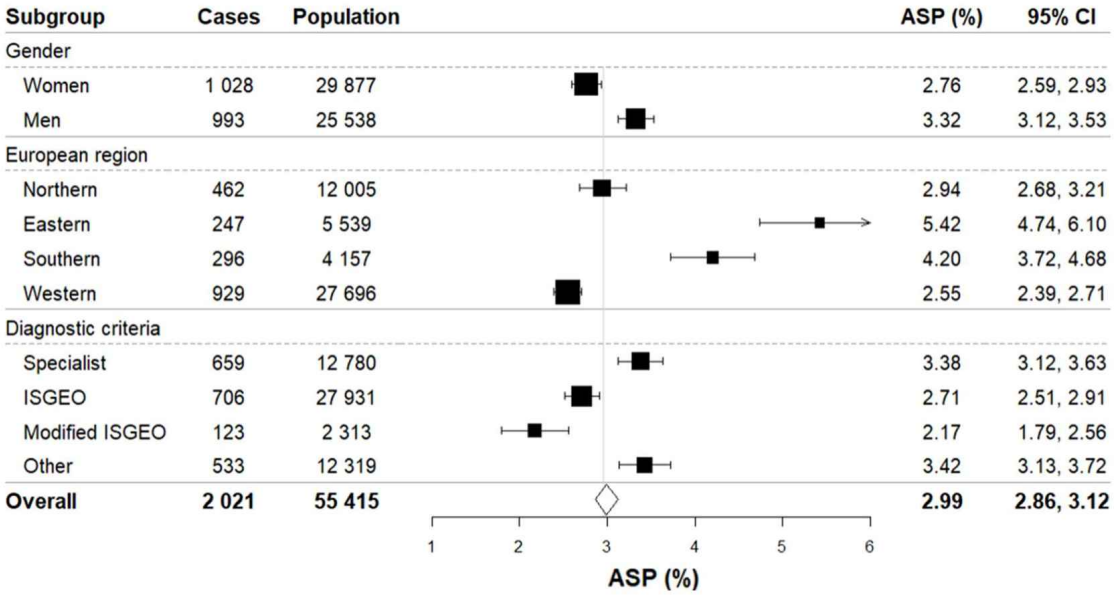

Figure 3.3 Forest plot of the results of the glaucoma prevalence subgroup analyses

ASP, age-standardised prevalence (European Standard Population, 2013 revision) for individuals aged 40 years and older; CI, confidence interval.

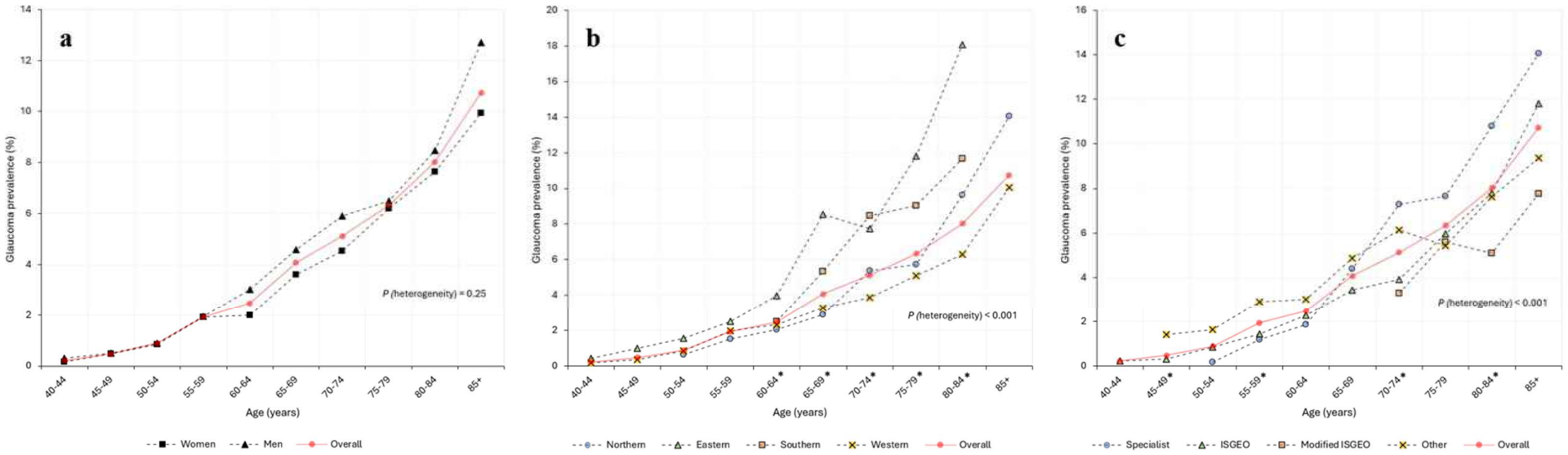

Figure 3.4 Subgroup analyses of European glaucoma prevalence estimates for individuals aged 40 years and older by (**a**) sex, (**b**) region, and (**c**) diagnostic criteria used

Sub-strata with less than 50 participants not included. * Indicates significant heterogeneity (P <0.05) for a particular age group.

3.1.4.3 Undiagnosed glaucoma

Overall, 56.4% of all glaucoma cases were previously undiagnosed. The proportion of undiagnosed disease was inversely related to age, declining from 84.6% in those aged less than 50 years to 42.4% in those aged 80 years and older (P <0.001) (**Figure 3.2** and **Table 3.3**). There was no difference by sex (women, 54.8%, men, 57.9%; P = 0.20). Despite lower rates of undiagnosed disease in older individuals, the prevalence of undiagnosed glaucoma was still found to increase with age, from 0.18% (95% CI, 0.02–0.46) in those aged 40–44 years to 3.84% (95% CI, 2.41–5.55) in those aged 85+ years (**Figure 3.5**).

3.1.4.4 Glaucoma subtypes

POAG accounted for 79.9% of all glaucoma cases, with an age-standardised prevalence of 2.51% (95% CI, 2.29–2.73). Estimates for PACG (9.1% of all cases) and secondary glaucoma (11.0% of all cases) were 0.21% (95% CI, 0.16–0.27) and 0.29% (95% CI, 0.22–0.36), respectively. All glaucoma subtypes demonstrated greater prevalence with increasing age (**Table 3.4**), although a decline in PACG prevalence was observed after the age of 80 years, with this subtype accounting for a relatively greater proportion of all glaucoma cases between the ages of 60–74 years (**Figure 3.6**).

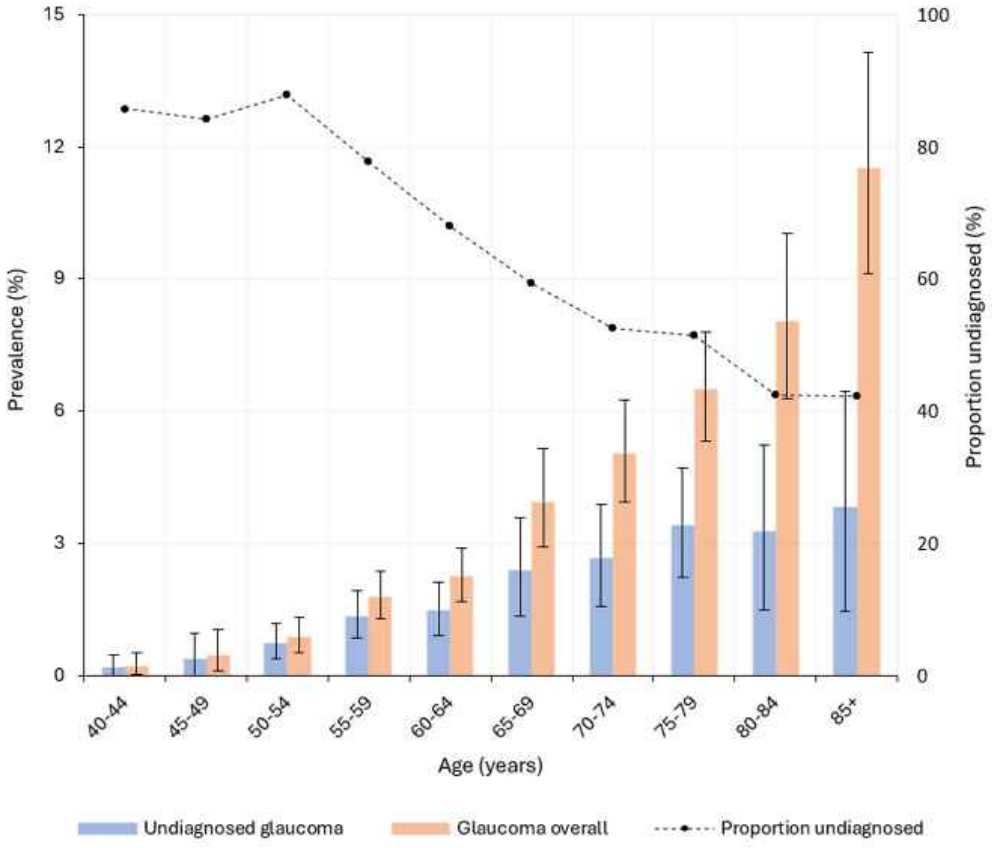

Figure 3.5 Age-standardised prevalence and proportion of undiagnosed glaucoma in individuals aged 40 years and older

Table 3.4 Age-specific and age-standardised European glaucoma prevalence estimates, by subtype

Age (years)	*N*	POAG		PACG		Secondary	
		Prevalence (%)	95% CI	Prevalence (%)	95% CI	Prevalence (%)	95% CI
Age-specific prevalence							
40–49	104	No estimate		No estimate		No estimate	
50–59	2 203	1.15	(0.69, 1.72)	0.01	(0.00, 0.16)	No estimate	
60–69	6 390	2.15	(1.59, 2.78)	0.29	(0.11, 0.55)	0.21	(0.04, 0.47)
70–79	4 568	4.55	(3.90, 5.26)	0.55	(0.34, 0.81)	0.70	(0.13, 1.65)
80+	1 294	8.80	(6.61, 11.25)	0.41	(0.07, 0.95)	0.79	(0.00, 2.48)
Age-standardised prevalence							
Overall	14 559	1.33	(1.21, 1.45)	0.11	(0.08, 0.14)	0.15	(0.12, 0.19)
≥40	14 559	2.51	(2.29, 2.73)	0.21	(0.16, 0.27)	0.29	(0.22, 0.36)
≥50	14 455	3.30	(3.01, 3.60)	0.29	(0.21, 0.36)	0.37	(0.29, 0.46)
≥60	12 252	4.48	(4.07, 4.89)	0.43	(0.31, 0.54)	0.50	(0.38, 0.62)
≥70	5 862	6.39	(5.70, 7.09)	0.55	(0.37, 0.73)	0.74	(0.53, 0.94)
≥80	1 294	9.69	(7.92, 11.45)	0.53	(0.14, 0.92)	0.77	(0.31, 1.23)

CI, confidence interval; *N*, sample size; PACG, primary angle-closure glaucoma; POAG, primary open-angle glaucoma.

Age-standardised prevalences based on the European Standard population (2013 revision).

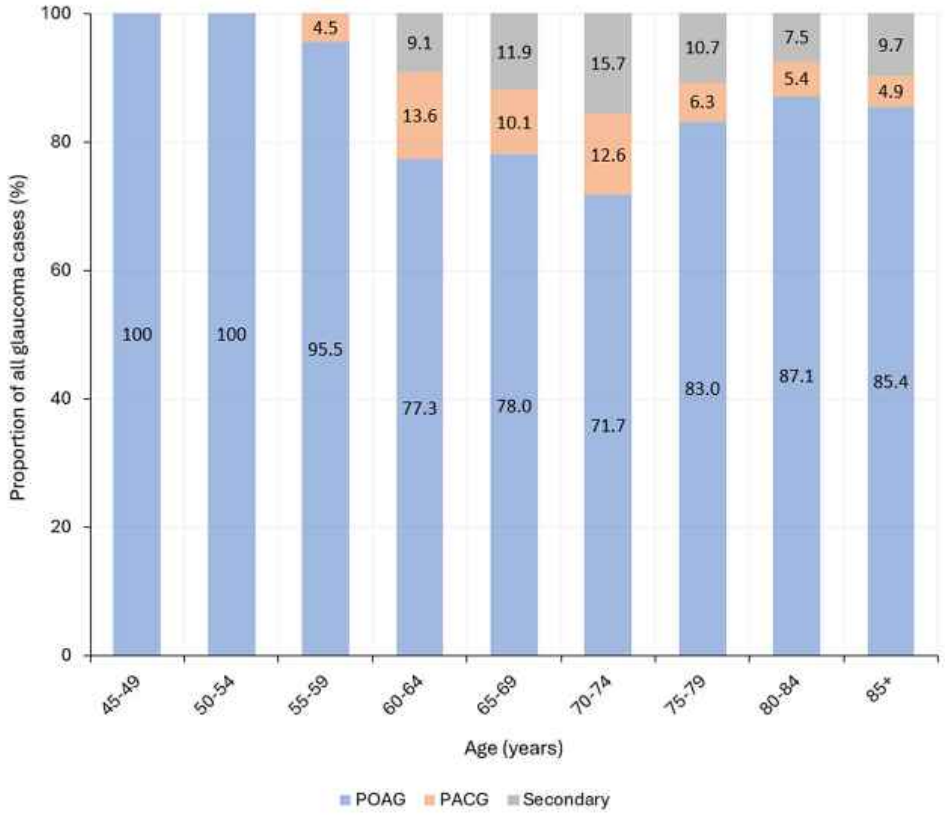

Figure 3.6 Subtype composition of all glaucoma cases, by age group

PACG, primary angle-closure glaucoma; POAG, primary open-angle glaucoma.

3.1.4.5 Meta-regression

In multiple-adjusted meta-regression models, glaucoma prevalence was significantly related to older age (OR, 1.32 per 5-year increase; 95% CI, 1.29–1.36; *P* <0.001) and male sex (OR, 1.18; 95% CI, 1.08–1.30; *P* <0.001). Compared to Western Europe, prevalence was higher in both Eastern (OR, 2.18; 95% CI, 1.17–4.05; *P* = 0.014) and Southern Europe (OR, 1.66; 95% CI, 1.06–2.60; *P* = 0.026). The use of ISGEO diagnostic criteria was associated with a lower glaucoma prevalence estimate (OR, 0.62; 95% CI, 0.39–0.97; *P* = 0.037) when compared to studies based on specialist opinion. No temporal trend was identified, with study year not related to glaucoma prevalence in both unadjusted and multiple-adjusted models. Full results of the meta-regression analyses are presented in **Table 3.5**.

Table 3.5 Results of the glaucoma prevalence meta-regression analyses

	Unadjusted			**Multiple adjusted***		
	Odds ratio	**95% CI**	***P*-value**	**Odds ratio**	**95% CI**	***P*-value**
Age, per 5-year increase	1.32	1.28, 1.36	**<0.001**	1.32	1.29, 1.36	**<0.001**
Sex						
Women		(Reference)			(Reference)	
Men	1.15	1.05, 1.26	**0.002**	1.18	1.08, 1.30	**<0.001**
Region						
Western		(Reference)			(Reference)	
Northern	1.03	0.47, 2.28	0.93	1.15	0.73, 1.82	0.54
Eastern	1.48	0.52, 4.26	0.47	2.18	1.17, 4.05	**0.014**
Southern	1.86	0.85, 4.10	0.12	1.66	1.06, 2.60	**0.026**
Diagnostic criteria						
Specialist		(Reference)			(Reference)	
ISGEO	0.57	0.28, 1.17	0.13	0.62	0.39, 0.97	**0.037**
Modified ISGEO	1.17	0.83, 2.11	0.70	0.54	0.29, 1.03	0.06
Other	0.63	0.28, 1.44	0.27	1.25	0.68, 2.30	0.48
Year of study, median	1.00	0.96, 1.04	0.86	0.99	0.96, 1.02	0.46

CI, confidence interval; ISGEO, International Society of Geographical and Epidemiological Ophthalmology.

* Adjusted for age, sex, and median year of study accordingly.

3.1.4.6 Sensitivity analyses

In total, 42 627 participants from 10 studies had available ethnicity data and were classified as European. This included 1 454 cases of glaucoma (crude prevalence, 3.41%) and restricting analyses to these participants only resulted in a slightly lower age-standardised prevalence (2.83%, 95% CI, 2.67–3.00) compared to the whole cohort.

3.1.4.7 Projections

In 2024, the total number of people in Europe with glaucoma is estimated to be 12.26 million. Of these, over 10 million (83.0%) are expected to have POAG, with approximately 1 million cases of both PACG (7.7%) and secondary glaucoma (9.4%) (**Table 3.6**). Despite projections of an overall decline in total population (-11.8%) over the next 26 years, the number of glaucoma cases is projected to increase by 10.3% to 13.52 million by 2050. The annual rate of change is expected to slow, however, with a peak of 13.63 million cases reached by 2045, before a decline in total case numbers is seen (**Figure 3.7**). Projections for both POAG and glaucoma overall are compared to figures derived from previous glaucoma prevalence meta-analyses in **Figure 3.8** and **Table 3.7**.

Table 3.6 Estimated number of people (millions) with glaucoma in Europe in 2024 and projections to 2050

	2024	2030	2040	2050
Population, millions	739.55	724.32	691.70	652.16
<40 years, %	45.2	43.1	42.1	41.8
40–69 years, %	40.6	41.1	40.0	39.0
≥70 years, %	14.2	15.8	18.0	19.2
Glaucoma cases, millions	12.26 (8.38–17.10)	12.86 (8.83–17.88)	13.56 (9.39–18.68)	13.52 (9.42–18.53)
POAG	10.17 (6.89–14.33)	10.66 (7.25–14.97)	11.26 (7.74–15.64)	11.22 (7.76–15.49)
PACG	0.94 (0.67–1.25)	0.98 (0.70–1.31)	1.01 (0.72–1.35)	1.01 (0.72–1.34)
Secondary	1.15 (0.83–1.51)	1.21 (0.88–1.60)	1.28 (0.93–1.69)	1.29 (0.94–1.70)

PACG, primary angle-closure glaucoma; POAG, primary open-angle glaucoma.

Population projections based on the 2022 United Nations Population Prospects for Europe.

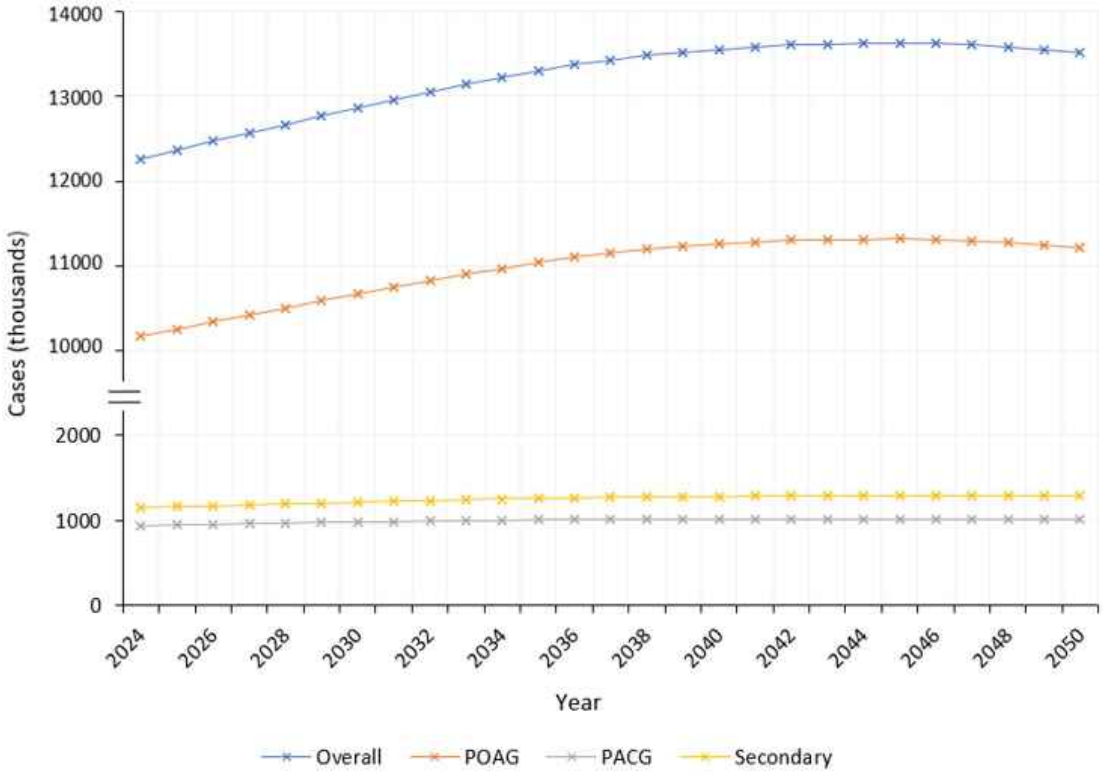

Figure 3.7 Projected number of glaucoma cases in Europe (2024–2050), overall and by subtype

PACG, primary angle-closure glaucoma; POAG, primary open-angle glaucoma.

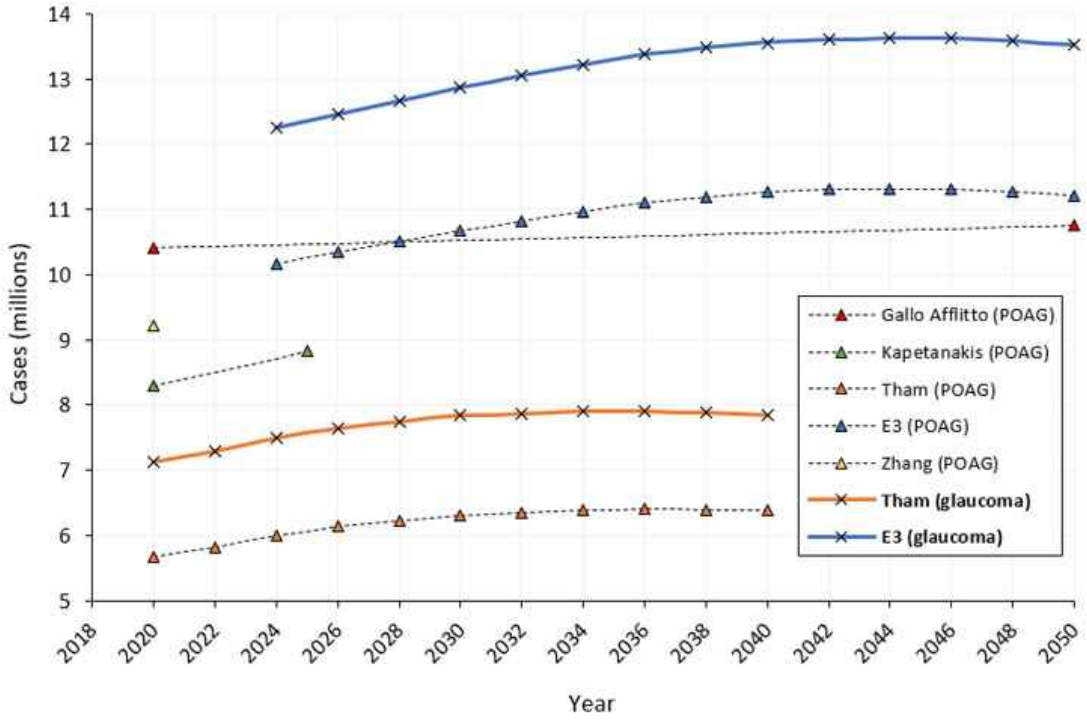

Figure 3.8 Comparison of previously reported primary open-angle glaucoma and overall glaucoma prevalence estimates

E3, European Eye Epidemiology consortium (this study); POAG, primary open-angle glaucoma. All estimates are based on the United Nations World Population Prospects and are for individuals aged ≥40 years with the exception of Tham, et al. (40–80 years).

Table 3.7 Comparison of previously reported POAG and glaucoma prevalence estimates

	UN World Population Prospects version	Prevalence (%)	Projected number of cases (millions)				
			2020	2024	2030	2040	2050
Glaucoma overall (40–80 years)							
Tham, et al. (2014)	2012	2.93	7.12	7.50	7.84	7.85	–
E3 consortium (this study)	2022	2.99	–	8.55	8.87	8.87	8.43
Primary open-angle glaucoma (40–80 years)							
Tham, et al. (2014)	2012	2.51	5.67	6.00	6.31	6.39	–
E3 consortium (this study)	2022	2.51	–	6.98	7.23	7.22	6.84
Primary open-angle glaucoma (≥40 years)							
Kapetanakis, et al. (2016)	2012	2.40	8.30	8.82*	–	–	–
Zhang, et al. (2021)	2019	2.30	9.21	–	–	–	–
Gallo Afflitto, et al. (2022)	2021	2.60	10.41	–	–	–	10.75
E3 consortium (this study)	2022	2.51	–	10.17	10.66	11.26	11.22

E3, European Eye Epidemiology; UN, United Nations.

* Estimated prevalence for 2025.

3.1.5 Discussion

This analysis provides updated age-standardised estimates of European glaucoma prevalence and future projections of glaucoma burden, based on pooled results from 14 population-based eye studies. Detailed subgroup analyses offer insight into demographic, regional, temporal, and diagnostic determinants of glaucoma prevalence estimates. Additionally, consideration of the burden of undiagnosed disease provides important data that may inform future public health policy and glaucoma screening strategies. I estimate that glaucoma affects three percent of European adults over the age of 40 years, with more than half of all cases remaining undiagnosed. This translates into a total glaucoma burden of more than 12 million individuals in 2024 – a figure projected to grow by more than one million cases over the next 20 years.

Several meta-analyses reporting European glaucoma prevalence estimates have been conducted in the last decade.[7,225,228,240] Importantly, previous studies have had to rely largely on published figures or summary statistics, as individual-level data are generally not available. Since glaucoma is a strongly age-related condition, prevalence estimates are largely dependent on the age structure of the population under study. For example, single-centre figures from Europe can range as widely as 1.34% in the Gutenberg Health Study (mean age, 52.2 years) to 8.38% in the MONTRACHET Study (mean age, 82.2 years).[231,232] This has important implications for direct comparability or pooling of results, and previous studies have often had to rely on statistical modelling or broad subgroup analyses to do so.

My estimates, based on individual-level data from more than 55 000 participants and standardised to European age structure, are in keeping with, or slightly higher than, previously reported figures for both POAG and glaucoma overall.[7,225,228,240] However,

the ability to apply directly-observed age- and sex-specific prevalence estimates to population figures suggests a substantially higher current glaucoma burden than previous reports. For example, when compared to the largest global glaucoma prevalence meta-analysis to date,[7] my findings suggest almost five million more affected individuals across Europe in 2024 (12.26 million versus 7.50 million). While this difference is largely due to Tham and colleagues restricting their prevalence estimates to individuals aged 40–80 years (I estimate that there are 3.71 million individuals aged >80 years with glaucoma across Europe), I still project more than one million additional glaucoma cases when restricting my analyses to this age range (see **Table 3.7**). In contrast to most world regions, the total population of Europe is expected to decline in coming decades.[239] Despite this overall reduction, ongoing demographic ageing means that this burden is expected to grow by more than one million cases by 2045.

In keeping with previous reports, I found a higher pooled prevalence of glaucoma in men relative to women.[7,240] This difference was apparent within all age groups and after age standardisation, suggesting a true difference that may reflect greater biological and environmental risk factors among men. It is important to acknowledge that this is primarily a reflection of a difference in POAG prevalence (crude prevalence, 4.01% and 2.82% for men and women, respectively; P <0.001), rather than PACG (0.36% and 0.40%, respectively: P = 0.73) or secondary glaucoma (0.57% and 0.38%, respectively; P = 0.08).

Higher prevalence was observed in studies from Eastern and Southern Europe, and this finding persisted after adjustment for age, sex, and year of study in the meta-regression model. While this may reflect regional differences in environmental risk factors for glaucoma, these subgroup estimates were based on a small number of

studies, and alternative explanations should be considered. For example, both studies from Southern Europe based their diagnosis on specialist opinion, a factor associated with higher glaucoma prevalence (see below); while the single study from Eastern Europe was characterised by high levels of ACG, a possible reflection of the ethnic diversity of the region which includes individuals of central Asian descent who are known to be at higher risk for angle-closure disease.[65,189,242] Restricting analyses to participants of European ethnicity consequently resulted in a slightly lower overall glaucoma prevalence estimate.

A previous review has demonstrated lower glaucoma prevalence estimates from older studies and hypothesised that this may relate to changing trends in study designs and diagnostic definitions, most notably the removal of IOP from glaucoma case definitions.[240] The adoption of ISGEO guidelines, published in 2002, aimed to improve and homogenise the diagnosis of glaucoma diagnosis in prevalence surveys and, while heterogeneity still remains, most studies included in this analysis were based on ISGEO criteria or specialist opinion, requiring evidence of both structural and functional glaucomatous damage independent of IOP.[34] The exception being the Rotterdam studies (the first cohort was assessed in the early 1990s), which relied on the presence of either glaucomatous optic neuropathy or visual field loss, based on local definitions.[230]

While I found no evidence of a temporal trend over the last three decades, I did observe lower prevalence estimates from studies based on ISGEO criteria relative to those based on the opinion of a glaucoma specialist. This is perhaps unsurprising given that ISGEO criteria rely on statistical cutoffs of vertical CDR and CDR asymmetry to define structural damage, potentially missing cases of early disease or other characteristic features of glaucomatous optic neuropathy. Particularly low age-

adjusted estimates were observed when modified ISGEO criteria were used – these studies lacked visual field data and could therefore not utilise category one of the ISGEO diagnostic criteria. As these studies comprised only 4% of the total cohort, excluding them did not substantially alter the overall prevalence estimates.

In line with a recent systematic review and meta-analysis, almost 60% of all glaucoma cases in this cohort were found to be previously undiagnosed.[5] Importantly, I show that this proportion also has a strong age relationship, with lower proportions of undiagnosed disease in older individuals. I suspect that this relates to a higher likelihood of opportunistic case detection occurring during routine examination for common age-related eye conditions, such as presbyopia and cataract, as well as a higher likelihood of symptomatic disease in older individuals, due to a longer disease course. Despite this relationship, the prevalence of previously undiagnosed disease was still found to increase with age. These factors are pertinent considerations when planning future public health interventions, the allocation of health care resources, and the formulation of glaucoma screening strategies.

POAG was found to constitute 80% of all glaucoma cases in this analysis and prevalence estimates were consistent with those from previous meta-analyses, reaffirming the known relative distribution of this subtype in European populations.[7,225,228,240] I estimate that the current POAG burden in Europe exceeds 10 million individuals, with a further one million cases projected by 2045. Overall PACG estimates were lower than previous reports, largely due to a decline in observed prevalence after the age of 80 years, but this subtype is still estimated to affect approximately one million people across Europe.[7,243] This finding may be a result of relatively small case numbers, with less precise estimates generated at

older ages. However, a relative increase in PACG cases between the ages of 60 and 74 may reflect the natural history of age-related cataract – an important determinant of PACG risk – and changing patterns of widespread cataract extraction across Europe which occurs at a mean age of 73 years.[244,245] The strict inclusion criteria for the glaucoma subtype analyses also allowed me to calculate prevalence estimates and future projections for secondary glaucoma, which may have been misclassified as primary forms of disease in previous reviews with less stringent case definitions.

The strengths of this study include the large sample size and access to individual-level data, allowing for calculation of detailed age- and sex-specific prevalence estimates necessary for age standardisation, accurate projections, and meta-regression modelling. This also facilitated relevant subgroup analyses and allowed me to additionally consider important factors, including previously undiagnosed disease and glaucoma subtypes. Included studies were population based and performed direct ophthalmic examination on all participants rather than relying on community diagnosis, minimising the risk for misclassification bias. The inclusion of studies with a variety of designs and case definitions allowed me to explore heterogeneity in glaucoma prevalence, while still allowing for sufficient sample size necessary for accurate estimates and projections.

Although I expected a high degree of ethnic homogeneity across studies, this means that my results are largely based on individuals of European ethnicity and may be less applicable to European centres with greater levels of ethnic diversity. I was also limited by relatively few studies having detailed and comparable glaucoma subtype data, with a subsequent loss of power for these analyses. Reassuringly however, these studies provided an almost identical glaucoma prevalence estimate to the overall analysis, suggesting that these results may be broadly reflective of the wider

cohort. While I used similar population projections and modelling assumptions employed in previous reviews, long-term trends are complex and difficult to predict, with resultant implications for accurate estimation of future glaucoma burden. Notable, I assumed glaucoma prevalence to remain stable over the next 26 years. While I found no evidence for a temporal trend in these data, this is assumption is difficult to quantify as it depends on several dynamic factors, including those that may change the true prevalence of disease over time (including changes in environmental risk factors) and those that affect the detection and diagnosis of disease (including varying case definitions and advances in diagnostic technology).

In summary, this study provides detailed estimates of glaucoma prevalence in Europe and updated projections of the future burden of disease. I corroborate many findings from previous reviews, but also offer novel insights into the burden of undiagnosed disease, as well as highlighting regional and diagnostic differences in glaucoma prevalence estimates. These findings may provide the groundwork for future epidemiological studies, facilitate the development of glaucoma screening strategies, and inform public health policy and healthcare resource allocation.

4

Alcohol

4.1 Systematic review and meta-analysis

The following section is a modified version of a paper published in *Ophthalmology*[32] and describes a systematic review and meta-analysis of the association of alcohol use with IOP and OAG. In accordance with widely adopted recommendations and guidelines,[208,209] certain aspects of this review were conducted independently by two researchers. I am grateful to Dr Kian Madjedi for his assistance with the study selection and quality control components of this analysis. I was responsible for all other aspects of the work. The relevant declaration form for previously published material is located in **Appendix A**. Supplementary material for this section can be found in **Appendix D**.

4.1.1 Abstract

Topic: This systematic review and meta-analysis summarises the existing evidence for the association of alcohol use with IOP and OAG.

Clinical relevance: Understanding and quantifying these associations may aid clinical guidelines or treatment strategies and shed light on disease pathogenesis. The role of alcohol, a modifiable factor, in determining IOP and OAG risk may also be of interest from an individual or public health perspective.

Methods: The study protocol was pre-registered in the Open Science Framework Registries (https://osf.io/z7yeg). Eligible articles (as of 14 May 2021) from three databases (PubMed, Embase, and Scopus) were independently screened and quality assessed by two reviewers. All case-control, cross-sectional, and cohort studies reporting a quantitative effect estimate and 95% CI for the association between alcohol use and either IOP or OAG were included. The evidence for the associations with both IOP and OAG were qualitatively summarised. Effect estimates

for the association with OAG were pooled using random-effects meta-analysis. Studies not meeting formal inclusion criteria for systematic review, but with pertinent results, were also appraised and discussed. Certainty of evidence was assessed using the GRADE framework.

Results: Thirty-four studies were included in the systematic review. Evidence from 10 studies reporting an association with IOP suggest that habitual alcohol use is associated with higher IOP and prevalence of OHT (IOP >21mmHg), although absolute effect sizes were small. Eleven of 26 studies, comprising 173 058 participants, that tested for an association with OAG met inclusion criteria for meta-analysis. Pooled effect estimates indicated a positive association between any use of alcohol and OAG (1.18; 95% CI, 1.02–1.36; $P = 0.03$; $I^2 = 40.5\%$), with similar estimates for both prevalent and incident OAG. The overall GRADE certainty of evidence was very low.

Conclusion: While this meta-analysis suggests a harmful association between alcohol use and OAG, these results should be interpreted cautiously given the weakness and heterogeneity of the underlying evidence base, the small absolute effect size, and the borderline statistical significance. Nonetheless, these findings may be clinically relevant and future research should focus on improving the quality of evidence.

4.1.2 Introduction

Alcohol use is implicated in a multitude of chronic diseases across various organ systems and is a leading cause of death and disability worldwide.[246–248] The acute effects of alcohol on the human eye include a transient, seemingly dose-dependent reduction in IOP[47–54] and increase in blood flow to the ONH,[53,55] theoretically

conferring a protective benefit against the development of glaucoma. Chronic alcohol use, however, is associated with a host of neurodegenerative, cardiovascular, and endocrine disorders, as well as systemic biochemical and physiological derangements, and the long-term or indirect roles these may play in glaucoma are unclear.[246,247]

In contrast to the short-term ocular hypotensive effects of alcohol, a number of epidemiological studies have reported cross-sectional associations between alcohol use and higher IOP or prevalence of OHT,[56–60] but this is not always a consistent finding.[62,63] There is also evidence to suggest that any association with IOP may be mediated by both sex and glaucoma status.[58,60] Additionally, most observational studies exploring the association between alcohol use and glaucoma have yielded non-significant results, with both cross-sectional[56,65–70] and longitudinal studies[71–73] failing to demonstrate a consistent association.

Existing reviews on the subject are limited to qualitative analyses within the context of broader review topics,[31,249–253] and, to the best of my knowledge, there has not been a published systematic review and meta-analysis exploring the potential role that alcohol may play in determining IOP and glaucoma risk. My research question, using the PECO framework, was therefore: in the general adult population (population), what is the effect of habitual alcohol consumption (exposure) on IOP and OAG (outcomes) compared to those who do not consume alcohol (comparison)?

A better understanding of these associations may offer insight into potential mechanisms of glaucomatous optic neuropathy, direct future research, and inform clinical advice or guidelines. It may also be of interest to individuals wishing to learn

how modifiable lifestyle factors, such as alcohol consumption, may influence IOP or the risk for glaucoma.

4.1.3 Methods

4.1.3.1 Guidelines and pre-registration

This study aimed to address the association between alcohol use with IOP and OAG in adults through systematic review and meta-analysis of observational studies. As such, it was conducted in accordance with MOOSE guidelines (**Figure D1)**.[209] The study protocol was pre-registered and published online in the Open Science Framework Registries (https://osf.io/z7yeg).[254]

4.1.3.2 Eligibility criteria

Alcohol use was defined as current or prior habitual consumption of any amount or type of alcohol. OAG was chosen as an outcome measure as many studies do not differentiate between primary and secondary forms of OAG. Given that the potential exclusion of these studies may have limited our findings and that POAG constitutes the majority of OAG cases, this expanded definition was considered appropriate. I aimed to include all relevant case-control, cross-sectional, and cohort studies.

4.1.3.3 Search methods

One investigator (KS) systematically conducted a search of three databases (PubMed, Embase, and Scopus) to identify relevant articles published up to 14 May 2021 using the search strategies described in **Figure D2**. Independent review of retrieved titles and abstracts was conducted by two investigators (KS and KM) and all articles deemed relevant to the research question were retrieved for full-text

review. A manual search of the reference lists of all included studies and previous reviews was also performed by the same two investigators. Any inconsistencies were resolved by consensus agreement or by consultation with a third investigator (AK), when necessary.

4.1.3.4 Study selection

Full-text articles were required to meet the following inclusion criteria for the purposes of the systematic review: (1) reported alcohol use in keeping with the exposure definition; (2) reported IOP or OAG as the outcome measure; (3) reported the measure of association as an effect estimate with a 95% CI or standard error, or allowed for the calculation of these measures from published raw data; and (4) study participants were 18 years of age or older.

Studies were excluded if they were: (1) reviews, letters, editorials, case reports, case series, conference abstracts, or animal studies; or (2) published in a non-English language. Articles not meeting formal criteria for systematic review, but which were relevant to the study question were reviewed in full and pertinent findings reported for context.

When multiple publications from the same study population were available, I included the study that best addressed the research question. Preference was given to: (1) studies with the correct exposure and outcome definitions, (2) prospective studies, (3) studies with a larger sample size, and (4) studies with greater adjustment for confounding variables.

4.1.3.5 Data collection and risk of bias assessment

For each included study, the following data were extracted using a standardised data collection tool: (1) first author name, (2) year of publication, (3) study name and country, (4) demographics of study participants, (5) study design, (6) number of study participants, (7) definition of alcohol exposure, (8) definition of IOP or OAG outcome, (9) effect estimate plus 95% CI or SE, and (10) confounding variables adjusted for.

Studies were grouped according to their main outcome measure/s: (1) IOP (as either a continuous or categorical measure), (2) OAG (as either prevalent or incident cases). If studies addressed more than one outcome, these were reported separately.

A risk of bias assessment was independently performed by two investigators (KS and KM), using a tool designed by the GRADE Working Group to assess the effects of environmental exposures on health outcomes.[255] This tool is modelled on the established ROBINS-I instrument,[256] and was designed by the ROBINS-E collaborative project to help guide the development of the final ROBINS-E instrument. Specific risk of bias domains assessed included: confounding, selection of participants, classification of exposure, departures from intended exposure, missing data, measurement of outcomes, and selection of reported results. Inconsistencies were resolved in the manner described previously.

4.1.3.6 Data synthesis and analysis

Due to considerable heterogeneity in the definition of both alcohol exposure and IOP across included studies, meta-analysis of this association was not deemed appropriate. Similarly, meta-analysis of the association between alcohol use and

OAG was limited to the comparison of any alcohol use (exposure group) with no alcohol use (reference group).

Studies reporting effect estimates for different levels or categories of alcohol exposure (e.g., former/current drinker, number of drinks per day/week, grams of alcohol consumed per day/week) were included and strata-specific results were pooled using inverse variance-weighted, fixed-effects meta-analysis to obtain a single effect estimate for each study. This model was chosen as it was assumed that there would be no statistical, clinical, or methodological heterogeneity between effect estimates derived from a single study.

Studies were excluded from meta-analysis if they met any of the following criteria: (1) did not provide a multivariable-adjusted effect estimate, or (2) the reference group was not comparable (either through inclusion of alcohol drinkers or exclusion of non-drinkers).

Effect estimates were pooled using inverse variance-weighted, random-effects meta-analysis (DerSimonian and Laird method)[257] and stratified according to whether they reported associations with prevalent or incident OAG. ORs and RRs were pooled in the final meta-analysis. A method for OR to RR conversion has been proposed,[258] but requires a baseline OAG risk, which was not available for every study, and is further complicated by the conversion of adjusted effect estimates. This method does, however, confirm that the OR is a close approximation of the RR, especially when baseline risk is <10% (the rare disease assumption) and effect estimates are small. Sensitivity analyses exploring the effect estimate derived from ORs and RRs separately were also performed.

Subgroup analyses to investigate the effects of study design (cross-sectional, case-control, and cohort) and study location/population (European/North American, African/African American, and Asian) on overall effect estimates were also performed. In addition, a number of post-hoc sensitivity analyses were conducted to assess the robustness of pooled estimates. These included: (1) further restriction of analysis to (a) only studies with POAG as the outcome, (b) only studies with multivariable adjustment for ≥5 covariables; (2) only studies reporting an effect estimate as (a) an OR, (b) a RR; (3) expanding analysis to (a) all studies with a multivariable effect estimate regardless of reference exposure group, (b) all studies included in the systematic review; (4) exclusion of studies assessed as having "critical" risk of bias; and (5) analysis of effect estimates from only the highest alcohol exposure level of each included study.

Dose-response meta-analysis was not considered appropriate given the significant heterogeneity in study design and exposure definition, as well as the small number of studies reporting multiple exposure levels.

Heterogeneity of effect estimates across studies and the effect of study heterogeneity on the pooled effect estimate were assessed using the Q statistic and the I^2 statistic, respectively.[237] The I^2 statistic was interpreted according to guidelines suggested by the Cochrane Collaboration: 0–40% (might not be important), 30–60% (may represent moderate heterogeneity), 50–90% (may represent substantial heterogeneity) and 75–100% (considerable heterogeneity).[210]

Publication bias was assessed graphically using a funnel plot and by means of the Egger[259] and Begg[260] tests. The trim and fill method, using the linear estimator L_0, was used to test and adjust for funnel plot asymmetry as an additional post-hoc

sensitivity analysis.[261] All analyses were conducted in Stata version 16.0 (StataCorp LLC, College Station, TX) using the *meta* programme.

The overall certainty of the evidence was assessed using the GRADE framework.[262] Findings from the risk of bias assessment were incorporated into the GRADE assessment using the methods described by Morgan, et al.[255]

4.1.4 Results

4.1.4.1 Study identification and selection

A total of 5 201 articles were identified from the initial database search (1 231 from PubMed, 2 338 from Embase, and 1 632 from Scopus). After removal of duplicates, 3 289 potentially eligible articles remained for title and abstract review. Of these, 120 articles underwent full text review and 29 contained results pertinent to the study question. Twelve studies from duplicate study populations were excluded during the full text review process (all for incorrect exposure or outcome definitions). One further cross-sectional study[99] was included in the IOP analysis but excluded from the OAG analysis, as a second study from the same population[71] provided prospective data with greater adjustment for confounding variables. A further five articles[65,66,69,73,263] were identified from a reference list search of all included studies and previous reviews for a total of 34 articles included in the systematic review. This included eight studies with IOP as the outcome, 24 with OAG as the outcome, and two with both IOP and OAG as outcomes. Funding and conflict of interest statements for all included studies are presented in **Table D1**.

Eleven studies reporting an association between alcohol and OAG met the criteria for meta-analysis. The full identification, screening, and selection process is detailed in **Figure 4.1**.

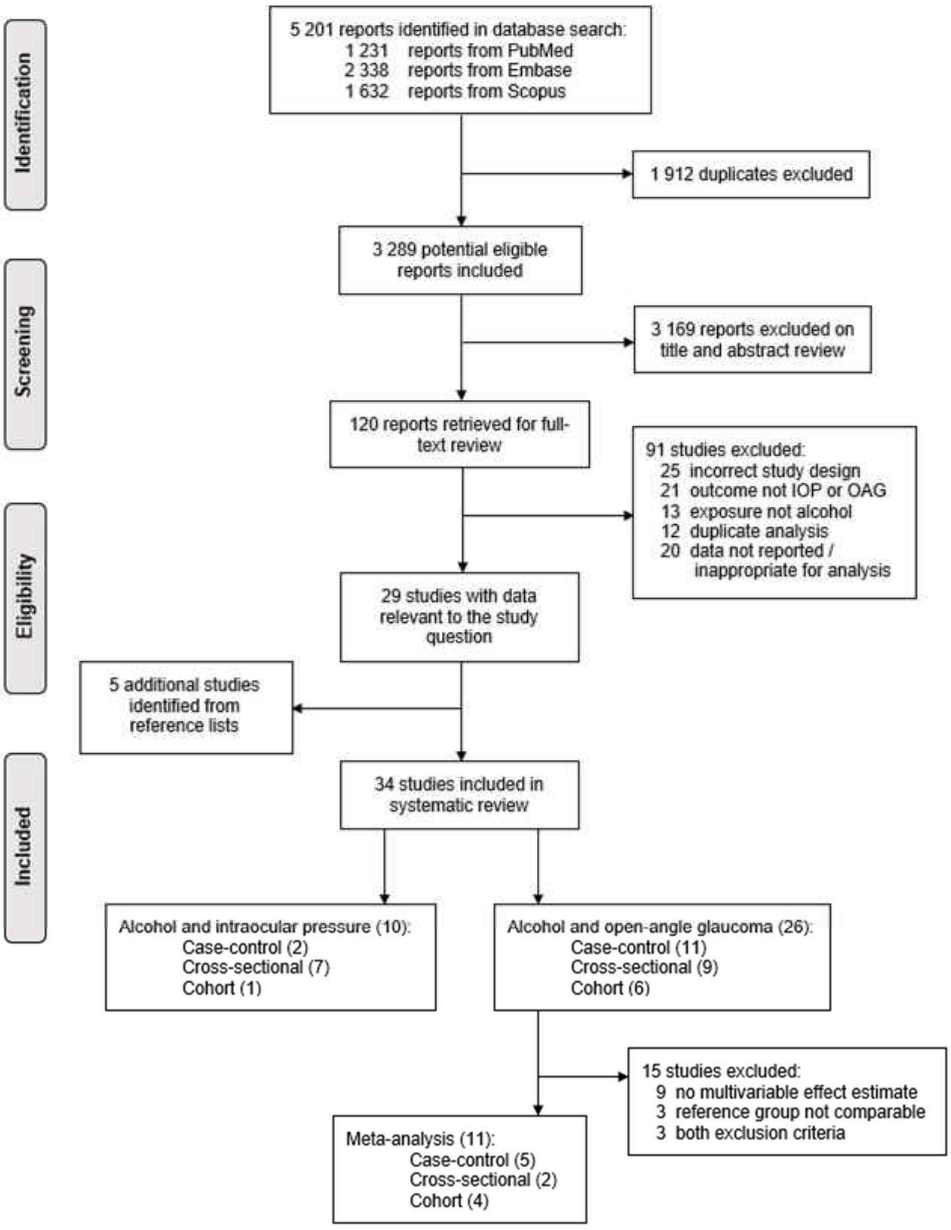

Figure 4.1 PRISMA flow diagram outlining the study identification, screening, and selection process for the systematic review and meta-analysis of the association of alcohol consumption with intraocular pressure and open-angle glaucoma

IOP, intraocular pressure; OAG, open-angle glaucoma.

4.1.4.2 Characteristics and results of studies

Intraocular pressure: The characteristics and main results of the ten studies reporting an association between alcohol and IOP are summarised in **Table 4.1**. This included six studies (five cross-sectional,[57–60,63] one prospective cohort[90]) with IOP as a continuous outcome and four studies (two cross-sectional,[99,264] two case-control[56,62]) with OHT as an outcome, comprising a total of 27 452 participants. OHT was defined as IOP >21 mmHg with no features of glaucomatous optic neuropathy by all studies using this as an outcome measure. IOP was measured by applanation tonometry in seven studies[56,58,59,62,63,90,99] and non-contact tonometry in three studies.[57,60,264] All studies limited their analyses to participants without glaucoma, or stratified outcomes by glaucoma status. Alcohol intake was assessed through either a standardised interview[56–60,62,63,99,264] or a semi-quantitative FFQ.[90]

Alcohol use was positively associated with IOP in two studies,[57,59] although the absolute difference between drinkers and non-drinkers (0.1 mmHg in both studies) was small. A further two studies found positive linear associations between alcohol intake and IOP in men, but not women, without glaucoma (IOP difference of 0.7–1.4 mmHg between highest intake group and no intake group).[58,60] In one of these studies, consumption of alcohol >4 times/week in women with glaucoma was associated with higher IOP (+2.8 mmHg) compared to non-drinkers, but with no evidence of linear trend.[58] Alcohol intake was not associated with IOP in one study[90] and negatively associated (IOP difference <0.1 mmHg) in previous, but not current, drinkers in another.[63]

Alcohol use was associated with OHT in one included study,[56] with no association reported in a further two studies.[99,264] A protective association with the use of liquor (but not other alcohol types) was found in the final study exploring this association.[62]

Within each outcome sub-group (IOP and OHT), further heterogeneity in exposure definition (including both continuous and categorical alcohol intake measures, as well as stratifications by sex, glaucoma status, alcohol type and flushing reaction) resulted in a limited number of studies with sufficiently similar results to allow for meaningful meta-analysis of the association between alcohol use and IOP.

Open-angle glaucoma: Twenty-six studies reported an association between alcohol use and OAG. The full case ascertainment criteria for these studies are presented in **Table D2**. Of these, 15 studies (comprising 41 123 participants) were excluded from meta-analysis due to lack of a multivariable effect estimate (*n* = 9), a reference exposure group that was not comparable (*n* = 3), or both (*n* = 3). The characteristics and main results of these excluded studies are presented in **Table D3**. In summary, of the excluded studies, one case-control study found a harmful association between alcohol and OAG,[265] 11 (seven cross-sectional,[88,263,266–270] two case-control,[94,95] two prospective cohort[90,271]) found no association and two case-control studies found protective associations.[93,272] A final case-control study reported a protective association in participants of African American descent but a harmful association in participants of European descent.[273]

Table 4.1 Summary of studies reporting an association between alcohol use and intraocular pressure included in systematic review

Author (year)	Location (study)	Design	Population	Size	Outcome	Result and effect estimate	Adjustments (exclusions)
Intraocular pressure							
Lin (2005)	Taiwan (1)	CS	≥65 years	1 292	NCT	Current and former alcohol use positively associated with IOP (+0.1 mmHg).	Age, sex, SBP, DM (glaucoma)
Ramdas (2011)	Netherlands (2)	C	≥55 years	3 939	AT	Alcohol intake (grams/day) not associated with IOP in men or women for any alcohol type (beer, wine, liquor, sherry).	Age, IOP treatment (OAG)
Song (2020)	South Korea (3)	CS	≥20 years	6 504	AT	Alcohol use 2–3 times/week (+0.6 mmHg) and ≥4 times/week (+0.7 mmHg) associated with higher IOP in men without glaucoma (P_{trend} = 0.01). Positive association in women with glaucoma consuming ≥4 times/week (+2.8 mmHg).	Age, sex, BMI, smoking, DM, HPT, cholesterol (ocular surgery or disease, treated glaucoma, non-OAG glaucoma, abnormal LFT)
Weih (2001)	Australia (4)	CS	≥40 years	4 576	AT	Previous, but not current, use of alcohol negatively associated with IOP (<-0.1 mmHg) in participants without glaucoma.	Rural residence, iris colour, vitamin E intake, SE (treated glaucoma)
Wu (1997)	West Indies (5)	CS	40–84 years	3 752	AT	Use of alcohol in the past year positively associated with IOP (+0.1 mmHg).	Age, sex, complexion, BMI, SBP, DM, smoking, PR, family history, ocular surgery or infection, examination season (glaucoma)
Yoshida (2003)	Japan	CS	29–79 years	569	NCT	Never or seldom alcohol use (-1.4 mmHg) and use several times per month (-0.8 mmHg) associated with lower IOP compared with daily use (P_{trend} <0.001) in men but not women.	BMI, SBP, smoking, exercise, coffee (HPT, OHT, glaucoma)
Ocular hypertension							
Doshi (2008)	USA (6)	CS	≥40 years	5 843	AT	Alcohol use: categorical (ex-/partial, current/heavy), grams/week (<40, 40–104, ≥105), type (wine, beer, liquor) not associated with OHT.	Age, Native American ancestry, employment status (glaucoma)
Lee (2019)	South Korea	CS	Males, <65 years, BMI ≥25	479	NCT	Any alcohol use not associated with OHT in participants with and without alcohol-induced flushing reaction. Evidence of effect mediation by total weekly alcohol intake.	Age, BMI, SBP, smoking, DM, cholesterol, CVD, thyroid function, ocular surgery (glaucoma)
Leske (1996)	USA (7)	CC	≥40 years	298	AT	Ever use of alcohol associated with OHT, OR 2.32 (95% CI, 1.15–4.69).	Age, sex, family history, HPT, smoking (glaucoma)
Seddon (1983)	USA	CC	Adults, age not defined	200	AT	No liquor intake (compared with daily intake) associated with OHT, OR 3.8 (95% CI, 1.4–10.4) with stronger association noted in men (OR 9.2). No association with other alcohol types.	Age, sex, family history, myopia, income, BP, stress, ocular surgery (glaucoma)

(1) Shihpai Eye Study, (2) Rotterdam Study, (3) Korea National Health and Nutrition Examination Survey, (4) Melbourne Visual Impairment Project, (5) Barbados Eye Study, (6) Los Angeles Latino Eye Study, (7) Long Island Glaucoma Case-Control Study Group.
CS, cross-sectional; CC, case-control; C, cohort; OR, odds ratio; CI, confidence interval; IOP, intraocular pressure; OHT, ocular hypertension; OAG, open-angle glaucoma; mmHg, millimetres of mercury; AT, applanation tonometry; NCT, non-contact tonometry; BMI, body mass index; SBP, systolic blood pressure; DM, diabetes mellitus; CVD, cardiovascular disease; HPT, hypertension; BP, blood pressure; PR, pulse rate; LFT, liver function test; SE, spherical equivalent.

Characteristics of the 11 studies (two cross-sectional,[65,66] five case-control,[56,67–70] four cohort[64,71–73]), comprising 173 058 participants, included in the meta-analysis of alcohol use and OAG are presented in **Table 4.2**. Seven reported associations with prevalent OAG[56,65–70] and four with incident OAG.[64,71–73] POAG was the outcome variable in seven of the studies.[64,66–68,70,72,73] The main results and effect estimates of these studies are presented in **Table 4.3**. Five studies reported multiple alcohol exposure levels and a single pooled effect estimate across all levels was calculated for use in meta-analysis.[64,68,70–72] Overall, 10 studies reported no association between any alcohol use and OAG,[56,65–73] with only one large cohort study of African American females reporting a harmful association.[64] Although there was a suggestion of a dose-response effect in those studies reporting ordinal alcohol exposure levels,[64,68,70,72] no study-specific test for trend reached statistical significance. Only three of these studies reported comparable, quantifiable alcohol exposure levels,[64,70,72] and further heterogeneity in study design (one cross-sectional, two longitudinal) precluded meaningful dose-response meta-analysis. There was also no evidence of an association by alcohol type[72] or OAG phenotype (normal-tension or high-tension)[66,68] in the included studies.

Table 4.2 Characteristics of studies included in the meta-analysis of the association between alcohol use and open-angle glaucoma

Author (year)	Location (study)	Design	Population	Size (cases)	Exposure	Outcome	Adjustment (covariates / matched variables)
Prevalent OAG							
Bikbov (2020)	Russia (Ural Eye and Medical Study)	CS	≥40 years	5 545 (177)	IAQ	OAG	Age
Bonomi (2000)	Italy (Egna-Neumarkt Study)	CS	≥40 years	4 147 (60)	IAQ	POAG	Sex
Charliat (1994)	Netherlands	CC	≥40 years	350 (175)	SAQ	POAG	Age, sex, type of healthcare
Chiam (2018)	Singapore (Singapore Chinese Eye Study)	CC	≥40 years	3 499 (2788)	IAQ	POAG	Age, sex, IHD, stroke, HPT, hyperlipidaemia, DM, migraine, smoking, family history, myopia, IOP, CCT
Leske (1996)	USA (Long Island Glaucoma Case-Control Study Group)	CC	≥40 years	312 (190)	IAQ	OAG	Age, sex, family history, HPT, smoking
Leske (2001)	West Indies (Barbados Family Study of Open-Angle Glaucoma)	CC	≥25 years	286 (219)	IAQ	OAG	Age, sex, sibling relation
Renard (2013)	France (Photograf Study)	CC	≥40 years	678 (339)	IAQ	POAG	Age, sex, duration of disease
Incident OAG							
Jiang (2012)	USA (Los Angeles Latino Eye Study)	C	≥40 years	3 772 (87)	IAQ	OAG	Age, IOP, AL, lack of vision insurance, WHR, CCT, smoking, SBP, OPP, DM, cataract surgery, family history
Kang (2007)	USA (Nurses' Health Study & Health Professionals Follow-Up Study)	C	≥40 years	12 0379 (856)	SQFFQ	POAG	Age, family history, African American heritage, HPT, DM, BMI, smoking, physical activity, caffeine, caloric intake
Pan (2017)	China (Yunnan Minority Eye Study)	C	≥50 years	1 520 (19)	IAQ	POAG	Age, sex, IOP, CCT, AL, myopia, BMI, education, HPT, DM, smoking
Wise (2011)	USA (Black Women's Health Study)	C	Females, 21–69 years	32 570 (366)	SAQ	POAG	Age, questionnaire cycle, education, smoking, HPT, physical activity, energy intake, BMI

IAQ, interviewer-administered questionnaire; SAQ, self-administered questionnaire; SQFFQ, semi-quantitative food frequency questionnaire; IHD, ischemic heart disease; HPT, hypertension; DM, diabetes mellitus; IOP, intraocular pressure; CCT, central corneal thickness; AL, axial length; WHR, waist:hip ratio; SBP, systolic blood pressure; OPP, ocular perfusion pressure; BMI, body mass index; CS, cross-sectional; CC, case-control; C, cohort; OAG, open-angle glaucoma; POAG, primary open-angle glaucoma.

Table 4.3 Results and effect estimates of studies included in the meta-analysis of the association between alcohol use and open angle glaucoma

Author (year)	Reference group	Exposure level/s	Effect estimate (95% CI)	Pooled effect estimate (95% CI)	Additional results
Prevalent OAG					
Bikbov (2020)	No consumption	Any consumption	OR 1.81 (0.99–3.31)	N/A	—
Bonomi (2000)	No consumption	Any consumption	OR 1.40 (0.80–2.20)	N/A	No association when stratified by HTG (>21 mmHg) or NTG (≤21 mmHg).
Charliat (1994)	No consumption	Any consumption	OR 1.00 (0.57–1.73)	N/A	—
Chiam (2018)	No consumption	<2 days/week ≥2 days/week	OR 1.08 (0.51–2.32) OR 1.27 (0.53–3.03)	OR 1.16 (0.65-2.05)	No association when stratified by HTG or NTG. No association with alcohol type in univariable analyses.
Leske (1996)	No consumption	Any consumption	OR 1.22 (0.66–2.24)	N/A	No association when OAG cases compared to OHT controls.
Leske (2001)	No consumption	Any consumption	OR 0.80 (0.34–1.88)	N/A	—
Renard (2013)	0 drinks/day	0–1 drinks/day 1–2 drinks/day 2–3 drinks/day >3 drinks/day	OR 0.85 (0.51–1.42) OR 0.75 (0.42–1.34) OR 1.35 (0.66–2.74) OR 0.81 (0.29–2.31)	OR 1.14 (0.93–1.40)	P_{trend} >0.10. No association with binge drinking (≥5 drinks/occasion).
Incident OAG					
Jiang (2012)	No consumption	Previous consumption Current consumption	OR 1.59 (0.95–2.64) OR 0.76 (0.28–2.06)	OR 1.36 (0.87–2.15)	—
Kang (2007)	0 grams/day	1–9 grams/day 10–19 grams/day 20–29 grams/day ≥30 grams/day	RR 0.99 (0.83–1.19) RR 0.96 (0.76–1.22) RR 0.95 (0.68–1.33) RR 0.71 (0.49–1.04)	RR 0.94 (0.83–1.07)	P_{trend} = 0.09. No association with alcohol type.
Pan (2017)	No consumption	Any consumption	OR 2.40 (0.80–7.50)	N/A	—
Wise (2011)	0 drinks/week	1–6 drinks/week ≥7 drinks/week	RR 1.28 (1.01–1.62) RR 1.60 (1.06–2.43)	RR 1.35 (1.10–1.66)	P_{trend} = 0.17. Stronger associations noted in women <50 years. Harmful association in current (RR 1.35, 95% CI 1.05–1.73) but not former drinkers. No association with total years of alcohol drinking.

CI, confidence interval; OR, odds ratio; RR, rate ratio; OAG, open-angle glaucoma; OHT, ocular hypertension; HTG, high-tension glaucoma; NTG, normal-tension glaucoma.

4.1.4.3 Meta-analysis

Meta-analysis of effect estimates from the 11 included studies showed that any consumption of alcohol was significantly associated with OAG (overall effect estimate 1.18; 95% CI, 1.02–1.36; $P = 0.03$; $I^2 = 40.5\%$) when compared to no consumption (**Figure 4.2**). Similar effect sizes were obtained for both prevalent (1.18; 95% CI, 1.01–1.38; $I^2 = 0.0\%$) and incident (1.22; 95% CI, 0.91–1.63; $I^2 = 74.9\%$) OAG, with no evidence of heterogeneity between groups ($P = 0.85$).

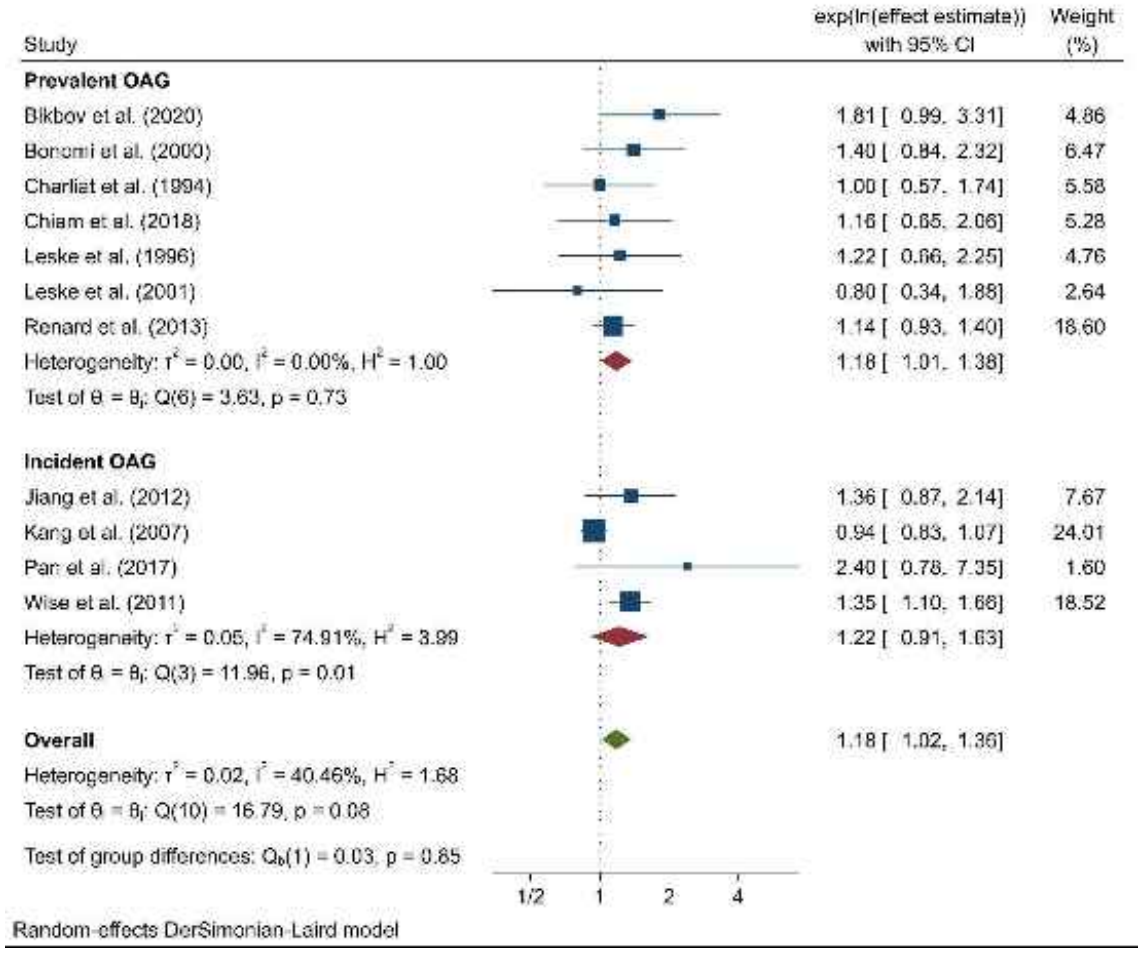

Figure 4.2 Meta-analysis of studies reporting an association between any consumption of alcohol and open-angle glaucoma

CI, confidence interval; OAG, open-angle glaucoma.

The strongest effect estimates were obtained for cross-sectional studies (1.56; 95% CI, 1.06–2.29; *n* = 2) and studies from Asia (1.53; 95% CI, 1.03–2.25; *n* = 3), although there was no evidence of heterogeneity by study design (*P* = 0.30) or study location/population (*P* = 0.20).

Effect estimates derived from various sensitivity analyses did not differ substantially from the main result (range 1.15–1.21), although loss of participant or study numbers often resulted in wider confidence intervals and loss of statistical significance. A slightly stronger effect was obtained from meta-analysis of studies reporting results as an OR (effect estimate 1.21; 95% CI,1.05–1.40). There was significant heterogeneity (*P* <0.01) between studies reporting a univariable effect estimate (0.86; 95% CI, 0.78–0.95), which suggest a protective effect, and those with a multivariable effect estimate (1.18; 95% CI, 1.04–1.34), which instead point to a harmful effect, included in this systematic review. Full details of subgroup and sensitivity analyses are reported in **Table 4.4**.

Although neither the Begg (*P* = 0.38) nor Egger (*P* = 0.51) tests suggested publication bias, there was an indication of funnel plot asymmetry with more studies appearing to the right of the pooled estimate. Stratified funnel plots showed symmetry of studies reporting associations with prevalent OAG, with the observed asymmetry arising from studies of incident OAG (**Figure 4.3**). Trim and fill analysis resulted in the imputation of two hypothetical studies both situated to the left of the pooled estimate (**Figure D3**). The updated effect estimate (based on 11 observed and two imputed studies) was slightly attenuated (1.14; 95% CI, 0.99–1.32).

Table 4.4 Meta-analysis of the association between alcohol use and open-angle glaucoma: subgroup and sensitivity analyses

Description (number of studies in meta-analysis)	Effect estimate (95% CI)		$P_{heterogeneity}$
Subgroup analyses			
Study design			0.30
Case-control (5)	1.12	(0.94–1.33)	
Cross-sectional (2)	**1.56**	**(1.06–2.29)**	
Cohort (4)	1.22	(0.91–1.63)	
Study location/population			0.20
European/North American (6)	1.06	(0.93–1.21)	
African/African American (2)	1.23	(0.84–1.82)	
Asian (3)	**1.53**	**(1.03–2.25)**	
Sensitivity analyses			
(1a) Include only studies with POAG as outcome (7)	1.15	(0.97–1.36)	
(1b) Include only studies with adjustment for ≥5 covariables (6)	1.19	(0.95–1.50)	
(2a) Include only studies with odds ratio as effect estimate (9)	**1.21**	**(1.05–1.40)**	
(2b) Include only studies with rate ratio as effect estimate (2)	1.12	(0.78–1.59)	
(3a) Include studies with different baseline reference category (14)	**1.18**	**(1.04–1.34)**	
(3b) Include all studies from systematic review			**<0.01**
Univariable effect estimate (12)	**0.86**	**(0.78–0.95)**	
Multivariable effect estimate (14)	**1.18**	**(1.04–1.34)**	
(4) Exclude studies with “critical” risk of bias (9)	**1.18**	**(1.01–1.39)**	
(5) Include only effect estimates from highest exposure level (11)	1.20	(0.97–1.50)	

CI, confidence interval; POAG, primary open-angle glaucoma.

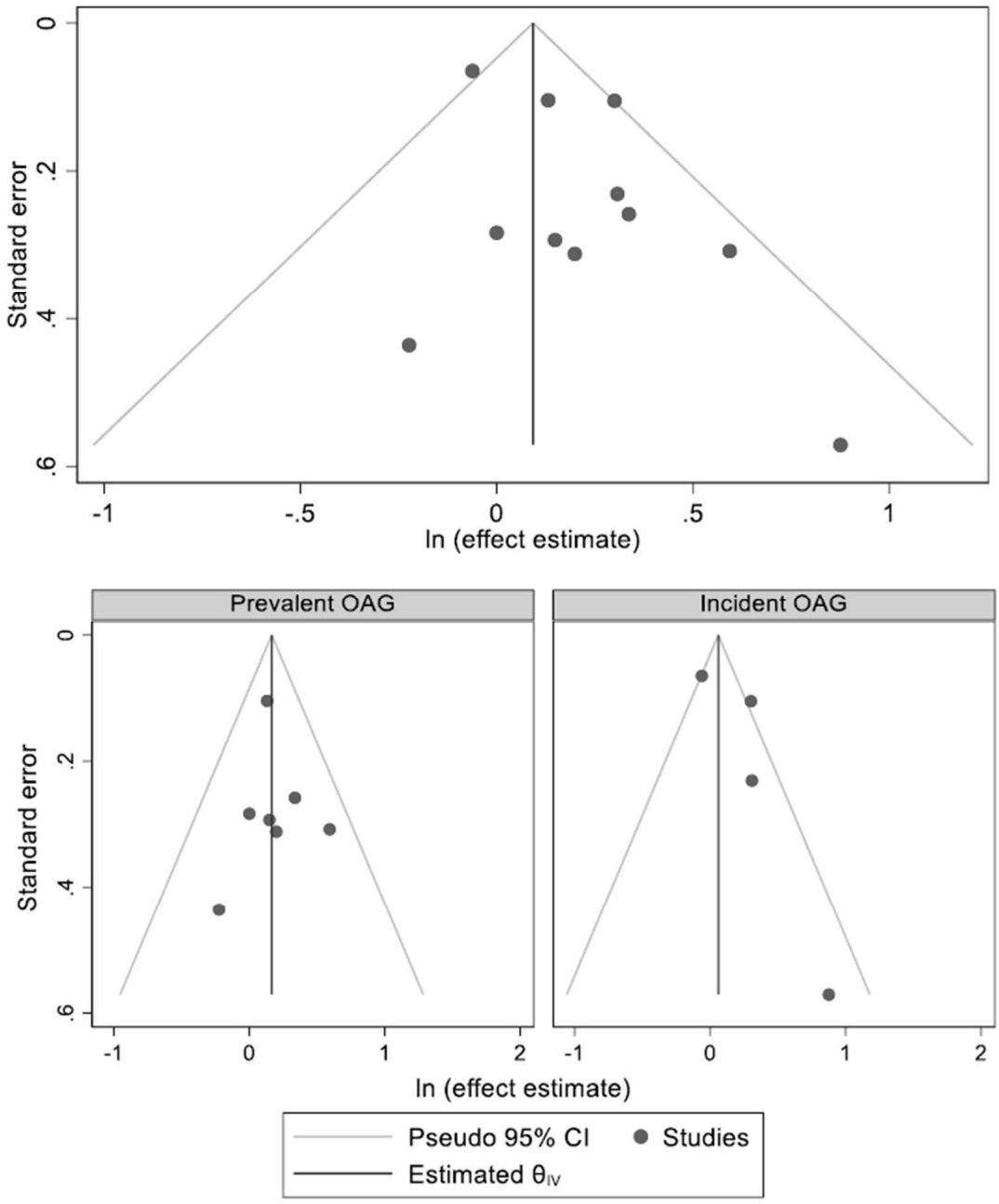

Figure 4.3 Overall (top) and stratified (bottom) funnel plots of studies included in the meta-analysis of alcohol use and open-angle glaucoma

CI, confidence interval; OAG, open-angle glaucoma.

4.1.4.4 Risk of bias and GRADE assessment

Assessment of study quality revealed residual confounding, exposure classification, and departures from exposure to be the greatest risks of bias across all included studies (**Figure 4.4**). Residual confounding was identified as a domain of particular concern, with most studies at "serious" or "critical" risk of bias. Overall, two studies were deemed to be at "critical" risk,[66,69] with only one study achieving a "moderate" risk of bias.[72]

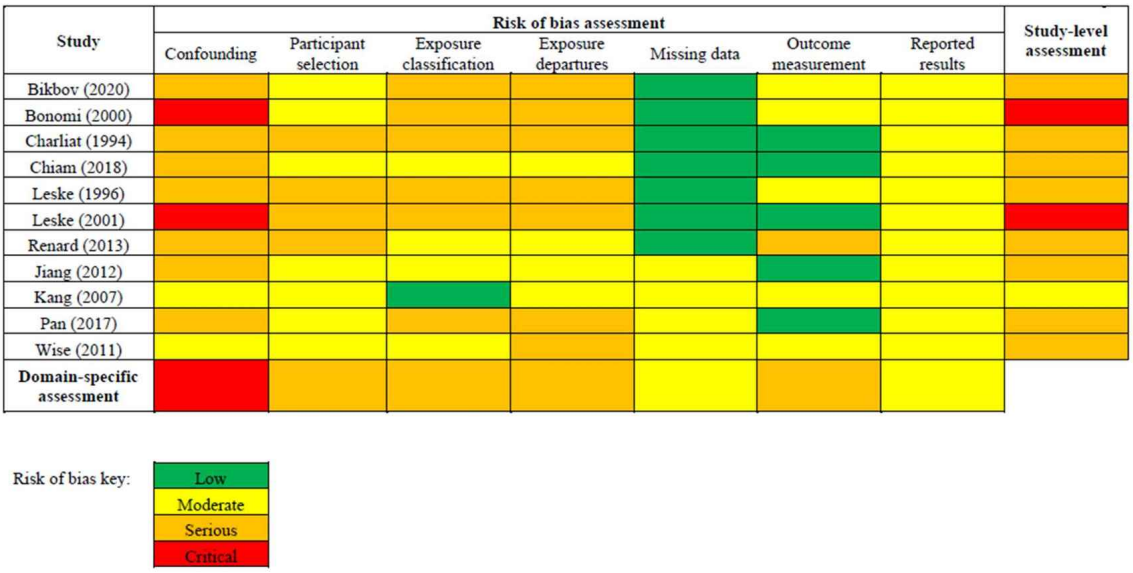

Figure 4.4 Risk of bias assessment of studies included in the meta-analysis of alcohol consumption and open-angle glaucoma

Although these risks varied between the included studies, assessment of study quality was not used as a weighting tool or exclusion criterion for the final meta-analysis. A post-hoc sensitivity analysis excluding studies with "critical" risk of bias, however, did not materially change the overall effect estimate.

The overall certainty of evidence assessment was "very low." Observational studies are assigned an initial "low" level of evidence, and this was further downgraded for

study limitations (risk of bias) and inconsistency (heterogeneity) in the evidence base. The assessment was upgraded one level as sensitivity analysis suggested that the plausible effect of residual confounding would be to strengthen the overall effect. Full details of the GRADE assessment are contained in **Table 4.5**.

Table 4.5 GRADE assessment of studies included in meta-analysis of alcohol use and open-angle glaucoma

		Factors that can reduce the quality of the evidence					**Factors that can increase the quality of the evidence**			
Number of studies	Design[1]	Study limitations[2]	Inconsistency[3]	Indirectness[4]	Imprecision[5]	Publication bias[6]	Large magnitude of effect[7]	Dose-response effect	Plausible effect of residual confounding[8]	**Overall quality of evidence**
11 (173 058 participants)	Observational	High	Present	None	None	None	None	None	Present	⨁◯◯◯
Evidence	*Low*	*-1*	*-1*	*0*	*0*	*0*	*0*	*0*	*+1*	Very low

[1] Observational studies are assigned a default "low" level of evidence, which can then be downgraded or upgraded further according to various factors.
[2] Assessed using a risk of bias tool designed for non-randomised studies of exposures. Downgraded one level due to "critical" limitation in one domain.
[3] Criteria for significant inconsistency of results were I^2 >50% or P <0.10 for the χ^2 test of heterogeneity.
[4] All studies assessed the association between self-reported alcohol consumption and a diagnosis of open-angle glaucoma.
[5] Not downgraded due to large sample size and 95% confidence intervals excluding no effect.
[6] The possibility of publication bias is not excluded but it was not considered sufficient to downgrade the quality of evidence.
[7] Defined as effect estimate >2.0 or <0.50, based on direct evidence with no plausible confounders.
[8] Sensitivity analysis revealed significant heterogeneity between studies reporting unadjusted and adjusted effect estimates, with the suggestion that further adjustment would result in a stronger effect.

4.1.5 Discussion

This section provides a systematic review of the current evidence for the association of habitual alcohol consumption with IOP and OAG. Although numerous identified studies provided quantitative estimates for these associations, very few were designed specifically to investigate these relationships. Consequently, there is considerable heterogeneity in the current evidence base and most results are limited to a simple binary comparison (drinkers versus non-drinkers), without further interrogation or sensitivity analyses.

This has important implications for direct comparability and meta-analytical approaches and, although I attempted to account for these limitations in analyses as far as possible, any pooled quantitative estimates should be viewed in the context of the largely questionable data strength of the underlying studies. Furthermore, the pooled effect estimate for the association with OAG was small and of borderline statistical significance. Although estimates were largely consistent across sensitivity analyses, the statistical evidence for these results was generally weaker, and it is conceivable that further adjustment for residual confounding factors would render the main finding non-significant.

Therefore, this meta-analysis should not in itself be considered strong evidence for a harmful association, but rather as an analytical approach to the synthesis of a widely heterogeneous evidence base which is best considered alongside the qualitative appraisal of the evidence that follows.

4.1.5.1 Physiology

The acute ocular hypotensive effects of alcohol have been known for at least 50 years,[52] although the precise physiological mechanism for the IOP reduction remains

unclear. Hypotheses include: a transient osmotic effect following alcohol consumption, suppression of anti-diuretic hormone (ADH) with a reduction in net ocular water movement, and a direct inhibitory effect on the secretory cells of the ciliary epithelium.[49,62,72]

This effect appears to be dose-dependent – a non-significant IOP reduction was noted following ingestion of <10 grams alcohol,[274] with absolute reductions of 1–4 mmHg after 10–30 grams,[47,48,51,53] and up to 6 mmHg with doses approaching 40 grams[52] – but is seemingly independent of alcohol concentration or total fluid volume. Equal quantities of alcohol administered in different concentrations (as beer or whiskey) produced similar IOP-lowering effects,[52] whilst administration of equal volumes of beer and water produced opposite effects.[54] Little to no effect on IOP was noted when alcohol was administered together with ADH or to individuals with abnormal posterior pituitary gland function.[49]

The peak ocular hypotensive effect is usually noted at 1–3 hours post-ingestion,[48–53,274] depending on the dose, and may last up to 5 hours.[52] Ocular hypotension can be maintained through repeated oral or intravenous alcohol doses[49] and a more pronounced effect is noted in eyes with a higher baseline IOP – absolute reductions of 12–30 mmHg have been reported in glaucomatous eyes.[49,52]

In addition to lowering IOP, alcohol also results in a significant increase in retrobulbar and ONH blood flow[53,55] and retinal artery diameter,[51] but does not appear to have an effect on OPP.[51,55]

4.1.5.2 Intraocular pressure

Although the short-term physiological effects of alcohol have been well established in experimental studies, this relationship does not translate to population-based

studies. Observational studies included in this systematic review generally show either a small positive association or no association between alcohol use and IOP[57–60,90] or OHT,[56,99,264] but this in itself is not a consistent result.[62,63] One further study excluded from this review also reported no association between alcohol use and IOP but did not present specific data for this finding.[88]

In addition, absolute IOP differences between drinkers and non-drinkers are often small (maximum difference in participants without glaucoma +1.4 mmHg), although most studies excluded participants with glaucoma from analysis. Given the strong association between IOP and glaucoma, exclusion of these individuals may have altered the IOP distribution in the remaining participants, potentially attenuating any observed IOP difference.

Females with untreated OAG consuming alcohol ≥4 times/week were found to have a higher IOP (+2.8 mmHg) than non-drinkers in a South Korean study,[58] but this relationship was not apparent in men nor was it demonstrated in an Australian study that also included participants with glaucoma in analysis.[63] Evidence of stronger effects and linear trend between alcohol intake and IOP also appear to be restricted to men, but this finding may be explained by a smaller number of female drinkers in these studies.[58,60]

There are numerous considerations when interpreting the available evidence for the association between alcohol use and IOP. If alcohol is not consumed at a frequency regular enough to result in sustained ocular hypotension or in the hours preceding IOP measurement, this physiological effect may not be apparent.

In addition, the direct short-term effects of alcohol may be outweighed by potential indirect or long-term IOP-raising effects. For example, both systolic and diastolic

blood pressure are positively associated with alcohol consumption and IOP.[246,275,276] Although most studies adjusted for blood pressure or hypertension in their analyses,[56–60,62,264] it is possible that any observed association may be due to residual confounding by various vascular (or other) risk factors. Alternatively, alcohol may have a true direct effect on IOP, albeit small and mediated via uncertain pathophysiological mechanisms.

4.1.5.3 Open-angle glaucoma

The earliest report of a harmful association between alcohol and OAG arose from the Framingham Eye Study in 1980 when formal diagnostic criteria for glaucoma were not yet established.[277] It was found that alcohol intake was associated with various definitions of OAG, largely based on visual field defects, but also with definitions encompassing IOP and cup-disc ratios.

Subsequently, numerous observational studies conducted during the 1980s and 1990s reported no association between alcohol use and OAG.[88,89,92,95,278] A number of these earlier studies,[89,92,278] as well as more recent studies,[279–281] however, did not report specific data or effect estimates for this association and were therefore excluded from this systematic review. Indeed, the vast majority of studies (10 of 11) included in the final meta-analysis reported no association between alcohol intake and prevalent or incident OAG.[56,65–73] Only when these results are meta-analysed does a significant harmful association become apparent.

Prospective evidence from the two largest studies exploring the association between alcohol intake and OAG report seemingly contradictory findings. Wise, et al. found a harmful association in a large cohort study of African American women (Black Women's Health Study, BWHS), especially in those consuming ≥7 drinks/week (RR

1.60; 95% CI, 1.06–2.43).[64] In contrast, Kang, et al. found that consumption of >30 grams of alcohol per day appeared to be protective for incident POAG (OR 0.71; 95% CI 0.49–1.04) in the Nurses' Health Study and Health Professionals Follow-Up Study (NHS/HPFS), although this result did not reach statistical significance. Various important differences between these two study populations need to be considered when interpreting this result.

Firstly, participants in the NHS/HPFS were approximately 20 years older than those in the BWHS. Given the significant association between alcohol intake and all-cause mortality,[248,282] competing events in the NHS/HPFS may have contributed to an underestimation of POAG risk, especially in older participants with the highest alcohol intake. However, since participants tended to be middle-aged (approximately 60 years) and moderate drinkers, a group not at increased risk for all-cause mortality,[282] this is unlikely to be a major contributory factor.

Secondly, the NHS/HPFS consisted entirely of health professionals, a group that is likely to differ substantially from the general population in various ways, including in factors related to alcohol intake behaviours, reporting of alcohol consumption, and general health status.

Finally, the BWHS consisted entirely of African American participants, but this group made up only 1% of participants in the NHS/HPFS. Similarly, females represented all participants in the BWHS but 65% of those in the NHS/HPFS. It is possible that any risk may be mediated by both race and sex, but there is currently no evidence to support this explanation. Only one small case-control study reported effect estimates stratified by race[95] and there was no suggestion of heterogeneity by study population/location in this meta-analysis. Similarly, findings from the NHS/HPFS

were consistent across sexes and sex was not found to be a significant factor in the only study reporting stratified results included in this systematic review.[88]

The overall effect estimate was robust across sensitivity analyses with the exception of studies reporting an univariable effect estimate, in which a significant protective association was observed. I hypothesise that this is due to the confounding effect of factors such as age and socio-economic status, which have associations with both alcohol intake and the occurrence or diagnosis of glaucoma.[283,284]

There are a number of possible explanations for the observed association between alcohol use and OAG in this meta-analysis. Alcohol may be directly implicated in OAG risk, although the exact pathophysiological mechanisms are not currently clear. Chronic alcohol use can lead to significant peripheral neuropathy and the proposed underlying mechanisms may play a similar role in glaucomatous optic neuropathy.[285] These include: oxidative stress leading to free radical damage to nerves, activation of the sympatho-adrenal and hypothalamo-pituitary-adrenal axes, nutritional deficiencies (especially thiamine), and direct toxic and pro-inflammatory effects.

Alternatively, alcohol may indirectly influence OAG risk through its association with a number of neurodegenerative and cardiovascular diseases and it is possible that residual confounding effects may be responsible for the observed association.

This systematic review also suggests a positive association between alcohol use and IOP which may further contribute to OAG risk.

4.1.5.4 Dose-response effects

An important consideration in the interpretation of observational studies of environmental or lifestyle exposures is evidence of a dose-response effect which, if present, supports the hypothesis of a causal relationship between associated

variables. Alcohol intake has a linear, logarithmic, or J-shaped association with a multitude of disease outcomes.[246,248]

Dose-dependent associations between alcohol and IOP were demonstrated in men without glaucoma in two studies,[58,60] but this was not a consistent finding. Although there was a suggestion of both harmful[64,68] and protective[72] dose-dependent linear relationships between alcohol intake and OAG, statistical significance was not demonstrated in any study included in this systematic review[64,70,72] and formal dose-dependent meta-analysis was not performed.

Furthermore, there was no consistent finding regarding the association in current and previous alcohol drinkers.[64,71] Future research should aim to better define the dose-response relationship between alcohol and various glaucoma-related outcomes and traits, including the possibility of a non-linear relationship.

4.1.5.5 Alcohol type

Aside from their ethyl alcohol content, there are considerable differences in the constituents and global consumption patterns of the wide variety of alcoholic beverages available.[248,286] It is therefore important to consider the possible confounding role of these factors when exploring any associations with alcohol consumption.

Of particular interest are the polyphenols, a group of compounds with anti-inflammatory and anti-oxidant properties, which are found in high levels in red wine and may play a promising role in improving visual function and slowing visual field loss in patients with OHT and glaucoma.[107] However, alcohol type,[68,72,90] and specifically red wine,[72] was not found to be associated with OAG in any study included in this systematic review. One case-control study reported a protective

association between daily liquor intake (but not intake of any other alcohol type) and OHT,[62] but this finding has not been reproduced in other studies.

4.1.5.6 Glaucoma and related outcomes

OCT measurement of the peripapillary and macular RNFL plays an important role in the diagnosis and management of glaucoma. Although alcohol intake was found not to be associated with peripapillary RNFL thickness in the EPIC-Norfolk Eye Study,[287] higher levels of alcohol consumption (females: >10 grams/day; males: >20 grams/day) was found to be associated with peripapillary RNFL thinning in the Gutenberg Health Study.[75]

In addition, high levels of alcohol consumption have been found to be associated with thinning of various macular inner retinal parameters, particularly the GCIPL in both the UK Biobank[74] and Beaver Dam Offspring[76] studies. This association does not appear to be limited only to population-based studies; alcohol intake was associated with GCIPL thinning in known POAG patients in a South Korean study.[77]

Although these findings suggest that alcohol may play a role in glaucoma severity and progression, there is limited other evidence in this regard. Alcohol use has not been associated with visual field defect deterioration in known glaucoma patients,[288] progression from POAG suspect to definite POAG,[289] or progression to blindness in high-tension POAG.[290] Alcohol consumption was also not found to be associated with incident self-reported glaucoma in the SUN cohort,[291] or with prevalent glaucoma in a German case-control study.[292]

4.1.5.7 Genetic considerations

A number of studies have explored the potential role and associations of gene-alcohol interactions with IOP and glaucoma. A particular focus has been the aldehyde dehydrogenase 2 (*ALDH2*) gene which plays a central role in alcohol metabolism.[293] ALDH2 converts acetaldehyde, a toxic byproduct of alcohol metabolism, to non-toxic acetic acid. Polymorphisms in the *ALDH2* gene, which are particularly common in East Asian populations, may result in an inactive form of ALDH2 and lead to a systemic accumulation of acetaldehyde when alcohol is consumed. Characteristic effects of ALDH2 enzyme deficiency include reduced alcohol tolerance, as well as alcohol-induced facial flushing, tachycardia, and palpitations.

A South Korean study found that drinking-related facial flushing in overweight men was associated with OHT at lower levels of alcohol consumption than in non-flushers.[264] An *ALDH2* polymorphism (rs671), however, was not found to be associated with peripapillary RNFL or GCIPL thickness in known POAG patients in another South Korean study, although gene-alcohol interactions were not analysed.[77] The alcohol-induced increase in retrobulbar blood flow has also been shown to be more pronounced in ALDH2- deficient individuals.[55]

Nitric oxide synthase 3 (NOS3), an enzyme that mediates luminal smooth muscle tone and found in both TM and ocular vascular endothelial cells, has previously been implicated as a potential factor in the pathogenesis of OAG.[176] However, the association between *NOS3* genetic variants and POAG was found not to be modified by alcohol consumption in a subsequent nested case-control study.[178]

Genetic variants of toll-like receptor 4 (*TLR4*), a transmembrane pathogen recognition receptor able to mediate the release of inflammatory cytokines, have been associated with POAG and NTG in the Japanese population. Significant gene-alcohol interaction has been reported in a Chinese study, with the highest POAG risk observed in alcohol drinkers carrying a *TLR4* polymorphism (rs2149356).[265]

The longevity-associated mitochondrial DNA 5178C polymorphism also has a reported interaction with alcohol. Daily consumption in Japanese men with an *mt5178C* polymorphism was found to be significantly associated with higher IOP.[294]

4.1.5.8 Strengths and limitations

This section represents the first systematic review and meta-analysis of the associations between alcohol, IOP, and OAG to date. There are, however, a number of important factors to consider when interpreting the study results, in addition to the limitations already discussed above.

As is the case with the study of most environmental exposures, evidence is limited to observational studies which have inherent weaknesses and risks of bias. Alcohol studies, in particular, are subject to further specific risks and methodological pitfalls.[295] Although well-conducted observational studies can minimise the potential biases introduced by factors such as participant selection, residual confounding, and reverse causality, it is possible that the findings of this systematic review and meta-analysis are influenced by study-specific and systematic biases. This was apparent in the findings of the risk of bias assessment, with domains relating to residual confounding and exposure ascertainment identified as particular areas of concern. In addition to heterogeneity, this risk of bias was deemed sufficient to further downgrade the overall GRADE certainty of evidence to “very low.”

There is currently no universally accepted standard or consensus for assessing risk of bias in observational studies and various concerns with early versions of the ROBINS-E tool have been raised.[296] Specific criticisms include: rating observational studies in comparison to an “ideal” randomised controlled trial when this is often not practically possible; failure to discriminate between studies with single or multiple risks of bias; equal weighting of all risk of bias domains; and serious limitations in determining whether confounders will bias study outcomes.

Therefore, although an important consideration in any systematic review and meta-analysis, given the current limitations, as well as the subjective nature of such an assessment, risk of bias was not used as a weighting tool or exclusion criterion for the final meta-analysis. Furthermore, the presence of other limitations in the current evidence base makes it unlikely that this would significantly alter the overall GRADE certainty of evidence.

Results did prove to be robust across the various sensitivity analyses, however, with the greatest risk of bias identified arising from univariable effect estimates. There was also no statistical evidence of publication bias despite a suggestion of funnel plot asymmetry. Trim and fill analysis, which detects and attempts to correct funnel plot asymmetry, resulted in slight attenuation of the overall effect estimate. It is important to note that this method is agnostic as to the reasons behind the funnel plot asymmetry and may underestimate a true positive effect if no publication bias is present.[297] Other possible explanations for the observed asymmetry include effect size heterogeneity across studies – especially considering the difference between estimates for prevalent (I^2 = 0.0%) and incident (I^2 = 74.9%) OAG – and chance.

Very few studies included in this systematic review were conducted specifically to explore the association between alcohol and IOP or OAG. Instead, most effect

estimates are derived from studies which examined either a different, specific exposure or multiple exposures.

Subsequently, the search strategy may have failed to detect similar relevant studies, especially if alcohol was not mentioned specifically in the article title, abstract or keywords. This was the case for the five additional studies identified during the manual search of the reference lists of included studies and previous reviews. All studies identified in this manner were epidemiological eye studies which collected alcohol intake data in addition to numerous other baseline characteristics. Although all studies reported associations with alcohol intake, this was not the primary study focus and all were indexed without specific reference to alcohol or related terms.

Although case ascertainment criteria for OAG were generally appropriately stringent, objective, and comparable across studies (most requiring a combination of direct visual field, ONH, and angle assessment), measurement of alcohol exposure was far more variable and may have led to significant misclassification bias. Most studies based their exposure assessment on self-reported alcohol consumption from a single questionnaire which, although practical, is subject to both recall and social desirability bias.

This was further complicated by variable definitions of “regular” alcohol intake as well as time periods under consideration. Even semi-quantitative FFQs, which are generally based on current or recent drinking behaviours, may not accurately reflect alcohol consumption over the life-course or drinking patterns such as binge drinking. Significant heterogeneity in categories or levels of alcohol exposure also precluded meaningful dose-response meta-analysis. This limitation in the evidence makes it difficult for health professionals to recommend a “safe dose” of alcohol consumption with regard to glaucoma risk.

4.1.5.9 Conclusion

In conclusion, findings from this systematic review suggest that alcohol consumption is positively associated with higher IOP, although the absolute effect size appears small. In addition, a possible association between alcohol consumption and OAG was demonstrated. This finding should be interpreted with caution, however, given the significant methodological heterogeneity and risk of bias present in the underlying evidence base, as well as the small absolute effect size and borderline statistical significance.

Further study is needed to better define and quantify these associations, but alcohol consumption should be considered a potential modifiable risk factor for the development of glaucoma. In particular, future research is needed to better define the dose-dependent associations of alcohol with various glaucoma-related outcomes and traits, as well as the gene-alcohol interactions underpinning these associations.

Large-scale observational studies and newer genetic epidemiological techniques also offer potential avenues for further investigation, including the use of genetic proxies of alcohol consumption (Mendelian randomisation),[217] objective structural glaucoma biomarkers (including inner retinal OCT measures and cup-disc ratios) and PRSs.[160]

As the global burden of glaucoma is projected to increase further over the coming decades, ongoing investigation into environmental risk factors, as well as gene-environment interactions, are necessary to improve our understanding of glaucoma pathogenesis and potentially lead to novel preventative measures and treatment strategies.

4.2 UK Biobank

Based on the findings of the systematic review and meta-analysis described in section 4.1 above, I conducted a detailed analysis of the relationship between alcohol consumption and glaucoma in the UK Biobank, with the goal of addressing several limitations of previous research studies. The following section is an adapted version of a paper published in *Ophthalmology Glaucoma*[61] and describes these analyses. I am grateful to Dr Marleen Lentjes for her advice and guidance in the derivation of the quantitative alcohol intake measure used in these analyses. I was responsible for all other aspects of the work. The relevant declaration form for previously published material is located in **Appendix A**. Supplementary material for this section can be found in **Appendix E**.

4.2.1 Abstract

Purpose: To examine the associations of alcohol consumption with glaucoma and related traits, and to assess whether a genetic predisposition to glaucoma modified these associations.

Design: Cross-sectional observational and gene-environment interaction analyses in the UK Biobank.

Participants: UK Biobank participants with data on IOP (*n* = 109 097), OCT-derived macular inner retinal layer thickness measures (*n* = 46 236), and glaucoma status (*n* = 173 407).

Methods: Participants were categorised according to self-reported drinking behaviours. Quantitative estimates of alcohol intake were derived from touchscreen questionnaires and food composition tables. A two-step analysis was performed, first

comparing categories of alcohol consumption (never, infrequent, regular, and former drinkers), before assessing for a dose-response effect in regular drinkers only. Multivariable linear, logistic, and restricted cubic spline regression, adjusted for key sociodemographic, medical, anthropometric, and lifestyle factors, were used to examine associations. Effect modification by a multi-trait glaucoma PRS was assessed for all associations.

Main outcome measures: IOP, mRNFL thickness, GCIPL thickness, and prevalent glaucoma.

Results: Compared to infrequent drinkers, regular drinkers had higher IOP (0.17 mmHg; 95% CI, 0.10–0.24; P <0.001) and thinner mGCIPL (-0.17 μm; 95% CI, -0.33 to 0.00; P = 0.049); while former drinkers had a higher prevalence of glaucoma (OR 1.53; 95% CI, 1.16–2.02; P = 0.002). In regular drinkers, alcohol intake was adversely associated with all outcomes in a dose-dependent manner (all P <0.001). RCS regression analyses suggested non-linear associations, with apparent threshold effects at approximately 50 grams/week, for mRNFL and GCIPL thickness. Significantly stronger alcohol-IOP associations were observed in participants at higher genetic susceptibility to glaucoma ($P_{interaction}$ <0.001).

Conclusions: Alcohol intake was consistently and adversely associated with glaucoma and related traits, and at levels below current UK and US guidelines. While causality is not definitively confirmed, these results may be of interest to people with, or at risk of, glaucoma and their advising physicians.

4.2.2 Introduction

Alcohol consumption is a leading cause of death and disability worldwide, responsible for an estimated 3 million deaths and 132 million disability-adjusted life

years lost in 2016 alone.[248,286] Alcohol use has been implicated in over 200 diverse health conditions, and it therefore represents a significant public health concern and an important modifiable lifestyle risk factor.[286] Despite these well-documented harms, it remains a highly prevalent behaviour in many populations, and particularly in Europe, where 60% of all adults are reported to be current alcohol drinkers.[286]

IOP remains the major modifiable risk factor for glaucoma but there is considerable interest in identifying other factors which may complement existing treatment strategies or guide lifestyle recommendations. Given the widespread prevalence of both alcohol consumption and glaucoma, an understanding the magnitude and shape of any underlying association may have important clinical and public health consequences.

The acute ophthalmological effects of alcohol consumption include transient ocular hypotension and an increase in blood flow to the optic nerve head, theoretically playing a protective role in the development of glaucoma.[47,53,55] However, alcohol has known neurotoxic properties and chronic use has been associated with multiple neurodegenerative conditions, which may have similar implications for glaucoma risk.[298]

Previous studies of the association between alcohol consumption and glaucoma have failed to yield consistent results, and although a recent systematic review and meta-analysis has suggested that habitual alcohol use is adversely associated with both IOP and open-angle glaucoma, firm conclusions are limited by marked heterogeneity and a high risk of bias.[32]

Observational studies of alcohol and glaucoma should be adequately powered to detect an association despite noise in the assessment variables; allow for

quantification of alcohol intake to explore possible dose-response and non-linear relationships; adjust for key covariates to limit residual confounding; and assess relationships with a variety of glaucoma-related traits to gauge the consistency of any observed associations.

The UK Biobank fulfils all the aforementioned criteria and represents an invaluable resource which may be leveraged to further understanding of the alcohol-glaucoma relationship. I utilised UK Biobank questionnaire, anthropometric, ocular, medical, and lifestyle data to explore the association of alcohol consumption with glaucoma and various glaucoma-related traits. I also used genetic data to consider possible modification of the alcohol-glaucoma association by a glaucoma PRS.

4.2.3 Methods

4.2.3.1 UK Biobank

See section 2.1.1.1.

4.2.3.2 Assessment and quantification of alcohol intake

Information on habitual alcohol consumption was assessed in the baseline questionnaire (2006–2010). Participants were asked how often they drank alcohol and were required to categorise their response as: “Daily/almost daily”, “3–4 times a week”, “1–2 times a week”, “1–3 times a month”, “Special occasions only”, or “Never”. If their alcohol consumption varied substantially, participants were asked to provide an average considering their intake over the last year.

Participants who reported a drinking frequency of “1–2 times a week” or greater were then asked to quantify their average weekly alcohol intake, whereas those reporting a frequency of “1–3 times a month” or “Special occasions only” were asked about

their average monthly intake, of each of the following: (1) “Glasses of red wine”, (2) “Glasses of white wine or champagne”, (3) “Pints of beer or cider”, (4) “Measures of spirits or liquors”, (5) “Glasses of fortified wine”, and (6) “Glasses of other alcoholic drinks”. These questions included definitions, examples and standard portion sizes for each of the six alcoholic beverage types.

Participants who reported a drinking frequency of “Never” to the first question were not asked to quantify their alcohol intake but were asked if they had previously drunk alcohol. Participants were additionally asked whether they usually consumed alcohol with meals.

For the purposes of this study, participants were categorised as never drinkers (frequency = “Never”; previously drunk alcohol = “No”), infrequent drinkers (frequency = “Special occasions only”), regular drinkers (frequency = “1–3 times a month” or greater), or former drinkers (frequency = “Never”; previously drunk alcohol = “Yes”).

I then calculated average total alcohol (ethanol) intake (grams/week) for all regular drinkers according to the formula:

$$\sum_{i=1}^{6} \text{number of portions}_{(i)} * \text{portion size (mL)}_{(i)} * \text{alcohol concentration (g/mL)}_{(i)} * k$$

where *i* represents the alcoholic beverage categories described above and *k* represents a conversion factor depending on whether an individual reported their average weekly (*k* = 1) or monthly (*k* = 0.23) alcohol intake. For those reporting a weekly intake, the conversion factor does not change the quantitative estimate, while for those reporting a monthly intake, the conversion factor represents:
(×12 months ÷365 days ×7 days).

The alcohol concentrations applied to each alcoholic beverage category were based on the same food composition tables and methodology used for the Oxford WebQ, a validated web-based food frequency questionnaire which has been used to calculate alcohol intake in UK Biobank 24-hour dietary follow-up assessments.[299–301] To handle implausibly low (e.g. regular drinkers reporting a weekly intake of 0 grams) and extreme upper values, I excluded total alcohol intake estimates in the top and bottom percentiles. Further details of the derivation of alcohol intake from the UK Biobank baseline questionnaire are available in **Figure E1** and **Table E1.**

4.2.3.3 Glaucoma-related outcome measures and case ascertainment

See section 2.2.1.1.

4.2.3.4 Genotyping and polygenic risk scores

See section 2.2.3.

4.2.3.5 Statistical analysis

Baseline characteristics, for each cohort (IOP, OCT, and glaucoma) and according to alcohol drinking status, were summarised as mean (SD) for continuous variables, and frequency (proportion) for categorical variables. Alcohol intake demonstrated a right-skewed distribution, and these data were summarised as median (IQR).

To assess the main associations between alcohol intake and the various glaucoma-related outcomes, I used multivariable linear (for IOP, mRNFL thickness, and mGCIPL thickness) and logistic (for glaucoma) regression models adjusted for key sociodemographic, medical, anthropometric, ocular, and lifestyle factors.

I included the following covariables (all of which were ascertained on the same day as the alcohol and ophthalmic assessments) based on previously reported risk factors and associations:[85] age, sex, self-reported ethnicity, Townsend deprivation index, BMI, height, SBP, SE, self-reported diabetes mellitus, smoking status, smoking intensity, physical activity, and assessment season. Full details of UK Biobank covariables can be found in section 2.2.4.

I first assessed associations in all available participants according to alcohol intake category. In epidemiological studies of alcohol intake, the use of low-volume drinkers as the reference group offers several advantages compared to the use of never drinkers.[302] I therefore used infrequent drinkers as the reference category for this step of the analysis.

Subsequent quantitative analyses were then restricted to regular drinkers only, as the inclusion of never and former drinkers, who tend to differ substantively from current drinkers, may introduce bias.[303] Additionally, since infrequent drinkers (who by definition consumed alcohol less than once a month) were asked to quantify their monthly alcohol intake, I deemed estimates of their alcohol intake less accurate than for regular drinkers and these participants were also excluded from subsequent analyses.

In the second step of our analysis, I aimed to assess for dose-response and non-linear associations. For the dose-response analyses, alcohol intake was analysed as both a continuous (grams/week) and categorical (quintiles of alcohol intake) variable. Trends across quintiles were examined by testing the median value of each group. Non-linear associations were assessed with restricted cubic spline regression models adjusted for the same covariates listed above. For each association, I considered 3–7 knots at fixed heuristic percentiles, as suggested by Harrell,[304] with

final model selection based on minimisation of the AIC. I used the natural logarithm of alcohol intake in these models, as this transformed variable was approximately normally distributed and aided graphical visualisation of inflection points occurring at relatively low quantities of alcohol intake.

I conducted the following sensitivity analyses: (1) sex-stratified analyses with tests for interaction; (2) analyses restricted to participants of European descent only; (3) analyses according to alcohol beverage type; (4) interaction analyses to assess whether associations were modified by frequency of alcohol consumption or drinking alcohol with meals; (5) exclusion of participants with glaucoma for analyses of IOP and OCT parameters; (6) analyses using different definitions for glaucoma (ICD-10 codes limited to POAG and undefined glaucoma); (7) analyses using different IOP measurements (IOPg and IOPcc without imputation of pre-treatment values); (8) analyses restricted to participants without hypertension (self-report or SBP ≥140 mmHg); and (9) analyses including additional covariates in the final regression models – caffeine intake (mg/day), total cholesterol (mmol/L), statin use, and oral beta-blocker use – based on recent results from similar analyses of glaucoma-related traits.[146,180,305]

To assess whether the relationship between alcohol intake and the various glaucoma-related traits were modified by the glaucoma MTAG PRS, I tested the significance of a multiplicative interaction term between alcohol intake and the genetic factor in the maximally adjusted regression models. The glaucoma MTAG PRS was included as a continuous variable in these models. Although UK Biobank participants were included in the original MTAG study from which the PRS weights were derived,[27] the independence of marginal and interaction effects in these models limits the risk of data overfitting.

4.2.4 Results

4.2.4.1 Participants

The number of UK Biobank participants eligible for, and included in, each of the analyses are presented in **Figure 4.5**. Overall, 81 324, 36 143, and 84 655 participants with complete data for the analyses of IOP, OCT-derived macular inner retinal thickness measures, and glaucoma status, were included, respectively. Participant characteristics for each of the three cohorts are summarised in **Table 4.6.** The mean age of participants was 56–57 years, with a slight female predominance (52–53%) and a majority of White participants (90–92%).

4.2.4.2 Alcohol intake

Overall, 80–81% of participants were classified as regular drinkers, with a median alcohol intake of slightly more than 90 grams/week. Among these participants, women were more likely to be red wine (38%) or white wine (29–30%) drinkers, while men were more likely to be beer/cider (44%) or red wine (24%) drinkers. By contrast, infrequent drinkers comprised only 12% of participants, with a median alcohol intake of less than 3 grams/week. Only 4–5% and 4% of the cohort were classified as never and former drinkers, respectively.

The distribution of alcohol intake among regular drinkers and stratified by sex is displayed in **Figure E2**. Further details of alcohol consumption according to cohort and sex are available in **Table E2**. Participant characteristics according to alcohol consumption category and quintile of alcohol intake for the glaucoma cohort (the largest of the three cohorts) are presented in **Table 4.7**. Crude average IOP, mRNFL thickness, GCIPL thickness, as well as glaucoma prevalence, according to the same categories are presented in **Table E3**.

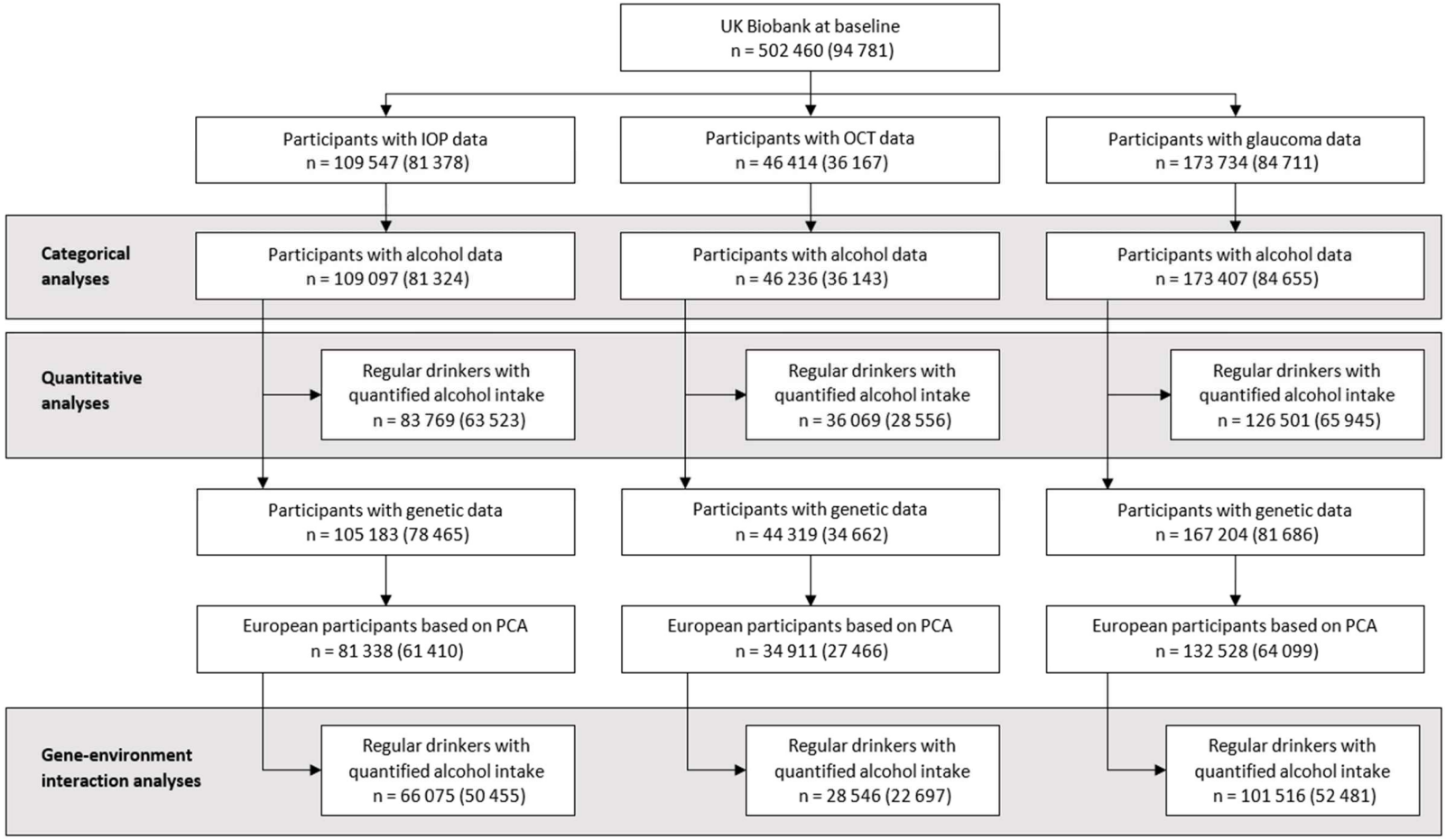

Figure 4.5 Flow diagram outlining eligible UK Biobank participants available for this study

Numbers in parentheses indicate participants with complete data for all covariables.
IOP, intraocular pressure; OCT, optical coherence tomography; PCA principal components analysis.

Table 4.6 Participant characteristics by cohort

	Analysis cohort		
	IOP	**OCT**	**Glaucoma**
Sample size	81 324	36 143	84 655
Age (years), mean (SD)	56.6 (8.1)	56.3 (8.1)	56.6 (8.1)
Sex, n (%)			
Women	43 214 (53.1)	18 835 (52.1)	44 970 (53.1)
Men	38 110 (46.9)	17 308 (47.9)	39 685 (46.9)
Ethnicity, n (%)			
White	73 548 (90.4)	33 081 (91.5)	76 677 (90.6)
Black	2 642 (3.3)	1 071 (3.0)	2 720 (3.2)
Other	5 134 (6.3)	1 991 (5.5)	5 258 (6.2)
Townsend deprivation index, mean (SD)	-1.1 (2.9)	-1.1 (2.9)	-1.1 (2.9)
Body mass index (kg/m^2), mean (SD)	27.3 (4.7)	27.2 (4.7)	27.3 (4.7)
Height (cm), mean (SD)	168.9 (9.3)	169.3 (9.2)	168.9 (9.3)
Systolic blood pressure (mmHg), mean (SD)	137.0 (18.3)	136.8 (18.3)	137.1 (18.3)
Spherical equivalent (D), mean (SD)	-0.4 (2.7)	0.0 (2.0)	-0.4 (2.7)
Diabetes, n (%)	4,411 (5.4)	1,782 (4.9)	4,616 (5.5)
Smoking status, n (%)			
Never	46 741 (57.5)	20 542 (56.8)	48 652 (57.5)
Previous	29 248 (36.0)	13 280 (36.7)	30 458 (36.0)
Current	5 335 (6.6)	2 321 (6.4)	5 545 (6.6)
Smoking intensity (cigarettes/day), mean (SD)			
Current smokers	14.5 (8.2)	13.8 (7.8)	14.5 (8.3)
Physical activity (MET-minutes/week), mean (SD)	2 669 (2 678)	2 692 (2 706)	2 666 (2 676)
Intraocular pressure (mmHg), mean (SD)	16.1 (3.4)	—	—
mRNFL thickness (µm), mean (SD)	—	28.9 (3.8)	—
GCIPL thickness (µm), mean (SD)	—	75.2 (5.2)	—
Glaucoma, n (%)	—	—	1,493 (1.8)
Alcohol consumption frequency, n (%)			
Never	3 906 (4.8)	1 536 (4.3)	4 077 (4.8)
Infrequent	9 700 (11.9)	4 184 (11.6)	10 097 (11.9)
Regular	64 803 (79.7)	29 136 (80.6)	67 421 (79.6)
Former	2 915 (3.6)	1 287 (3.6)	3 060 (3.6)
Alcohol intake quantity (g/week), median (IQR)			
Infrequent	2.8 (0.0–7.9)	2.8 (0.0–8.1)	2.8 (0.0–7.9)
Regular	91.3 (43.3–170.9)	92.8 (44.6–173.5)	91.8 (43.8–171.6)
Glaucoma MTAG, mean (SD)[1]	-0.04 (1.04)	-0.05 (1.04)	-0.04 (1.04)

[1] *n* = 50 455 (IOP), *n* = 22 697 (OCT), *n* = 52 481 (glaucoma).

D, dioptre; IOP, intraocular pressure; OCT, optical coherence tomography; SD, standard deviation; MET, metabolic equivalent of task; mRNFL, macular retinal nerve fibre layer; GCIPL, ganglion cell-inner plexiform layer; IQR, interquartile range; MTAG, multi-trait analysis of GWAS (genome-wide association study).

Table 4.7 Participant characteristics by alcohol consumption frequency and alcohol intake quintile

	Never	Infrequent	Regular					Former
			Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
Sample size	4 077	10 097	13 534	13 544	13 508	13 170	13 189	3 060
Age (years), mean (SD)	55.9 (8.7)	56.6 (8.3)	56.4 (8.2)	56.5 (8.1)	56.7 (8.0)	56.9 (7.9)	57.0 (7.8)	56.7 (8.0)
Sex, n (%)								
Women	2 822 (69.2)	6 980 (69.1)	9 429 (69.7)	8 486 (62.7)	6 550 (52.4)	5 510 (41.8)	2 964 (22.5)	1 601 (52.3)
Men	1 255 (30.8)	3 117 (30.9)	4 105 (30.3)	5 058 (37.3)	5 958 (47.6)	7 660 (58.2)	10 225 (77.5)	1 459 (47.7)
Ethnicity, n (%)								
White	2 273 (55.8)	8 103 (80.3)	12 291 (90.8)	12 679 (93.6)	11 868 (94.9)	12 668 (96.2)	12 787 (97.0)	2 640 (86.3)
Black	392 (9.6)	772 (7.7)	489 (3.6)	343 (2.5)	248 (2.0)	180 (1.4)	112 (0.9)	138 (4.5)
Other	1 412 (34.6)	1 222 (12.1)	754 (5.6)	522 (3.9)	392 (3.1)	322 (2.4)	290 (2.2)	282 (9.2)
Townsend deprivation index, mean (SD)	0.1 (3.3)	-0.4 (3.1)	-1.2 (2.9)	-1.4 (2.8)	-1.4 (2.8)	-1.4 (2.8)`	-1.2 (2.9)	0.0 (3.2)
Body mass index (kg/m^2), mean (SD)	27.8 (5.3)	28.3 (5.6)	27.1 (4.9)	26.6 (4.5)	26.7 (4.4)	27.0 (4.2)	27.7 (4.3)	27.9 (5.4)
Height (cm), mean (SD)	164.1 (9.0)	165.8 (8.8)	166.7 (8.8)	167.9 (9.0)	169.4 (9.0)	170.9 (9.0)	173.6 (8.4)	168.4 (9.1)
Systolic blood pressure (mmHg), mean (SD)	135.9 (18.4)	136.2 (18.5)	135.3 (18.6)	135.5 (18.5)	136.1 (18.0)	137.9 (17.7)	141.6 (17.7)	134.9 (18.0)
Spherical equivalent (D), mean (SD)	-0.3 (2.6)	-0.3 (2.7)	-0.5 (2.9)	-0.5 (2.8)	-0.5 (2.8)	-0.4 (2.8)	-0.3 (2.6)	-0.2 (2.7)
Diabetes, n (%)								
Yes	414 (10.2)	925 (9.2)	664 (4.9)	559 (4.1)	485 (3.9)	525 (4.0)	663 (5.0)	306 (10.0)
Smoking status, n (%)								
Never	3 474 (85.2)	6 542 (64.8)	9 170 (67.8)	8 477 (62.6)	7 235 (57.8)	6 583 (50.0)	5 051 (38.3)	1 429 (46.7)
Previous	449 (11.0)	2 741 (27.2)	3 687 (27.2)	4 517 (33.4)	4 676 (37.4)	5 810 (44.1)	6 735 (51.1)	1 281 (41.9)
Current	154 (3.8)	814 (8.1)	677 (5.0)	550 (4.1)	597 (4.8)	777 (5.9)	1 403 (10.6)	350 (11.4)
Smoking intensity (cigarettes/day), mean (SD)								
Current smokers	14.5 (9.6)	14.8 (7.7)	13.1 (6.7)	13.3 (7.2)	13.2 (7.7)	13.1 (7.4)	15.7 (9.1)	16.6 (9.4)
Physical activity (MET-minutes/week), mean (SD)	2 504 (2 764)	2 690 (2 793)	2 578 (2 570)	2 597 (2 497)	2 657 (2 587)	2 685 (2 647)	2 812 (2 817)	2 738 (2 947)

Details of alcohol intake quintiles are reported in **Table E2**. Summary statistics exclude 1 476 regular drinkers with missing alcohol intake data.

SD, standard deviation; D, dioptre; MET, metabolic equivalent of task.

Total alcohol intake demonstrated strong associations with known alcohol-associated biochemical parameters, including HDL-C and MCV, after adjustment for all covariates used in the main analyses (both P <0.001).[306]

4.2.4.3 Categorical analyses

In the maximally adjusted multivariable linear and logistic regression models (**Table 4.8**), when compared to infrequent drinkers, regular drinkers had higher IOP (0.17 mmHg; 95% CI 0.10–0.24; P <0.001) and thinner GCIPL (-0.17 μm; 95% CI, -0.33 to 0.00; P = 0.049), but no difference in mRNFL thickness (-0.10 μm; 95% CI, -0.23 to 0.02; P = 0.11) or prevalence of glaucoma (OR 1.13; 95% CI, 0.95–1.34; P = 0.16). Former drinkers had a higher prevalence of glaucoma (OR 1.53; 95% CI, 1.16–2.02; P = 0.002) and, interestingly, lower IOP (-0.15 mmHg; 95% CI, -0.28 to -0.01; P = 0.03). These results were materially unchanged when combining never and infrequent drinkers as the reference category.

4.2.4.4 Quantitative analyses

When considering regular drinkers only, consistent linear dose-response relationships between alcohol intake and all the glaucoma-related outcomes were observed. Each additional SD increase in alcohol intake (111–112 grams/week) was associated with higher IOP (0.08 mmHg; 95% CI, 0.05–0.11), thinner mRNFL (-0.17 μm; 95% CI, -0.22 to -0.12), thinner GCIPL (-0.34 μm; 95% CI, -0.40 to -0.27), and higher prevalence of glaucoma (OR 1.11; 95% CI, 1.05–1.18) (all P <0.001). Similarly, when compared to the lowest alcohol intake quintile (median 18–19 grams/week), those in the highest alcohol intake quintile (median 278–280 grams/week) had higher IOP (0.27 mmHg; 95% CI, 0.19–0.36), thinner mRNFL (-0.41 μm; 95% CI, -0.56 to -0.27), thinner GCIPL (-0.83 μm; 95% CI, -1.02 to -0.63),

and higher prevalence of glaucoma (OR 1.36; 95% CI, 1.12–1.66) (all P_{trend} ≤0.001). Full details of the main analyses are presented in **Table 4.8**.

Maximally adjusted restricted cubic spline regression models suggested the presence of non-linear associations (**Figure 4.6**). While there was a clear log-linear relationship with IOP and glaucoma, there appeared to be a threshold effect of the log of alcohol intake on mRNFL thickness and GCIPL thickness, with adverse associations only apparent after approximately 50 grams/week. The same threshold effect on the inner retinal OCT parameters was apparent when modelling associations with an untransformed alcohol intake variable.

Importantly, adverse associations with all glaucoma-related outcomes were demonstrated at quantities below current recommended UK (<112 grams/week) and US (women <98 grams/week; men <196 grams/week) drinking guidelines.[307,308] When including all participants, with the exception of former drinkers, in these analyses (never drinkers were assigned an alcohol intake of 0 grams/week), a similar threshold effect was additionally observed for glaucoma, but not for IOP (**Figure E3**). Full details of the restricted cubic spline regression analyses and model selection are available in **Table E4**.

Table 4.8 Association of alcohol consumption frequency and alcohol intake quantity with intraocular pressure, inner retinal OCT measures, and glaucoma

	IOP (mmHg)			mRNFL (µm)			GCIPL (µm)			Glaucoma (%)		
	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value	OR	95% CI	*P*-value
Alcohol consumption frequency												
Never	0.09	(-0.04, 0.21)	0.17	-0.08	(-0.31, 0.14)	0.46	-0.08	(-0.38, 0.21)	0.57	1.23	(0.94, 1.62)	0.13
Infrequent		Reference			Reference			Reference			Reference	
Regular	**0.17**	**(0.10, 0.24)**	**<0.001**	-0.10	(-0.23, 0.02)	0.11	**-0.17**	**(-0.33, 0.00)**	**0.049**	1.13	(0.95, 1.34)	0.16
Former	-0.15	(-0.28, -0.01)	0.03	-0.21	(-0.45, 0.02)	0.08	-0.06	(-0.37, 0.25)	0.69	**1.53**	**(1.16, 2.02)**	**0.002**
Alcohol intake quantity (g/week)												
Per SD increase	**0.08**	**(0.05, 0.11)**	**<0.001**	**-0.17**	**(-0.22, -0.12)**	**<0.001**	**-0.34**	**(-0.40, -0.27)**	**<0.001**	**1.11**	**(1.05, 1.18)**	**<0.001**
Quintiles												
Quintile 1		Reference			Reference			Reference			Reference	
Quintile 2	0.09	(0.01, 0.17)	0.02	0.01	(-0.13, 0.14)	0.91	0.04	(-0.14, 0.22)	0.65	1.07	(0.88, 1.31)	0.48
Quintile 3	**0.15**	**(0.07, 0.23)**	**<0.001**	**-0.12**	**(-0.26, 0.02)**	**0.09**	-0.18	(-0.36, 0.01)	0.06	1.10	(0.90, 1.34)	0.37
Quintile 4	**0.18**	**(0.09, 0.26)**	**<0.001**	**-0.25**	**(-0.39, -0.11)**	**<0.001**	**-0.34**	**(-0.53, -0.15)**	**<0.001**	**1.22**	**(1.00, 1.48)**	**0.05**
Quintile 5	**0.27**	**(0.19, 0.36)**	**<0.001**	**-0.41**	**(-0.56, -0.27)**	**<0.001**	**-0.83**	**(-1.02, -0.63)**	**<0.001**	**1.36**	**(1.12, 1.66)**	**0.002**
P_{trend}			**<0.001**			**<0.001**			**<0.001**			**0.001**

Alcohol intake quantified in regular drinkers only. Details of alcohol intake quintiles for each cohort are reported in **Table E2**. All models adjusted for age (years), sex (women, men), ethnicity (White, Black, Other), Townsend deprivation index, assessment season (Summer, Autumn, Winter, Spring), body mass index (kg/m^2), height (cm), systolic blood pressure (mmHg), spherical equivalent (dioptres), diabetes (yes, no), smoking status (never, previous, current), smoking intensity (number of cigarettes smoked/day), physical activity (MET-minutes/week). One standard deviation increase in alcohol intake is equivalent to an additional 111–112 grams/week.

OCT, optical coherence tomography; IOP, intraocular pressure; mRNFL, macular retinal nerve fibre layer; GCIPL, ganglion cell-inner plexiform layer; β, beta coefficient; CI, confidence interval; OR, odds ratio; SD, standard deviation.

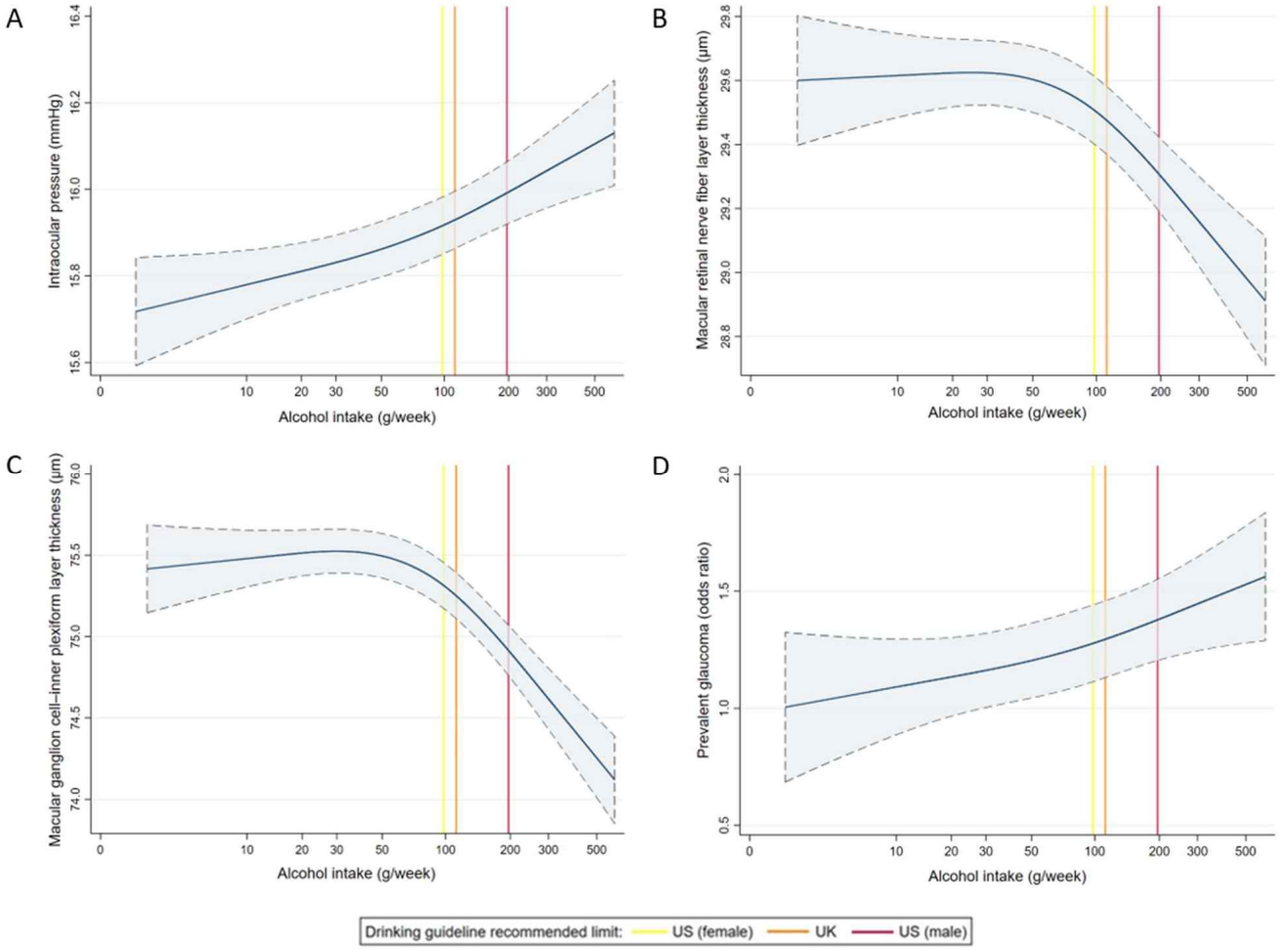

Figure 4.6 Maximally adjusted restricted cubic spline regression models for the association between alcohol intake and (**a**) intraocular pressure, (**b**) macular retinal nerve fibre layer thickness, (**c**) ganglion cell-inner plexiform layer thickness, and (**d**) glaucoma in regular drinkers

Vertical lines represent current UK (112 grams/week) and US (women 98 grams/week; men 196 grams/week) recommended alcohol drinking guidelines.

4.2.4.5 Sensitivity analyses

There was no evidence for a differential effect or interaction by sex (**Table E5** and **Table E6**). Results were materially unchanged when restricting analyses to participants of European descent or those without hypertension. Results were generally consistent across all alcoholic beverage types (**Table E7**) and there was no evidence for interaction according to frequency of alcohol consumption or drinking alcohol with meals. Exclusion of participants with glaucoma and the use of different glaucoma definitions did not yield different results, and similarly, results were largely unchanged when using different IOP definitions, although larger effect sizes and a null IOP association with former drinkers were noted with IOPg (**Table E8**). The inclusion of additional covariables did not materially change the results, although there was a loss of statistical power due to fewer participants with complete data (**Table E9**).

4.2.4.6 Gene-environment interaction analyses

The glaucoma MTAG PRS was found to significantly modify the association between alcohol intake and IOP ($P_{interaction}$ <0.001), but not mRNFL, GCIPL, or glaucoma (all $P \geq 0.21$). No association was observed in participants in the lowest quintile of genetic risk, with progressively stronger associations noted in subsequent quintiles (**Figure 4.7**). Specifically, for those in the highest glaucoma MTAG PRS quintile, each SD increase in alcohol intake was associated with 0.15 mmHg (95% CI, 0.07–0.24) higher IOP, compared to 0.00 mmHg (95% CI, -0.06 to 0.06), 0.04 mmHg (95% CI, -0.04 to 0.12), 0.08 mmHg (95% CI, -0.01 to 0.16), and 0.11 mmHg (95% CI, 0.03–0.20) for those in quintiles 1–4, respectively.

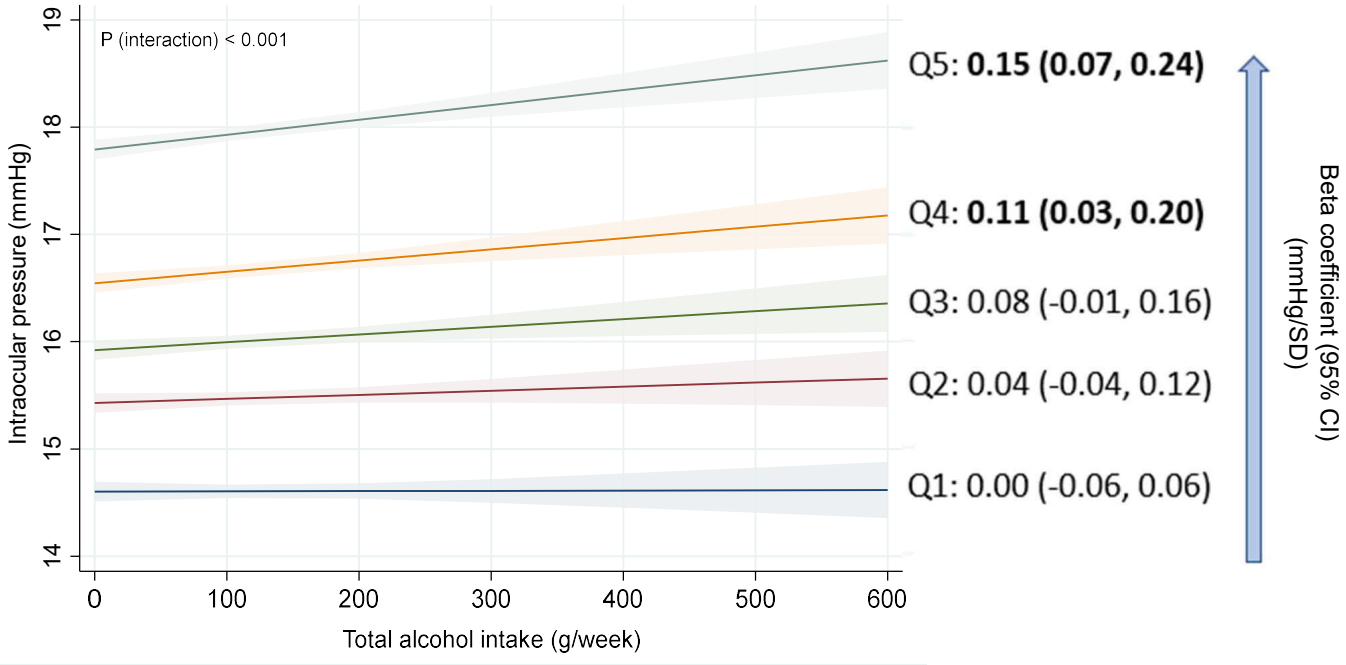

Figure 4.7 Gene-environment interaction analysis for the effect of the glaucoma MTAG PRS on the association between alcohol intake and intraocular pressure in regular drinkers of European ancestry

MTAG, multi-trait analysis of genome-wide association studies; PRS, polygenic risk score; Q, quintile; SD, standard deviation; CI, confidence interval.

4.2.5 Discussion

In this study, I utilised data from the UK Biobank to explore the association between alcohol consumption and various glaucoma-related traits, using a combination of observational and gene-environment analyses. Overall, strong and consistent adverse dose-response associations were observed for all glaucoma-related outcomes, which proved robust to a variety of sensitivity analyses. Although there was evidence for a threshold effect, specifically for inner retinal OCT measures, no quantity of alcohol consumption was found to confer a protective association with any outcome. Importantly, all adverse associations were apparent at alcohol intake below current recommended UK (112 grams/week) and US (women 98 grams/week; men 196 grams/week) drinking guidelines.[307,308] Additionally, the alcohol-IOP association was found to be modified by a glaucoma MTAG PRS, with the strongest associations noted in participants with the highest genetic susceptibility to glaucoma, a finding recently replicated in an independent cohort.[309]

Although previous studies have demonstrated adverse associations of alcohol consumption with IOP[57,59] and glaucoma,[64] results have generally been non-significant or inconsistent. Importantly, most studies have not been designed specifically to explore these relationships or suffer from multiple limitations and potential biases. The evidence for inner retinal measures is more consistent, with multiple studies demonstrating adverse associations with alcohol intake.[74–77]

Epidemiological studies of alcohol consumption, in general, are prone to additional biases and methodological pitfalls and no single study is ideal.[295] However, in the absence of randomised control trials, observational studies represent the best current approach to gauging these associations. The UK Biobank, in particular, with its large sample size and wealth of glaucoma-related, phenotypic, and genotypic

information, represents an unparalleled resource. The availability of objective structural glaucoma biomarkers, including IOP and inner retinal OCT measures, greatly increases statistical power and minimises the risk of misclassification bias in the outcome variables.

This study is the first to simultaneously assess the association of alcohol with multiple glaucoma-related outcomes in the same cohort and the largest of the alcohol-IOP association. It is also the first to assess whether these relationships are modified by background genetic risk of glaucoma. Notably, I found strong dose-dependent adverse associations across all outcomes. These relationships remained significant even after adjustment for multiple potential confounding variables and proved robust to a variety of sensitivity analyses. While causality cannot be definitively inferred, these results are supportive of a true underlying association, rather than a case of residual confounding or reverse causality.

In contrast to previous studies which have suggested that adverse associations with IOP may be restricted to men,[58,60] I found no differential effect or evidence of sex interaction for any outcome. This previously reported finding may be due to a relatively lower proportion of female drinkers in non-European populations.[286] Despite evidence for the neuroprotective properties of polyphenols,[107] a group of anti-inflammatory and anti-oxidant compounds found in high concentrations in red wine, I found no evidence for a differential or protective effect of any alcoholic beverage. This is consistent with previous studies[72,90] and may be explained by the detrimental effects of alcohol on glaucoma outweighing any potential beneficial properties.

While the reported effect estimates for the glaucoma-related traits may seem small, it is useful to contextualise these findings. It is important to emphasise that I am

comparing *between* participants, rather than *within* participants, and this always reduces effect sizes due to variability from other differences among individuals. For example, systemic beta-blockers are known to have a profound IOP-lowering effect within individuals (which led to the development of topical beta-blockers, a mainstay of glaucoma management), yet the difference in IOP between users and non-users of systemic beta-blockers in the UK Biobank was only 0.54 mmHg, which is similar to other population-based studies.[146,310] Therefore, the 0.27 mmHg difference between top and bottom quintile of alcohol consumption (even excluding non-drinkers) is considerable and suggests potentially highly clinically significant effects of alcohol within individuals. Similarly, on a population level, the effect estimates for mRNFL and GCIPL thickness are equivalent to the average difference seen between participants separated by 10 and 5 years of age, respectively.[74]

Despite predominantly detrimental health associations, alcohol exhibits a J-shaped relationship with certain cardiovascular outcomes, with a protective effect observed at low intake. This relationship is thought to be partly accentuated by the inclusion of never drinkers in analyses and various other biases.[303] The restricted cubic spline regression analyses provided evidence for a threshold effect on inner retinal OCT measures, but no quantity of alcohol intake was found to be protective for any glaucoma-related outcome in this study. There was a suggestion of a threshold effect on glaucoma when including all participants, but this disappeared when restricting analyses to regular drinkers only, highlighting this potential epidemiological artefact.

There are numerous plausible biological mechanisms underlying the observed associations between alcohol and glaucoma-related traits. Chronic alcohol use is associated with various biochemical and physiological derangements, as well as a host of neurodegenerative, cardiovascular, and endocrine disorders,[246,247,286] and it is

possible that the associations represent a combination of causative factors, rather than a single mechanism. Alternatively, glaucoma-related outcome measures may be influenced by different underlying pathways, and this may account for the observed difference in the modelled associations between alcohol with IOP or glaucoma (log-linear effect), and mRNFL thickness or GCIPL thickness (threshold effect) in this study.

It is well-established that alcohol has neurotoxic properties, with habitual consumption associated with decreased brain volumes,[311] peripheral neuropathy,[285] and neurodegenerative disorders, including Alzheimer's and Parkinson's diseases.[298] Since the retina represents an extension of the central nervous system, with known associations of retinal layer thickness and brain volumes,[312] this may constitute a major etiological factor. Proposed underlying mechanisms for these associations include: oxidative stress leading to free radical damage to nerves, activation of the sympatho-adrenal and hypothalamo-pituitary-adrenal axes, nutritional deficiencies (especially thiamine), and direct toxic and pro-inflammatory effects.[285]

Similarly, oxidative stress-mediated damage to the TM may account for the observed alcohol-IOP association, which may further contribute to glaucoma risk through traditional IOP-dependent mechanisms. The gene-environment interaction analyses showed that this association was stronger in individuals with a higher genetic risk of glaucoma. A similar interaction has been demonstrated for caffeine intake,[180] suggesting the hypothesis that these dietary associations may reflect a combination of environmental exposure and genetically determined functional reserve in the aqueous outflow pathways.

Additionally, the observed associations may be related to the detrimental cardiovascular effects of heavy drinking, including hypertension and

atherosclerosis,[313] which may have implications for glaucomatous neurodegeneration through IOP-independent mechanisms.[314] Although all associations were noted to attenuate after adjustment for SBP in analyses, this did not account for a significant difference in the overall results and results were materially unchanged when restricting analyses to participants without hypertension.

It is important to acknowledge several limitations of the study. The UK Biobank response rate was only 5.5% and it has been reported that participants drank less alcohol and had lower rates of disease than the general population.[315] Despite this "healthy volunteer" selection bias, the fact that an alcohol-glaucoma association was observed may imply that the true association in the general population is even stronger and does not negate the internal validity of our findings.

Exposure ascertainment through self-reported alcohol consumption from a single questionnaire is subject to both recall and social desirability bias and may lead to significant misclassification. Furthermore, this measure may not accurately reflect alcohol consumption over the life course or specific drinking patterns. However, the alcohol intake measure did demonstrate expected associations with known alcohol-related biochemical parameters, including HDL-C and MCV, providing a measure of construct validity. The presence of systemic misclassification bias (i.e. underreporting) would also not necessarily negate any observed associations, although it may have implications for quantifying threshold effects or degrees of risk and may have contributed to the finding that a higher risk was observed at alcohol intake below current recommended drinking guidelines.

The cross-sectional study design evaluated all outcomes at a single timepoint, which limits the ability to make causal inferences. The definition of glaucoma was not specific and relied largely on participant self-report, which may again result in biases

related to outcome misclassification. Finally, the results may not be generalisable to other populations and ethnic groups, as the vast majority of the study cohort were of European descent, although this does not necessarily impact the internal validity of our findings.

In conclusion, this study implicates alcohol consumption as a potentially modifiable risk factor for glaucoma, with adverse associations noted at quantities below current UK and US drinking guideline recommendations. Although it would be important for these results to be replicated in independent cohorts and ethnically diverse populations, in the absence of viable alternative study designs, these findings may be of particular interest to people with, or at risk of, glaucoma and their advising physicians.

The presence of an underlying causal association may have important clinical and public implications and may lead to targeted lifestyle recommendations for glaucoma. This study also adds to the growing body of literature implicating gene-environment interactions in glaucoma,[180] raising the possibility of precision nutrition and dietary recommendations based on genomic data in the future.[316] This may be of particular importance as a preventative strategy in healthy individuals identified to be at high genetic risk of glaucoma, but before the development of disease.

4.3 Mendelian randomisation

In addition to the analyses described above, I also performed MR analyses to probe the potential causal relationship of alcohol consumption with glaucoma and related traits. These analyses were reported in the same *Ophthalmology Glaucoma*[61] paper referenced in section 4.2 above, but are presented separately here. The relevant declaration form for previously published material is located in **Appendix A**. Supplementary material for this section can be found in **Appendix F**.

4.3.1 Introduction

Traditional epidemiological studies of alcohol consumption are typically prone to various biases and methodological pitfalls.[295] Important considerations include recall, social desirability, and misclassification bias; reverse causality; and confounding by a multitude of interrelated lifestyle risk factors. Additionally, assessment of alcohol intake is generally assessed at a single timepoint, which may not accurately reflect lifetime exposure or capture harmful drinking patterns, such as binge drinking.

MR offers an alternative approach to gauging such relationships.[317] The technique may be less prone to various biases and reverse causality, and may provide a better indication of lifetime exposure and dose-response effects, than classical observational approaches. A summary of the rationale and key assumptions of MR is provided in section 2.3.5. Additionally, variants in key genes related to alcohol metabolism – *ALDH2* (see section 4.1.5.7) and *ADH1B* (see section 4.3.2.2) – are known to strongly associate with alcohol intake on a population level, making them particularly promising instrumental variables.[170,318]

Leveraging data from large-scale genetic consortia, I performed MR analyses of genetically determined alcohol consumption on a variety of glaucoma-related traits.

4.3.2 Methods

4.3.2.1 Study design

See section 2.3.5 for full details of the two-sample MR study design. These analyses were conducted in accordance with STROBE-MR guidelines (**Figure F1**).

4.3.2.2 Instrumental variable selection

I used results from the most recent GWAS of alcohol intake from the GSCAN consortium to guide construction of the instrumental variables.[197] The study identified 99 conditionally independent, genome-wide significant (P <5 ×10^{-8}) SNPs in a sample of 941 280 European participants. These genetic variants explain <1% of the variance in alcohol intake with a one SD increase in the genetic instrument representing one additional alcoholic drink per week.[197] Genetic principal components, population stratification, and participant relatedness were adjusted for in the original GWAS.

At loci with multiple genome-wide significant SNPs, I excluded those with LD R^2 >0.001 and within 10 000 kb, retaining only the SNP with the lowest P-value, using the 1000 Genomes Project European reference population.[319] Palindromic SNPs with MAF >0.42, or when allele frequencies were not reported, were excluded. Effect alleles were harmonised across exposure and outcome datasets.

The rs1229984 variant in the alcohol dehydrogenase 1B (*ADH1B*) gene region is consistently and strongly associated with lower alcohol intake in European populations.[170,318,320] Alcohol consumption in the presence of this genetic variant, however, leads to rapid accumulation of toxic intermediate metabolites and it is therefore also associated with higher levels of alcohol-related tissue damage.[320] Given these biological associations and the large effect size on alcohol intake

compared to other SNPs (see **Figures F2–F6**), inclusion of this SNP in an IV may bias MR results.

I therefore considered two alcohol intake IVs in these analyses: a full instrument, comprised of all genetic variants from the GSCAN GWAS including rs1229984; and a restricted instrument, comprising the same variants but excluding rs1229984. The number of SNPs included in the full and restricted IV for each outcome are reported in **Table F1** and full details of these SNPs are presented in **Table F2**.

While common in East Asian populations, the rs671 variant in *ALDH2* (discussed in section 4.1.5.7) is virtually absent in European populations and was therefore not considered in these analyses.[321]

4.3.2.3 Outcome data sources

See section 2.1.2.

4.3.2.4 Statistical analyses

The main MR analyses were performed using a multiplicative random-effects IVW method.[322] This method provides precise and efficient estimates but is sensitive to invalid IVs and pleiotropy.[323] I therefore conducted a variety of sensitivity analyses using four alternative MR methods: weighted median,[221] weighted mode,[220] MR-Egger[219] and MR-PRESSO.[324]

I additionally performed multivariable MR,[325] adjusting for genetically-determined smoking initiation, using 378 conditionally independent, genome-wide significant SNPs associated with smoking initiation (a binary phenotype defined as any history of regular smoking) in a sample of 1 232 091 European participants from the GSCAN GWAS.[197] These genetic variants explain 2.3% of the variance in smoking initiation

with a one SD increase in genetically predicted smoking initiation corresponding to a 10% increased risk of smoking.[197]

Each method makes different assumptions about the nature of pleiotropy and consistent estimates across methods strengthens causal inferences.[326] The weighted median method gives consistent estimates if the majority of IVs are valid, while the weighted mode method assumes that a plurality of IVs are valid. The MR-Egger and MR-PRESSO methods can test and correct for directional pleiotropy.

Under the IVW method, I calculated the mean *F* statistic as an indicator of instrument strength (a value >10 is usually considered a strong instrument).[327] I assessed for heterogeneity with the I^2 and Cochran's *Q* statistics in the IVW model and with Rucker's *Q'* statistic in MR-Egger regression. The I^2_{GX} statistic is an indicator of expected relative bias (or dilution) of the MR-Egger causal estimate.[328] In MR-Egger regression, a significant difference of the intercept from zero is evidence for average directional horizontal pleiotropy.[219] The MR-PRESSO global test evaluates for horizontal pleiotropy, the outlier test detects specific SNP outliers, and the distortion test evaluates whether there is a significant difference in the causal estimate before and after adjusting for outliers.[324]

MR estimates are presented as unit change in the outcome per one SD increase in the genetic instrument. All analyses were performed in R version 4.1.1 (https://www.R-project.org) using the *TwoSampleMR*, *MendelianRandomization,* and *MRPRESSO* packages.

4.3.3 Results

4.3.3.1 Full instrument

IVW MR analyses using the full alcohol genetic instrument (all genetic variants, including rs1229984) provided evidence for a causal effect of alcohol intake on GCIPL thickness (-1.52 μm per SD increase in the instrument; 95% CI, -2.55 to -0.50; $P = 0.004$) but not IOP, mRNFL thickness, CDR, or POAG (all $P \geq 0.13$). The main GCIPL result was supported by both the MR-PRESSO and multivariable MR methods (**Table 4.9**).

4.3.3.2 Restricted instrument

Similar MR analyses using the restricted alcohol instrument (all genetic variants, excluding rs1229984) provided stronger evidence for a causal association with GCIPL, with a stronger IVW estimate (-2.07 μm per SD increase in the instrument; 95% CI, -3.22 to -0.93; $P < 0.001$) and consistent, generally significant, results across all alternative MR methods (**Table 4.9**).

Additionally, this approach provided weak evidence for a causal association with mRNFL thickness, with a marginally significant IVW estimate (-0.98 μm per SD increase in the instrument; 95% CI, -1.89 to -0.07; $P = 0.04$) and consistent, albeit insignificant, estimates across all alternative MR methods. Although there was no evidence for a causal relationship with CDR under the IVW method, multivariable MR yielded a marginally significant result (0.03 increase in CDR per SD increase in the instrument; 95% CI, 0.00 to 0.06; $P = 0.03$).

4.3.3.3 Supplementary tests and statistics

With respect to the GCIPL estimates, despite evidence for global heterogeneity for both the full and restricted alcohol instruments (Cochran's *Q* statistic, *P* = 0.02 and *P* = 0.04, respectively), the MR-Egger intercept test did not suggest average directional pleiotropy (*P* = 0.06 and *P* = 0.55, respectively).

Full results of the MR tests of heterogeneity, directional pleiotropy, and regression dilution statistics are available in **Table F3.** Scatter plots of all MR analyses are available in **Figures F2–F11.**

Table 4.9 Results of Mendelian randomisation analyses for alcohol intake on glaucoma-related traits

MR method	IOP (mmHg)		mRNFL thickness (μm)		GCIPL thickness (μm)		CDR		POAG (log odds)	
	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P* -value	Estimate (95% CI)	*P* -value	Estimate (95% CI)	*P* -value
Full instrument (including rs1229984)										
IVW	-0.21 (-0.69, 0.28)	0.40	-0.63 (-1.43, 0.18)	0.13	**-1.52 (-2.55, -0.50)**	**0.004**	0.02 (-0.01, 0.04)	0.22	-0.17 (-0.51, 0.16)	0.32
Weighted median	-0.21 (-0.70, 0.29)	0.41	0.11 (-0.96, 1.19)	0.84	-0.59 (-1.99, 0.80)	0.41	0.00 (-0.05, 0.04)	0.97	-0.21 (-0.65, 0.23)	0.36
Weighted mode	-0.06 (-0.57, 0.45)	0.82	0.11 (-1.09, 1.31)	0.86	-0.29 (-2.10, 1.53)	0.76	0.01 (-0.04, 0.06)	0.69	-0.20 (-0.59, 0.19)	0.31
MR-Egger	0.05 (-0.86, 0.96)	0.92	0.17 (-1.34, 1.67)	0.83	0.02 (-1.86, 1.91)	0.98	0.01 (-0.04, 0.05)	0.73	-0.07 (-0.67, 0.53)	0.82
MR-PRESSO	-0.32 (-0.73, 0.09)	0.13	N/A	-	**-1.53 (-2.45, -0.60)**	**0.002**	N/A	-	-0.16 (-0.42, 0.11)	0.26
Multivariable MR	0.05 (-0.27, 0.37)	0.77	-0.37 (-1.06, 0.33)	0.30	**-1.05 (-2.00, -0.10)**	**0.03**	0.02 (0.00, 0.04)	0.08	-0.20 (-0.46, 0.07)	0.14
Restricted instrument (excluding rs1229984)										
IVW	-0.21 (-0.76, 0.35)	0.47	**-0.98 (-1.89, -0.07)**	**0.04**	**-2.07 (-3.22, -0.93)**	**<0.001**	0.02 (-0.01, 0.06)	0.13	-0.16 (-0.56, 0.24)	0.43
Weighted median	-0.16 (-0.70, 0.38)	0.56	-1.19 (-2.44, 0.06)	0.06	**-2.45 (-4.09, -0.82)**	**0.003**	0.04 (-0.01, 0.09)	0.10	-0.25 (-0.74, 0.24)	0.32
Weighted mode	0.51 (-0.34, 1.36)	0.25	-1.37 (-3.32, 0.58)	0.17	-2.66 (-5.48, 0.16)	0.07	0.08 (0.00, 0.15)	0.06	-0.23 (-0.97, 0.52)	0.55
MR-Egger	0.62 (-1.04, 2.26)	0.46	-0.93 (-3.54, 1.67)	0.48	-1.14 (-4.44, 2.13)	0.50	0.05 (-0.05, 0.14)	0.31	0.30 (-0.88, 1.49)	0.62
MR-PRESSO	-0.30 (-0.77, 0.16)	0.20	N/A	-	**-2.09 (-3.11, -1.06)**	**<0.001**	N/A	-	-0.14 (-0.46, 0.18)	0.40
Multivariable MR	0.12 (-0.25, 0.49)	0.51	-0.65 (-1.44, 0.15)	0.11	**-1.51 (-2.61, -0.41)**	**0.007**	**0.03 (0.00, 0.06)**	**0.03**	-0.22 (-0.53, 0.18)	0.18

No estimate is generated under the MR-PRESSO method if significant outliers are not detected. Multivariable MR adjusted for genetically determined smoking initiation.

mRNFL, macular retinal nerve fibre layer; GCIPL, ganglion cell-inner plexiform layer; IOP, intraocular pressure; CDR, cup-disc ratio; POAG, primary open-angle glaucoma; CI, confidence interval; IVW, inverse variance weighted; MR, Mendelian randomisation; MR-PRESSO, Mendelian Randomisation-Pleiotropy Residual Sum and Outlier; N/A, not applicable.

4.3.4 Discussion

Two-sample MR analyses, using the largest available GWAS summary statistics for alcohol intake and various glaucoma-related traits, provided consistent evidence for a causal relationship of higher levels of alcohol consumption with a thinner GCIPL. Additionally, there was weaker and less consistent evidence for a thinner mRNFL and a greater CDR. No relationship with IOP or POAG was observed.

The neural retina is an extension of the central nervous system, being derived from an outpouching of the primitive forebrain.[329] It is well established that alcohol has neurotoxic properties – chronic use has been associated with decreased brain volumes,[311] and is causally implicated in peripheral neuropathies,[285] and neurodegenerative disorders, including Alzheimer's and Parkinson's diseases.[298] More recently, multiple epidemiological studies have demonstrated a relationship between greater alcohol intake and thinner inner retinal layers.[74–77] These MR analyses add to the existing biological and epidemiological evidence supporting a detrimental role of alcohol on retinal neural tissue and are consistent with the observational analyses described in section 4.2.

However, it is important to note that results were not consistent across all MR methods or glaucoma-related traits, and no relationship with IOP or glaucoma status was observed. Alcohol consumption is a complex trait, likely influenced by a combination of environmental, genetic, and societal factors. Despite being the largest genetic association study of alcohol intake to date, the GSCAN GWAS explained very little variance in this phenotype. Given the limitations of selecting IVs based purely on statistical associations (see section 2.3.5), these analyses may be influenced by violations of the IV assumptions, particularly horizontal pleiotropy (see **Table F3**). For example, the alcohol intake IV may be more reflective of an

underlying genetic propensity to addictive behaviours, potentially implicating multiple alternative pathways and accounting for the observed discrepancy. When applying a biological approach and using the single genetic variant in *ADH1B* (rs1229984) as an IV, no significant results were observed (see **Table F2** and **Figures F2–F6**).

In summary, although these MR analyses provide evidence supporting a causal role underlying the previously reported observational association between greater levels of alcohol intake and a thinner inner retina, they should be interpreted in the context of several limitations. While the results may have implications and biological relevance to glaucoma, the experiments for both POAG status and IOP did not support a causal relationship.

5

Smoking

5.1 UK Biobank and CLSA

The following section is a modified version of a paper published in *Investigative Ophthalmology & Visual Science*[330] and describes analyses of the associations of smoking with corneal biomechanical properties and glaucoma-related traits in two large population-based cohorts. I was responsible for all aspects of this work. The relevant declaration form for previously published material is located in **Appendix A**. Supplementary material for this section can be found in **Appendix G**.

5.1.1 Abstract

Purpose: Smoking may influence measured IOP through an effect on corneal biomechanics, but it is unclear whether this translates into an increased risk for glaucoma. This study aimed to examine the association of cigarette smoking with corneal biomechanical properties and glaucoma-related traits.

Methods: Cross-sectional analyses within the UK Biobank and CLSA cohorts. Multivariable linear and logistic regression models were used to assess associations of smoking (status, intensity, and duration) with CH, CRF, IOP, inner retinal thicknesses, and glaucoma.

Results: Overall, 68 738 UK Biobank (mean age 56.7 years, 54.7% women) and 22 845 CLSA (mean age 62.7 years, 49.1% women) participants were included. Compared to non-smokers, smokers had higher CH (UK Biobank: +0.48 mmHg; CLSA: +0.57 mmHg; P <0.001) and CRF (UK Biobank: +0.47 mmHg; CLSA: +0.60 mmHg; P <0.001) with evidence of a dose-response effect in both studies. Differential associations with IOPg (UK Biobank: +0.25 mmHg; CLSA: +0.36 mmHg; P <0.001) and IOPcc (UK Biobank: -0.28 mmHg; CLSA: -0.32 mmHg; P ≤0.001)

were observed. Smoking was not associated with inner retinal thicknesses or glaucoma status in either study.

Conclusions: Cigarette smoking appears to increase corneal biomechanical resistance to deformation, but there was little evidence to support a relationship with glaucoma. This may result in an artefactual association with measured IOP and could account for discordant results with glaucoma in previous epidemiological studies.

5.1.2 Introduction

Tobacco smoking is a leading cause of global morbidity and mortality, and has been implicated as a risk factor for several ocular diseases, including cataract, age-related macular degeneration, and thyroid eye disease.[331–334] Evidence for the role of smoking in glaucoma, however, is less clear. Despite multiple population-based studies demonstrating higher IOP in smokers relative to non-smokers, associations with glaucoma are inconsistent and inconclusive.[78,82–84]

Exposure to tobacco smoke has been shown to have detrimental effects on the ocular surface and to induce collagen crosslinking in experimental models.[80,335] These physiological and biochemical changes may lead to altered corneal biomechanical properties in habitual smokers, and it has been suggested that this could account for an apparent protective effect on keratoconus and other corneal ectasias.[336,337]

Methods of IOP estimation based on corneal applanation are inherently affected by variability in ocular surface and corneal characteristics, such as tear film adhesion and central corneal thickness.[338,339] Any external factor that influences corneal parameters may therefore induce an artefactual association with IOP, independent of

any true effect on ocular tension. Smoking has been implicated as one such factor that may influence measured IOP through an effect on corneal biomechanical properties, and this may explain the lack of a consistent association with glaucoma in epidemiological studies.[85]

To better understand these relationships, I assessed the association of smoking with corneal biomechanical and glaucoma-related parameters in two large population-based cohorts – the UK Biobank and CLSA.

5.1.3 Methods

5.1.3.1 UK Biobank

See section 2.1.1.1.

5.1.3.2 Canadian Longitudinal Study on Aging

See section 2.1.1.3.

5.1.3.3 Smoking-related exposure measures

In both the UK Biobank and CLSA, self-reported smoking exposures were derived from a questionnaire administered as part of the baseline assessment. Participants answered several questions relating to their current and past smoking behaviours, including details of frequency, intensity, type, duration, and pattern of use. Full details of these assessments, including questionnaire flow and possible responses, are available online for both UKB (https://biobank.ndph.ox.ac.uk/showcase/) and CLSA (https://www.clsa-elcv.ca/data-collection).

Smoking status (never, former, current) was defined according to a lifetime exposure to at least 100 cigarettes (**Figure G1** and **Figure G2**).[340] In both studies, quantifiable

smoking data were only available for regular (daily or almost daily) cigarette smokers. I therefore excluded non-regular and/or non-cigarette smokers from the main analyses but included these participants in sensitivity analyses of overall smoking status. Smoking intensity (cigarettes per day) was available as a continuous measure in the UK Biobank and was categorised (≤5, 6–10, 11–15, 16–20, >20) for both former and current smokers to align with CLSA data. Smoking duration (years) was categorised separately for former (≤10, 11–20, 21–30, 31–40, >40) and current (≤30, 31–40, >40) smokers in both studies.

Pack years, a quantification of an individual's lifetime exposure to tobacco smoke (one pack year is equivalent to 7 300 cigarettes), was calculated in the UK Biobank as smoking intensity (packs [20 cigarettes] per day) multiplied by smoking duration (years), and was categorised (<10, 10–19, 20–29, 30–39, ≥40) for both former and current smokers. Passive ("second-hand") smoke exposure (hours per week) in never smokers was calculated in the UK Biobank as the sum of household and work exposure to other people's tobacco smoke (0, ≤2, 3–10, >10).

5.1.3.4 Corneal biomechanical measures

A subset of approximately 115 000 UK Biobank participants and all approximately 30 000 comprehensive cohort CLSA participants underwent a detailed ophthalmic examination as part of the baseline assessment. The ORA (Reichert Corp., Philadelphia, PA, USA), used as part of these assessments, is a non-invasive device that provides measures of both IOP and corneal biomechanics.[203] A rapid air pulse flattens the cornea, causing an initial inward applanation (P1), followed by an outward applanation event (P2) as the cornea returns to its original shape. An

electro-optical system measures the air pressures at these two applanation events and combines them to create four different parameters (**Figure 5.1**).

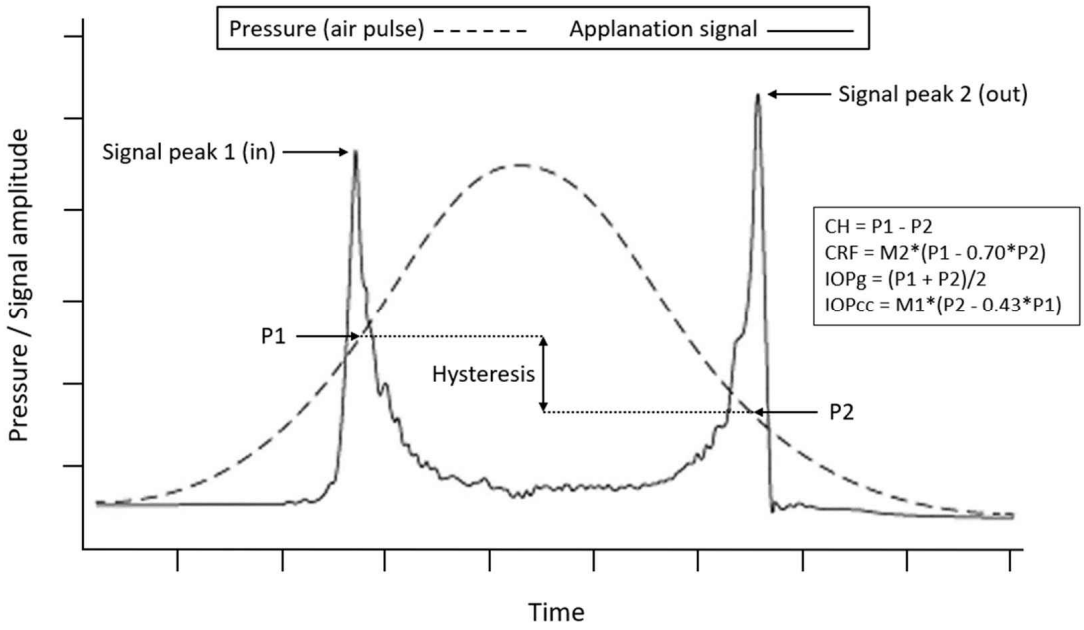

Figure 5.1 Ocular Response Analyzer pressure profile, illustrating the derivation of the corneal biomechanical (CH, CRF) and intraocular pressure (IOPg, IOPcc) parameters utilised in this study

P1, applanation pressure 1; P2, applanation pressure 2; CH, corneal hysteresis; CRF, corneal resistance factor; IOPg, Goldmann-correlated intraocular pressure; IOPcc, corneal-compensated intraocular pressure. M1 and M2 are industry calibration constants derived from clinical correlation with Goldmann applanation tonometry.

The mean of P1 and P2 is calibrated to provide a measure of IOP closely correlated with Goldmann applanation tonometry (IOPg). A second measure, IOPcc, is derived from a linear combination of P1 and P2, and aims to account for corneal biomechanical properties to provide a better reflection of true IOP.[341] CH, the difference between P1 and P2, is a measure of the viscoelastic dampening property of the cornea, and reflects the ability of the cornea to absorb and dissipate energy. CRF, a complementary measure to IOPcc, is also derived from a linear combination

of P1 and P2, and aims to provide a measure of corneal resistance independent of IOP.[341]

Although the ORA aims to provide independent measures, the biological assumptions and formulae underlying these calculations are based on a small cohort of select individuals, and widespread validity has not been demonstrated.

In both studies, individual-level ORA parameters (CH and CRF) were calculated as the mean of available right and left eye values, and extreme values in the top and bottom 0.5 percentiles of the distribution were excluded. I excluded participants using ocular hypotensive medication (available in both studies), and those with a history of glaucoma surgery, laser therapy, corneal graft, refractive surgery, or visually-significant ocular trauma (only available in the UK Biobank), or recent eye surgery (only available in CLSA), as these may all influence IOP and/or corneal biomechanical properties.

5.1.3.5 Glaucoma-related outcome measures and case ascertainment

See section 2.2.1.1 and section 2.2.1.3.

5.1.3.6 Covariables

To account for potential confounding bias, I considered a range of factors that may be related to both smoking habits and corneal- or glaucoma-related measures. These variables, selected a priori based on previously reported associations,[85,342,343] were ascertained as part of the baseline assessment in both studies, but varied slightly depending on data availability. Both studies: age, sex, self-reported ethnicity, BMI, SBP, HbA1c, total cholesterol,[305] alcohol intake,[61] assessment season. UK Biobank only: Townsend deprivation index and SE. CLSA only: highest level of

education and total household income. Full details of UK Biobank and CLSA covariables can be found in section 2.2.4.

5.1.3.7 Statistical analysis

Baseline participant characteristics were summarised as mean (SD) or median (IQR) for continuous variables and frequency (proportion) for categorical variables. Normality of continuous data was assessed graphically with histograms and *P-P* plots. Differences in participant characteristics by cohort were tested with a two-sample t-test, Wilcoxon rank-sum test, or z-test of proportion, as appropriate.

To assess the associations of the smoking-related exposures with the various corneal- and glaucoma-related outcomes, I used multivariable linear (for CH, CRF, IOPg, IOPcc, mRNFL, and GCIPL) and logistic (for glaucoma) regression models, with adjustment for all covariables described above. In the analyses of smoking status, former and current smokers were compared to those who had never smoked.

Subsequent analyses of smoking intensity (cigarettes per day) and smoking duration (years) were performed separately in former and current smokers, using those with the lowest exposure as the reference category. Trends across ordinal categories were examined by testing the median value of each group. Statistical tests were two-sided, and all analyses were performed using Stata (Version 17.0. StataCorp LLC. 2021. College Station, TX, USA).

5.1.3.8 Sensitivity analyses

I repeated the analyses of smoking status, including all non-regular and non-cigarette smokers who were excluded from the main analyses. I additionally considered associations with total lifetime smoking exposure (pack years) and

passive smoke exposure (hours per week) in the UK Biobank. To assess the impact of ethnicity on the results, I performed the main analyses of smoking status separately in White and Black participants from both studies.

5.1.4 Results

5.1.4.1 Participants

Overall, I included 68 738 participants from UKB and 22 845 participants from CLSA. The study selection process is highlighted in **Figure 5.2** and baseline participant characteristics by cohort are summarised in **Table 5.1**. On average, CLSA participants were older (62.7 ±10.1 years vs. 56.7 ±8.0 years), more likely to be men (50.9% vs. 45.3%) and of self-reported White ethnicity (94.8% vs. 92.5%) than those from the UK Biobank (*P* <0.001 for all). CLSA had a higher proportion of former (41.6% vs. 27.1%, *P* <0.001) and slightly lower proportion of current (7.3% vs. 8.0%, *P* = 0.001) smokers. The distribution of study participants in each smoking intensity and smoking duration category are available in **Table 5.2**, **Table 5.3**, and **Table 5.4**.

5.1.4.2 Associations with corneal biomechanics

Compared to never smokers, current smokers had higher CH (UK Biobank: 0.48 mmHg; 95% CI, 0.43–0.53; *P* <0.001. CLSA: 0.57 mmHg; 95% CI, 0.48–0.66; *P* <0.001) and CRF (UK Biobank: 0.47 mmHg; 95% CI, 0.42–0.53; *P* <0.001. CLSA: 0.60 mmHg; 95% CI, 0.50–0.69; *P* <0.001). Similar associations, but of smaller magnitude, were observed in former smokers. In both studies, there was consistent evidence of a dose-response relationship between greater smoking intensity and smoking duration with higher CH and CRF, in both former and current smokers. Full results of these analyses are presented in **Table 5.2**.

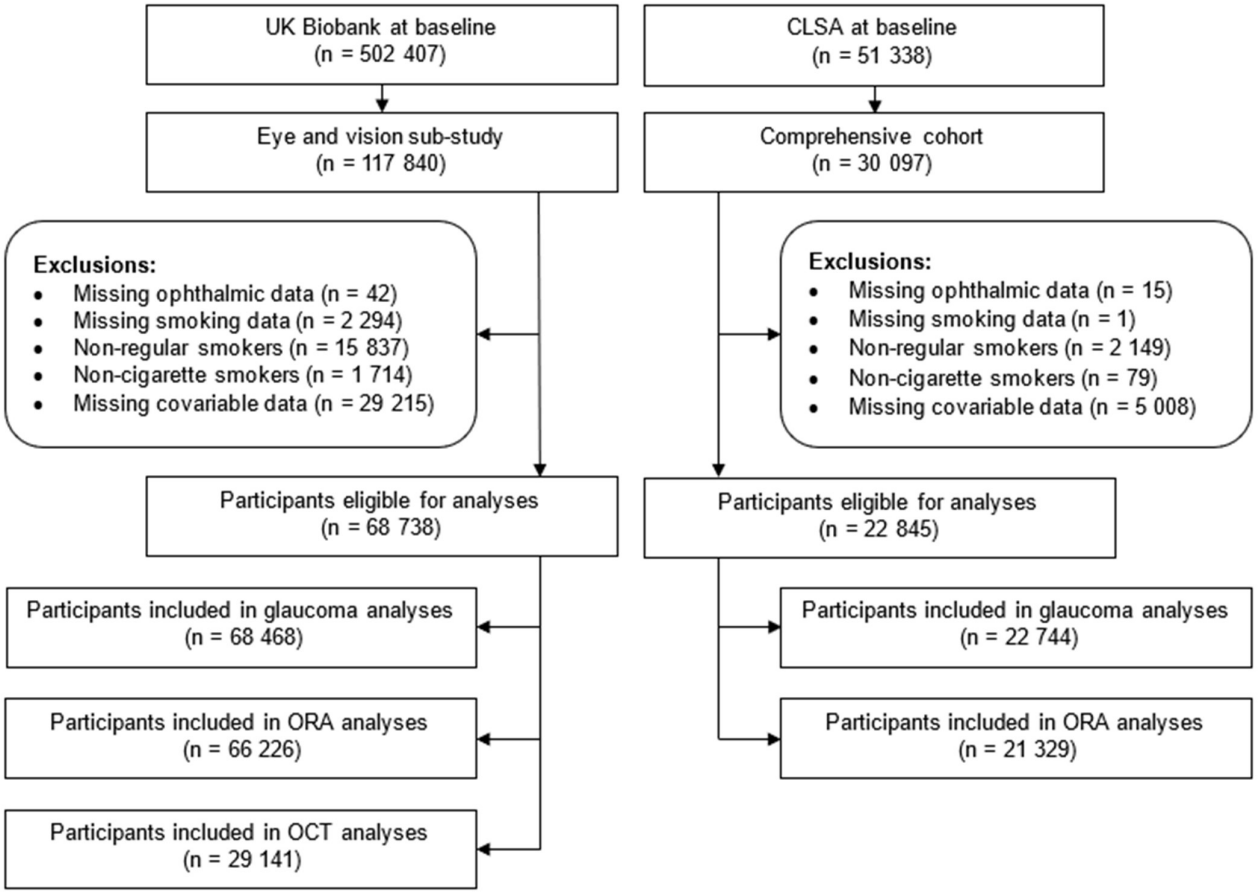

Figure 5.2 Participant selection and study flow in the UK Biobank and Canadian Longitudinal Study on Aging

CLSA, Canadian Longitudinal Study on Aging; OCT, optical coherence tomography, ORA, Ocular Response Analyzer; UK, United Kingdom.

Table 5.1 Participant characteristics by cohort

Characteristic	UKB	CLSA	*P*-value
Sample size, *n*	68 738	22 845	
Age (years)	56.7 (8.0)	62.7 (10.1)	**<0.001**
Sex, *n* (%)			**<0.001**
Women	37 595 (54.7)	11 211 (49.1)	
Men	31 143 (45.3)	11 634 (50.9)	
Ethnicity, *n* (%)			
White	63 610 (92.5)	21 646 (94.8)	**<0.001**
Black	1 833 (2.7)	173 (0.8)	**<0.001**
Other	3 295 (4.8)	1 026 (4.5)	0.06
Townsend Deprivation Index	-1.1 (2.9)	–	–
Highest level of education, *n* (%)			–
Less than tertiary	–	5 024 (22.0)	
Tertiary	–	17 821 (78.0)	
Total household income (C$), *n* (%)			–
<50 000	–	6 231 (27.3)	
50 000–150 000	–	12 654 (55.4)	
>150 000	–	3 960 (17.3)	
Body mass index (kg/m^2)	27.3 (4.7)	28.0 (5.3)	**<0.001**
Systolic blood pressure (mmHg)	137.4 (18.3)	121.0 (16.6)	**<0.001**
Glycated haemoglobin (mmol/mol)	36.1 (6.5)	38.2 (8.2)	**<0.001**
Total cholesterol (mmol/L)	5.7 (1.1)	5.1 (1.1)	**<0.001**
Alcohol intake (g/week), median (IQR)	69.9 (130.4)	40.4 (94.2)	**<0.001**
Spherical equivalent (dioptres)	-0.4 (2.7)	–	–
Smoking status, *n* (%)			
Never smoker	44 636 (64.9)	11 672 (51.1)	**<0.001**
Former smoker	18 600 (27.1)	9 501 (41.6)	**<0.001**
Current smoker	5 502 (8.0)	1 672 (7.3)	**0.001**
Corneal hysteresis (mmHg)	10.6 (1.7)	10.1 (1.7)	**<0.001**
Corneal resistance factor (mmHg)	10.7 (1.8)	10.0 (1.8)	**<0.001**
Goldmann-correlated IOP (mmHg)	15.8 (3.3)	15.1 (3.4)	**<0.001**
Corneal-compensated IOP (mmHg)	16.0 (3.2)	16.0 (3.4)	**0.029**
mRNFL thickness (μm)	28.9 (3.8)	–	–
GCIPL thickness (μm)	75.2 (5.2)	–	–
Glaucoma prevalence, *n* (%)	1 128 (1.7)	1 130 (5.0)	**<0.001**

All values represent mean (standard deviation), unless otherwise specified.

UKB, UK Biobank; CLSA, Canadian Longitudinal Study on Aging; IQR, interquartile range; IOP, intraocular pressure; mRNFL, macular retinal nerve fibre layer; GCIPL, ganglion cell inner plexiform layer.

Table 5.2 Association of smoking status, smoking intensity, and smoking duration with corneal hysteresis and corneal resistance factor

	Corneal hysteresis (mmHg)						Corneal resistance factor (mmHg)					
	UKB			CLSA			UKB			CLSA		
	N	β (95% CI)	*P*	*N*	β (95% CI)	*P*	*N*	β (95% CI)	*P*	*N*	β (95% CI)	*P*
Smoking status												
Never smokers	42 986	Reference		10 899	Reference		42 980	Reference		10 898	Reference	
Former smokers	17 873	**0.10 (0.07, 0.13)**	**<0.001**	8 823	**0.10 (0.05, 0.15)**	**<0.001**	17 880	**0.12 (0.09, 0.15)**	**<0.001**	8 820	**0.11 (0.06, 0.16)**	**<0.001**
Current smokers	5 283	**0.48 (0.43, 0.53)**	**<0.001**	1 574	**0.57 (0.48, 0.66)**	**<0.001**	5 281	**0.47 (0.42, 0.53)**	**<0.001**	1 573	**0.60 (0.50, 0.69)**	**<0.001**
Smoking intensity												
Former smokers												
≤5 cigarettes/day	1 032	Reference		1 408	Reference		1 032	Reference		1 407	Reference	
6–10 cigarettes/day	3 712	0.05 (-0.06, 0.16)	0.41	1 725	0.04 (-0.07, 0.16)	0.45	3 711	0.02 (-0.10, 0.14)	0.74	1 723	**0.13 (0.01, 0.25)**	**0.033**
11–15 cigarettes/day	3 081	0.09 (-0.03, 0.20)	0.13	1 487	**0.19 (0.07, 0.31)**	**0.002**	3 082	0.02 (-0.10, 0.15)	0.72	1 487	**0.21 (0.08, 0.34)**	**0.001**
16–20 cigarettes/day	6 292	**0.12 (0.01, 0.23)**	**0.027**	1 800	0.04 (-0.08, 0.15)	0.51	6 294	0.06 (-0.06, 0.18)	0.31	1 800	0.10 (-0.02, 0.22)	0.11
>20 cigarettes/day	3 650	**0.16 (0.04, 0.27)**	**0.007**	2 403	**0.22 (0.11, 0.32)**	**<0.001**	3 655	0.11 (-0.01, 0.24)	0.08	2 403	**0.20 (0.08, 0.31)**	**0.001**
P (trend)			**<0.001**			**<0.001**			**0.015**			**0.010**
Current smokers												
≤5 cigarettes/day	703	Reference		217	Reference		702	Reference		217	Reference	
6–10 cigarettes/day	1 438	**0.18 (0.03, 0.33)**	**0.019**	368	-0.19 (-0.47, 0.10)	0.20	1 436	**0.20 (0.03, 0.36)**	**0.018**	368	-0.10 (-0.41, 0.21)	0.53
11–15 cigarettes/day	1 293	**0.34 (0.19, 0.50)**	**<0.001**	351	-0.06 (-0.35, 0.22)	0.66	1 293	**0.35 (0.18, 0.52)**	**<0.001**	351	-0.06 (-0.37, 0.25)	0.72
16–20 cigarettes/day	1 197	**0.49 (0.33, 0.65)**	**<0.001**	326	0.12 (-0.17, 0.41)	0.43	1 198	**0.43 (0.25, 0.60)**	**<0.001**	324	0.27 (-0.05, 0.58)	0.10
>20 cigarettes/day	611	**0.66 (0.48, 0.85)**	**<0.001**	312	**0.58 (0.28, 0.88)**	**<0.001**	611	**0.64 (0.44, 0.85)**	**<0.001**	313	**0.60 (0.28, 0.93)**	**<0.001**
P (trend)			**<0.001**			**<0.001**			**<0.001**			**<0.001**
Smoking duration												
Former smokers												
≤10 years	3 435	Reference		2 915	Reference		3 438	Reference		2 913	Reference	
11–20 years	5 777	-0.01 (-0.08, 0.06)	0.75	2 607	0.08 (-0.01, 0.17)	0.07	5 775	0.02 (-0.05, 0.10)	0.56	2 606	0.07 (-0.02, 0.16)	0.14
21–30 years	4 409	**0.08 (0.00, 0.15)**	**0.039**	1 748	**0.16 (0.06, 0.26)**	**0.001**	4 413	0.06 (-0.02, 0.14)	0.13	1 747	**0.15 (0.04, 0.25)**	**0.005**
31–40 years	2 832	**0.18 (0.10, 0.27)**	**<0.001**	1 031	**0.26 (0.14, 0.38)**	**<0.001**	2 834	**0.19 (0.10, 0.28)**	**<0.001**	1 030	**0.29 (0.16, 0.41)**	**<0.001**
> 40 years	1 267	**0.21 (0.10, 0.32)**	**<0.001**	484	**0.26 (0.09, 0.42)**	**0.002**	1 267	**0.23 (0.11, 0.34)**	**<0.001**	486	**0.19 (0.02, 0.37)**	**0.028**
P (trend)			**<0.001**			**<0.001**			**<0.001**			**<0.001**
Current smokers												
≤30 years	1 460	Reference		420	Reference		1 458	Reference		420	Reference	
31–40 years	1 834	**0.26 (0.12, 0.39)**	**<0.001**	533	**0.25 (0.02, 0.47)**	**0.031**	1 833	**0.19 (0.05, 0.34)**	**0.010**	533	**0.30 (0.06, 0.55)**	**0.013**
>40 years	1 920	**0.26 (0.07, 0.46)**	**0.009**	613	0.21 (-0.07, 0.48)	0.14	1 921	0.19 (-0.02, 0.41)	0.08	613	**0.39 (0.09, 0.69)**	**0.012**
P (trend)			**0.006**			0.11			0.06			**0.009**

UKB, UK Biobank; CLSA, Canadian Longitudinal Study on Aging; *N*, sample size; β, beta coefficient; CI, confidence interval.

5.1.4.3 Associations with intraocular pressure

Compared to never smokers, current smokers had higher IOPg (UK Biobank: 0.25 mmHg; 95% CI, 0.15–0.34; *P* <0.001. CLSA: 0.36 mmHg; 95% CI, 0.18–0.55; *P* <0.001) but lower IOPcc (UK Biobank: -0.28 mmHg; 95% CI, -0.38 to -0.19; *P* <0.001. CLSA: -0.32 mmHg; 95% CI, -0.50 to -0.14; *P* = 0.001). There was no association of smoking intensity or smoking duration with IOPg in either study. Dose-response associations of greater smoking intensity and smoking duration with lower IOPcc, apparent in the UK Biobank, were not consistently replicated in CLSA. Full results of these analyses are presented in **Table 5.3**.

5.1.4.4 Associations with glaucoma

Smoking status was not associated with glaucoma status in either study, or with inner retinal thickness in the UK Biobank. There was also no evidence for a dose-response relationship with either smoking intensity or smoking duration, except for an association between greater smoking duration and thinner mRNFL in former smokers in the UK Biobank. Full results of these analyses are presented in **Table 5.4**.

Table 5.3 Association of smoking status, smoking intensity, and smoking duration with Goldmann-correlated and corneal-compensated IOP

	Goldmann-correlated intraocular pressure (mmHg)						Corneal-compensated intraocular pressure (mmHg)					
	UKB			CLSA			UKB			CLSA		
	N	β (95% CI)	*P*	*N*	β (95% CI)	*P*	*N*	β (95% CI)	*P*	*N*	β (95% CI)	*P*
Smoking status												
Never smokers	42 955	Reference		10 894	Reference		42 983	Reference		10 901	Reference	
Former smokers	17 867	**0.11 (0.05, 0.17)**	**<0.001**	8 820	0.09 (-0.01, 0.19)	0.07	17 858	-0.01 (-0.06, 0.05)	0.85	8 827	-0.02 (-0.12, 0.08)	0.70
Current smokers	5 283	**0.25 (0.15, 0.34)**	**<0.001**	1 569	**0.36 (0.18, 0.55)**	**<0.001**	5 282	**-0.28 (-0.38, -0.19)**	**<0.001**	1 573	**-0.32 (-0.50, -0.14)**	**0.001**
Smoking intensity												
Former smokers												
≤5 cigarettes/day	1 033	Reference		1 405	Reference		1 031	Reference		1 406	Reference	
6–10 cigarettes/day	3 705	-0.04 (-0.26, 0.19)	0.76	1 723	**0.42 (0.18, 0.66)**	**0.001**	3 707	-0.04 (-0.26, 0.17)	0.69	1 726	**0.33 (0.09, 0.56)**	**0.008**
11–15 cigarettes/day	3 080	-0.13 (-0.36, 0.10)	0.27	1 488	0.21 (-0.04, 0.46)	0.10	3 082	-0.20 (-0.42, 0.02)	0.08	1 487	-0.01 (-0.26, 0.24)	0.93
16–20 cigarettes/day	6 290	-0.07 (-0.29, 0.14)	0.51	1 803	**0.27 (0.03, 0.51)**	**0.029**	6 284	-0.18 (-0.39, 0.03)	0.09	1 804	0.20 (-0.04, 0.44)	0.10
>20 cigarettes/day	3 653	-0.05 (-0.28, 0.18)	0.67	2 401	0.12 (-0.11, 0.35)	0.29	3 648	-0.20 (-0.42, 0.03)	0.08	2 404	-0.11 (-0.34, 0.12)	0.35
P (trend)			0.73			0.85			**0.016**			0.06
Current smokers												
≤5 cigarettes/day	702	Reference		216	Reference		703	Reference		216	Reference	
6–10 cigarettes/day	1 437	0.11 (-0.19, 0.41)	0.48	365	0.08 (-0.52, 0.67)	0.80	1 436	-0.07 (-0.35, 0.22)	0.65	368	0.20 (-0.37, 0.78)	0.48
11–15 cigarettes/day	1 295	0.15 (-0.15, 0.46)	0.33	350	-0.13 (-0.73, 0.47)	0.68	1 296	-0.21 (-0.51, 0.08)	0.15	350	-0.05 (-0.63, 0.52)	0.86
16–20 cigarettes/day	1 197	-0.07 (-0.38, 0.24)	0.65	326	0.54 (-0.07, 1.16)	0.08	1 194	**-0.51 (-0.81, -0.21)**	**0.001**	327	0.25 (-0.34, 0.84)	0.40
>20 cigarettes/day	611	0.16 (-0.21, 0.53)	0.40	312	0.17 (-0.45, 0.80)	0.59	612	**-0.61 (-0.96, -0.25)**	**0.001**	312	-0.59 (-1.19, 0.02)	0.06
P (trend)			0.93			0.22			**<0.001**			0.07
Smoking duration												
Former smokers												
≤10 years	3 436	Reference		2 913	Reference		3 435	Reference		2 916	Reference	
11–20 years	5 773	0.11 (-0.03, 0.25)	0.13	2 607	-0.03 (-0.21, 0.15)	0.72	5 774	0.11 (-0.03, 0.24)	0.11	2 610	-0.09 (-0.27, 0.09)	0.32
21–30 years	4 408	-0.01 (-0.15, 0.14)	0.94	1 745	0.06 (-0.15, 0.26)	0.54	4 402	-0.09 (-0.23, 0.05)	0.21	1 746	-0.13 (-0.33, 0.08)	0.22
31–40 years	2 834	0.08 (-0.08, 0.25)	0.32	1 033	0.21 (-0.04, 0.46)	0.10	2 832	-0.12 (-0.28, 0.04)	0.14	1 033	-0.10 (-0.35, 0.14)	0.41
>40 years	1 263	0.12 (-0.10, 0.34)	0.30	485	-0.12 (-0.46, 0.22)	0.50	1 262	-0.13 (-0.34, 0.09)	0.24	484	**-0.39 (-0.73, -0.05)**	**0.023**
P (trend)			0.56			0.42			**0.007**			**0.046**
Current smokers												
≤30 years	1 460	Reference		418	Reference		1 459	Reference		420	Reference	
31–40 years	1 835	-0.14 (-0.40, 0.13)	0.31	532	0.19 (-0.27, 0.65)	0.42	1 833	**-0.46 (-0.71, -0.20)**	**<0.001**	533	-0.10 (-0.55, 0.34)	0.65
>40 years	1 918	-0.12 (-0.51, 0.27)	0.54	611	0.54 (-0.04, 1.11)	0.07	1 921	**-0.43 (-0.80, -0.06)**	**0.023**	612	0.16 (-0.39, 0.72)	0.57
P (trend)			0.51			0.07			**0.015**			0.62

UKB, UK Biobank; CLSA, Canadian Longitudinal Study on Aging; *N*, sample size; β, beta coefficient; CI, confidence interval.

Table 5.4 Association of smoking status, smoking intensity, and smoking duration with mRNFL thickness, GCIPL thickness, and glaucoma

	mRNFL thickness (µm)			GCIPL thickness (µm)			Glaucoma status					
	UKB			UKB			UKB			CLSA		
	N	β (95% CI)	*P*	*N*	β (95% CI)	*P*	*N*	OR (95% CI)	*P*	*N*	OR (95% CI)	*P*
Smoking status												
Never smokers	18 601	Reference		18 562	Reference		44 459	Reference		11 625	Reference	
Former smokers	7 998	-0.03 (-0.13, 0.08)	0.61	7 982	-0.01 (-0.14, 0.13)	0.93	18 533	1.10 (0.96, 1.26)	0.17	9 458	1.02 (0.89, 1.16)	0.78
Current smokers	2 227	-0.09 (-0.26, 0.08)	0.28	2 208	0.07 (-0.15, 0.30)	0.53	5 476	1.13 (0.90, 1.43)	0.30	1 661	1.22 (0.94, 1.57)	0.14
Smoking intensity												
Former smokers												
≤5 cigarettes/day	446	Reference		445	Reference		1 060	Reference		1 520	Reference	
6–10 cigarettes/day	1 676	0.13 (-0.26, 0.52)	0.51	1 674	0.16 (-0.36, 0.68)	0.54	3 850	1.25 (0.70, 2.24)	0.45	1 833	1.02 (0.75, 1.38)	0.91
11–15 cigarettes/day	1 394	-0.17 (-0.56, 0.23)	0.40	1 389	-0.06 (-0.59, 0.47)	0.82	3 178	1.20 (0.66, 2.18)	0.55	1 583	0.80 (0.57, 1.12)	0.19
16–20 cigarettes/day	2 847	0.01 (-0.36, 0.38)	0.96	2 842	-0.06 (-0.56, 0.44)	0.81	6 534	1.51 (0.87, 2.65)	0.15	1 939	0.86 (0.63, 1.17)	0.33
>20 cigarettes/day	1 592	-0.03 (-0.43, 0.36)	0.87	1 590	-0.07 (-0.60, 0.47)	0.81	3 801	1.42 (0.79, 2.53)	0.24	2 583	1.08 (0.82, 1.44)	0.57
P (trend)			0.49			0.26			0.12			0.73
Current smokers												
≤5 cigarettes/day	326	Reference		326	Reference		731	Reference		229	Reference	
6–10 cigarettes/day	636	0.11 (-0.38, 0.61)	0.65	628	0.35 (-0.32, 1.03)	0.30	1 486	1.34 (0.59, 3.03)	0.49	390	0.83 (0.35, 1.99)	0.68
11–15 cigarettes/day	543	0.07 (-0.44, 0.58)	0.79	538	0.16 (-0.54, 0.86)	0.65	1 336	1.67 (0.74, 3.77)	0.22	372	1.77 (0.81, 3.88	0.13
16–20 cigarettes/day	490	0.19 (-0.33, 0.71)	0.48	488	0.58 (-0.13, 1.29)	0.11	1 252	1.78 (0.79, 4.04)	0.17	337	1.04 (0.44, 2.48)	0.92
>20 cigarettes/day	219	-0.36 (-1.01, 0.29)	0.27	215	-0.45 (-1.34, 0.43)	0.32	627	0.99 (0.36, 2.73)	0.99	333	1.55 (0.67, 3.58)	0.30
P (trend)			0.61			0.90			0.59			0.23
Smoking duration												
Former smokers												
≤10 years	1 539	Reference		1 536	Reference		3 564	Reference		3 095	Reference	
11–20 years	2 638	0.01 (-0.22, 0.24)	0.94	2 624	**-0.51 (-0.82, -0.19)**	**0.001**	5 987	1.01 (0.74, 1.38)	0.96	2 785	**1.43 (1.12, 1.84)**	**0.005**
21–30 years	1 989	-0.19 (-0.44, 0.06)	0.13	1 989	-0.29 (-0.63, 0.04)	0.08	4 564	1.00 (0.72, 1.40)	0.99	1 892	1.16 (0.88, 1.54)	0.30
31–40 years	1 259	**-0.31 (-0.59, -0.03)**	**0.030**	1 261	**-0.49 (-0.87, -0.11)**	**0.011**	2 949	1.07 (0.75, 1.53)	0.69	1 111	1.33 (0.98, 1.82)	0.07
>40 years	514	-0.32 (-0.70, 0.06)	0.10	513	-0.35 (-0.86, 0.16)	0.18	1 311	1.07 (0.70, 1.62)	0.76	533	1.32 (0.91, 1.91)	0.14
P (trend)			**0.004**			0.12			0.66			0.20
Current smokers												
≤30 years	649	Reference		645	Reference		1 492	Reference		444	Reference	
31–40 years	744	0.15 (-0.30, 0.60)	0.51	736	0.19 (-0.42, 0.80)	0.54	1 899	1.35 (0.65, 2.81)	0.43	556	1.35 (0.67, 2.71)	0.40
>40 years	806	0.32 (-0.32, 0.97)	0.33	799	-0.25 (-1.14, 0.63)	0.58	2 013	1.21 (0.47, 3.09)	0.69	652	0.88 (0.40, 1.92)	0.75
P (trend)			0.33			0.65			0.77			0.68

mRNFL, macular retinal nerve fibre layer; GCIPL, ganglion cell inner plexiform layer; UKB, UK Biobank; CLSA, Canadian Longitudinal Study on Aging; N, sample size; β, beta coefficient; CI, confidence interval; OR, odds ratio.

5.1.4.5 Sensitivity analyses

Associations of smoking status were not materially changed when including all non-regular and non-cigarette smokers (**Table G1**). In the UK Biobank, greater total lifetime smoking exposure (pack years) was associated with higher CH, higher CRF, and lower IOPcc, in both former and current smokers (*P* trend <0.001 for all), but not with IOPg.

Similar associations with CH, CRF, and IOPcc were also apparent for passive smoke exposure in never smokers (*P* trend <0.013 for all) (**Table 5.5**). These analyses also provided evidence for a dose-response association of greater passive smoke exposure with thinner mRNFL and GCIPL in never smokers (**Table 5.6**).

Associations with smoking status were unchanged when restricting analyses to White participants only (**Table G2**). Consistent with the overall results, among Black UKB participants (*n* <2 000), smoking status was associated with higher CH and CRF (in both former and current smokers), but not IOP, inner retinal thickness, or glaucoma status.

Table 5.5 Association of lifetime smoking exposure and current passive smoke exposure with CH, CRF, IOPcc, and IOPg

	Corneal hysteresis			Corneal resistance factor			Goldmann-correlated IOP			Corneal-compensated IOP		
	N	β (95% CI)	*P*	*N*	β (95% CI)	*P*	*N*	OR (95% CI)	*P*	*N*	OR (95% CI)	*P*
Lifetime smoking exposure												
Former smokers												
≤10 pack years	5 323	Reference		5 325	Reference		5 323	Reference		5 324	Reference	
11–20 pack years	5 056	0.04 (-0.02, 0.10)	0.19	5 055	0.04 (-0.03, 0.11)	0.23	5 052	0.04 (-0.09, 0.17)	0.51	5 051	-0.02 (-0.14, 0.11)	0.80
21–30 pack years	3 138	**0.13 (0.06, 0.20)**	**<0.001**	3 141	**0.11 (0.03, 0.19)**	**0.008**	3 136	0.01 (-0.14, 0.16)	0.92	3 132	-0.15 (-0.30, -0.01)	**0.035**
31–40 pack years	1 882	**0.18 (0.09, 0.27)**	**<0.001**	1 883	**0.16 (0.06, 0.25)**	**0.001**	1 883	0.03 (-0.15, 0.21)	0.75	1 881	**-0.18 (-0.36, -0.01)**	**0.034**
>40 pack years	1 960	**0.28 (0.20, 0.37)**	**<0.001**	1 962	**0.26 (0.17, 0.36)**	**<0.001**	1 960	0.04 (-0.14, 0.22)	0.69	1 957	**-0.27 (-0.45, -0.10)**	**0.002**
P (trend)			**<0.001**			**<0.001**			0.77			**<0.001**
Current smokers												
≤10 pack years	828	Reference		826	Reference		827	Reference		827	Reference	
11–20 pack years	1 287	**0.26 (0.11, 0.41)**	**0.001**	1 286	**0.28 (0.12, 0.45)**	**0.001**	1 285	0.14 (-0.16, 0.42)	0.37	1 285	-0.15 (-0.43, 0.13)	0.28
21–30 pack years	1 205	**0.28 (0.13, 0.43)**	**<0.001**	1 205	**0.34 (0.18, 0.51)**	**<0.001**	1 209	0.24 (-0.06, 0.54)	0.11	1 209	-0.08 (-0.36, 0.21)	0.59
31–40 pack years	905	**0.59 (0.43, 0.76)**	**<0.001**	905	**0.51 (0.33, 0.69)**	**<0.001**	903	-0.02 (-0.34, 0.31)	0.92	902	**-0.57 (-0.88, -0.26)**	**<0.001**
>40 pack years	954	**0.72 (0.55, 0.89)**	**<0.001**	955	**0.69 (0.50, 0.87)**	**<0.001**	954	0.12 (-0.22, 0.45)	0.49	955	**-0.68 (-1.00, -0.36)**	**<0.001**
P (trend)			**<0.001**			**<0.001**			0.87			**<0.001**
Passive smoke exposure												
Never smokers												
0 hours/week	40 082	Reference		40 076	Reference		40 048	Reference		40 073	Reference	
≤2 hours/week	834	-0.04 (-0.15, 0.07)	0.49	834	-0.10 (-0.22, 0.02)	0.11	834	-0.17 (-0.39, 0.05)	0.13	836	-0.08 (-0.29, 0.13)	0.47
3–10 hours/week	527	0.11 (-0.02, 0.25)	0.11	527	0.11 (-0.04, 0.26)	0.16	527	0.03 (-0.25, 0.31)	0.82	527	-0.07 (-0.33, 0.20)	0.63
>10 hours/week	563	**0.29 (0.16, 0.43)**	**<0.001**	563	**0.21 (0.07, 0.36)**	**0.004**	563	-0.07 (-0.34, 0.20)	0.63	564	**-0.41 (-0.67, -0.15)**	**0.002**
P (trend)			**<0.001**			**0.013**			0.46			**0.003**

IOP, intraocular pressure; *N*, sample size; β, beta coefficient; CI, confidence interval; OR, odds ratio.

Table 5.6 Association of lifetime smoking exposure and current passive smoke exposure with mRNFL thickness, GCIPL thickness, and glaucoma

	mRNFL thickness			GCIPL thickness			Glaucoma status		
	N	β (95% CI)	*P*	*N*	β (95% CI)	*P*	*N*	OR (95% CI)	*P*
Lifetime smoking exposure									
Former smokers									
≤10 pack years	2 410	Reference		2 401	Reference		5 505	Reference	
11–20 pack years	2 315	-0.08 (-0.29, 0.13)	0.47	2 314	-0.28 (-0.56, 0.00)	0.05	5 245	1.21 (0.90, 1.62)	0.22
21–30 pack years	1 412	-0.24 (-0.49, 0.00)	0.05	1 415	-0.24 (-0.57, 0.09)	0.15	3 271	**1.53 (1.12, 2.09)**	**0.008**
31–40 pack years	831	**-0.41 (-0.71, -0.12)**	**0.007**	827	-0.33 (-0.73, 0.07)	0.10	1 946	1.16 (0.79, 1.70)	0.45
>40 pack years	819	-0.29 (-0.60, 0.02)	0.07	816	-0.34 (-0.76, 0.07)	0.11	2 037	1.27 (0.88, 1.82)	0.20
P (trend)			**0.004**			0.07			0.19
Current smokers									
≤10 pack years	376	Reference		374	Reference		857	Reference	
11–20 pack years	584	-0.16 (-0.64, 0.32)	0.52	582	0.00 (-0.66, 0.66)	0.99	1 322	1.66 (0.64, 4.30)	0.36
21–30 pack years	491	0.27 (-0.24, 0.78)	0.29	484	0.02 (-0.68, 0.71)	0.96	1 256	**2.77 (1.12, 6.86)**	**0.027**
31–40 pack years	365	-0.24 (-0.79, 0.32)	0.40	361	-0.15 (-0.91, 0.61)	0.70	935	2.31 (0.89, 6.01)	0.09
>40 pack years	372	0.11 (-0.47, 0.769	0.72	368	0.10 (-0.69, 0.89)	0.81	996	1.71 (0.64, 4.56)	0.29
P (trend)			0.73			0.97			0.38
Passive smoke exposure									
Never smokers									
0 hours/week	17 379	Reference		17 347	Reference		41 439	Reference	
≤2 hours/week	370	-0.35 (-0.74, 0.04)	0.08	365	-0.20 (-0.71, 0.31)	0.44	863	0.87 (0.47, 1.59)	0.65
3–10 hours/week	224	-0.07 (-0.56, 0.43)	0.80	223	-0.32 (-0.97, 0.34)	0.34	551	0.68 (0.30, 1.54)	0.36
>10 hours/week	230	-0.42 (-0.91, 0.06)	0.09	231	**-0.93 (-1.57, -0.29)**	**0.004**	582	0.57 (0.23, 1.54)	0.22
P (trend)			**0.043**			**0.003**			0.11

mRNFL, macular retinal nerve fibre layer; GCIPL, ganglion cell inner plexiform layer; *N*, sample size; β, beta coefficient; CI, confidence interval; OR, odds ratio.

5.1.5 Discussion

In this cross-sectional study of two large population-based eye studies, I examined the association of habitual cigarette smoking with corneal biomechanics and glaucoma-related traits. Overall, smoking was consistently associated with higher CH (greater ability to absorb and dissipate energy) and higher CRF (greater overall "resistance") in a dose-dependent manner, with a more pronounced effect in current smokers relative to former smokers.

There was also a dose-dependent association of smoking with lower IOPcc in the UK Biobank, although this was not consistently replicated in CLSA. Conversely, smoking status was associated with higher IOPg in both studies but with no evidence of a dose-response effect. Smoking was not associated with inner retinal thicknesses or glaucoma status in either study. Similar associations were demonstrated when examining total lifetime smoking exposure (in former and current smokers) and passive smoke exposure (in never smokers) in the UK Biobank.

Acute exposure to tobacco smoke has been shown to have detrimental effects on the ocular surface and tear film function, and certain byproducts of cigarette smoke - including nitrogen oxides, nitrate, and formaldehyde - have been shown to induce collagen cross-linking in experimental models.[80,335,344,345] This may lead to permanent corneal changes, with several studies demonstrating altered corneal biomechanical properties in habitual smokers compared to non-smokers.[336,346] This study provides consistent large-scale evidence replicating this association on a population level and strong dose-dependent associations provide additional evidence to support a causal relationship.

Conversely, cigarette smoke appears to have little short-term effect on IOP, the major modifiable risk factor for glaucoma, or ONH perfusion.[81] Chronic exposure to harmful compounds found in tobacco smoke has been theorised to influence glaucoma risk though ischaemic or oxidative mechanisms, but nicotine has also been hypothesised to be protective through nitric oxide-induced vasodilatory properties.[1]

Although smoking is consistently associated with higher IOP in population-based studies, associations with glaucoma are conflicting and inconclusive.[1,78,82–84] Since applanation-based methods of IOP measurement may be influenced by structural and functional properties of the cornea, it is possible that smoking-related corneal changes could result in an artefactual association with measured IOP, potentially accounting for the lack of a consistent association with glaucoma.[85,338,339]

Consistent with previous reports, current smokers were found to have higher IOPg than never smokers. Smoking was also found to be inversely associated with IOPcc in a dose-dependent manner. This differential IOP association has also been reported for several other factors – including ethnicity, height, and diabetes – and suggests that these may be particularly related to corneal biomechanical properties.[85] Similar to diabetes, smoking represents a source of advanced glycosylation end products, which have been shown to induce connective tissue cross-linking and increase tissue rigidity, especially in the presence of glucose.[347,348]

It is important to acknowledge that measured IOP and corneal biomechanics are inextricably linked, and disentangling these interrelated measures is complex, especially given that all measures are derived from the same device. Although a dose-dependent relationship with lower IOPcc was observed in this study, and also in previous MR analyses, this may be an artefact related to the ORA's correction for corneal biomechanical properties.[349] While it remains possible that smoking may

have an independent effect on IOP, I found no evidence to support an association between smoking and glaucoma (either adverse or protective) in either cohort, which may have been expected if this were the case.

Interestingly, passive smoke exposure, which has a different chemical composition to that inhaled by active smokers, was found to be adversely associated with inner retinal thickness, especially the GCIPL, in UK Biobank never smokers.[350] It is possible that the compounds found in passive smoke may have a toxic effect on neural retinal tissue, however, I was unable to replicate these findings in CLSA due to a lack of OCT data, and given the relatively small participant numbers for these analyses, may represent a chance finding.

In recent years, there has also been significant interest in the role that corneal biomechanics, most notably CH, may play in glaucoma. Individuals with glaucoma have been shown to have lower CH than healthy controls, and lower CH is associated with an increased risk of glaucoma progression based on visual fields or structural biomarkers, including in those with apparently well-controlled IOP.[351] Similar to the limitations discussed above, interpretation of these results is complicated by the influence of IOP (inversely related to CH) and topical hypotensive medications on CH measurements, although CH has also been demonstrated to be lower in treatment-naïve, NTG patients compared with healthy subjects with a similar IOP.[351]

Strengths of this study include the large sample size and detailed participant phenotyping available in both the UK Biobank and CLSA, allowing for a simultaneous assessment of associations in two independent cohorts, and across multiple measures of smoking exposure, corneal biomechanics, and glaucoma. This enabled me to conduct detailed subgroup and sensitivity analyses, assess for dose-

response relationships, and account for important lifestyle and medical factors, such as alcohol consumption and metabolic parameters, that may have biased the results.[32,305]

While the main findings of this study were consistent across cohorts, certain results, especially those from analyses involving multiple subgroups and from CLSA in general, were less so. Greater variability in these estimates is likely a result of smaller participant numbers available for these analyses.

Although both studies included a detailed smoking questionnaire, this method of exposure ascertainment may be subject to recall and social desirability biases and may not be an accurate reflection of lifetime smoking patterns or behaviours. I was also limited by the method of glaucoma case ascertainment, based on a combination of self-report and electronic medical records, which may be prone to misclassification bias, although this was partly overcome by the availability of quantitative structural OCT biomarkers for a subset of participants.

While the cross-sectional study design limited my ability to assess temporal relationships and make causal inferences, I was able to perform dose-response and MR analyses (see section 5.2), which provide alternative approaches to gauge such relationships.

Lastly, the findings in predominantly middle-aged European-descent participants (>90% White ethnicity in both studies) may not be generalisable to other ethnicities or population groups. There are notable regional and ethnic differences in both patterns and methods of tobacco use, and Black individuals in particular have a higher burden of glaucoma and different corneal biomechanical properties relative to White individuals.[7,352,353] This may account for disparate results observed in this study when

compared to those conducted in other regions or in more diverse cohorts. Although I did observe suggestive associations between smoking status and corneal biomechanics among Black UK Biobank participants, these analyses were conducted on a relatively small sample (<2 000 participants), and it would be important for these results to be replicated in larger cohorts.

Although cigarette smoking is undoubtably detrimental to overall health, this study found little evidence to support an association with glaucoma. Instead, strong associations with CH and CRF, and differential associations with IOPg and IOPcc, suggest a predominant effect on corneal biomechanics which may induce an artefactual association with measured IOP.

Clinicians should be cognisant of this relationship when interpreting applanation-based IOP measures, especially in current smokers. Future research may aim to assess whether similar associations are apparent in e-cigarettes users, especially considering the increasing popularity of this form of smoking in recent years. Recent advances in the development of implantable IOP biosensors may provide further insights into the complex relationship between corneal biomechanics and IOP, by providing a measure of ocular tension independent of potential corneal artefact.[354]

5.2 Mendelian randomisation

Based on the findings described in section 5.1 above, I performed MR analyses to further probe the causal relationship between smoking traits and corneal biomechanical properties. These results were included in the same paper as the observational results from the UK Biobank and CLSA but are presented separately here.[330] Additionally, I was involved in a collaborative project, led by Dr Jessica Tran and Professor Louis Pasquale, examining the genetic associations between smoking- and glaucoma-related traits, published in *Translational Vision Science & Technology*.[349] My role was to perform the MR experiments and these are presented here. The relevant declaration forms for previously published material are located in **Appendix A**. Supplementary material for this section can be found in **Appendix H**.

5.2.1 Introduction

The rationale for the use of MR to assess causal relationships of environmental exposures is highlighted in section 4.3.1. Given the challenges associated with traditional observational studies and the inability to perform interventional studies of harmful exposures, MR has been used extensively to study the role of smoking on human health and disease.[355] Previous studies have implicated smoking as a causal risk factor for age-related macular degeneration and senile cataract,[355,356] but its role in glaucoma is less clear. Here I perform MR experiments on the same glaucoma-related traits considered in the alcohol analyses, with additional consideration given to corneal biomechanical properties, based on the findings of the observational studies described in section 5.1.

5.2.2 Methods

5.2.2.1 Study design

See section 2.3.5 for full details of the two-sample MR study design. These analyses were conducted in accordance with STROBE-MR guidelines.

5.2.2.2 Instrumental variable selection

Smoking-related IVs were selected using results from GSCAN for smoking initiation (a binary phenotype indicating whether an individual had ever smoked regularly, *n* = 1 232 091) and smoking intensity (cigarettes per day, *n* = 337 334).[197] The GSCAN GWAS identified 378 and 55 conditionally independent, genome-wide significant SNPs associated with smoking initiation (2.3% of variance explained) and smoking intensity (1.1% of variance explained), respectively. To avoid participant overlap, which may bias MR estimates, I also utilised summary statistics excluding participants from UK Biobank and 23andMe (due to data sharing restrictions) for the analyses of corneal biomechanics. IV construction was performed using the same methods described in section 4.3.2.2.

5.2.2.3 Outcome data sources

See section 2.1.2 for details of glaucoma-related outcomes. Corneal biomechanical summary statistics were drawn from a recent GWAS for CH (*n* = 106 041) and CRF (*n* = 106 030) in the UK Biobank.[202]

5.2.2.4 Statistical analyses

Statistical analyses were performed according to the same methods described in section 4.3.2.4. For the analyses of glaucoma-related traits, I additionally performed

multivariable MR,[325] adjusting for genetically-determined alcohol (drinks per day)[197] and caffeine (cups per day) consumption,[198] given their moderate genetic correlations with smoking phenotypes.[197,357]

5.2.3 Results

5.2.3.1 Corneal biomechanical properties

All genetic variants (derived from the GSCAN GWAS excluding UK Biobank and 23andMe) included in the smoking initiation and smoking intensity IVs had an *F* statistic >10 (mean 36.2 and 100.4, respectively), suggesting sufficient IV strength. Under the IVW method, genetically predicted smoking initiation was associated with higher CH (0.26 mmHg per SD increase in the IV; 95% CI, 0.13 to 0.38; *P* <0.001). This result was supported by both the weighted median and weighted mode approaches. Although the IVW method did not demonstrate a significant association between smoking initiation and CRF, there was evidence for global heterogeneity in this analysis (Cochran's *Q* statistic *P* = 0.025), and alternative approaches able to account for IV heterogeneity (weighted median and MR-PRESSO) generated consistent and significant results. Genetically predicted smoking intensity was associated with CH under the weighted median and weighted mode methods, but not with CRF under any approach. Full results of the MR analyses are presented in **Table 5.7** and relevant test statistics in **Table H1**. Estimates derived from the full GSCAN GWAS were attenuated, but generally consistent, with those from the main MR analysis, and provided further evidence to support a causal relationship with CH (**Table H2**).

Table 5.7 Results of Mendelian randomisation analyses for smoking-related traits on corneal biomechanical properties

MR method	Corneal hysteresis (mmHg)		Corneal resistance factor (mmHg)	
	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value
Smoking initiation				
IVW	**0.26 (0.13, 0.38)**	**<0.001**	0.17 (-0.02, 0.37)	0.08
Weighted median	**0.32 (0.15, 0.49)**	**<0.001**	**0.26 (0.05, 0.47)**	**0.016**
Weighted mode	**0.36 (0.06, 0.66)**	**0.044**	0.42 (-0.08, 0.93)	0.13
MR-Egger	-0.56 (-1.45, 0.33)	0.22	-0.82 (-2.13, 0.50)	0.22
MR-PRESSO	–	–	**0.25 (0.07, 0.43)**	**0.024**
Smoking intensity				
IVW	0.12 (-0.01, 0.26)	0.07	0.08 (-0.07, 0.22)	0.29
Weighted median	**0.17 (0.02, 0.32)**	**0.022**	0.12 (-0.04, 0.27)	0.14
Weighted mode	**0.22 (0.07, 0.37)**	**0.021**	0.12 (-0.04, 0.28)	0.17
MR-Egger	0.21 (-0.08, 0.49)	0.16	0.07 (-0.25, 0.39)	0.66
MR-PRESSO	–	–	–	–

MR estimates expressed per unit change in the instrumental variable.

CI, confidence interval; IV, instrumental variable; SNP, single nucleotide polymorphism; IVW, inverse variance weighted; MR, Mendelian randomization; PRESSO, pleiotropy residual sum and outlier.

No MR-PRESSO estimate is calculated if no significant outliers are detected.

5.2.3.2 Glaucoma-related traits

Details of the SNPs included in the smoking initiation and smoking intensity IVs are available in **Table H3** and **Table H4**. MR did not support a causal relationship between smoking initiation and POAG, mRNFL, GCIPL, CDR, or AI-CDR ($P \geq 0.14$ for all). However, the smoking initiation IV was associated with lower IOP (-0.18 mmHg per SD increase in the IV; 95% CI, -0.30 to -0.06; $P = 0.003$) under the IVW method (**Table 5.8**). This was supported by MR-PRESSO and multivariable MR, but not by the other MR analyses. There was significant global heterogeneity in the smoking initiation IV, although no evidence for directional pleiotropy from the MR-Egger intercept test (**Table H5**). The smoking intensity IV was significantly associated with POAG (OR, 0.74; 95% CI, 0.61–0.90; $P = 0.002$) under the IVW method, with similar results yielded from all other MR methods (**Table 5.8**), and no evidence of global heterogeneity (**Table H5)**. There were no other significant associations between the smoking intensity IV and other glaucoma-related traits after adjusting for multiple comparisons. Results were materially unchanged when using GSCAN summary statistics excluding the UK Biobank and 23andMe (**Table H6**). Scatter plots of all MR analyses are available in **Figure H1** and **Figure H2**.

Table 5.8 Results of Mendelian randomisation analyses for smoking initiation and smoking intensity on glaucoma-related traits

MR method	POAG (OR)		IOP (mmHg)		CDR		AI-CDR		mRNFL (μm)		GCIPL (μm)	
	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Smoking initiation												
IVW	0.97 (0.87, 1.08)	0.58	**-0.18 (-0.30, -0.06)**	**0.003**	0.00 (-0.01, 0.01)	0.63	0.00 (-0.01, 0.00)	0.37	-0.05 (-0.32, 0.23)	0.74	-0.17 (-0.56, 0.21)	0.38
Weighted median	0.91 (0.79, 1.04)	0.18	-0.09 (-0.22, 0.05)	0.21	0.00 (-0.01, 0.02)	0.70	0.00 (-0.01, 0.01)	0.99	0.18 (-0.17, 0.53)	0.32	-0.07 (-0.53, 0.40)	0.78
Weighted mode	0.83 (0.59, 1.15)	0.26	0.11 (-0.28, 0.51)	0.58	0.00 (-0.03, 0.04)	0.86	0.00 (-0.01, 0.00)	0.62	0.69 (-0.38, 1.77)	0.21	0.50 (-0.79, 1.79)	0.66
MR-Egger	0.91 (0.58, 1.45)	0.71	-0.31 (-0.80, 0.18)	0.22	-0.02 (-0.05, 0.03)	0.47	0.00 (-0.02, 0.01)	0.79	0.63 (-0.53, 1.79)	0.28	0.02 (-1.61, 1.65)	0.98
MR-PRESSO	0.96 (0.86, 1.06)	0.41	**-0.19 (-0.30, -0.08)**	**<0.001**	0.00 (-0.01, 0.01)	0.75	0.00 (-0.01, 0.00)	0.14	-0.06 (-0.31, 0.20)	0.67	-0.21 (-0.56, 0.14)	0.25
Multivariable MR	0.98 (0.88, 1.09)	0.77	**-0.19 (-0.32, -0.06)**	**0.003**	0.00 (-0.01, 0.01)	0.77	0.00 (-0.01, 0.00)	0.28	-0.01 (-0.28, 0.27)	0.96	-0.07 (-0.45, 0.31)	0.72
Smoking intensity												
IVW	**0.74 (0.61, 0.90)**	**0.002**	-0.08 (-0.34, 0.18)	0.54	0.00 (-0.02, 0.02)	0.82	0.00 (-0.01, 0.01)	0.60	0.10 (-0.45, 0.66)	0.71	-0.76 (-1.50, -0.03)	0.04
Weighted median	**0.60 (0.46, 0.78)**	**<0.001**	-0.06 (-0.32, 0.20)	0.65	-0.01 (-0.04, 0.02)	0.49	0.00 (-0.01, 0.01)	0.63	-0.10 (-0.85, 0.66)	0.81	-1.08 (-2.07, -0.09)	0.03
Weighted mode	**0.64 (0.50, 0.84)**	**0.002**	-0.03 (-0.26, 0.19)	0.76	-0.01 (-0.04, 0.01)	0.30	0.00 (-0.01, 0.00)	0.55	0.06 (-0.68, 0.80)	0.88	-0.95 (-1.86, -0.04)	0.05
MR-Egger	**0.60 (0.44, 0.83)**	**0.002**	0.10 (-0.35, 0.54)	0.67	-0.01 (-0.05, 0.03)	0.57	0.00 (-0.02, 0.01)	0.74	0.56 (-0.41, 1.53)	0.26	-0.66 (-1.95, 0.63)	0.32
MR-PRESSO	-	-	-0.13 (-0.35, 0.08)	0.23	-	-	0.00 (-0.01, 0.00)	0.43	-	-	-	-
Multivariable MR	0.86 (0.77, 0.96)	0.006	-0.05 (-0.19, 0.10)	0.54	0.00 (-0.01, 0.01)	0.62	0.00 (-0.01, 0.00)	0.47	0.05 (-0.21, 0.32)	0.69	-0.36 (-0.70, -0.02)	0.04

No estimate is generated under the MR-PRESSO method if significant outliers are not detected. Multivariable MR adjusted for genetically determined smoking initiation.

mRNFL, macular retinal nerve fibre layer; GCIPL, ganglion cell-inner plexiform layer; IOP, intraocular pressure; CDR, cup-disc ratio; POAG, primary open-angle glaucoma; CI, confidence interval; AI, artificial intelligence; IVW, inverse variance weighted; MR, Mendelian randomisation; MR-PRESSO, Mendelian Randomisation-Pleiotropy Residual Sum and Outlier; N/A, not applicable.

5.2.4 Discussion

In support of the observational findings in both the UK Biobank and CLSA, genetic instruments related to smoking initiation, and to a lesser extent smoking intensity, were found to be consistently associated with higher CH under a variety of MR approaches. There was also less consistent evidence for an association between greater smoking intensity and higher CRF. These results add to the strong dose-dependent relationships observed in the epidemiological studies and provide additional evidence for a causal role of smoking on corneal biomechanical properties. As discussed previously, these results should be considered in the context of certain inherent limitations. However, in contrast to the alcohol analyses, results were generally more consistent across different MR methods and there was less evidence for global heterogeneity, suggesting that results may be less influenced by pleiotropic variants.

Overall, results for glaucoma-related traits were null with the exception of two significant findings. Firstly, smoking initiation, but not intensity, was found to be related to lower IOP. Interestingly, the IOP phenotype used in the original GWAS was predominantly based on IOPcc and this relationship mirrors the results from the observational analyses where both former and current smokers had lower IOPcc when compared to never smokers. A genetic risk score derived from the smoking initiation GWAS was also found to be associated with lower IOP in the independent Rotterdam Study.[349] However, the smoking initiation IV was not related to any other glaucoma-related trait in these MR analyses, which may have been expected if there were a true effect on IOP. Again, these results are consistent with the observational findings in which smoking-related traits were not found to be associated with any other glaucoma-related phenotype. This may lend further support to the hypothesis

that the relationship with IOPcc is artefactual and driven by changes to corneal biomechanics (see section 5.1 above).

Additionally, the smoking intensity IV was found to be inversely related to POAG, suggesting a protective effect of greater levels of smoking on glaucoma. While this would be consistent with the MR result between smoking initiation and lower IOP, results for all other glaucoma-related traits were null and a smoking intensity GRS was not found to be associated with OAG status in the Rotterdam Study.[349] Observational studies of the relationship between smoking and glaucoma have been conflicting and inconclusive. For example, while the randomised placebo-controlled United Kingdom Glaucoma Treatment Study (UKGTS) found an association between smoking initiation and decreased rates of glaucoma progression based on visual field testing,[98] a recent retrospective study reported an adverse association between smoking intensity, but not smoking initiation, and increased visual field loss.[358] A 2016 systematic review and meta-analysis found little evidence for a link between smoking and glaucoma, although excludes several more recent studies on the topic.[78]

In conclusion, these MR analyses provide further evidence for a causal relationship between smoking and corneal biomechanical properties, with a possible artefactual relationship with lower IOP. Despite an apparent protective effect of smoking intensity on POAG, there was little other evidence for a clear relationship between smoking and glaucoma-related traits.

6

Dietary salt

6.1 UK Biobank

The following section is a modified version of a paper currently under revision in *Ophthalmology Glaucoma*[359] and describes analyses of the association of urinary sodium excretion, a biomarker of dietary intake, with glaucoma and related traits. I was responsible for all aspects of this work. The relevant declaration form is located in **Appendix A**. Supplementary material for this section can be found in **Appendix I**.

6.1.1 Abstract

Objective: Excessive dietary sodium intake has known adverse effects on intravascular fluid volume and systemic blood pressure, which may influence IOP and glaucoma risk. This study aimed to assess the association of urinary sodium excretion, a biomarker of dietary intake, with glaucoma and related traits, and to determine whether this relationship is modified by genetic susceptibility to disease.

Design: Cross-sectional observational and gene-environment interaction analyses in the population-based UK Biobank study.

Participants: Up to 103 634 individuals (mean age 57 years, 51% women) with complete urinary, ocular, and covariable data.

Methods: Urine sodium:creatinine ratio (UNa:Cr; mmol:mmol) was calculated from a midstream urine sample. Ocular parameters were measured as part of a comprehensive eye examination and glaucoma case ascertainment was through a combination of self-report and linked national hospital records. Genetic susceptibility to glaucoma was calculated based on a glaucoma PRS comprising 2 673 common genetic variants. Multivariable linear and logistic regression, adjusted for key

sociodemographic, medical, anthropometric, and lifestyle factors, were used to model associations and gene-environment interactions.

Main outcome measures: Corneal-compensated IOP, OCT-derived mRNFL and GCIPL thickness, and prevalent glaucoma.

Results: In maximally adjusted regression models, a one SD increase in UNa:Cr was associated with higher IOP (0.14 mmHg; 95% CI, 0.12–0.17; *P* <0.001) and greater prevalence of glaucoma (OR, 1.11; 95% CI, 1.07–1.14; *P* <0.001), but not mRNFL or GCIPL thickness. Compared to those with UNa:Cr in the lowest quintile, those in the highest quintile had significantly higher IOP (0.45 mmHg; 95% CI, 0.36–0.53, *P* <0.001) and prevalence of glaucoma (OR, 1.30; 95% CI, 1.17–1.45; *P* <0.001). Stronger associations with glaucoma (*P* interaction = 0.001) were noted in participants with a higher glaucoma PRS.

Conclusions: Urinary sodium excretion, a biomarker of dietary intake, may represent an important modifiable risk factor for glaucoma, especially in individuals at high underlying genetic risk. These findings warrant further investigation as they may have important clinical and public health implications.

6.1.2 Introduction

Excessive dietary sodium intake is an important cardiovascular risk factor, estimated to cause five million deaths per annum worldwide, through an association with elevated blood pressure.[360] This relationship is thought to be mediated primarily through alterations in intravascular fluid volume, adverse vascular remodelling, and autonomic nervous dysfunction.[361] Although systemic hypertension has previously been implicated as a potential risk factor for glaucoma, the association between dietary sodium intake and glaucoma is less clear.[275] Self-reported dietary salt

consumption was recently reported to be adversely associated with prevalent POAG, but only among hypertensive medication users, in the Thessaloniki Eye Study.[120]

The aetiology of glaucoma is complex and multifactorial, with numerous genetic and environmental determinants thought to play a role.[1] Recent advances in glaucoma genetic discovery and PRS development have now made it possible to identify high-risk individuals before the clinical onset of disease, and the identification of environmental factors that could potentially modify genetic risk is a particular research priority.[1,27]

The estimation of sodium intake based on dietary analysis is difficult and the validity generally low.[362,363] Since the majority of dietary sodium is excreted via the kidneys, urinary sodium excretion represents an objective and reliable biomarker of dietary intake.[360,364] The purpose of this study was therefore to assess the association of urinary sodium excretion with glaucoma and related traits, including IOP and OCT-derived measures of inner retinal thickness, on a population level, as a better understanding of these relationships may have important clinical and public health implications.

6.1.3 Methods

6.1.3.1 UK Biobank

See section 2.1.1.1.

6.1.3.2 Assessment of urinary sodium excretion

From 2006–2010, approximately 485 000 UK Biobank participants provided a midstream urine sample as part of the baseline assessment.[181] Specimens were packaged and refrigerated according to protocol before being transported overnight

by a dedicated commercial courier to a central laboratory. Samples were then processed, and 9 mL urine aliquots stored in ultra-low temperature archives.

A pre-defined panel of biomarkers – including sodium (CV, 1%), potassium (CV, 1%), and creatinine (CV, 2%) – were assayed using a single Beckman Coulter AU5400 clinical chemistry analyser (Beckman Coulter UK, Ltd.) using the manufacturer's reagents and calibrators. The Beckman Coulter AU5400 series uses a potentiometric measurement for the determination of sodium and potassium concentrations, and a photometric measurement for the determination of creatinine concentration.

Each assay was validated against the manufacture's performance information and linearity experiments determined the reportable range. For each assay, the observed reportable range covered the manufacture's analytical range (sodium, 10–400 mmol/L; potassium, 2–200 mmol/L; creatinine, 88–44 200 μmol/L).

To account for variable urine concentration, I calculated the UNa:Cr from these specimens. In a steady state, renal excretion of creatinine remains relatively constant, and the urinary creatinine concentration therefore provides a measure of the state of dilution or concentration of the urine. This approach is widely used to estimate 24-hour excretion of sodium and other analytes, such as albumin and catecholamines, from spot urine samples.[365] UNa:Cr in the top and bottom percentiles of the distribution were excluded. Full details of the urine assays and quality control information for the urinary biomarker data are available online (https://biobank.ndph.ox.ac.uk/ukb/ukb/docs/urine_assay.pdf).

In addition, a subset of approximately 70 000 participants completed a 24-hour dietary assessment (Oxford WebQ questionnaire) as part of their baseline assessment.[299] Estimated nutrient intake, including dietary sodium (mg), has been

calculated for these participants using food composition data from the United Kingdom Nutrient Databank, and was used to assess the relationship between urinary sodium excretion and reported dietary intake.[366]

6.1.3.3 Glaucoma-related outcome measures and case ascertainment

See section 2.2.1.1.

6.1.3.4 Genotyping and polygenic risk scores

See section 2.2.3.

6.1.3.5 Covariables

I considered a range of sociodemographic, medical, anthropometric, and lifestyle factors in my analyses based on previously reported risk factors for glaucoma, associations with IOP, or determinants of urinary sodium excretion. All covariables used in this analysis were ascertained at the time of the baseline assessment and on the same day as the urine collection and ophthalmic assessment. These included: age, sex, self-reported ethnicity, Townsend deprivation index, height, weight, SBP, HbA1c, total cholesterol, smoking status, alcohol intake,[61] physical activity,[367] assessment season, time of urine collection, and urinary potassium concentration. Full details of these variables are available in section 2.2.4.

6.1.3.6 Statistical analysis

Baseline participant characteristics were summarised as mean (SD) for continuous variables, and frequency (proportion) for categorical variables. The linear-by-linear and Cochrane-Armitage tests were used to assess trends across UNa:Cr quintiles, as appropriate.

To assess the main associations between urinary sodium excretion and the various glaucoma-related outcomes, I used multivariable linear (for IOP, mRNFL thickness, and GCIPL thickness) and logistic (for glaucoma) regression models adjusted for the covariables described above. Given the strong causal relationship between dietary salt intake and hypertension, and to assess whether any associations may be mediated through blood pressure, I considered multivariable regression models both without, and with, adjustment for SBP. All other covariables were considered potential confounders and were included in both sets of regression models.

Urinary sodium excretion was analysed as both a continuous (standardised UNa:Cr) and categorical (quintiles of UNa:Cr) variable. Trends across quintiles were examined by testing the median value of each group.

To assess whether any associations were modified by the glaucoma PRS, I tested the significance of a multiplicative interaction term between the standardised UNa:Cr and standardised PRS in the final multivariable models using the Wald test. Gene-environment interaction analyses were restricted to participants of European ancestry based on principal components analysis. All analyses were performed using Stata (Version 17.0. StataCorp LLC. 2021. College Station, TX, USA).

6.1.3.7 Sensitivity analyses

Given that urinary sodium excretion may be influenced by antihypertensive medication use or renal impairment, I performed stratified analyses by self-reported use of any blood pressure medication and eGFR categories. The eGFR calculations were based on the revised 2021 Chronic Kidney Disease Epidemiology Collaboration formulae.[368]

I also performed sex-stratified analyses, as women have been shown to have a greater susceptibility to salt-sensitive hypertension than men, and additionally adjusted all models for systemic beta-blocker use and caffeine intake, based on previously reported associations.[146,180,369]

6.1.4 Results

6.1.4.1 Participants

The study flow and participant selection process are summarised in **Figure 6.1**. After exclusions for missing data and outliers, 71 075, 29 965, and 103 634 individuals were eligible for the analyses of IOP, OCT-derived inner retinal thickness measures, and glaucoma status, respectively. As there was considerable overlap between cohorts, demographic features and baseline characteristics were largely similar. In keeping with the overall UK Biobank, mean participant age was 56–57 years, with a slight predominance of women (51–52%), and a majority of White participants (91–92%) (**Table 6.1**). Further restriction to European participants with genetic data left 55 178, 23 487, and 82 359 individuals for the respective gene-environment interaction analyses.

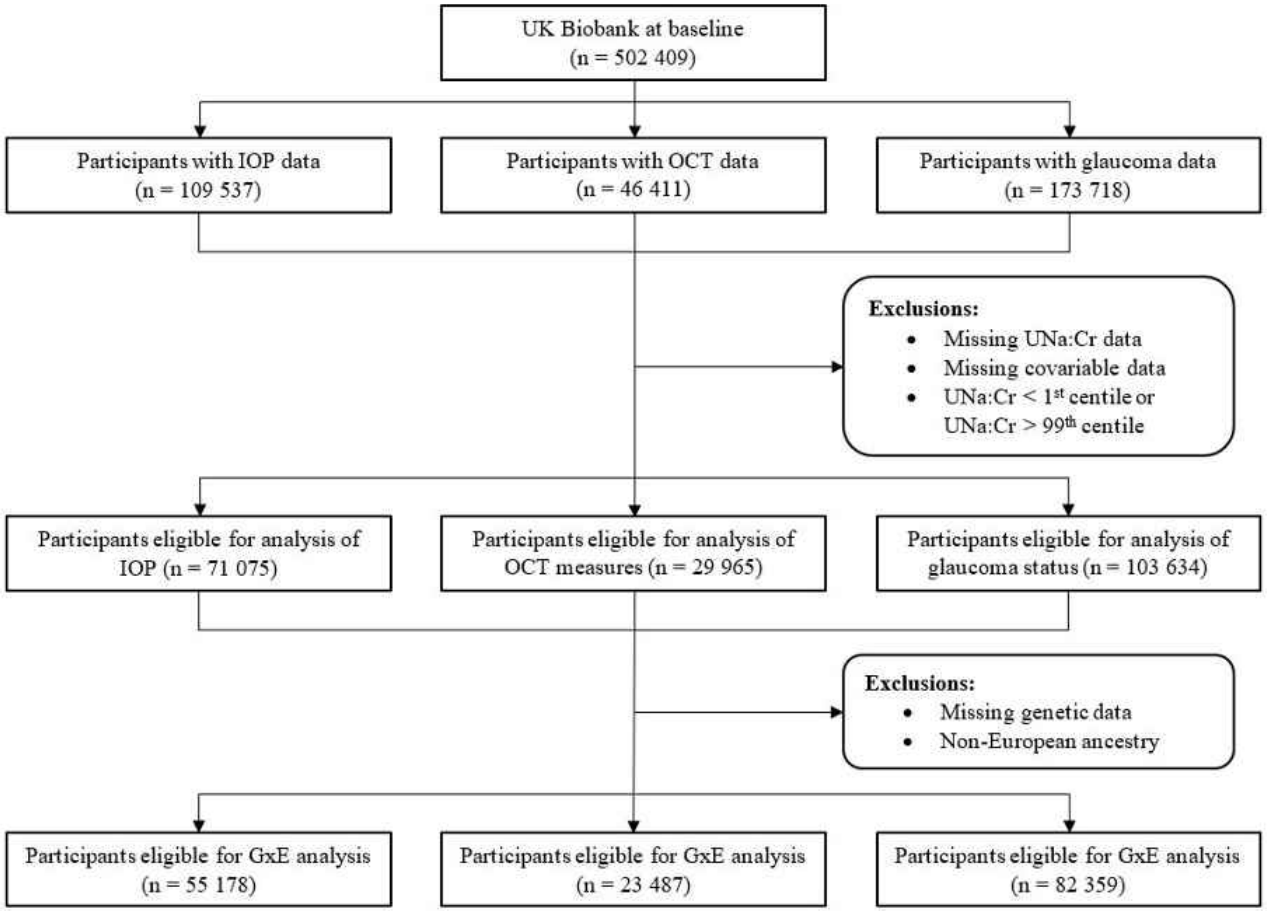

Figure 6.1 Flow diagram outlining the participant selection process for this study in the UK Biobank

GxE, gene-environment interaction; IOP, intraocular pressure; OCT, optical coherence tomography; UNa:Cr, urine sodium:creatinine ratio.

Table 6.1 Baseline characteristics of eligible UK Biobank participants

Characteristic (unit of measurement)	Analysis cohort		
	IOP	OCT	Glaucoma
Sample size, *n*	71 075	29 965	103 634
Age (years)	56.7 (8.1)	56.2 (8.2)	56.9 (8.1)
Sex, *n* (%)			
Women	36 713 (51.7)	15 171 (50.6)	52 991 (51.1)
Men	34 362 (48.3)	14 794 (49.4)	50 643 (48.9)
Ethnicity, *n* (%)			
White	64 762 (91.1)	27 655 (92.3)	95 682 (92.3)
Asian	2 760 (3.9)	907 (3.0)	3 457 (3.3)
Black	1 970 (2.8)	737 (2.5)	2 401 (2.3)
Other/Mixed	1 583 (2.2)	666 (2.2)	2 094 (2.0)
Townsend Deprivation Index	-1.1 (2.9)	-1.1 (2.9)	-1.1 (3.0)
Height (cm)	169.2 (9.3)	169.5 (9.2)	169.1 (9.3)
Weight (kg)	78.2 (15.9)	78.4 (15.7)	78.3 (15.9)
Body mass index (kg/m^2)	27.2 (4.6)	27.2 (4.6)	27.3 (4.7)
Systolic blood pressure (mmHg)	137.0 (18.2)	136.7 (18.3)	137.5 (18.4)
HbA1c (mmol/mol)	36.1 (6.6)	35.9 (6.6)	36.2 (7.0)
Total cholesterol (mmol/L)	5.7 (1.1)	5.7 (1.1)	5.7 (1.1)
Smoking status, *n* (%)			
Never smoker	39 265 (55.2)	16 316 (54.5)	56 107 (54.1)
Current smoker	6 857 (9.7)	2 916 (9.7)	10 311 (10.0)
Former smoker	24 953 (35.1)	10 733 (35.8)	37 216 (35.9)
Alcohol intake (g/week)	107.5 (129.4)	109.0 (128.5)	114.1 (133.3)
Physical activity (MET-hours/week)	44.7 (44.6)	45.2 (45.1)	44.5 (44.8)
Urine sodium concentration (mmol/L)	72.8 (40.7)	72.3 (40.4)	73.9 (41.7)
Urine potassium concentration (mmol/L)	59.8 (31.4)	60.0 (31.5)	61.0 (32.2)
Urine creatinine concentration (mmol/L)	8.4 (5.2)	8.5 (5.3)	8.6 (5.4)
Urine sodium:creatinine ratio (mmol:mmol)	10.2 (5.3)	10.0 (5.2)	10.3 (5.3)
Quintile 1, range	<5.7	<5.6	<5.7
Quintile 2, range	5.7–8.1	5.6–7.9	5.7–8.1
Quintile 3, range	8.1–10.6	7.9–10.4	8.1–10.7
Quintile 4, range	10.6–14.2	10.4–13.9	10.7–14.3
Quintile 5, range	>14.2	>13.9	>14.3
eGFR (mL/min/1.73m^2)	94.4 (12.8)	94.4 (12.7)	94.3 (13.0)
Intraocular pressure (mmHg)	16.1 (3.4)	–	–
mRNFL thickness (μm)	–	28.9 (3.8)	–
GCIPL thickness (μm)	–	75.2 (5.2)	–
Glaucoma prevalence, *n* (%)	–	–	4 045 (3.9)

All values represent mean (standard deviation), unless otherwise specified.

IOP, intraocular pressure; OCT, optical coherence tomography; HbA1c, glycated haemoglobin; MET, metabolic equivalent of task; mRNFL, macular retinal nerve fibre layer; GCIPL, ganglion cell-inner plexiform layer; eGFR, estimated glomerular filtration rate.

6.1.4.2 Urinary sodium excretion

Participants characteristics stratified by UNa:Cr quintile for individuals included in the analysis of glaucoma status (the largest of the three cohorts) are reported in **Table 6.2**. There were notable linear trends of estimated 24-hour dietary sodium intake (Q1: 1 773 mg; Q5: 2 046 mg), SBP (Q1: 135.5 mmHg; Q5: 139.9 mmHg), eGFR (Q1: 91.0 mL/min/1.73m^2; Q5: 97.7 mL/min/1.73m^2), and urine potassium concentration (Q1: 79.8 mmol/L; Q5: 44.8 mmol/L) across UNa:Cr quintiles (*P* trend ≤0.001 for all), which persisted after adjustment for all covariables considered in the main analyses (**Figure 6.2)**. Similar results for the cohorts of IOP and OCT-derived inner retinal thickness measures are presented in **Table I1** and **Table I2.**

6.1.4.3 Association with glaucoma and related traits

In maximally adjusted multivariable regression models, a one SD increase in UNa:Cr was associated with higher IOP (0.14 mmHg; 95% CI, 0.12–0.17; *P* <0.001) and greater prevalence of glaucoma (OR, 1.11; 95% CI, 1.07–1.14; *P* <0.001), but not mRNFL or GCIPL thickness (**Table 6.3, Model A**).

There was evidence of a dose-response relationship across UNa:Cr quintiles for IOP and glaucoma (*P* trend <0.001 for both), but not for the OCT-derived inner retinal parameters (**Table 6.3, Model A**). Compared to those in the lowest quintile, those in the highest UNa:Cr quintile had higher IOP (0.45 mmHg; 95% CI, 0.36–0.53; *P* <0.001) and higher prevalence of glaucoma (OR, 1.30; 95% CI, 1.17–1.45; *P* <0.001).

Further adjustment of the final regression models for SBP resulted in attenuation of the IOP association but did not materially affect the other associations (**Table 6.1.3, Model B**).

Table 6.2 Baseline characteristics of eligible UK Biobank participants by urine sodium:creatinine ratio quintile (glaucoma cohort)

Characteristic (unit of measurement)	Urine sodium:creatinine ratio quintile (mmol:mmol) (*n* = 103 634)					*P* (trend)
	Quintile 1 (<5.7)	Quintile 2 (5.7–8.1)	Quintile 3 (8.1–10.7)	Quintile 4 (10.7–14.3)	Quintile 5 (>14.3)	
Age (years)	57.4 (8.0)	57.0 (8.1)	56.8 (8.1)	56.8 (8.1)	56.7 (8.2)	**<0.001**
Sex (women), *n* (%)	8 888 (42.9)	9 356 (45.1)	10 099 (48.7)	11 156 (53.8)	13 492 (65.1)	**<0.001**
Ethnicity (White), *n* (%)	19 344 (93.2)	19 446 (93.8)	19 232 (92.8)	19 126 (92.3)	18 534 (89.4)	**<0.001**
Townsend deprivation index	-1.2 (3.0)	-1.2 (3.0)	-1.2 (3.0)	-1.1 (3.0)	-0.9 (3.0)	**<0.001**
Height (cm)	170.9 (9.3)	170.4 (9.2)	169.6 (9.2)	168.4 (9.0)	166.2 (8.9)	**<0.001**
Weight (kg)	81.2 (16.1)	79.6 (15.6)	78.5 (15.7)	77.3 (15.6)	74.8 (15.6)	**<0.001**
Body mass index (kg/m^2)	27.7 (4.7)	27.3 (4.5)	27.2 (4.5)	27.2 (4.6)	27.0 (4.8)	**<0.001**
Systolic blood pressure (mmHg)	135.5 (17.8)	136.4 (18.0)	137.3 (18.0)	138.3 (18.4)	139.9 (19.3)	**<0.001**
HbA1c (mmol/mol)	36.4 (7.5)	36.2 (7.0)	36.2 (6.8)	36.1 (6.6)	36.3 (6.9)	0.32
Total cholesterol (mmol/L)	5.6 (1.2)	5.7 (1.1)	5.7 (1.1)	5.7 (1.1)	5.7 (1.1)	**<0.001**
Smoking status (current smoker), *n* (%)	2 150 (10.4)	2 072 (10.0)	1 993 (9.6)	2 026 (9.8)	2 070 (10.0)	0.16
Alcohol intake (g/week)	123.7 (144.8)	120.9 (137.7)	115.1 (132.3)	109.6 (126.0)	101.3 (123.4)	**<0.001**
Physical activity (MET-hours/week)	41.2 (42.5)	42.9 (43.0)	45.3 (45.6)	45.9 (45.8)	47.1 (47.0)	**<0.001**
Urine sodium concentration (mmol/L)	51.5 (26.5)	67.6 (34.4)	76.4 (40.0)	83.1 (44.2)	91.0 (48.1)	**<0.001**
Urine potassium concentration (mmol/L)	79.8 (36.2)	67.2 (32.2)	59.9 (29.4)	53.4 (27.3)	44.8 (23.0)	**<0.001**
Urine creatinine concentration (mmol/L)	13.0 (6.5)	9.8 (5.0)	8.2 (4.3)	6.8 (3.6)	5.0 (2.8)	**<0.001**
eGFR (mL/min/1.73m^2)	91.0 (14.1)	92.9 (13.1)	94.4 (12.6)	95.5 (12.3)	97.7 (11.8)	**<0.001**
Intraocular pressure (mmHg) [a]	15.9 (3.4)	16.0 (3.4)	16.1 (3.4)	16.1 (3.4)	16.1 (3.4)	**<0.001**
mRNFL thickness (µm) [b]	28.9 (3.9)	29.0 (3.8)	28.9 (3.8)	29.0 (3.8)	28.9 (3.8)	0.49
GCIPL thickness (µm) [c]	75.1 (5.3)	75.2 (5.2)	75.3 (5.3)	75.3 (5.2)	75.3 (5.1)	**0.004**
Glaucoma prevalence, *n* (%)	845 (4.1)	766 (3.7)	793 (3.8)	801 (3.9)	840 (4.1)	0.13
Estimated sodium intake (mg, 24-hour recall) [d]	1 773 (871)	1 888 (881)	1 945 (932)	1 997 (929)	2 046 (985)	**<0.001**

All values represent mean (standard deviation), unless otherwise specified. [a] *n* = 70 793. [b] *n* = 29 616. [c] *n* = 29 532. [d] *n* = 35 566.

HbA1c, glycated haemoglobin; MET, metabolic equivalent of task; eGFR, estimated glomerular filtration rate; mRNFL, macular retinal nerve fibre layer; GCIPL, ganglion cell-inner plexiform layer.

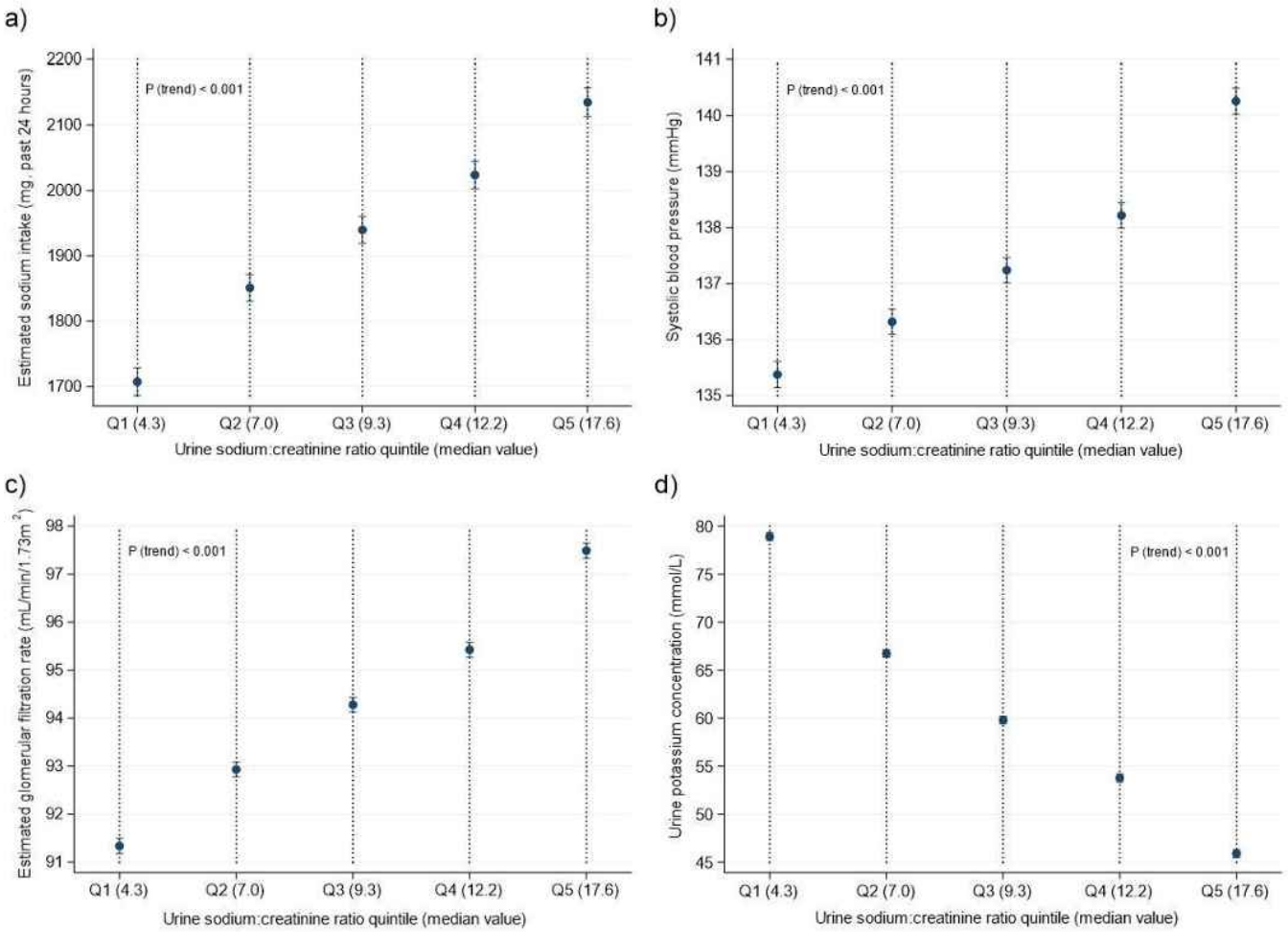

Figure 6.2 Associations of urinary sodium excretion with (**a**) estimated sodium intake in the past 24 hours, (**b**) systolic blood pressure, (**c**) estimated glomerular filtration rate, and (**d**) urine potassium concentration in UK Biobank participants

Models adjusted for: age (years), sex (women, men), Townsend deprivation index, height (cm), weight (kg), glycated haemoglobin (mmol/mol), total cholesterol (mmol/L), smoking status (never, current, former), alcohol intake (g/day), physical activity (MET-minutes/week), assessment season (Summer, Autumn, Winter, Spring), time of urine collection (morning, afternoon, evening), and urinary potassium concentration (**a–c** only). Q, quintile.

Table 6.3 Results of multivariable regression analyses for the association of urinary sodium excretion with glaucoma and related traits

Urine sodium:creatinine ratio	Intraocular pressure (mmHg) (*n* = 71 075)			mRNFL thickness (µm) (*n* = 29 660)			GCIPL thickness (µm) (*n* = 29 577)			Glaucoma prevalence (%) (*n* = 103 634)		
	Beta	**95% CI**	***P*-value**	**Beta**	**95% CI**	***P*-value**	**Beta**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Model A (without SBP) [a]												
Continuous												
Per SD increase	**0.14**	**0.12, 0.17**	**<0.001**	-0.03	-0.08, 0.01	0.17	0.03	-0.03, 0.10	0.32	**1.11**	**1.07, 1.14**	**<0.001**
Quintiles [b]												
Quintile 1		Reference			Reference			Reference			Reference	
Quintile 2	**0.15**	**0.07, 0.23**	**<0.001**	0.06	-0.08, 0.20	0.39	0.09	-0.10, 0.27	0.37	0.99	0.90, 1.10	0.91
Quintile 3	**0.30**	**0.22, 0.38**	**<0.001**	-0.03	-0.17, 0.11	0.68	0.10	-0.09, 0.29	0.29	1.10	0.99, 1.21	0.09
Quintile 4	**0.33**	**0.25, 0.42**	**<0.001**	-0.03	-0.17, 0.11	0.66	0.11	-0.08, 0.30	0.26	**1.15**	**1.03, 1.28**	**0.009**
Quintile 5	**0.45**	**0.36, 0.53**	**<0.001**	-0.08	-0.22, 0.07	0.30	0.16	-0.04, 0.36	0.12	**1.30**	**1.17, 1.45**	**<0.001**
P (trend)			**<0.001**			0.14			0.15			**<0.001**
Model B (with SBP) [c]												
Continuous												
Per SD increase	**0.09**	**0.06, 0.12**	**<0.001**	-0.03	-0.08, 0.02	0.20	0.05	-0.02, 0.11	0.17	1.10	1.06, 1.14	**<0.001**
Quintiles [b]												
Quintile 1		Reference			Reference			Reference			Reference	
Quintile 2	**0.12**	**0.04, 0.20**	**0.002**	0.06	-0.08, 0.20	0.38	0.09	-0.09, 0.28	0.33	0.99	0.90, 1.10	0.87
Quintile 3	**0.24**	**0.16, 0.32**	**<0.001**	-0.03	-0.17, 0.11	0.69	0.11	-0.08, 0.30	0.24	1.09	0.98, 1.21	0.10
Quintile 4	**0.24**	**0.16, 0.32**	**<0.001**	-0.03	-0.17, 0.11	0.68	0.13	-0.06, 0.32	0.18	**1.14**	**1.03, 1.27**	**0.013**
Quintile 5	**0.30**	**0.21, 0.38**	**<0.001**	-0.07	-0.22, 0.07	0.33	0.19	-0.01, 0.39	0.06	**1.29**	**1.16, 1.44**	**<0.001**
P (trend)			**<0.001**			0.16			0.07			**<0.001**

[a] Model A adjusted for: age (years), sex (women, men), ethnicity (White, Asian, Black, Other/Mixed), Townsend deprivation index, height (cm), weight (kg), glycated haemoglobin (mmol/mol), total cholesterol (mmol/L), smoking status (never, current, former), alcohol intake (g/day), physical activity (MET-minutes/week), assessment season (Summer, Autumn, Winter, Spring), time of urine collection (morning, afternoon, evening), and urinary potassium concentration (mmol/L). [b] Details of urine sodium:creatinine ratio quintiles for each cohort are available in **Table 6.1.1**. [c] Model B adjusted for: as for Model A, plus systolic blood pressure (mmHg).

mRNFL, macular retinal nerve fibre layer; GCIPL, ganglion cell-inner plexiform layer; CI, confidence interval; OR, odds ratio; SD, standard deviation; SBP, systolic blood pressure.

6.1.4.4 Gene-environment interaction analyses

There was no evidence of a gene-environment interaction for IOP (*P* interaction = 0.95), mRNFL thickness (*P* interaction = 0.32), or GCIPL thickness (*P* interaction = 0.49) (**Figure 6.3a–c**). The glaucoma PRS modified the relationship of urinary sodium excretion with glaucoma prevalence (*P* interaction = 0.001), however, with the strongest associations noted in participants at the highest underlying genetic risk (**Figure 6.3d**).

While the association between urinary sodium excretion and IOP was the same at all levels of genetic risk, the same relationship was not observed for glaucoma. For those in the lowest PRS quartile, urinary sodium excretion was not significantly associated with glaucoma prevalence, with progressively stronger associations noted in subsequent quartiles. For those in the highest PRS quartile, glaucoma prevalence increased from 8.5% to 13.2% across the range of urinary sodium excretion. Further adjustment for SBP did not materially change the results of these analyses (**Figure I1**).

6.1.4.5 Sensitivity analyses

Results for all outcomes were consistent by sex and antihypertensive medication status (**Table 6.4**). Associations also persisted when restricting analyses to participants without renal impairment (eGFR >90 ml/min/1.73m^2) (**Table 6.4**). Additional adjustment for systemic beta-blocker use and caffeine intake did not materially change the overall results (**Table I3**).

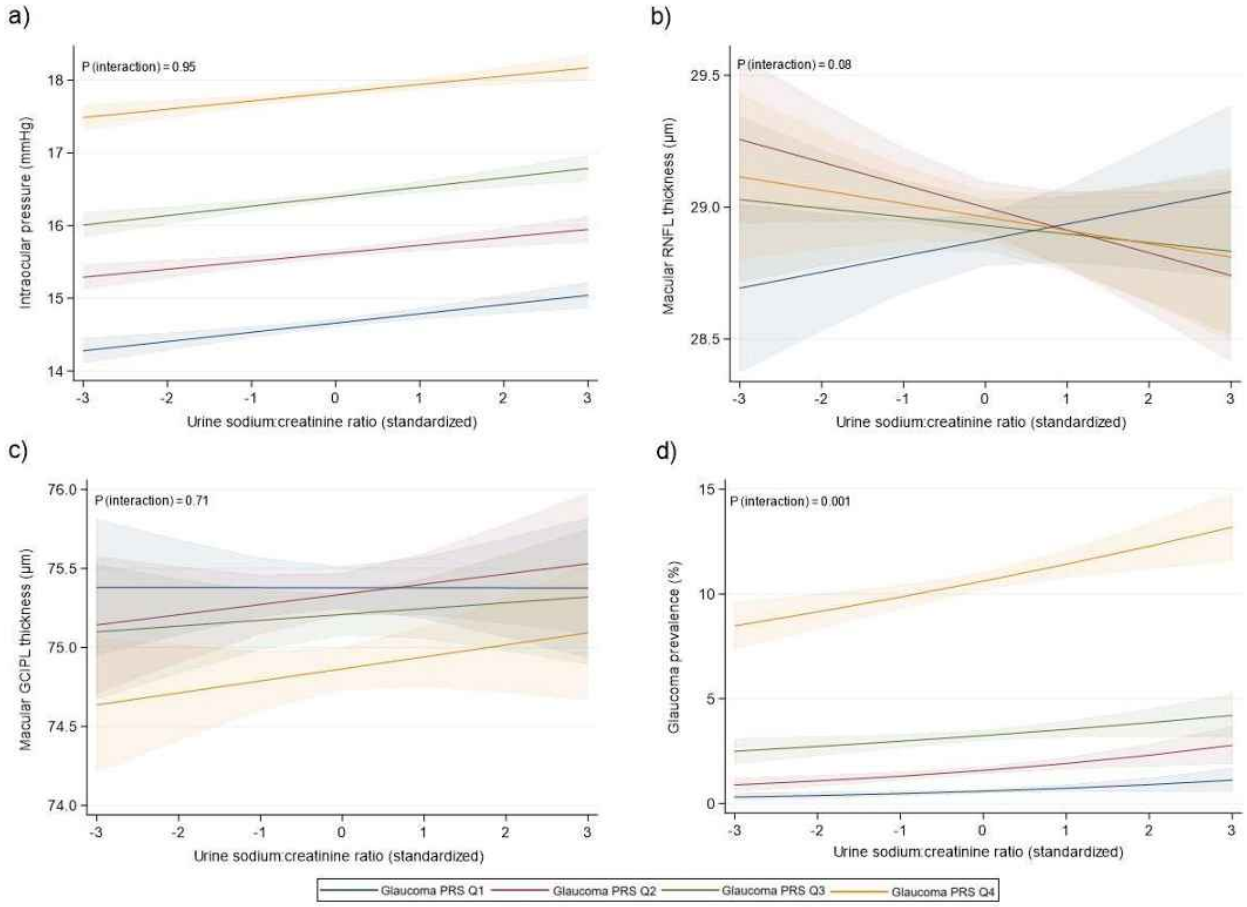

Figure 6.3 Gene-environment interaction analyses illustrating the effect of the glaucoma PRS on the association of urinary sodium excretion with (**a**) intraocular pressure, (**b**) macular retinal nerve fibre layer thickness, (**c**) ganglion cell-inner plexiform layer thickness, and **(d)** glaucoma status in European UK Biobank participants

Models adjusted for: age (years), sex (women, men), Townsend deprivation index, height (cm), weight (kg), glycated haemoglobin (mmol/mol), total cholesterol (mmol/L), smoking status (never, current, former), alcohol intake (g/day), physical activity (MET-minutes/week), assessment season (Summer, Autumn, Winter, Spring), time of urine collection (morning, afternoon, evening), and urinary potassium concentration (mmol/L). RNFL, retinal nerve fibre layer; GCIPL, ganglion cell-inner plexiform layer; PRS, polygenic risk score; Q, quartile.

Table 6.4 Results of multivariable regression analyses for the association of urinary sodium excretion with glaucoma and related traits, stratified by renal function, antihypertensive medication use, and sex

Urine sodium:creatinine ratio (per SD increase)	Intraocular pressure (mmHg)				mRNFL thickness (µm)				GCIPL thickness (µm)				Glaucoma prevalence (%)			
	n	Beta	95% CI	*P*-value	*n*	Beta	95% CI	*P*-value	*n*	Beta	95% CI	*P*-value	*n*	OR	95% CI	*P*-value
Model A (without SBP)																
eGFR (mL/min/1.73m²)																
≥90	48 633	**0.16**	**0.13, 0.19**	**<0.001**	20 295	-0.05	-0.10, 0.01	0.10	20 243	0.04	-0.04, 0.12	0.29	70 970	**1.13**	**1.09, 1.18**	**<0.001**
60-<90	21 411	**0.09**	**0.04, 0.14**	**0.001**	8 977	0.01	-0.08, 0.09	0.90	8 948	-0.02	-0.15, 0.09	0.63	31 043	1.04	0.98, 1.10	0.20
<60	965	0.13	-0.12, 0.38	0.32	355	-0.17	-0.64, 0.30	0.48	354	-0.15	-0.79, 0.48	0.63	1 534	1.00	0.78, 1.28	0.99
Antihypertensive use																
No	56 702	**0.14**	**0.11, 0.17**	**<0.001**	23 977	-0.04	-0.09, 0.02	0.18	23 916	0.04	-0.03, 0.11	0.25	81 609	**1.11**	**1.06, 1.16**	**<0.001**
Yes	14 373	**0.15**	**0.09, 0.22**	**<0.001**	5 683	0.01	-0.10, 0.12	0.88	5 661	0.05	-0.10, 0.20	0.54	22 025	**1.10**	**1.04, 1.18**	**0.003**
Sex																
Women	36 713	**0.12**	**0.08, 0.15**	**<0.001**	15 012	-0.02	-0.08, 0.05	0.65	15 009	0.00	-0.09, 0.09	0.99	52 991	**1.10**	**1.05, 1.16**	**<0.001**
Men	34 362	**0.18**	**0.14, 0.22**	**<0.001**	14 648	-0.05	-0.12, 0.01	0.10	14 568	0.07	-0.02, 0.17	0.12	50 643	**1.10**	**1.06, 1.15**	**<0.001**
Model B (with SBP)																
eGFR (mL/min/1.73m²)																
≥90	48 633	**0.10**	**0.07, 0.13**	**<0.001**	20 295	-0.05	-0.10, 0.01	0.12	20 243	0.05	-0.02, 0.13	0.18	70 970	**1.13**	**1.08, 1.18**	**<0.001**
60-<90	21 411	0.03	-0.02, 0.08	0.20	8 977	0.00	-0.08, 0.09	0.93	8 948	-0.01	-0.13, 0.10	0.81	31 043	1.04	0.98, 1.10	0.25
<60	965	0.14	-0.11, 0.39	0.27	355	-0.20	-0.68, 0.28	0.41	354	-0.09	-0.73, 0.56	0.79	1 534	1.00	0.78, 1.28	0.99
Antihypertensive use																
No	56 702	**0.09**	**0.06, 0.11**	**<0.001**	23 977	-0.04	-0.09, 0.02	0.19	23 916	0.05	-0.02, 0.13	0.13	81 609	**1.10**	**1.06, 1.15**	**<0.001**
Yes	14 373	**0.11**	**0.05, 0.18**	**0.001**	5 683	0.01	-0.10, 0.12	0.84	5 661	0.05	-0.10, 0.20	0.47	22 025	**1.11**	**1.04, 1.18**	**0.002**
Sex																
Women	36 713	**0.06**	**0.02, 0.09**	**0.001**	15 012	-0.02	-0.08, 0.05	0.61	15 009	0.01	-0.07, 0.10	0.76	52 991	**1.09**	**1.04, 1.15**	**0.001**
Men	34 362	**0.13**	**0.09, 0.17**	**<0.001**	14 648	-0.05	-0.12, 0.02	0.14	14 568	0.09	-0.01, 0.18	0.06	50 643	**1.11**	**1.06, 1.15**	**<0.001**

[a] Model A adjusted for: age (years), sex (women, men), ethnicity (White, Asian, Black, Other), Townsend deprivation index, height (cm), weight (kg), glycated haemoglobin (mmol/mol), total cholesterol (mmol/L), smoking status (never, current, former), alcohol intake (g/day), physical activity (MET-minutes/week), assessment season (Summer, Autumn, Winter, Spring), time of urine collection (morning, afternoon, evening), and urinary potassium concentration (mmol/L). [b] Model B adjusted for: as for Model A, plus systolic blood pressure (mmHg).

SD, standard deviation, mRNFL, macular retinal nerve fibre layer; GCIPL, ganglion cell-inner plexiform layer; CI, confidence interval; OR, odds ratio; SBP, systolic blood pressure.

6.1.5 Discussion

In this large population-based study, I investigated the association of urinary sodium excretion, a biomarker of dietary sodium intake, with prevalent glaucoma and various glaucoma-related traits. Overall, consistent adverse dose-response relationships were observed for IOP and glaucoma, but not with mRNFL or GCIPL thickness. The relationship with IOP appeared to be partially mediated through SBP, while the association with glaucoma prevalence was modified by a glaucoma PRS, with the strongest associations noted in those at the highest underlying genetic risk.

Results remained robust to stratified analyses by sex and antihypertensive medication status, and associations also persisted when excluding participants in whom urinary sodium excretion may have been altered from physiological levels by kidney disease.

Urine-based estimations offer an objective and reliable alternative to dietary methods for quantifying sodium intake and the large-scale availability of this biomarker data is a particular strength of the current study.[360,362,364] Although quantification methods based on multiple 24-hour urine collections are considered the gold standard, numerous technical and practical challenges have limited their uptake in large epidemiological studies.

Spot urinary sodium measurements are far easier to obtain, have demonstrated expected associations with blood pressure, and provide a good indication of mean dietary sodium intake on a population level.[364,370] They are also widely used to estimate 24-hour sodium excretion through a variety of regression-based equations and, importantly, my analyses included adjustment for all the variables central to these formulae: age, sex, weight, height, urinary creatinine concentration, and

urinary potassium concentration..[371–373] I was also able to validate the exposure measure by assessing associations with relevant dietary data and clinical parameters.

While the analyses were further strengthened by the large sample size, extensive phenotyping, detailed ocular data, and availability of genetic information in the UK Biobank, it is important to consider certain limitations. Spot urine sodium concentration may reflect recent dietary sodium intake but may not be an accurate representation of long-term salt consumption or capture past changes in dietary behaviour. Similarly, the use of these measures is likely to be less accurate than quantification methods based on 24-hour urine collection.

I was also limited by the method of glaucoma case ascertainment, which relied on a combination of self-report and ICD codes, although this limitation was partly overcome by the ability to simultaneously assess associations with continuous objective glaucoma-related parameters. The cross-sectional study design limits the ability to assess temporal relationships and make causal inferences. While I was able to adjust for multiple important confounders in the analyses, the observed associations may represent residual confounding by unknown or unconsidered factors.

Finally, the findings in UK Biobank participants, where >90% are of self-reported White ethnicity, may not be generalisable to other populations. Multiple studies have demonstrated notable ethnic differences in average dietary intake and urinary excretion of sodium, salt sensitivity, and glaucoma prevalence. It would therefore be important for the findings of this study to be replicated in different cohorts with a greater representation of non-White ethnicities.[7,374,375]

The characteristics of the subset of UK Biobank participants undergoing IOP measurement and OCT imaging have been described in detail previously.[158] Although largely similar to the overall UK Biobank cohort, those undergoing ophthalmic assessment were more likely to be of non-White ethnicity and have a more positive Townsend Deprivation Index (indicating greater relative deprivation).[158]

It is also important to note that UK Biobank participants (response rate, 5.5%) were more likely to be older, female, live in less socioeconomically deprived area, and have lower rates of disease when compared to the general UK population (a healthy volunteer effect).[315] Therefore, although the UK Biobank is not suitable for deriving generalisable estimates of disease prevalence and incidence, the large sample size and heterogeneity of exposures provide for valid assessments of exposure-disease associations that may be generalisable to other populations.[315]

To the best of my knowledge, this is the first population-based study to assess the relationship between urinary sodium excretion and glaucoma. A higher frequency of self-reported dietary salt intake has recently been reported to be adversely associated with prevalent POAG in the Thessaloniki Eye Study (TES), but only in those using antihypertensive medication.[120]

Important limitations of TES include a relatively small sample size and the use of self-report to assess dietary salt intake, which may have resulted in misclassification bias and limited the investigators' ability to explore dose-response relationships. Notably, as more than 70% of TES participants reported using blood pressure medication, the study may have been underpowered to detect an effect in non-users (292 participants). Alternatively, differences in the exposure (self-reported dietary salt versus urinary sodium excretion) and population under investigation may mean that

the two studies are not directly comparable and could account for the disparate results observed.

These results suggest that urinary sodium excretion, and by extension, dietary sodium intake, may represent a modifiable risk factor for glaucoma, potentially through an IOP-dependent mechanism, and that this effect may be more pronounced in those with a higher glaucoma PRS. Sodium plays a central role in volume homeostasis and increased salt consumption may provoke water retention, leading to a state of high flow in arterial blood vessels.[361] Fluid overload, increased plasma osmolality and higher blood pressure, leading to increased aqueous humour production and higher episcleral venous pressures, are plausible biological mechanisms underpinning the relationship between urinary sodium excretion and IOP in this study.[275] Blood pressure is consistently associated with IOP in epidemiological studies, with a pooled mean IOP 0.26 mmHg higher per 10 mmHg higher SBP, while the acute effect of changes in intravascular fluid volume and concentration have been studied in patients undergoing hemodialysis.[275,376] It is also possible that vascular and autonomic changes could further influence glaucoma risk through IOP-independent mechanisms.

Current World Health Organisation guidelines recommend consuming <5 grams of salt (equivalent to <2 000 mg dietary sodium) daily.[377] Although I was unable to directly translate UNa:Cr into a measure of dietary intake, only participants in quintile 5 had a mean 24-hour sodium intake exceeding this threshold. While dietary patterns of UK Biobank participants are healthier than those of the general population, the fact that adverse associations were apparent across the range of UNa:Cr values, suggests a continuous relationship rather than one occurring beyond a particular threshold.[378]

Despite adverse associations with IOP and glaucoma, urinary sodium excretion was not found to be associated with mRNFL or GCIPL thickness. It is possible that glaucoma-related inner retinal thinning may be masked by sodium-mediated changes in total body water or extracellular fluid volume. For example, higher levels of markers related to body fluid status are correlated with a thicker retinal central subfield in patients with diabetic retinopathy, while mean retinal thickness has been shown to decrease significantly after dialysis in patients with end-stage kidney disease.[379,380]

While adverse associations with IOP were apparent at all levels of genetic risk, progressively stronger associations with prevalent glaucoma were noted in participants with a higher glaucoma PRS. This may suggest that the glaucoma PRS could partly reflect an individual's susceptibility to IOP-mediated glaucomatous neurodegeneration. Similar interactions have been noted for other dietary factors, including caffeine and alcohol, potentially implicating a combination of environmental exposure and genetically determined functional reserve in the aqueous outflow pathways.[61,180]

It would be important for the results of this study to be replicated in independent cohorts and for the sodium-IOP relationship to be probed further in experimental studies, as the presence of an underlying causal association may have important clinical and public health implications, and may lead to targeted lifestyle recommendations for glaucoma.[1] The presence of a significant gene-environment interaction highlights the role that an individual's underlying genetic architecture may play in determining their susceptibility to lifestyle and environmental risk factors, and raises the possibility of precision nutrition and dietary recommendations based on genomic data in the future.[316]

7

Systemic medication

7.1 EPIC-Norfolk

As part of the investigation into the association of systemic medication use with glaucoma and related traits, I was involved in a large collaborative project within the E3 consortium. This exploratory study, jointly led by Dr Joëlle Vergroesen and Dr Alexander Schuster, aimed to meta-analyse the association of systemic medication use with IOP and glaucoma using data from population-based European eye studies. Results of this work were published in *Ophthalmology*.[154] My role was to perform the association analyses within the EPIC-Norfolk Eye Study, results of which were then used for the overall meta-analysis, and these findings are briefly presented here. I am grateful to Dr Robert Luben for his assistance with data curation and for his work on categorising systemic medication use within the cohort. The relevant declaration form for previously published material is located in **Appendix A**. Supplementary material for this section can be found in **Appendix J**.

7.1.1 Introduction

Increasing age is an important risk factor for glaucoma, with prevalence estimates exceeding 10% in individuals aged 85 years and older (see section 3.1). Many other chronic medical conditions are also more common in older individuals, and glaucoma patients, therefore, often suffer from multiple comorbidities, such as hypertension and diabetes mellitus (DM).[381] This, combined with ongoing demographic ageing, means that polypharmacy (the use of multiple medications) has become increasingly prevalent, especially in older individuals,[45,46] and understanding the role that this may play in glaucoma is a particular research priority.

Several classes of medication are known or suspected to modulate the risk of glaucoma, either through an effect on IOP or via IOP-independent mechanisms.[45]

Corticosteroid-induced OHT is a well-established cause of secondary OAG in susceptible individuals,[144,145] while systemic beta-blockers are thought to be protective through an ocular hypotensive effect.[146,147] Certain medications – including statins, metformin, selective serotonin reuptake inhibitors, and postmenopausal hormones – have been implicated as potentially protective in glaucoma, although the evidence for these agents is less consistent.[45] Conversely, associations with higher IOP have been reported for angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), and sulfonylureas.[310] Many of the reported associations, however, are inconsistent and previous studies have often failed to account for important confounders, such as ethnicity, or polypharmacy. For example, the association between statin use and lower IOP may be confounded by concurrent beta blocker use.[146]

Here, I describe a hypothesis-free association study of commonly used systemic medications with IOP and glaucoma in the EPIC-Norfolk Eye Study. These results then contributed to a pooled analysis of 11 independent population-based cohorts, the findings of which I briefly discuss.

7.1.2 Methods

7.1.2.1 EPIC-Norfolk

See section 2.1.1.2.

7.1.2.2 Assessment of systemic medication use

Study participants were asked to bring all their current medication and related documentation with them to the health examination. These were then recorded by a trained research nurse using an electronic case record form.[146] Free text systemic

medication lists were then matched to drug classes using the British National Formulary (BNF) and categorised according to the Anatomical Therapeutic Chemical (ATC) classification system.[382] A full list of medication classes and subgroups used for these analyses are available in **Table J1**. In total, associations were assessed for 11 classes of antihypertensive medications, three lipid-lowering classes, three antidepressant classes, and three antidiabetic medication classes.

7.1.2.3 Assessment of IOP and glaucoma case ascertainment

See section 2.2.1.2. This analysis used Goldmann-correlated IOP to allow for consistency across studies.

7.1.2.4 Assessment of covariables

A standardised set of covariables was selected a priori and these were used by all studies contributing to the overall meta-analysis. These included: age, sex, BMI, SBP, total cholesterol, and DM status. In EPIC-Norfolk,[146,184] height and weight was measured with participants wearing light clothes and no shoes. Height was measured to 0.1 cm using a stadiometer, and weight was measured to the nearest 0.1 kg using digital scales (Tanita UK Ltd, Middlesex, UK). BMI was calculated as weight/height^2. SBP was measured twice with the participant seated at rest using an objective measurement device (Accutorr Plus; Datascope Patient Monitoring, Mindray UK Ltd, Huntington, UK) and the mean of the two measurements was used. Total cholesterol was determined from a blood sample taken at the baseline examination. Individuals were considered to have DM if they met any of the following criteria: self-reported history of a diagnosis of DM, use of antidiabetic medication, or an average HbA1c ≥6.5%. Ethnicity was based on self-report.

7.1.2.5 Statistical analysis

Multivariable linear and logistic regression models were used for the analyses of IOP and glaucoma status, respectively, with current medication use as the explanatory variable. Each medication class or subgroup (according to ATC codes) was initially modelled separately from other medications. For antihypertensive medication, additional analyses were also performed for broader medication subgroups (diuretics, systemic beta blockers, CCBs, and renin-angiotensin system inhibitors). Four sets of regression models were used, with increasing adjustment for covariables. Model 1 was adjusted for age and sex. Model 2 (considered the maximally adjusted model) was adjusted for age, sex, BMI, and DM status. Model 3 included additional adjustment of Model 2 with SBP. Model 4 was only performed for lipid-lowering medications and included further adjustment of Model 2 with total cholesterol. For the analyses of antidiabetic medications, analyses were only performed in individuals with DM. Sensitivity analyses included additional adjustment of Model 2 with ethnicity, combining multiple drug classes in same model, and modelling associations with OAG status (rather than overall glaucoma status).

7.1.3 Results

7.1.3.1 Participants

Overall, 8 623 participants were included in the analysis of glaucoma status and 7 958 in the analysis of IOP. Baseline characteristics for these two cohorts are presented in **Table 7.1**. Participants had a mean age of 68.7 years with a slight predominance of women (55.2%) and were almost exclusively of White ethnicity (99.4%). Glaucoma prevalence was 4.2% with a mean IOP of 16.1 mmHg. The most commonly used medications were statins (20.7%) and ACEIs (12.9%).

Table 7.1 Participant characteristics by cohort

Characteristic	IOP cohort	Glaucoma cohort
Sample size, n	7 958	8 623
Age (years), mean (SD)	68.7 (7.9)	68.7 (8.1)
Sex, n (%)		
Women	4 421 (55.6)	4 762 (55.2)
Men	3 537 (44.4)	3 861 (44.8)
Ethnicity, n (%)		
White	7 912 (99.4)	8 572 (99.4)
Non-White	22 (0.3)	26 (0.3)
Unknown	24 (0.3)	25 (0.3)
Systolic blood pressure (mmHg), mean (SD)	136.1 (16.6)	136.2 (16.6)
Body mass index (kg/m^2), mean (SD)	26.8 (4.3)	26.8 (4.3)
HbA1c (%), mean (SD)	5.8 (0.6)	5.8 (0.6)
Total cholesterol (mmol/L), mean SD	5.4 (1.1)	5.4 (1.1)
Diabetes mellitus, n (%)	238 (3.0)	262 (3.0)
IOP (mmHg), mean (SD)	16.1 (3.7)	–
Glaucoma, n (%)	–	363 (4.2)
Medication use (ATC code), n (%)		
Alpha agonists (C02A)	12 (0.2)	15 (0.2)
Low-ceiling diuretics, thiazides (C03A)	852 (10.7)	920 (10.7)
Low-ceiling diuretics, other (C03B)	23 (0.3)	24 (0.3)
High-ceiling diuretics (C03C)	257 (3.2)	287 (3.3)
Aldosterone antagonists (C03D)	53 (0.7)	61 (0.7)
Nonselective beta blockers (C07AA)	73 (0.9)	78 (0.9)
Selective beta blockers (C07AB)	655 (8.2)	709 (8.2)
Selective CCBs, vascular effects (C08CA)	478 (6.0)	501 (5.8)
Selective CCBs, direct cardiac effects (C08D)	20 (0.3)	23 (0.3)
Angiotensin-converting enzyme inhibitors (C09A)	1 029 (12.9)	1 115 (12.9)
Angiotensin II receptor blockers (C09C)	396 (5.0)	416 (4.8)
Statins (C10AA)	1 693 (21.3)	1 782 (20.7)
Fibrates (C10AB)	15 (0.2)	18 (0.2)
Other lipid-lowering medications (C10AX)	50 (0.6)	52 (0.6)
Nonselective monoamine reuptake inhibitors (N06AA)	203 (2.6)	220 (2.6)
Selective serotonin reuptake inhibitors (N06AB)	235 (3.0)	250 (2.9)
Other antidepressants (N06AX)	46 (0.6)	47 (0.6)
Insulin (A10A)	4 (0.1)	4 (0.1)
Biguanides (A10BA)	176 (2.2)	181 (2.1)
Sulfonylureas (A10BB)	120 (1.5)	131 (1.5)

IOP, intraocular pressure; SD, standard deviation; HbA1c, glycated haemoglobin; ATC, Anatomical Therapeutic Chemical classification; CCB, calcium channel blocker.

7.1.3.2 Associations with IOP

In maximally adjusted regression models, both antihypertensive medication use (-0.27 mmHg; 95% CI, -0.45 to -0.09; *P* = 0.004) and lipid-lowering medication use (-0.37 mmHg; 95% CI, -0.57 to -0.16; *P* <0.001) were associated with lower IOP. The association with antihypertensive medication was largely driven by an association with beta blocker use (-1.07 mmHg; 95% CI, -1.35 to -0.79; *P* <0.001), with similar associations for both selective and nonselective agents. There was also a suggestive association with high ceiling diuretic use (-0.46 mmHg; 95% CI, -0.92 to 0.00; *P* =0.05). The association with lipid-lowering medication use was primarily related to an association with statin use (-0.36 mmHg; 95% CI, -0.57 to -0.16; *P* <0.001). No other significant associations were observed.

Further adjustment for SBP and total cholesterol did not materially alter the results. Similar findings were obtained when restricting analyses to White participants only. In models accounting for polypharmacy, the association with statin use was no longer significant when accounting for concurrent beta blocker use, as has been described previously.[146] Full results of these analyses are presented in **Table 7.2**.

7.1.3.3 Associations with glaucoma status

In maximally adjusted regression models, only 'other antidepressant' use was found to be associated with glaucoma (OR, 3.26; 95% CI,1.25–8.47; *P* = 0.016). Additionally, there was also a suggestive association with selective CCBs with vascular effects (OR, 1.43; 95% CI, 1.00–2.05; *P* =0.05). No other medication class was found to be associated with glaucoma status.

Results were materially unchanged in the various sensitivity analyses, although the association with selective CCBs with vascular effects strengthened after additional

adjustment for SBP and when restricting analyses to White participants only. Full results of these analyses are presented in **Table 7.3**.

Table 7.2 Association of systemic medication use with IOP in EPIC-Norfolk

ATC code	Description	Beta (95% CI)	*P*-value
Antihypertensives			
C02A	Alpha agonists	0.29 (-1.76, 2.36)	0.78
	Diuretics	-0.02 (-0.26, 0.22)	0.87
C03A	Low-ceiling diuretics, thiazides	0.08 (-0.18, 0.35)	0.53
C03B	Low-ceiling diuretics, other	0.07 (-1.43, 1.57)	0.93
C03C	High-ceiling diuretics	-0.46 (-0.92, 0.00)	0.05
C03D	Aldosterone antagonists	-0.53 (-1.52, 0.47)	0.30
	Beta blockers	**-1.07 (-1.35, -0.79)**	**<0.001**
C07AA	Nonselective beta blockers	**-1.29 (-2.13, -0.45)**	**0.003**
C07AB	Selective beta blockers	**-1.05 (-1.35, -0.76)**	**<0.001**
	Calcium channel blockers	-0.15 (-0.49, 0.18)	0.37
C08CA	Selective CCBs, vascular effects	-0.15 (-0.49, 0.19)	0.39
C08D	Selective CCBs, direct cardiac effects	-0.22 (-1.82, 1.38)	0.79
	Renin-angiotensin system	-0.09 (-0.31, 0.12)	
C09A	Angiotensin-converting enzyme inhibitors	-0.04 (-0.29, 0.20)	0.74
C09C	Angiotensin II receptor blockers	-0.16 (-0.54, 0.21)	0.39
	Any antihypertensive	**-0.27 (-0.45, -0.09)**	**0.004**
Lipid-lowering medication			
C10AA	Statins	**-0.36 (-0.57, -0.16)**	**0.001**
C10AB	Fibrates	0.43 (-1.42. 2.29)	0.65
C10AX	Other lipid-lowering medications	-0.54 (-1.56, 0.47)	0.30
	Any lipid-lowering	**-0.37 (-0.57, -0.16)**	**<0.001**
Antidepressants			
N06AA	Nonselective monoamine reuptake inhibitors	-0.22 (-0.73, 0.29)	0.41
N06AB	Selective serotonin reuptake inhibitors	-0.18 (-0.65, 0.30)	0.47
N06AX	Other antidepressants	-0.58 (-1.64, 0.48)	0.28
	Any antidepressant	-0.24 (-0.58, 0.11)	0.18
Antidiabetic medication			
A10A	Insulin	-1.20 (-5.25, 2.84)	0.56
A10BA	Biguanides	0.37 (-0.60, 1.34)	0.45
A10BB	Sulfonylureas	-0.07 (-1.09, 0.95)	0.89
	Any antidiabetic	0.72 (-0.22, 1.66)	0.13

ATC, Anatomical therapeutic Chemical classification; CI, confidence interval; CCB, calcium channel blocker.

Table 7.3 Association of systemic medication use with glaucoma in EPIC-Norfolk

ATC code	Description	Odds ratio (95% CI)	*P*-value
Antihypertensives			
C02A	Alpha agonists	1.97 (0.25, 15.40)	0.52
	Diuretics	1.00 (0.75, 1.33)	0.99
C03A	Low-ceiling diuretics, thiazides	0.88 (0.63, 1.22)	0.43
C03B	Low-ceiling diuretics, other	0.97 (0.13, 7.37)	0.98
C03C	High-ceiling diuretics	1.29 (0.82, 2.03)	0.28
C03D	Aldosterone antagonists	1.03 (0.36, 2.91)	0.96
	Beta blockers	0.86 (0.60, 1.22)	0.40
C07AA	Nonselective beta blockers	1.39 (0.55, 3.50)	0.48
C07AB	Selective beta blockers	0.79 (0.54, 1.16)	0.23
	Calcium channel blockers	1.37 (0.96, 1.97)	0.09
C08CA	Selective CCBs, vascular effects	**1.43 (1.00, 2.05)**	**0.05**
C08D	Selective CCBs, direct cardiac effects	–	–
	Renin-angiotensin system	0.92 (0.70, 1.21)	0.56
C09A	Angiotensin-converting enzyme inhibitors	0.84 (0.62, 1.15)	0.27
C09C	Angiotensin II receptor blockers	1.07 (0.68, 1.66)	0.78
	Any antihypertensive	1.00 (0.80, 1.26)	0.98
Lipid-lowering medication			
C10AA	Statins	0.98 (0.76, 1.27)	0.90
C10AB	Fibrates	1.55 (0.20, 11.99)	0.68
C10AX	Other lipid-lowering medications	0.38 (0.05, 2.82)	0.35
	Any lipid-lowering	0.98 (0.76, 1.26)	0.85
Antidepressants			
N06AA	Nonselective monoamine reuptake inhibitors	0.64 (0.28, 1.45)	0.28
N06AB	Selective serotonin reuptake inhibitors	0.89 (0.43, 1.83)	0.75
N06AX	Other antidepressants	**3.26 (1.25, 8.47)**	**0.016**
	Any antidepressant	0.94 (0.57, 1.53)	0.79
Antidiabetic medication			
A10A	Insulin	–	–
A10BA	Biguanides	0.61 (0.06, 6.22)	0.67
A10BB	Sulfonylureas	4.84 (0.48, 48.78)	0.18
	Any antidiabetic	–	–

ATC, Anatomical therapeutic Chemical classification; CCB, calcium channel blocker.

7.1.4 Discussion

In this cross-sectional analysis of the EPIC-Norfolk cohort, the use of systemic beta blockers was consistently and significantly associated with lower IOP, confirming the known ocular hypotensive effect of this medication class.[45] This relationship was first reported more than 50 years ago,[383] and formed the basis for the subsequent development of topical beta blockers for the treatment of glaucoma. Beta blockers exert their IOP-lowering effect by blockade of sympathetic activity at the ciliary epithelium, resulting in a reduction of aqueous humour production. This association persisted in the overall E3 meta-analysis, and was robust to various sensitivity analyses accounting for ethnicity, SBP, mono- and polypharmacy, and different glaucoma case definitions.[154]

The suggestive association between high-ceiling diuretics and lower IOP was also observed in the E3 meta-analysis, but results did not persist in sensitivity analyses. Although associated with lower IOP in the EPIC-Norfolk cohort, statins were not associated with IOP in the overall meta-analysis. All remaining medication classes were also not found to be associated with IOP in the E3 meta-analysis.

The apparent association between ‘other antidepressants’ and higher glaucoma prevalence in EPIC-Norfolk did not persist in the E3 meta-analysis and, given the small number of users, may represent a chance finding. However, the suggestive adverse association with CCBs was replicated in the overall meta-analysis, with CCB use associated with a 23% higher prevalence of glaucoma.[154] Similar associations were observed for both CCB subgroups and these results were robust to sensitivity analyses accounting for ethnicity, SBP, mono- and polypharmacy, and different glaucoma case definitions.

Overall, higher glaucoma prevalence was also found with RAS inhibitors, statins, nonselective monoamine reuptake inhibitors, and insulin; however, none of the relationships persisted in sensitivity analyses.

In conclusion, findings from the EPIC-Norfolk, with subsequent replication in the E3 consortium, confirm and quantify the known association of systemic beta blockers with lower IOP. Additionally, an adverse association with CCB use was identified. This finding is in keeping with several previous epidemiological studies,[147,152,153] and is examined in more detail in section 7.2 below.

7.2 UK Biobank

In parallel with the E3 meta-analysis described in the section above, I conducted a detailed analysis of the association of CCB use with glaucoma and related traits in the UK Biobank. The following section is a modified version of a paper published in *JAMA Ophthalmology*[384] and describes these analyses. This topic was originally addressed by Dr Alan Kastner and the results presented at the 2020 ARVO annual meeting.[385] I am grateful to Dr Kastner for allowing me the opportunity to build on this work and for providing me with his code list used to identify CCB users in the UK Biobank. I was responsible for performing all the revised analyses described below, and for drafting and revising the published manuscript. The relevant declaration form for previously published material is located in **Appendix A**. Supplementary material for this section can be found in **Appendix K**.

7.2.1 Abstract

Importance: CCB use has been associated with an increased risk of glaucoma in exploratory studies.

Objective: To examine the association of systemic CCB use with glaucoma and related traits in the UK Biobank.

Design: Cross-sectional study (2006–2010).

Setting: Population-based.

Participants: I included 427 480, 97 100, and 41 023 participants with complete data for the analyses of glaucoma status, IOP, and OCT-derived inner retinal layer thicknesses, respectively.

Exposure: CCB use assessed in a baseline touchscreen questionnaire and confirmed during a trained nurse-led interview.

Main outcome measures: Glaucoma status, corneal-compensated IOP, mRNFL thickness, and GCIPL thickness.

Results: Among all included participants (median age 58 years, 54.1% women, 94.8% White), 33 175 (7.8%) were CCB users. After adjustment for key sociodemographic, medical, anthropometric, and lifestyle factors, the use of CCBs (but not other antihypertensives) was associated with greater odds of glaucoma (OR, 1.39; 95% CI, 1.14–1.69; *P* = 0.001). CCB use was also associated with thinner GCIPL (-0.34 μm; 95% CI, -0.54 to -0.15: *P* = 0.001) and thinner mRNFL (-0.16 μm; 95% CI, -0.30 to -0.02; *P* = 0.03), but not IOP (-0.01 mmHg; 95% CI, -0.09 to 0.07; *P* = 0.84).

Conclusion and relevance: I identified an adverse association between CCB use and glaucoma, with CCB users, on average, having 39% higher odds of glaucoma. CCB use was also associated with a thinner mRNFL and GCIPL, providing a structural basis that supports the association with glaucoma. The lack of an association with IOP suggests that an IOP-independent mechanism of glaucomatous neurodegeneration may be involved. Although a causal relationship has not been established, CCB replacement or withdrawal may be a consideration should a glaucoma patient continue to progress despite optimal care.

7.2.2 Introduction

CCBs are a commonly used class of medication, frequently prescribed in the management of various cardiovascular diseases, particularly hypertension. Up to

40% of patients with hypertension are prescribed a CCB and, across all medication classes, CCBs account for almost 4% of all primary care prescriptions in UK).[386,387]

CCB use has been associated with incident glaucoma requiring a procedural treatment in a large exploratory study of insurance claims data in the US.[151] Although the study was limited by a lack of detailed clinical findings and was not able to account for potentially important confounding factors, including ethnicity and comorbidities, this result is consistent with several previous population-based studies which have demonstrated similar associations.[147,152–154]

Given the global prevalence of both hypertension and glaucoma,[7,388] and the fact that the two conditions frequently co-exist,[152,381] this association may have important clinical implications for millions of individuals worldwide and warrants further investigation. This may be particularly relevant in ageing and elderly populations, such as the UK and US, where multimorbidity is a common occurrence.[389]

Limited experimental data have suggested that CCBs may have an acute ocular hypotensive effect, especially in individuals with glaucoma.[390,391] It would therefore also be important to assess whether CCB use is associated with IOP on a population level, as this may offer insights into potential underlying pathophysiological mechanisms. Additionally, the use of objective structural glaucoma-related biomarkers may mitigate misclassification bias and help validate any observed associations with glaucoma.

I therefore aimed to examine the association of CCB use with glaucoma in a large cohort using data from the UK Biobank data resource. I further explored associations with IOP and two OCT-derived inner retinal thickness parameters.

7.2.3 Methods

7.2.3.1 Reporting guidelines

This study is reported in accordance with the STROBE guidelines. The completed checklist is available in **Figure K1**.

7.2.3.2 Study population

See section 2.1.1.1.

7.2.3.3 Assessment of calcium channel blocker use

CCB use was assessed in the baseline UK Biobank questionnaire (2006–2010). All self-reported medications were recorded and subsequently confirmed by a trained nurse in an interview conducted during the same visit. Medications were then matched to a comprehensive drug list obtained from the British National Formulary (78th edition).

Antihypertensives were grouped according to the following classes: CCBs (dihydropyridine, phenylalkylamine, benzothiazepine, and other), diuretics (thiazide, loop, and potassium-sparing), RAS inhibitors (angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers), and systemic beta blockers. The full code list comprising the CCB medication class and its subtypes is available online is available in **Table K1**. No information was recorded regarding the dosage, frequency, or time each medication was in use.

7.2.3.4 Glaucoma-related outcome measures and case ascertainment

See section 2.2.1.1.

7.2.3.5 Covariables

I also considered a variety of demographic, lifestyle, and systemic health status variables in my analyses to account for potential confounding bias. These variables were selected a priori and included: age, sex, self-reported ethnicity, education level, Townsend deprivation index, diabetes, BMI, total cholesterol, smoking status, and alcohol consumption frequency. Full details of UK Biobank covariables are available in section 2.2.4.

7.2.3.6 Statistical analysis

Baseline participant characteristics, stratified by CCB use, were described and compared using a two-sample t-test or test of proportion, where appropriate. I examined the association of CCB use with glaucoma prevalence using multivariable logistic regression, adjusted for all the covariables described above. I then performed similar analyses for any antihypertensive medication use and for the other major antihypertensive medication classes (diuretics, RAS inhibitors, and systemic beta blockers) to gauge whether the observed CCB association represented a class-specific effect or a general effect across all antihypertensive medications.

To aid direct comparability of results, associations with IOP, GCIPL, and mRNFL were assessed using multivariable linear regression models adjusted for the same covariables as used in the glaucoma analysis. To address potential confounding by indication, I assessed the effect of further adjustment for mean SBP. Finally, I considered all associations according to three CCB subtypes (dihydropyridines, phenylalkylamines, and benzothiazepines).

All statistical analyses were performed using Stata (Version 17.0. StataCorp LLC. 2021. College Station, TX, USA). *P*-values were two sided and were not adjusted for multiple comparisons.

7.2.3.7 Sensitivity analyses

I performed sensitivity analyses using alternative case definitions, including: any ICD-coded glaucoma; ICD-coded POAG only; self-report and/or any ICD-coded glaucoma; self-report and/or ICD-10 coded POAG/unspecified glaucoma; and self-report and/or ICD-coded POAG. I additionally assessed whether the main association with glaucoma was modified by hypertension, sex, or ethnicity.

To address the possibility that the association with IOP may be influenced by ocular hypotensive medication, I excluded all participants reporting topical glaucoma therapy use. Lastly, I repeated the primary analyses with further adjustment for refractive error (mean SE) and a glaucoma PRS.[27]

7.2.4 Results

7.2.4.1 Participants

The participant selection process is outlined in **Figure 7.1**. I included 427 480, 97 100, 40 486, and 40 583 participants with complete data for the analyses of glaucoma status, IOP, GCIPL thickness, and mRNFL thickness, respectively. Median age at baseline was 58 years (IQR, 50–63 years), with a predominance of female (54.1%) and White (94.8%) participants. Of all included participants, 114 311 (26.7%) had a history of physician-diagnosed systemic hypertension and there were 33 175 (7.8%) CCB users (29 508 with hypertension [89.0%] and 3 667 without hypertension [11.0%]).

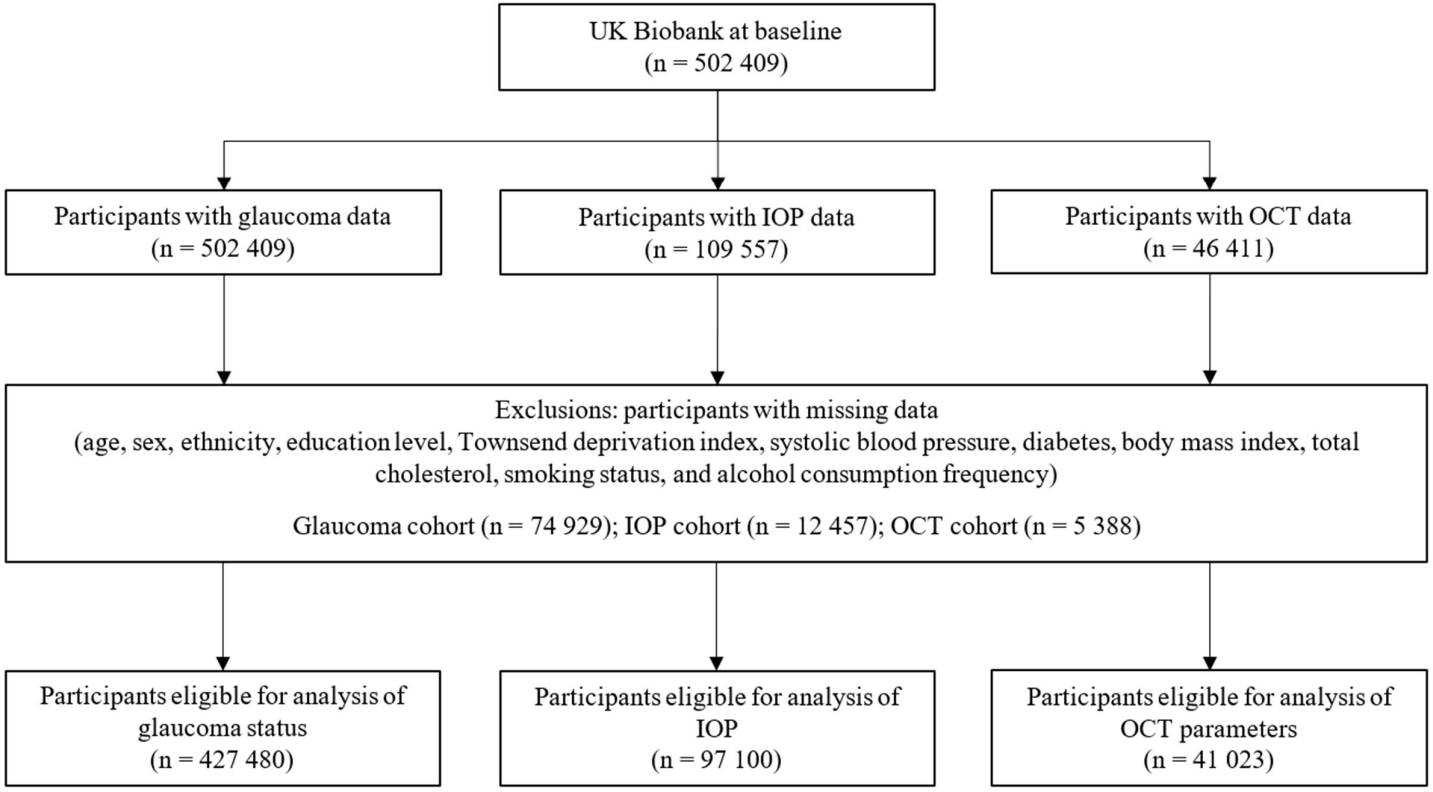

Figure 7.1 Flowchart outlining eligible participants for this study in the UK Biobank

IOP, intraocular pressure; OCT, optical coherence tomography.

Baseline participant characteristics, stratified by CCB use, are presented in **Table 7.4**. CCB users were more likely to be older, men, Black, less educated, more deprived, hypertensive, diabetic, have higher SBP and BMI, and lower total cholesterol than non-users. Lower average total cholesterol levels in CCB users may be the result of a difference in statin use between groups (CCB users, 52.1%; non-users, 14.5%; *P* <0.001). Participants reporting CCB use also had a higher glaucoma prevalence, higher average IOP, thinner average GCIPL thickness, and thinner average mRNFL thickness than non-users.

7.2.4.2 Association of antihypertensive medication use with glaucoma status

In maximally-adjusted regression models, antihypertensive medication use was adversely associated with glaucoma (OR, 1.29; 95% CI, 1.10–1.52; *P* = 0.002). This association appeared to be driven by CCB use (OR, 1.39; 95% CI, 1.14–1.69; *P* = 0.001), with no association demonstrated for diuretic (35 099 users; OR, 1.03; 95% CI, 0.84–1.28; *P* = 0.75), RAS inhibitor (55 983 users; OR, 1.12; 95% CI, 0.93–1.34; *P* = 0.24), or systemic beta blocker (29 818 users; OR, 0.93; 95% CI, 0.74–1.18; *P* = 0.56) use (**Table 7.5**). Associations were materially unchanged when additionally adjusting for SBP and concurrent use of more than one antihypertensive medication class.

Table 7.4 Characteristics of eligible UK Biobank participants by calcium channel blocker use

Description	CCB user (n = 33 175)	CCB non-user (n = 394 305)	Difference (95% CI)	*P*-value
Age (years), mean (SD)	61.2 (6.2)	56.1 (8.1)	5.0 (4.9, 5.1)	**<0.001**
Sex				
Women	13 473 (40.6)	217 860 (55.3)	-14.6 (-15.2, -14.1)	**<0.001**
Men	19 702 (59.4)	176 445 (44.7)	14.6 (14.1, 15.2)	**<0.001**
Ethnicity				
White	30 548 (92.1)	374 853 (95.1)	-3.0 (-3.3, -2.7)	**<0.001**
Asian	814 (2.5)	7 058 (1.8)	0.7 (0.5, 0.8)	**<0.001**
Black	1 211 (3.7)	5 406 (1.4)	2.3 (2.1, 2.5)	**<0.001**
Other/Mixed	602 (1.8)	6 988 (1.8)	0.0 (-0.1, 0.2)	0.57
Education level				
Less than O-level	14 975 (45.1)	131 830 (33.4)	11.7 (11.1, 12.3)	**<0.001**
O-level	6 792 (20.5)	85 765 (21.8)	-1.3 (-1.7, -0.8)	**<0.001**
A-level	3 064 (9.2)	45 083 (11.4)	-2.2 (-2.5, -1.9)	**<0.001**
Degree	8 344 (25.2)	131 627 (33.4)	-8.2 (-8.7, -7.7)	**<0.001**
Townsend deprivation index, mean (SD)	-1.0 (3.2)	-1.4 (3.0)	0.4 (0.4, 0.4)	**<0.001**
Hypertension				
No	3 667 (11.1)	309 502 (78.5)	-67.4 (-67.8, -67.1)	**<0.001**
Yes	29 508 (88.9)	84 803 (21.5)	67.4 (67.1, 67.8)	**<0.001**
Diabetes				
No	27 635 (83.3)	377 109 (95.6)	-12.3 (-12.7, -11.9)	**<0.001**
Yes	5 540 (16.7)	17 196 (4.4)	12.3 (11.9, 12.7)	**<0.001**
Systolic blood pressure (mmHg), mean (SD)	145.8 (17.1)	137.1 (18.6)	8.7 (8.5, 8.9)	**<0.001**
Body mass index (kg/m^2), mean (SD)	29.4 (4.8)	27.2 (4.4)	2.2 (2.2, 2.3)	**<0.001**
Total cholesterol (mmol/L), mean (SD)	5.2 (1.2)	5.7 (1.1)	-0.6 (-0.6, -0.5)	**<0.001**
Smoking status				
Never	15 659 (47.2)	218 226 (55.3)	-8.1 (-8.7, -7.6)	**<0.001**
Former	14 321 (43.2)	135 058 (34.3)	8.9 (8.3, 9.5)	**<0.001**
Current	3 195 (9.6)	41 021 (10.4)	-0.8 (-1.1, -0.4)	**<0.001**
Alcohol consumption frequency				
Never or special occasions only	7 591 (22.9)	73 792 (18.7)	4.2 (3.7, 4.6)	**<0.001**
1–3 times per month	3 208 (9.7)	44 222 (11.2)	-1.5 (-1.9, -1.2)	**<0.001**
1–2 times per week	7 730 (23.3)	102 561 (26.0)	-2.7 (-3.2, -2.2)	**<0.001**
3–4 times per week	7 014 (21.1)	92 701 (23.5)	-2.4 (-2.8, -1.9)	**<0.001**
Daily or almost daily	7 632 (23.0)	81 029 (20.6)	2.5 (2.0, 2.9)	**<0.001**
Statin use	17 294 (52.1)	56 983 (14.5)	37.7 (37.1, 38,2)	**<0.001**
Glaucoma prevalence	137 (0.4)	652 (0.2)	0.2 (0.2, 0.3)	**<0.001**
Intraocular pressure (mmHg), mean (SD)[1]	16.4 (3.7)	16.0 (3.4)	0.4 (0.3, 0.5)	**<0.001**
GCIPL thickness (µm), mean (SD)[2]	74.2 (5.3)	75.3 (5.2)	-1.1 (-0.9, 1.3)	**<0.001**
mRNFL thickness (µm), mean (SD)[3]	28.2 (3.8)	29.0 (3.8)	-0.8 (-0.9, -0.6)	**<0.001**

[1] *N* = 97 100; [2] *N* = 40 486; [3] *N* = 40 583.

Figures represent counts (n) and percentages (%), unless otherwise stated.
CCB, calcium channel blocker; CI, confidence interval; GCIPL, ganglion cell-inner plexiform layer; mRNFL, macular retinal nerve fibre layer; SD, standard deviation.

Table 7.5 Association of antihypertensive medication use with glaucoma in the UK Biobank

Description	Model A[1]			Model B[2]		
	Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Any antihypertensive medication	**1.29**	**1.10, 1.52**	**0.002**	N/A	N/A	N/A
Antihypertensive medication class						
Calcium channel blockers	**1.39**	**1.14, 1.69**	**0.001**	**1.39**	**1.13, 1.70**	**0.001**
Diuretics	1.03	0.84, 1.28	0.75	0.96	0.77, 1.20	0.75
Renin angiotensin system inhibitors	1.12	0.93, 1.34	0.24	1.07	0.88, 1.30	0.47
Systemic beta blockers	0.93	0.74, 1.18	0.56	0.90	0.71, 1.14	0.39

[1] Model A adjusted for: age (years), sex (women, men), self-reported ethnicity (White, Asian, Black, Other/Mixed), education level (less than O-level, O-level, A-level, degree), Townsend deprivation index (units), diabetes (no, yes), body mass index (kg/m^2), total cholesterol (mmol/L), smoking status (never, former, current), and alcohol consumption frequency (never or special occasion only, 1–3 times per month, 1–2 times per week, 3–4 times per week, daily or almost daily).

[2] Model B adjusted for: as for Model A, plus additional adjustment for systolic blood pressure (mmHg), and simultaneous use of other antihypertensive medications.

CI, confidence interval; N/A, not applicable.

7.2.4.3 Association of CCB use with glaucoma and related traits

Results for the association of CCB use with glaucoma and related traits are presented in **Table 7.6**. The main association with glaucoma status (OR, 1.39; 95% CI, 1.14–1.69; *P* = 0.001) was unchanged by the inclusion of SBP to the model. CCB use was also associated with thinner OCT-derived inner retinal parameters, with only slight attenuation of the associations after further adjustment for SBP. Those reporting the use of CCBs had thinner GCIPL (-0.34 µm; 95% CI, -0.54 to -0.15; *P* = 0.001) and mRNFL (-0.16 µm; 95% CI, -0.30 to -0.02; *P* = 0.03) than non-users. In maximally-adjusted regression models, CCB use was not associated with IOP (-0.01 mmHg; 95% CI -0.09 to 0.07; *P* = 0.84). Further adjustment for SBP, however, resulted in an association with lower IOP (-0.15 mmHg; 95% CI -0.23 to -0.07; *P* <0.001). The complete results of the models for glaucoma status, IOP, and OCT-derived inner retinal parameters are available in **Table K2** and **Table K3.**

7.2.4.4 Association of CCB subtypes with glaucoma and related traits

Dihydropyridines (e.g., amlodipine) were by far the most used CCB subtype (n = 29 314, 88.4%), followed by benzothiazepines (e.g., diltiazem, n = 3 022, 9.1%) and phenylalkylamines (e.g., verapamil, n = 951, 2.9%). There were no 'other CCB' users. The associations for dihydropyridine users were consistent with the results of the main analyses (**Table 7.7**). Benzothiazepine users had higher odds of glaucoma (OR, 1.80; 95% CI, 1.14–2.86; *P* = 0.01) and lower IOP (-0.51 mmHg; 95% CI -0.77 to -0.24; *P* <0.001), but no association with GCIPL or mRNFL thickness. There were no associations for phenylalkylamine users.

Table 7.6 Association of calcium channel blocker use with glaucoma and related traits in the UK Biobank

Outcome (unit)	Sample size	Model A[1]			Model B[2]		
		Effect estimate	95% CI	*P*-value	Effect estimate	95% CI	*P*-value
Glaucoma (odds ratio)	427 480	**1.39**	**1.14, 1.69**	**0.001**	**1.39**	**1.14, 1.69**	**0.001**
Intraocular pressure (mmHg)	97 100	-0.01	-0.09, 0.07	0.84	**-0.15**	**-0.23, -0.07**	**<0.001**
GCIPL thickness (μm)	40 486	**-0.34**	**-0.54, -0.15**	**0.001**	**-0.31**	**-0.50, -0.11**	**0.001**
mRNFL thickness (μm)	40 583	**-0.16**	**-0.30, -0.02**	**0.03**	**-0.14**	**-0.29, 0.00**	**0.049**

[1] Model A adjusted for: age (years), sex (women, men), self-reported ethnicity (White, Asian, Black, Other/Mixed), education level (less than O-level, O-level, A-level, degree), Townsend deprivation index (units), diabetes (no, yes), body mass index (kg/m^2), total cholesterol (mmol/L), smoking status (never, former, current), and alcohol consumption frequency (never or special occasion only, 1–3 times per month, 1–2 times per week, 3–4 times per week, daily or almost daily).

[2] Model B adjusted for: as for Model A, plus additional adjustment for systolic blood pressure (mmHg).

CI, confidence interval; GCIPL, ganglion cell-inner plexiform layer; mRNFL, macular retinal nerve fibre layer.

Table 7.7 Association of calcium channel blocker subtypes with glaucoma and related traits in the UK Biobank

Outcome (unit)	Dihydropyridine CCBs (29 314 users)			Phenylalkylamine CCBs (951 users)			Benzothiazepine CCBs (3 022 users)		
	Effect estimate	95% CI	*P*-value	Effect estimate	95% CI	*P*-value	Effect estimate	95% CI	*P*-value
Model A[1]									
Glaucoma (odds ratio)	**1.33**	**1.08, 1.63**	**0.007**	0.99	0.32, 3.09	0.99	**1.80**	**1.14, 2.86**	**0.01**
IOP (mmHg)	0.03	-0.05, 0.11	0.45	0.17	-0.28, 0.63	0.46	**-0.51**	**-0.77, -0.24**	**<0.001**
GCIPL thickness (μm)	**-0.36**	**-0.57, -0.16**	**<0.001**	-0.78	-1.82, 0.25	0.14	0.13	-0.52, 0.77	0.70
mRNFL thickness (μm)	**-0.17**	**-0.32, -0.02**	**0.02**	0.01	-0.75, 0.77	0.98	-0.10	-0.57, 0.37	0.68
Model B[2]									
Glaucoma (odds ratio)	**1.33**	**1.08, 1.64**	**0.006**	0.99	0.32, 3.09	0.99	**1.80**	**1.14, 2.86**	**0.01**
IOP (mmHg)	**-0.12**	**-0.20, -0.04**	**0.005**	0.11	-0.34, 0.56	0.62	**-0.50**	**-0.76, -0.23**	**<0.001**
GCIPL thickness (μm)	**-0.32**	**-0.53, -0.12**	**0.002**	-0.76	-1.80, 0.27	0.15	0.12	-0.53, 0.76	0.73
mRNFL thickness (μm)	**-0.16**	**-0.30, -0.01**	**0.04**	0.01	-0.74, 0.77	0.97	-0.11	-0.58, 0.37	0.66

[1] Model A adjusted for: age (years), sex (women, men), self-reported ethnicity (White, Asian, Black, Other/Mixed), education level (less than O-level, O-level, A-level, degree), Townsend deprivation index (units), diabetes (no, yes), body mass index (kg/m^2), total cholesterol (mmol/L), smoking status (never, former, current), and alcohol consumption frequency (never or special occasion only, 1–3 times per month, 1–2 times per week, 3–4 times per week, daily or almost daily).

[2] Model B adjusted for: as for Model A, plus additional adjustment for systolic blood pressure (mmHg).

CCB, calcium channel blocker; CI, confidence interval; IOP, intraocular pressure; GCIPL, ganglion cell-inner plexiform layer; mRNFL, macular retinal nerve fibre layer.

7.2.4.5 Sensitivity analyses

Sensitivity analyses using alternative glaucoma case definitions are presented in **Table K4**. Overall, analyses including self-report as a component of the case definition showed weaker associations than those based on ICD-codes alone. Of the various glaucoma definitions used, only the narrowest ICD-coded definition of POAG (476 cases) did not demonstrate an association with CCB use.

There was evidence that the association between CCB use and glaucoma was modified by a history of physician-diagnosed hypertension (**Figure 7.2**). In the maximally-adjusted regression model, including adjustment for baseline SBP, CCB use in those *without* hypertension (OR, 2.01; 95% CI, 1.26–3.21; *P* = 0.003) was associated with higher odds of glaucoma than CCB use in those *with* hypertension (OR, 1.47; 95% CI, 1.18–1.84; *P* = 0.001) (OR for interaction, 0.59; 95% CI, 0.35–0.98; *P* = 0.04).

There was no evidence of a differential effect by sex or ethnicity for the association with glaucoma. Results for IOP were materially unchanged when restricting analyses to participants not using ocular hypotensive agents (-0.06 mmHg; 95% CI, -0.13 to 0.01; *P* = 0.15). Further adjustment for spherical equivalent and a glaucoma PRS resulted in a substantial sample size reduction (*n* = 84 924), but a similar adverse association with glaucoma (OR, 1.59; 95% CI, 1.04–2.45; *P* = 0.03).

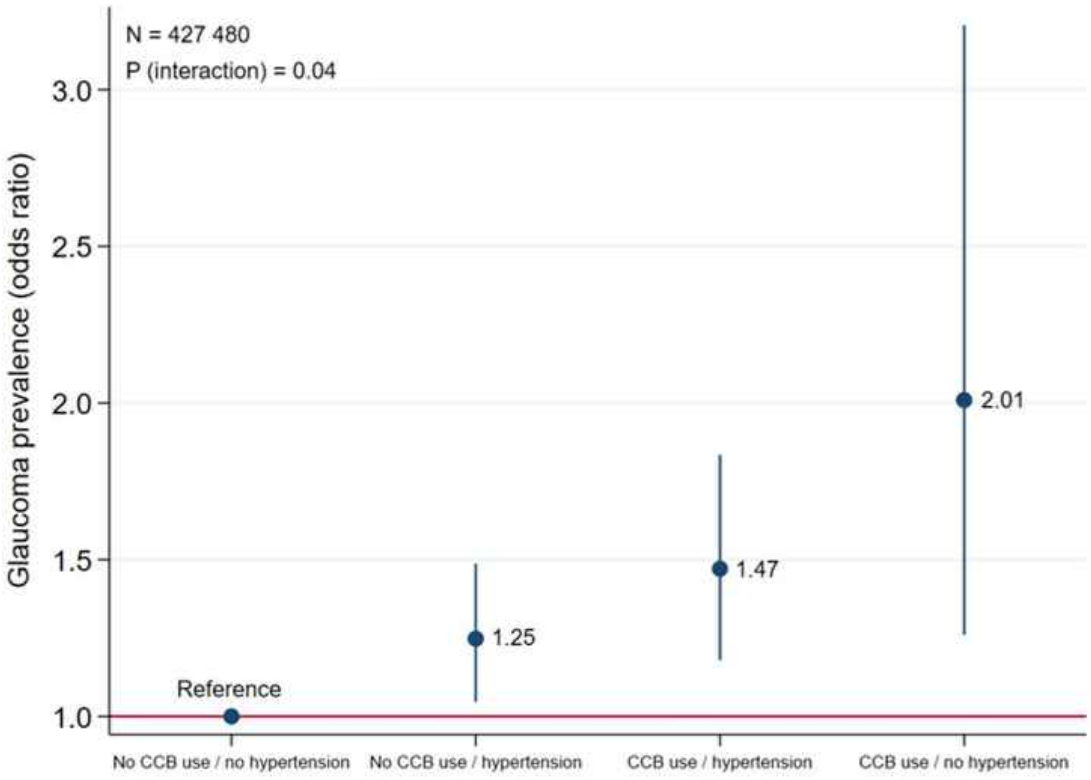

Figure 7.2 Interaction of calcium channel blocker use and hypertension for the association with glaucoma in the UK Biobank

Based on a multivariable logistic regression model including a multiplicative interaction term between calcium channel blocker use and a history of physician-diagnosed hypertension, and adjusted for: age (years), sex (women, men), self-reported ethnicity (White, Asian, Black, Other/Mixed), education level (less than O-level, O-level, A-level, degree), Townsend deprivation index (units), diabetes (no, yes), body mass index (kg/m^2), total cholesterol (mmol/L), smoking status (never, former, current), alcohol consumption frequency (never or special occasion only, 1–3 times per month, 1–2 times per week, 3–4 times per week, daily or almost daily), and systolic blood pressure (mmHg).

CCB, calcium channel blocker.

7.2.5 Discussion

In this large population-based study, I found that CCB users had, on average, 39% higher odds of glaucoma than non-users, after controlling for multiple potential confounders. Consistent with this finding, I also demonstrated that GCIPL and mRNFL (both objective structural glaucoma-related parameters) were thinner in CCB users. CCB use was not found to be associated with IOP.

An adverse association between CCB use and glaucoma has previously been demonstrated in both cross-sectional and longitudinal studies.[147,151–153] In a large US insurance claims study, CCBs demonstrated the strongest adverse statistical association with glaucoma of 423 different medication classes.[151] Similarly, amlodipine (a dihydropyridine CCB) was found to have the strongest statistical association with glaucoma of all 1 723 unique generic medications studied.[151] This analysis was, however, limited by a lack of data on potential confounders which may have resulted in biased results. For example, participant ethnicity was not available and the observed association may have been driven by a higher prevalence of CCB use among individuals of African descent (an important risk factor for glaucoma), in whom CCBs are standard first-line therapy.[392]

These analyses provide further large-scale evidence supporting these previously reported associations and suggest that the adverse association between CCB use and glaucoma risk may act via IOP-independent mechanisms. While the primary analyses were based on a strict case definition which is likely to underestimate true prevalence, sensitivity analyses using less specific glaucoma definitions and conducted in up to 7 000 cases (including more than 900 CCB users) demonstrated similar associations.

To the best of my knowledge, there has been no published report of an adverse association between CCB use and glaucoma-related inner retinal parameters. A previous study of antihypertensive use from southeast Asia found no association between CCBs with average GCIPL or pRNFL thickness.[393] While these reported effect estimates for GCIPL and mRNFL thicknesses may seem small, on a population-level they are equivalent to the average difference seen between participants separated by four years in age.[74]

While limited experimental data have suggested that systemic CCBs may have an acute ocular hypotensive effect, especially in individuals with glaucoma,[390,391] this is not always a consistent finding.[394] I found no difference in average IOP between CCB users and non-users, however, this may be related to IOP assessment being limited to a single measurement, and I cannot fully exclude the possibility of a small effect on IOP. This result is consistent with a recent large meta-analysis of European population-based eye studies which also found an adverse association between CCB use and glaucoma status, but no relationship with IOP.[154]

It is also important to note that this study lacked data on length, frequency, or dosage of CCB use, and whether the medication was taken on the day of IOP assessment, and the findings may therefore not fully account for the potential effect of CCBs on IOP. Although an association with lower IOP was observed after additional adjustment for baseline SBP, this may be the result of collider bias. Both CCB use (through a direct effect) and higher IOP (indirectly via treatment with topical beta blockers) may influence SBP, and adjusting for this factor may induce an artificial relationship between the two.

The implication that CCBs have a direct detrimental effect on retinal tissue is contrary to the general view of these agents being neuroprotective. In vitro studies

have shown that CCBs exert protective effects on neurons undergoing apoptosis and necrosis, and these effects have also been documented in RGCs and photoreceptors in experimental animal models.[395] This is thought to be related to the inhibition of calcium influx-mediated apoptotic pathways. Additionally, several small interventional studies have demonstrated that CCBs increase retrobulbar and ONH blood flow, improve colour contrast sensitivity, and may stabilise visual field loss in individuals with NTG.[148,150,396,397]

While the reasons for this apparent discrepancy are unclear, a simple explanation has been proposed: in vitro studies do not account for the blood pressure-lowering effects of CCBs, and the CCBs investigated in the visual field studies had no appreciable effect on blood pressure in glaucoma cases. It may be that the detrimental effects of CCBs are only manifest when coupled with the hypotensive and/or vasodilatory properties of certain CCBs, such as amlodipine.[395] This hypothesis may be supported by my interaction sensitivity analysis, in which I found that CCB use was associated with higher odds of glaucoma in those without hypertension, compared to those with hypertension, suggesting that a history of higher blood pressure may partially ameliorate the adverse association with glaucoma.

While adverse associations with glaucoma were demonstrated for both dihydropyridine and benzothiazepine users, I found no evidence for an adverse association with phenylalkylamine CCBs (which are relatively selective for the myocardium and have little effect on SBP), although these analyses may have been limited by reduced statistical power due to a relatively small number of users. Alternatively, changes in calcium homeostasis may affect mitochondrial function

which may make neurons more vulnerable to processes such as oxidative stress.[398,399]

The strengths of this study include the large sample size, allowing for the detection of small, but meaningful differences between CCB users and non-users. The wealth of participant data allowed me to adjust for multiple important confounders, which may have limited previous study designs. I was also able to account for the concurrent use of other systemic medication classes with known effects on IOP or previously reported adverse associations with glaucoma. In addition, I was able to simultaneously explore the associations of CCB use with glaucoma, IOP, and inner retinal thickness, thus providing a plausible anatomic and mechanistic basis for the observed association.

The study is limited by glaucoma case ascertainment in the UK Biobank, which relies on a combination of self-report and linked ICD-codes. Although the primary case definition, based on ICD-codes alone, is likely to be relatively specific, it may fail to detect a significant proportion of true glaucoma cases, who may not be captured on a hospital-based database. Self-report, on the other hand, may identify more cases, but poses a risk of misclassification and/or recall bias.

Another limitation is that I was not able to analyse the duration or dosage of CCB use, which may play an important role in the association with glaucoma. Together with the cross-sectional study design, this precluded me from examining for dose-response and temporal effects, further restricting the ability to make causal inferences. Although I adjusted for multiple important confounders, the observed associations might represent residual confounding by unknown or unconsidered factors. Lastly, the findings in UK Biobank participants, where almost 95% are of White ethnicity, may not be generalisable to other populations.

In keeping with other smaller population-based studies, this study adds further evidence to support an adverse association between CCB use and glaucoma, despite no apparent relationship with IOP. These findings warrant further investigation to determine whether the associations are causal and to probe potential underlying biological mechanisms.

Although the current evidence is not strong enough to generically influence systemic hypertension management in patients with, or at risk of, glaucoma, should a patient on a CCB continue to progress despite optimal care, it may be helpful to discuss their hypertension management with the relevant physician and consider a medication change despite the lack of definitive causal evidence.

8

Physical activity

8.1 UK Biobank and Mendelian randomisation

The last modifiable risk factor examined as part of this thesis was the role of physical activity in glaucoma. This work was led by Dr Kian Madjedi and final results were published in *Ophthalmology*.[400] My role in the project was to perform the gene-environment interaction analyses within the UK Biobank cohort and to conduct the Mendelian randomisation analyses. This section provides an overview of these analyses and a brief discussion of the main findings of the overall work. The relevant declaration form for previously published material is located in **Appendix A**. Supplementary material for this section can be found in **Appendix L**.

8.1.1 Introduction

Physical activity is an important modifiable lifestyle factor, with well-established benefits on a range of chronic medical conditions.[401–403] Neuroprotective effects have also been reported,[404] and several studies have examined the role that physical activity may play in ocular health.[140,405] Bouts of physical activity are well documented to cause a transient reduction in IOP in both healthy individuals[124–134] and glaucoma patients,[129,134] as well as an increase in ocular blood flow and perfusion of the ONH and retina.[133,135–137] Fewer studies have assessed the association of habitual physical activity with IOP[138,139] and glaucoma.[140–143] While protective associations have been reported for both greater levels of physical activity and greater cardiovascular fitness,[138,139,141,142] this is not always a consistent finding in epidemiological studies.[140,143]

The existing literature, however, is often limited by small sample sizes and self-reported measures of physical activity. Accelerometry is considered the gold standard for objective assessment of physical activity and overcomes the limitations

of recall and misclassification bias in self-reported questionnaires.[406,407] Newer accelerometers are lightweight, wearable devices that provide a valid and reliable way to convert triaxial acceleration data into summary measures of total physical activity.[408,409] These devices are increasingly available in large cohort studies and have already been utilised in glaucoma-related research.[410–412] Additionally, with emerging evidence that lifestyle factors may only be evident in individuals at the highest genetic risk for glaucoma,[1] investigation of underlying gene-environment interactions for physical activity requires further attention.

In this UK Biobank study, the relationship between both self-reported and accelerometry-derived physical activity with glaucoma and related traits is explored. Additional consideration is given to gene-environment interactions and Mendelian randomisation to probe causal effects.

8.1.2 Methods

8.1.2.1 UK Biobank

See section 2.1.1.1.

8.1.2.2 Assessment of physical activity

The baseline UK Biobank assessment included several questions related to self-reported physical activity based on an adapted version of the validated International Physical Activity Questionnaire (IPAQ).[413] Participants were asked to provide information on their frequency of participation in a variety of activities, classified as sedentary (e.g., driving, watching TV), light (e.g., walking), moderate (e.g., bicycling at a regular pace), and heavy (e.g., aerobics). These data were then processed in line with IPAQ guidelines to arrive at an objective measurement of the ratio of energy

expenditure rate to an individual's mass (metabolic equivalent of task, MET).[367] Average METs per week across all physical activity levels were then summed to arrive at a quantitative measure of an individual's usual weekly physical activity level.

A subset of approximately 100 000 UK Biobank participants were also invited to wear a commercial triaxial accelerometer (Axivity AX3; Axivity Ltd) on the wrist of their dominant arm continuously for seven days.[407] Accelerometers were returned by mail and, after calibration, raw data were collected and processed according to methods described previously.[407] Summary metrics are then calculated and provide a measure of the time spent within a range of different mean acceleration values as a marker of physical activity intensity.[414]

8.1.2.3 Glaucoma-related outcome measures and case ascertainment

See section 2.2.1.1.

8.1.2.4 Genotyping and polygenic risk scores

See section 2.2.3.

8.1.2.5 Covariables

Covariables collected at the time of the baseline assessment and used in this study included: age, sex, self-reported ethnicity, Townsend deprivation index, height, BMI, SBP, self-reported history of DM, smoking status, alcohol intake,[61] and SE. Full details of these variables are available in section 2.2.4.

8.1.2.6 Statistical analysis

Associations were assessed using multivariable linear (for IOP, mRNFL thickness, and GCIPL thickness) and logistic (for glaucoma) regression models adjusted for the

covariables described in the section above. To assess whether any associations were modified by the glaucoma PRS, I tested the significance of a multiplicative interaction term between total self-reported physical activity and the standardised PRS in the final multivariable models using the Wald test. Gene-environment interaction analyses were restricted to participants of European ancestry based on principal components analysis.

8.1.2.7 Mendelian randomisation

See section 2.3.5 for full details of the two-sample MR study design.

For this analysis, I used published data from a recent large GWAS meta-analysis (including the UK Biobank) of physical activity to guide construction of the IVs.[199] The study identified 89 and 11 independent genetic variants associated (at $P < 5 \times 10^{-9}$) with 'leisure screen time' (LST) and 'moderate-to-vigorous physical activity' (MVPA), respectively. I included only significant SNPs from the primary meta-analyses of European ancestry participants (*n* up to 606 820 for LST, and *n* up to 526 725 for MVPA), using the same methods described in section 4.3.2.2. Details of the sources used for the glaucoma-related outcome measures are available in section 2.1.2.

Statistical analyses were performed according to the same methods described in section 4.3.2.4. I applied a conservative Bonferroni-corrected significance threshold of $P < 0.005$ (to account for tests between two exposures and five outcomes).

8.1.3 Results

8.1.3.1 Associations with glaucoma and related traits

The main observational associations of this study are available in the published article.[400] In summary, no associations between physical activity level or time spent

in physical activity (both IPAQ and accelerometry) with glaucoma status were observed. Higher self-reported physical activity was associated with a very modestly higher IOP, but this finding was not replicated in the accelerometry data. Higher levels of physical activity (both IPAQ and accelerometry) were associated with a thicker GCIPL, but no relationship with mRNFL was observed.

8.1.3.2 Gene-environment interaction analyses

These analyses were restricted to genetically European participants based on principal components analysis, and included 65 598 participants with data on glaucoma status, 72 355 participants with data on IOP, and 27 532 participants with data on mRNFL and GCIPL thickness. There was no evidence for an interaction of genetic risk with physical activity on glaucoma status (P = 0.07), IOP (P = 0.57), mRNFL thickness (P = 0.34), or GCIPL thickness (P = 0.87) (**Figure 8.1**).

8.1.3.3 Mendelian randomisation analyses

Full details of the SNPs included in the physical activity IVs and their associations with glaucoma and related traits are presented in **Table L1**. Primary MR analyses did not support a causal relationship between LST and any glaucoma-related outcome (P >0.12 for all), with similar null associations for all sensitivity analyses (**Table 8.1**). There was a suggestive association (not meeting the Bonferroni-corrected significance threshold) between MVPA and lower IOP (P = 0.014). Although this finding was supported by both the weighted median and MR-Egger methods, there was evidence for significant directional pleiotropy (MR-Egger intercept test P-value = 0.016). Similarly, significant associations between MVPA and POAG for the weighted median and MR-Egger methods were marked by significant global heterogeneity

($P < 0.001$) and directional pleiotropy ($P = 0.046$), suggesting a violation of the exclusion restriction assumption (**Table L2**).

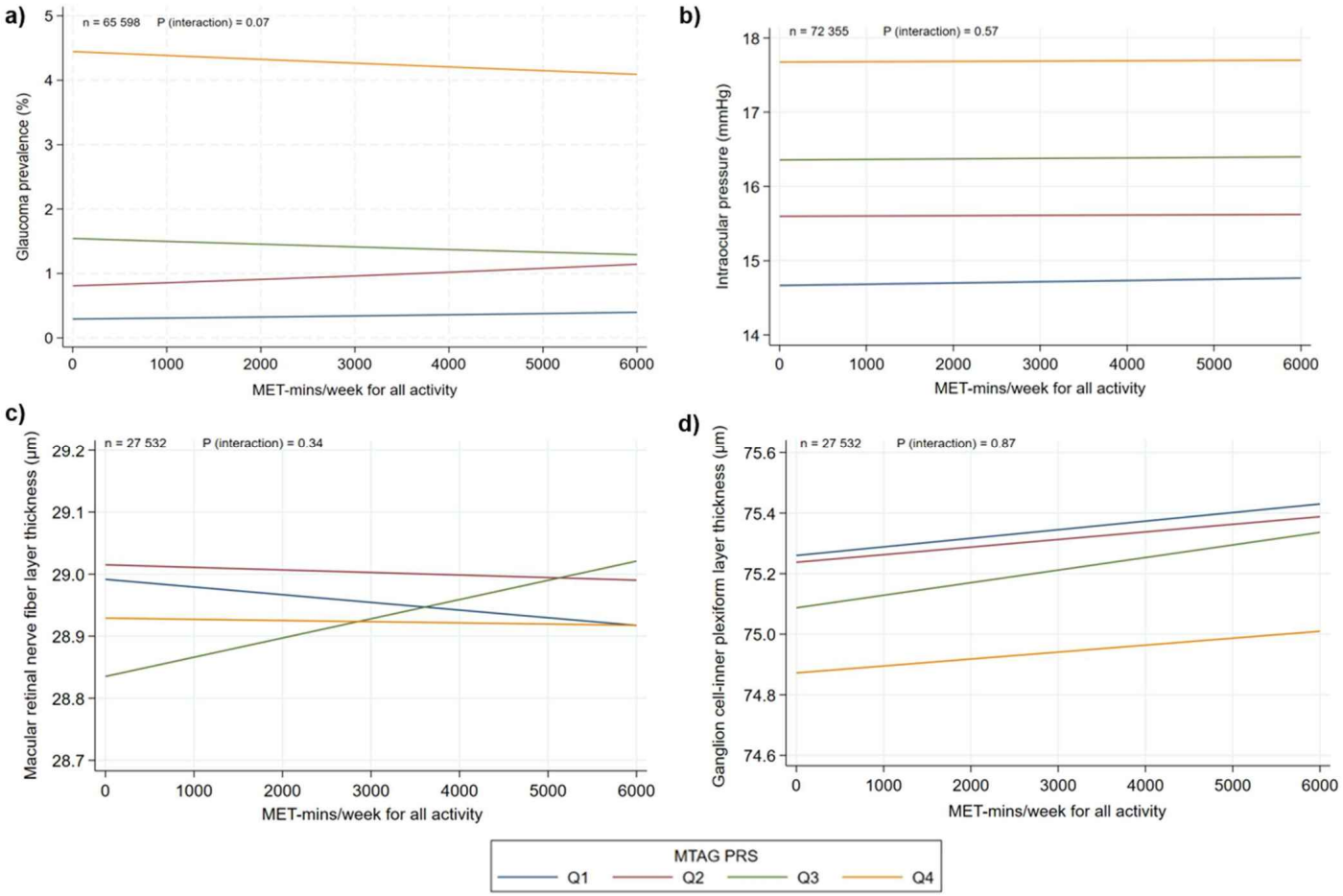

Figure 8.1 Gene-environment interaction analyses illustrating the effect of the glaucoma PRS on the association of physical activity with (**a**) glaucoma, (**b**) intraocular pressure, (**c**) macular retinal nerve fibre layer thickness, and (**d**) ganglion cell-inner plexiform layer thickness in European UK Biobank participants

MET, metabolic equivalent of task; MTAG, multitrait analysis of GWAS (genome-wide association study); PRS, polygenic risk score; Q, quartile.

Table 8.1 Results of Mendelian randomisation analyses for physical activity on glaucoma and related traits

	IOP (mmHg)		mRNFL (µm)		GCIPL (µm)		CDR		POAG (OR)	
	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
LST										
IVW	-0.15 (-0.39, 0.09)	0.22	0.00 (-0.57, 0.57)	0.99	0.06 (-0.60, 0.72)	0.86	-0.01 (-0.01, 0.00)	0.12	0.92 (0.75, 1.13)	0.44
Weighted median	-0.01 (-0.25, 0.24)	0.97	-0.07 (-0.64, 0.51)	0.82	-0.42 (-1.18, 0.34)	0.28	0.00 (-0.01, 0.01)	0.61	0.87 (0.69, 1.10)	0.24
MR-Egger	0.34 (-0.81, 1.49)	0.56	0.48 (-2.38, 3.34)	0.74	1.56 (-1.73, 4.85)	0.35	0.00 (-0.04, 0.04)	0.91	1.02 (0.37, 2.85)	0.97
MR-PRESSO	-0.05 (-0.26, 0.16)	0.66	-0.20 (-0.61, 0.21)	0.34	-0.23 (-0.80, 0.33)	0.42	0.00 (-0.01, 0.00)	0.16	0.88 (0.72, 1.07)	0.20
MVPA										
IVW	-0.62 (-1.11, -0.12)	0.014	0.10 (-1.01, 1.20)	0.86	0.45 (-2.15, 3.06)	0.73	0.01 (-0.03, 0.04)	0.66	0.60 (0.21, 1.71)	0.34
Weighted median	**-0.82 (-1.37, -0.26)**	**0.004**	-0.25 (-1.63, 1.13)	0.72	-0.47 (-2.50, 1.56)	0.65	0.00 (-0.02, 0.01)	0.72	**0.33 (0.18, 0.62)**	**0.001**
MR-Egger	**-3.07 (-5.11, -1.03)**	**0.003**	-0.45 (-6.12, 5.22)	0.88	-2.61 (-17.21, 11.99)	0.73	-0.13 (-0.32, 0.06)	0.19	**0.01 (0.00, 0.60)**	**0.028**
MR-PRESSO	–	–	–	–	-0.25 (-2.66, 2.16)	0.85	0.03 (-0.02, 0.08)	0.50	0.68 (0.18, 2.54)	0.67

MR estimates expressed per unit change in the instrumental variable.

CI, confidence interval; IV, instrumental variable; SNP, single nucleotide polymorphism; IVW, inverse variance weighted; MR, Mendelian randomisation; PRESSO, pleiotropy residual sum and outlier; LST, leisure screen time; MVPA, moderate-to-vigorous physical activity; IOP, intraocular pressure; mRNFL, macular retinal nerve fibre layer; GCIPL, ganglion cell-inner plexiform layer; CDR, cup-disc ratio; POAG, primary open-angle glaucoma; OR, odds ratio.

No MR-PRESSO estimate is calculated if no significant outliers are detected.

8.1.4 Discussion

In this large study of UK Biobank participants, overall habitual levels of physical activity and duration of time spent in physical activity were not found to be associated with glaucoma status. A modest association between self-reported physical activity and higher IOP was also identified, although there was no evidence of a dose-response relationship and this finding was not replicated in the accelerometer data. Greater levels of physical activity, ascertained through both self-report and accelerometry, however, were found to be associated with a thicker GCIPL, although not with mRNFL thickness. There was no evidence for any underlying gene-environment interactions and two-sample MR did not support a causal relationship between physical activity and glaucoma-related traits.

Previous studies of the relationship between physical activity and glaucoma have often been based on a small number of participants and results have been inconsistent.[140–143] This large population-based study, backed by MR analyses, found no evidence to support such an association. Although bouts of physical activity are well-established to cause a transient reduction in IOP,[124–134] this study also found no evidence to support a long-term IOP lowering effect of habitual physical activity. Findings from this study suggest that higher levels of habitual physical activity may be associated with a thicker GCIPL in the general population. This relationship was not found to be mediated by DM or glycaemic traits (known to affect GCIPL thickness), and is hypothesised to relate to the potentially neuroprotective effects of physical activity,[404,415,416] which may extend to RGCs.[417,418]

While limited by the cross-sectional study design, particular strengths of this study include the large sample size and the availability of both self-reported and accelerometry-derived physical activity data. This, combined with the availability of

multiple glaucoma-related traits and genetic data, makes this study one of the largest and most robust of the relationship between physical activity and glaucoma to date. Although MR analyses lent further support to the observational findings, a particular limitation was the significant participant overlap between exposure and outcome datasets, which may result in biased results in the presence of weak genetic instruments.[419] However, in a two-sample setting, the direction of this bias is away from the null, and while this may account for the few significant findings, it suggests that the identified null associations are truly nonsignificant. Furthermore, a suggestive association between MVPA and lower IOP was characterised by evidence of directional pleiotropy, suggesting that any association with IOP may not be mediated through physical activity.

In conclusion, the gene-environment interaction and MR analyses presented here do not support a role of physical activity in glaucoma. These results are generally in keeping with the main observational findings from the UK Biobank, which suggest only a modest association with a thicker GCIPL on a population level.

PART IV

DISCUSSION

9

Discussion

9.1 Summary of main findings

The work presented in this thesis leveraged datasets from multiple large-scale epidemiological eye studies and international genetics consortia to (i) quantify and characterise the burden of glaucoma in Europe, and (ii) explore the relationship of several important modifiable factors – including dietary components, lifestyle behaviours, and systemic medication use – with glaucoma and related traits. I report novel findings that may inform future epidemiological studies and public health policy, and that may have implications for dietary recommendations and targeted lifestyle advice for glaucoma patients in the future.

In Chapter 3, I performed a detailed meta-analysis of glaucoma prevalence using individual-level data from 14 population-based European eye studies. In addition to confirming many known epidemiological characteristics of glaucoma, the study provided updated age-, sex-, and subtype-stratified estimates of European glaucoma prevalence. The burden and characteristics of undiagnosed glaucoma were better defined, with a relationship between younger age and a higher proportion of undiagnosed disease identified. The analysis revealed that prevalence estimates vary according to diagnostic criteria used and this factor should be considered when comparing or pooling results in future. Importantly, the ability to apply granular prevalence estimates to European population projections suggests a significantly higher burden of disease than previously reported.

In Chapter 4, I explored the relationship between alcohol consumption and glaucoma, beginning with a systematic review and meta-analysis of existing literature. Although this analysis suggested an adverse association of alcohol with both IOP and glaucoma, it also highlighted the significant limitations and weakness

of the current evidence base, reinforcing the need for additional studies and highlighting potential avenues for future research. This was followed by a detailed analysis within the UK Biobank, including the derivation of a new quantitative alcohol intake variable, that revealed consistent dose-dependent adverse associations between alcohol consumption and glaucoma-related traits. Building on previous work suggesting that lifestyle factors may only play a detrimental role in glaucoma in those at high levels of underlying genetic risk, this study also demonstrated a significant gene-environment interaction for alcohol. Since the publication of this analysis, several other studies have reported similar adverse associations with alcohol, including replication of the reported gene-environment interaction in an independent cohort.[309,420–423] The chapter concluded with MR analyses that provided further evidence for a causal role of alcohol on inner retinal thinning.

In Chapter 5, I performed cross-sectional analyses within two large population-based cohorts, showing that smoking is strongly related to corneal biomechanical parameters in a dose-dependent fashion, and that this may result in an artefactual association with higher IOP. No relationship with other glaucoma-related traits was observed and these findings may explain why cigarette smoking is consistently associated with IOP, but not glaucoma, in epidemiological studies. The chapter concluded with MR analyses that lend further support to a causal role of smoking on corneal biomechanics, but not on glaucoma and related traits.

In Chapter 6, I explored the association of dietary salt with glaucoma by using urinary sodium excretion as a biomarker for dietary intake, this being the first study to report on such a relationship. I showed that urinary sodium excretion correlates strongly with self-reported dietary salt intake and systolic blood pressure, validating its utility as a biomarker. Urinary sodium excretion was found to be related to both

IOP and glaucoma in a dose-dependent manner, implicating dietary salt as a novel modifiable risk factor for disease. The study also demonstrated a gene-environment interaction for this relationship, lending further support to the findings demonstrated in Chapter 4 and providing additional evidence that lifestyle factors may only influence glaucoma risk in those at high underlying genetic risk.

In Chapter 7, I examined the association of systemic medication with glaucoma and related traits by conducting an exploratory analysis within the EPIC-Norfolk cohort, the findings of which contributed to a large European meta-analysis. This study confirmed and quantified the known relationship between systemic beta blockers and lower IOP, but also identified a consistent adverse association between calcium channel blocker use and glaucoma. No other common medications were found to be related to IOP or glaucoma. I then explored the relationship with calcium channel blockers in further detail in the UK Biobank cohort, replicating the findings of the meta-analysis, and additionally demonstrating adverse associations with inner retinal glaucoma-related biomarkers. In both studies, calcium channel blockers were not found to be associated with IOP, suggesting that any effect on glaucoma may be mediated through IOP-independent mechanisms.

Lastly, in Chapter 8, I reported the results of gene-environment interaction and MR analyses performed as part of a larger investigation into the association of physical activity with glaucoma and related traits. These analyses did not provide convincing evidence to support a role of physical activity in glaucoma risk, although there may be a modest relationship with inner retinal thickness on a population level.

9.2 Impact and directions for future research

Outputs from this thesis have already been utilised in several complementary research projects and certain findings may inform future lines of research.

- The results of the glaucoma prevalence meta-analysis have been provided to working groups of the European Glaucoma Society and the Royal College of Ophthalmologists and will form the basis of proposed glaucoma screening models and workplace planning strategies. It is also envisioned that the results will contribute to future glaucoma-related epidemiological studies and public health policy.
- Alcohol and dietary salt have been implicated as potentially modifiable risk factors for glaucoma. While several recent studies have reported similar findings for alcohol,[309,420–423] the role of dietary salt needs to be explored further and the findings replicated in independent cohorts. Future studies should consider constructing environmental risk scores, combining multiple putative risk factors (in a similar manner to a PRS), as the cumulative impact of these may prove to be substantial and clinically meaningful. Interventional studies of alcohol and/or salt restriction should consider including glaucoma-related endpoints to assess whether modifying exposure to these factors has any impact on disease course.
- The quantitative alcohol intake measure derived in this project has already been employed as a covariable in multiple UK Biobank analyses and the comprehensive glaucoma code list has been provided to external research groups currently engaged in a variety of research projects, including the utility of genetic risk scores in eye disease and the role of phosphodiesterase inhibitors in glaucoma.

- This thesis built on existing research suggesting that gene-environment interactions may underlie the relationship between common environmental factors and glaucoma. The possibility that any association may be modified by genetic risk should be considered in future research and previously reported associations, null or otherwise, should be revisited. These findings may lead to the development of targeted dietary recommendations and lifestyle advice for individuals at high genetic risk for glaucoma, especially with widespread population-level genotyping fast becoming a reailty.[1]
- The role of calcium channel blockers in glaucoma requires further attention and future basic science and animal studies should consider probing possible biological mechanisms underlying this relationship.

9.3 Conclusion

Large-scale population-based cohort studies and new epidemiological techniques provide an opportunity to revisit and better characterise the relationship between modifiable environmental risk factors and glaucoma, offering insights into disease pathogenesis and potentially leading to novel therapeutic approaches in the future.

REFERENCES

1. Stuart KV, Pasquale LR, Kang JH, Foster PJ, Khawaja AP. Towards modifying the genetic predisposition for glaucoma: An overview of the contribution and interaction of genetic and environmental factors. Mol Aspects Med. 2023;93:101203.

2. Stein JD, Khawaja AP, Weizer JS. Glaucoma in Adults—Screening, Diagnosis, and Management. JAMA. 2021;325(2):164.

3. Killer HE, Pircher A. Normal tension glaucoma: review of current understanding and mechanisms of the pathogenesis. Eye. 2018;32(5):924–30.

4. Chua J, Baskaran M, Ong PG, Zheng Y, Wong TY, Aung T, et al. Prevalence, Risk Factors, and Visual Features of Undiagnosed Glaucoma: The Singapore Epidemiology of Eye Diseases Study. JAMA Ophthalmol. 2015;133(8):938–46.

5. Soh Z, Yu M, Betzler BK, Majithia S, Thakur S, Tham YC, et al. The Global Extent of Undetected Glaucoma in Adults: A Systematic Review and Meta-analysis. Ophthalmology. 2021;128(10):1393–404.

6. Quigley HA. Number of people with glaucoma worldwide. Br J Ophthalmol. 1996;80(5):389–93.

7. Tham Y-C, Li X, Wong TY, Quigley HA, Aung T, Cheng C-Y. Global Prevalence of Glaucoma and Projections of Glaucoma Burden through 2040. Ophthalmology. 2014;121(11):2081–90.

8. Cheng J-W, Zong Y, Zeng Y-Y, Wei R-L. The prevalence of primary angle closure glaucoma in adult Asians: a systematic review and meta-analysis. PLoS One. 2014;9(7):e103222.

9. Bourne RRA, Taylor HR, Flaxman SR, Keeffe J, Leasher J, Naidoo K, et al. Number of people blind or visually impaired by glaucoma worldwide and in world regions 1990 - 2010: A meta-analysis. PLoS One. 2016;11(10):1–16.

10. Prager AJ, Liebmann JM, Cioffi GA, Blumberg DM. Self-reported Function, Health Resource Use, and Total Health Care Costs Among Medicare Beneficiaries With Glaucoma. JAMA Ophthalmol. 2016;134(4):357–65.

11. Varma R, Lee PP, Goldberg I, Kotak S. An assessment of the health and economic burdens of glaucoma. Am J Ophthalmol. 2011;152(4):515–22.

12. Feldman RM, Cioffi GA, Liebmann JM, Weinreb RN. Current Knowledge and Attitudes Concerning Cost-Effectiveness in Glaucoma Pharmacotherapy: A Glaucoma Specialists Focus Group Study. Clin Ophthalmol. 2020;14:729–39.

13. Allison K, Patel D, Alabi O. Epidemiology of Glaucoma: The Past, Present, and Predictions for the Future. Cureus. 2020;12(11):e11686.

14. Wright C, Tawfik MA, Waisbourd M, Katz LJ. Primary angle-closure glaucoma: an update. Acta Ophthalmol. 2016;94(3):217–25.

15. Krishnadas R, Ramakrishnan R. Secondary glaucomas: the tasks ahead. Community Eye Heal. 2001;14(39):40–2.

16. Khawaja AP, Cooke Bailey JN, Wareham NJ, Scott RA, Simcoe M, Igo RP, et al. Genome-wide analyses identify 68 new loci associated with intraocular

pressure and improve risk prediction for primary open-angle glaucoma. Nat Genet. 2018;50(6):778–82.

17. Kass MA, Heuer DK, Higginbotham EJ, Johnson CA, Keltner JL, Miller JP, et al. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. Arch Ophthalmol. 2002;120(6):701–13.

18. Heijl A, Leske MC, Bengtsson B, Hyman L, Bengtsson B, Hussein M, et al. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. Arch Ophthalmol. 2002;120(10):1268–79.

19. Collaborative Normal-Tension Glaucoma Study Group. Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. Am J Ophthalmol. 1998;126(4):487–97.

20. Wang K, Gaitsch H, Poon H, Cox NJ, Rzhetsky A. Classification of common human diseases derived from shared genetic and environmental determinants. Nat Genet. 2017;49(9):1319–25.

21. Wolfs RC, Klaver CC, Ramrattan RS, van Duijn CM, Hofman A, de Jong PT. Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. Arch Ophthalmol. 1998;116(12):1640–5.

22. Lewis CJ, Hedberg-Buenz A, DeLuca AP, Stone EM, Alward WLM, Fingert JH. Primary congenital and developmental glaucomas. Hum Mol Genet. 2017;26(R1):R28–36.

23. Sears NC, Boese EA, Miller MA, Fingert JH. Mendelian genes in primary open angle glaucoma. Exp Eye Res. 2019;186:107702.

24. Wiggs JL, Pasquale LR. Genetics of glaucoma. Hum Mol Genet. 2017;26(R1):R21–7.

25. Choquet H, Wiggs JL, Khawaja AP. Clinical implications of recent advances in primary open-angle glaucoma genetics. Eye. 2020;34(1):29–39.

26. Gharahkhani P, Jorgenson E, Hysi P, Khawaja AP, Pendergrass S, Han X, et al. Genome-wide meta-analysis identifies 127 open-angle glaucoma loci with consistent effect across ancestries. Nat Commun. 2021;12(1):1258.

27. Craig JE, Han X, Qassim A, Hassall M, Cooke Bailey JN, Kinzy TG, et al. Multitrait analysis of glaucoma identifies new risk loci and enables polygenic prediction of disease susceptibility and progression. Nat Genet. 2020;52(2):160–6.

28. Pasquale LR, Kang JH. Lifestyle, nutrition, and glaucoma. J Glaucoma. 2009;18(6):423–8.

29. Wiggs JL. The cell and molecular biology of complex forms of glaucoma: updates on genetic, environmental, and epigenetic risk factors. Invest Ophthalmol Vis Sci. 2012;53(5):2467–9.

30. Kumar DM, Agarwal N. Oxidative stress in glaucoma: a burden of evidence. J Glaucoma. 2007;16(3):334–43.

31. Al Owaifeer AM, Al Taisan AA. The Role of Diet in Glaucoma: A Review of the Current Evidence. Ophthalmol Ther. 2018;7(1):19–31.

32. Stuart KV, Madjedi K, Luben RN, Chua SYL, Warwick AN, Chia M, et al. Alcohol, Intraocular Pressure, and Open-Angle Glaucoma: A Systematic Review and Meta-analysis. Ophthalmology. 2022;129(6):637–52.

33. Wolfs RC, Borger PH, Ramrattan RS, Klaver CC, Hulsman CA, Hofman A, et al. Changing views on open-angle glaucoma: definitions and prevalences--The Rotterdam Study. Invest Ophthalmol Vis Sci. 2000;41(11):3309–21.

34. Foster PJ, Buhrmann R, Quigley HA, Johnson GJ. The definition and classification of glaucoma in prevalence surveys. Br J Ophthalmol. 2002;86(2):238–42.

35. Strimbu K, Tavel JA. What are biomarkers? Curr Opin HIV AIDS. 2010;5(6):463–6.

36. Coleman AL, Miglior S. Risk factors for glaucoma onset and progression. Surv Ophthalmol. 2008;53(Suppl1):S3-10.

37. Gandhi M, Dubey S. Evaluation of the Optic Nerve Head in Glaucoma. J Curr glaucoma Pract. 7(3):106–14.

38. Garway-Heath DF, Ruben ST, Viswanathan A, Hitchings RA. Vertical cup/disc ratio in relation to optic disc size: its value in the assessment of the glaucoma suspect. Br J Ophthalmol. 1998;82(10):1118–24.

39. Kim KE, Park KH. Macular imaging by optical coherence tomography in the diagnosis and management of glaucoma. Br J Ophthalmol. 2018;102(6):718–24.

40. Oddone F, Lucenteforte E, Michelessi M, Rizzo S, Donati S, Parravano M, et al. Macular versus Retinal Nerve Fiber Layer Parameters for Diagnosing Manifest Glaucoma: A Systematic Review of Diagnostic Accuracy Studies. Ophthalmology. 2016;123(5):939–49.

41. Choquet H, Thai KK, Yin J, Hoffmann TJ, Kvale MN, Banda Y, et al. A large multi-ethnic genome-wide association study identifies novel genetic loci for intraocular pressure. Nat Commun. 2017;8(1):2108.

42. Han X, Steven K, Qassim A, Marshall HN, Bean C, Tremeer M, et al. Automated AI labeling of optic nerve head enables insights into cross-ancestry glaucoma risk and genetic discovery in >280,000 images from UKB and CLSA. Am J Hum Genet. 2021;108(7):1204–16.

43. GBD 2019 Risk Factors Collaborators. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020;396(10258):1223–49.

44. Narayan KMV, Ali MK, Koplan JP. Global noncommunicable diseases--where worlds meet. N Engl J Med. 2010;363(13):1196–8.

45. Wu A, Khawaja AP, Pasquale LR, Stein JD. A review of systemic medications that may modulate the risk of glaucoma. Eye (Lond). 2020;34(1):12–28.

46. Kantor ED, Rehm CD, Haas JS, Chan AT, Giovannucci EL. Trends in Prescription Drug Use Among Adults in the United States From 1999-2012. JAMA. 2015;314(17):1818–31.

47. Buckingham T, Young R. The Rise and Fall of Intra-Ocular Pressure: the Influence of Physiological Factors. Ophthalmic Physiol Opt. 1986;6(1):95–9.

48. Harris A, Swartz D, Engen D, Beck D, Evans D, Caldemeyer K, et al. Ocular Hemodynamic Effects of Acute Ethanol Ingestion. Ophthalmic Res. 1996;28(3):193–200.

49. Houle RE, Grant WM. Alcohol, vasopressin, and intraocular pressure. Investig Ophthalmol Vis Sci. 1967;6(2):145–54.

50. Giurlani BP, Obie LG, Petersen CG, Presley DD. Alcohol and open angle glaucoma - influence on detection, IOP, BP/IOP ratios. J Am Optom Assoc. 1978;49(4):409–16.

51. Luksch A, Resch H, Weigert G, Sacu S, Schmetterer L, Garhöfer G. Acute effects of intravenously administered ethanol on retinal vessel diameters and flicker induced vasodilatation in healthy volunteers. Microvasc Res. 2009;78(2):224–9.

52. Peczon JD, Grant WM. Glaucoma, Alcohol, and Intraocular Pressure. Arch Ophthalmol. 1965;73:495–501.

53. Weber A, Remky A, Bienert M, der Velden KH van, Kirschkamp T, Rennings C, et al. Retrobulbar blood flow and visual field alterations after acute ethanol ingestion. Clin Ophthalmol. 2013;7:1641–6.

54. Yamada K, Hayasaka S, Matsuoka Y. Changes in intraocular pressure after drinking beer in normal eyes and in those with ocular hypertension. Ann Ophthalmol. 1995;27(2):85–8.

55. Kojima S, Sugiyama T, Kojima M, Azuma I, Ito S. Effect of the Consumption of Ethanol on the Microcirculation of the Human Optic Nerve Head in the Acute Phase. Jpn J Ophthalmol. 2000;44(3):318–9.

56. Leske MC, Warheit-Roberts L, Wu SY. Open-angle glaucoma and ocular hypertension: The Long Island Glaucoma Case-control Study. Ophthalmic Epidemiol. 1996;3(2):85–96.

57. Lin HY, Hsu WM, Chou P, Liu CJ, Chou JC, Tsai SY, et al. Intraocular Pressure Measured With a Noncontact Tonometer in an Elderly Chinese Population: The Shihpai Eye Study. Arch Ophthalmol. 2005;123(3):381–6.

58. Song JE, Kim JM, Lee MY, Jang HJ, Park KH. Effects of Consumption of Alcohol on Intraocular Pressure: Korea National Health and Nutrition Examination Survey 2010 to 2011. Nutrients. 2020;12(8):1–15.

59. Wu S-Y, Leske MC, for the Barbados Eye Study Group. Associations With Intraocular Pressure in the Barbados Eye Study. Arch Ophthalmol. 1997;115:1572–6.

60. Yoshida M, Ishikawa M, Kokaze A, Sekine Y, Matsunaga N, Uchida Y, et al. Association of Life-style with Intraocular Pressure in Middle-aged and Older

Japanese Residents. Jpn J Ophthalmol. 2003;47(2):191–8.

61. Stuart KV, Luben RN, Warwick AN, Madjedi KM, Patel PJ, Biradar MI, et al. The Association of Alcohol Consumption with Glaucoma and Related Traits: Findings from the UK Biobank. Ophthalmol Glaucoma. 2023;6(4):366–79.

62. Seddon JM, Schwartz B, Flowerdew G. Case-Control Study of Ocular Hypertension. Arch Ophthalmol. 1983;101:891–4.

63. Weih LM, Mukesh BN, McCarty CA, Taylor HR. Association of Demographic, Familial, Medical, and Ocular Factors with Intraocular Pressure. Arch Ophthalmol. 2001;119(6):875–80.

64. Wise LA, Rosenberg L, Radin RG, Mattox C, Yang EB, Palmer JR, et al. A Prospective Study of Diabetes, Lifestyle Factors, and Glaucoma Among African-American Women. Ann Epidemiol. 2011;21(6):430–9.

65. Bikbov MM, Gilmanshin TR, Zainullin RM, Kazakbaeva GM, Arslangareeva II, Panda-Jonas S, et al. Prevalence and associated factors of glaucoma in the Russian Ural Eye and Medical Study. Sci Rep. 2020;10(1):1–14.

66. 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(7):1287–93.

67. Charliat G, Jolly D, Blanchard F. Genetic risk factor in primary open-angle glaucoma: a case-control study. Ophthalmic Epidemiol. 1994;1(3):131–8.

68. Chiam N, Baskaran M, Li Z, Perera S, Goh D, Husain R, et al. Social, health and ocular factors associated with primary open-angle glaucoma amongst Chinese Singaporeans. Clin Exp Ophthalmol. 2018;46(1):25–34.

69. Leske MC, Nemesure B, He Q, Wu SY, Fielding Hejtmancik J, Hennis A. Patterns of Open-angle Glaucoma in the Barbados Family Study. Ophthalmology. 2001;108(6):1015–22.

70. Renard JP, Rouland JF, Bron A, Sellem E, Nordmann JP, Baudouin C, et al. Nutritional, lifestyle and environmental factors in ocular hypertension and primary open-angle glaucoma: An exploratory case-control study. Acta Ophthalmol. 2013;91(6):505–13.

71. Jiang X, Varma R, Wu S, Torres M, Azen SP, Francis BA, et al. Baseline Risk Factors that Predict the Development of Open-Angle Glaucoma in a Population: The Los Angeles Latino Eye Study. Ophthalmology. 2012;119(11):2245–53.

72. Kang JH, Willett WC, Rosner BA, Hankinson SE, Pasquale LR. Prospective Study of Alcohol Consumption and the Risk of Primary Open-Angle Glaucoma. Ophthalmic Epidemiol. 2007;14(3):141–7.

73. Pan CW, Yang WY, Hu DN, Xu JG, Niu ZQ, Yuan YS, et al. Longitudinal Cohort Study on the Incidence of Primary Open-Angle Glaucoma in Bai Chinese. Am J Ophthalmol. 2017;176:127–33.

74. Khawaja AP, Chua SYL, Hysi PG, Georgoulas S, Currant H, Fitzgerald TW, et al. Comparison of Associations with Different Macular Inner Retinal Thickness

Parameters in a Large Cohort: The UK Biobank. Ophthalmology. 2020;127(1):62–71.

75. Lamparter J, Schmidtmann I, Schuster AK, Siouli A, Wasielica-Poslednik J, Mirshahi A, et al. Association of ocular, cardiovascular, morphometric and lifestyle parameters with retinal nerve fibre layer thickness. PLoS One. 2018;13(5):1–11.

76. Paulsen AJ, Pinto A, Merten N, Chen Y, Fischer ME, Huang G-H, et al. Factors Associated with the Macular Ganglion Cell–Inner Plexiform Layer Thickness in a Cohort of Middle-aged U.S. Adults. Optom Vis Sci. 2021;98(3):295–305.

77. Han YS, Kim YW, Kim YJ, Park KH, Jeoung JW. Alcohol consumption is associated with glaucoma severity regardless of ALDH2 polymorphism. Sci Rep. 2020;10(1):1–9.

78. Jain V, Jain M, Abdull MM, Bastawrous A. The association between cigarette smoking and primary open-angle glaucoma: a systematic review. Int Ophthalmol. 2017;37(1):291–301.

79. Toda N, Nakanishi-Toda M. Nitric oxide: ocular blood flow, glaucoma, and diabetic retinopathy. Prog Retin Eye Res. 2007;26(3):205–38.

80. Latif N, Naroo SA. Transient effects of smoking on the eye. Cont Lens Anterior Eye. 2022;45(5):101595.

81. Tamaki Y, Araie M, Nagahara M, Tomita K, Matsubara M. The acute effects of cigarette smoking on human optic nerve head and posterior fundus circulation in light smokers. Eye. 2000;14(1):67–72.

82. Lee AJ, Rochtchina E, Wang JJ, Healey PR, Mitchell P. Does smoking affect intraocular pressure? Findings from the Blue Mountains Eye Study. J Glaucoma. 2003;12(3):209–12.

83. Yoshida M, Take S, Ishikawa M, Kokaze A, Karita K, Harada M, et al. Association of smoking with intraocular pressure in middle-aged and older Japanese residents. Environ Health Prev Med. 2014;19(2):100–7.

84. Lee CS, Owen JP, Yanagihara RT, Lorch A, Pershing S, Hyman L, et al. Smoking Is Associated with Higher Intraocular Pressure Regardless of Glaucoma: A Retrospective Study of 12.5 Million Patients Using the Intelligent Research in Sight (IRIS®) Registry. Ophthalmol Glaucoma. 3(4):253–61.

85. Chan MPY, Grossi CM, Khawaja AP, Yip JLY, Khaw KT, Patel PJ, et al. Associations with Intraocular Pressure in a Large Cohort: Results from the UK Biobank. Ophthalmology. 2016;123(4):771–82.

86. Juronen E, Tasa G, Veromann S, Parts L, Tiidla A, Pulges R, et al. Polymorphic glutathione S-transferase M1 is a risk factor of primary open-angle glaucoma among Estonians. Exp Eye Res. 2000;71(5):447–52.

87. Kang JH, Pasquale LR, Rosner BA, Willett WC, Egan KM, Faberowski N, et al. Prospective study of cigarette smoking and the risk of primary open-angle glaucoma. Arch Ophthalmol. 2003;121(12):1762–8.

88. Klein BEK, Klein R, Ritter LL. Relationship of Drinking Alcohol and Smoking to Prevalence of Open-angle Glaucoma: The Beaver Dam Eye Study. Ophthalmology. 1993;100(11):1609–13.

89. Quigley HA, Enger C, Katz J, Sommer A, Scott R, Gilbert D. Risk Factors for the Development of Glaucomatous Visual Field Loss in Ocular Hypertension. Arch Ophthalmol. 1994;112(5):644.

90. Ramdas WD, Wolfs RCW, Hofman A, De Jong PTVM, Vingerling JR, Jansonius NM. Lifestyle and Risk of Developing Open-Angle Glaucoma: The Rotterdam Study. Arch Ophthalmol. 2011;129(6):767–72.

91. Wang D, Huang Y, Huang C, Wu P, Lin J, Zheng Y, et al. Association analysis of cigarette smoking with onset of primary open-angle glaucoma and glaucoma-related biometric parameters. BMC Ophthalmol. 2012;12(1):59.

92. Wilson MR, Hertzmark E, Walker AM, Childs Shaw K, Epstein DL. A Case-Control Study of Risk Factors in Open Angle Glaucoma. Arch Ophthalmol. 1987;105(8):1066–71.

93. Fan B, Leung Y, Wang N, Lam S, Liu Y, Tam O, et al. Genetic and environmental risk factors for primary open-angle glaucoma. Chin Med J (Engl). 2004;117(5):706–10.

94. Kaimbo Wa Kaimbo D, Buntinx F, Missotten L. Risk factors for open-angle glaucoma: A case-control study. J Clin Epidemiol. 2001;54(2):166–71.

95. Katz J, Sommer A. Risk Factors for Primary Open Angle Glaucoma. Am J Prev Med. 1988;4(2):110–4.

96. Le A, Mukesh BN, McCarty CA, Taylor HR. Risk factors associated with the incidence of open-angle glaucoma: the visual impairment project. Invest Ophthalmol Vis Sci. 2003;44(9):3783–9.

97. Buys YM, Harasymowycz P, Gaspo R, Kwok K, Hutnik CML, Blondeau P, et al. Comparison of newly diagnosed ocular hypertension and open-angle glaucoma: ocular variables, risk factors, and disease severity. J Ophthalmol. 2012;2012:757106.

98. Founti P, Bunce C, Khawaja AP, Doré CJ, Mohamed-Noriega J, Garway-Heath DF, et al. Risk Factors for Visual Field Deterioration in the United Kingdom Glaucoma Treatment Study. Ophthalmology. 2020;127(12):1642–51.

99. Doshi V, Ying-Lai M, Azen SP, Varma R. Sociodemographic, Family History, and Lifestyle Risk Factors for Open-angle Glaucoma and Ocular Hypertension: The Los Angeles Latino Eye Study. Ophthalmology. 2008;115(4).

100. Chung HS, Harris A, Kristinsson JK, Ciulla TA, Kagemann C, Ritch R. Ginkgo biloba extract increases ocular blood flow velocity. J Ocul Pharmacol Ther. 1999;15(3):233–40.

101. Hirooka K, Tokuda M, Miyamoto O, Itano T, Baba T, Shiraga F. The Ginkgo biloba extract (EGb 761) provides a neuroprotective effect on retinal ganglion cells in a rat model of chronic glaucoma. Curr Eye Res. 2004;28(3):153–7.

102. Eckert A, Keil U, Scherping I, Hauptmann S, Müller WE. Stabilization of

mitochondrial membrane potential and improvement of neuronal energy metabolism by Ginkgo biloba extract EGb 761. Ann N Y Acad Sci. 2005;1056:474–85.

103. Park JW, Kwon HJ, Chung WS, Kim CY, Seong GJ. Short-term effects of Ginkgo biloba extract on peripapillary retinal blood flow in normal tension glaucoma. Korean J Ophthalmol. 2011;25(5):323–8.

104. Shim SH, Kim JM, Choi CY, Kim CY, Park KH. Ginkgo biloba extract and bilberry anthocyanins improve visual function in patients with normal tension glaucoma. J Med Food. 2012;15(9):818–23.

105. Quaranta L, Bettelli S, Uva MG, Semeraro F, Turano R, Gandolfo E. Effect of Ginkgo biloba extract on preexisting visual field damage in normal tension glaucoma. Ophthalmology. 2003;110(2):359–62.

106. Lee J, Sohn SW, Kee C. Effect of Ginkgo biloba extract on visual field progression in normal tension glaucoma. J Glaucoma. 2013;22(9):780–4.

107. Patel S, Mathan JJ, Vaghefi E, Braakhuis AJ. The effect of flavonoids on visual function in patients with glaucoma or ocular hypertension: a systematic review and meta-analysis. Graefe's Arch Clin Exp Ophthalmol. 2015;253(11):1841–50.

108. Kang JH, Ivey KL, Boumenna T, Rosner B, Wiggs JL, Pasquale LR. Prospective study of flavonoid intake and risk of primary open-angle glaucoma. Acta Ophthalmol. 2018;96(6):e692–700.

109. Coleman AL, Stone KL, Kodjebacheva G, Yu F, Pedula KL, Ensrud KE, et al. Glaucoma risk and the consumption of fruits and vegetables among older women in the study of osteoporotic fractures. Am J Ophthalmol. 2008;145(6):1081–9.

110. Giaconi JA, Yu F, Stone KL, Pedula KL, Ensrud KE, Cauley JA, et al. The association of consumption of fruits/vegetables with decreased risk of glaucoma among older African-American women in the study of osteoporotic fractures. Am J Ophthalmol. 2012;154(4):635–44.

111. Kang JH, Willett WC, Rosner BA, Buys E, Wiggs JL, Pasquale LR. Association of Dietary Nitrate Intake With Primary Open-Angle Glaucoma: A Prospective Analysis From the Nurses' Health Study and Health Professionals Follow-up Study. JAMA Ophthalmol. 2016;134(3):294–303.

112. Wang SY, Singh K, Lin SC. Glaucoma and vitamins A, C, and E supplement intake and serum levels in a population-based sample of the United States. Eye. 2013;27(4):487–94.

113. Garcia-Medina JJ, Garcia-Medina M, Garrido-Fernandez P, Galvan-Espinosa J, Garcia-Maturana C, Zanon-Moreno V, et al. A two-year follow-up of oral antioxidant supplementation in primary open-angle glaucoma: an open-label, randomized, controlled trial. Acta Ophthalmol. 2015;93(6):546–54.

114. Moreno-Montañés J, Gándara E, Moreno-Galarraga L, Hershey MS, López-Gil JF, Kales S, et al. ACE-Vitamin Index and Risk of Glaucoma: The SUN Project. Nutrients. 2022;14(23):5129.

115. Hui F, Tang J, Williams PA, McGuinness MB, Hadoux X, Casson RJ, et al. Improvement in inner retinal function in glaucoma with nicotinamide (vitamin B3) supplementation: A crossover randomized clinical trial. Clin Experiment Ophthalmol. 2020;48(7):903–14.

116. Taechameekietichai T, Chansangpetch S, Peerawaranun P, Lin SC. Association between Daily Niacin Intake and Glaucoma: National Health and Nutrition Examination Survey. Nutrients. 2021;13(12):4263.

117. Ren H, Magulike N, Ghebremeskel K, Crawford M. Primary open-angle glaucoma patients have reduced levels of blood docosahexaenoic and eicosapentaenoic acids. Prostaglandins Leukot Essent Fatty Acids. 2006;74(3):157–63.

118. Pérez de Arcelus M, Toledo E, Martínez-González MÁ, Sayón-Orea C, Gea A, Moreno-Montañés J. Omega 3:6 ratio intake and incidence of glaucoma: the SUN cohort. Clin Nutr. 2014;33(6):1041–5.

119. Kang JH, Pasquale LR, Willett WC, Rosner BA, Egan KM, Faberowski N, et al. Dietary fat consumption and primary open-angle glaucoma. Am J Clin Nutr. 2004;79(5):755–64.

120. Tseng VL, Topouzis F, Yu F, Keskini C, Pappas T, Founti P, et al. Association Between Dietary Salt Intake and Open Angle Glaucoma in the Thessaloniki Eye Study. J Glaucoma. 2022;31(7):494–502.

121. Miller VJ, Villamena FA, Volek JS. Nutritional Ketosis and Mitohormesis: Potential Implications for Mitochondrial Function and Human Health. J Nutr Metab. 2018;2018:5157645.

122. Hanyuda A, Rosner BA, Wiggs JL, Willett WC, Tsubota K, Pasquale LR, et al. Low-carbohydrate-diet scores and the risk of primary open-angle glaucoma: data from three US cohorts. Eye. 2020;34(8):1465–75.

123. Vergroesen JE, de Crom TOE, van Duijn CM, Voortman T, Klaver CCW, Ramdas WD. MIND diet lowers risk of open-angle glaucoma: the Rotterdam Study. Eur J Nutr. 2023;62(1):477–87.

124. Yan X, Li M, Song Y, Guo J, Zhao Y, Chen W, et al. Influence of Exercise on Intraocular Pressure, Schlemm's Canal, and the Trabecular Meshwork. Invest Ophthalmol Vis Sci. 2016;57(11):4733–9.

125. Read SA, Collins MJ. The short-term influence of exercise on axial length and intraocular pressure. Eye. 2011;25(6):767–74.

126. Avunduk AM, Yilmaz B, Sahin N, Kapicioglu Z, Dayanir V. The comparison of intraocular pressure reductions after isometric and isokinetic exercises in normal individuals. Ophthalmologica. 1999;213(5):290–4.

127. Ashkenazi I, Melamed S, Blumenthal M. The effect of continuous strenuous exercise on intraocular pressure. Invest Ophthalmol Vis Sci. 1992;33(10):2874–7.

128. Martin B, Harris A, Hammel T, Malinovsky V. Mechanism of exercise-induced ocular hypotension. Invest Ophthalmol Vis Sci. 1999;40(5):1011–5.

129. Natsis K, Asouhidou I, Nousios G, Chatzibalis T, Vlasis K, Karabatakis V. Aerobic exercise and intraocular pressure in normotensive and glaucoma patients. BMC Ophthalmol. 2009;9(9):6.

130. Leighton DA, Phillips CI. Effect of moderate exercise on the ocular tension. Br J Ophthalmol. 1970;54(9):599–605.

131. Harris A, Malinovsky V, Martin B. Correlates of acute exercise-induced ocular hypotension. Invest Ophthalmol Vis Sci. 1994;35(11):3852–7.

132. Conte M, Baldin AD, Russo MRRR, Storti LR, Caldara AA, Cozza HFP, et al. Effects of high-intensity interval vs. continuous moderate exercise on intraocular pressure. Int J Sports Med. 2014;35(10):874–8.

133. Price EL, Gray LS, Humphries L, Zweig C, Button NF. Effect of exercise on intraocular pressure and pulsatile ocular blood flow in a young normal population. Optom Vis Sci. 2003;80(6):460–6.

134. Qureshi IA. The effects of mild, moderate, and severe exercise on intraocular pressure in glaucoma patients. Jpn J Physiol. 1995;45(4):561–9.

135. Vo Kim S, Semoun O, Pedinielli A, Jung C, Miere A, Souied EH. Optical Coherence Tomography Angiography Quantitative Assessment of Exercise-Induced Variations in Retinal Vascular Plexa of Healthy Subjects. Invest Ophthalmol Vis Sci. 2019;60(5):1412–9.

136. Alnawaiseh M, Lahme L, Treder M, Rosentreter A, Eter N. Short-term effects of exercise on optic nerve and macular perfusion measured by optical coherence tomography angiography. Retina. 2017;37(9):1642–6.

137. Li S, Pan Y, Xu J, Li X, Spiegel DP, Bao J, et al. Effects of physical exercise on macular vessel density and choroidal thickness in children. Sci Rep. 2021;11(1):2015.

138. Qureshi IA, Xi XR, Wu XD, Zhang J, Shiarkar E. The effect of physical fitness on intraocular pressure in Chinese medical students. Chin Med J (Engl). 1996;58(5):317–22.

139. Fujiwara K, Yasuda M, Hata J, Yoshida D, Kishimoto H, Hashimoto S, et al. Long-term regular exercise and intraocular pressure: the Hisayama Study. Graefes Arch Clin Exp Ophthalmol. 2019;257(11):2461–9.

140. Wang YX, Wei W Bin, Xu L, Jonas JB. Physical activity and eye diseases. The Beijing Eye Study. Acta Ophthalmol. 2019;97(3):325–31.

141. Williams PT. Relationship of incident glaucoma versus physical activity and fitness in male runners. Med Sci Sports Exerc. 2009;41(8):1566–72.

142. Meier NF, Lee DC, Sui X, Blair SN. Physical Activity, Cardiorespiratory Fitness, and Incident Glaucoma. Med Sci Sports Exerc. 2018;50(11):2253–8.

143. Lin S-C, Wang SY, Pasquale LR, Singh K, Lin SC. The relation between exercise and glaucoma in a South Korean population-based sample. PLoS One. 2017;12(2):e0171441.

144. Johnson D, Gottanka J, Flügel C, Hoffmann F, Futa R, Lütjen-Drecoll E.

Ultrastructural changes in the trabecular meshwork of human eyes treated with corticosteroids. Arch Ophthalmol. 1997;115(3):375–83.

145. Fini ME, Schwartz SG, Gao X, Jeong S, Patel N, Itakura T, et al. Steroid-induced ocular hypertension/glaucoma: Focus on pharmacogenomics and implications for precision medicine. Prog Retin Eye Res. 2017;56:58–83.

146. Khawaja AP, Chan MPY, Broadway DC, Garway-Heath DF, Luben R, Yip JLY, et al. Systemic medication and intraocular pressure in a British population: the EPIC-Norfolk Eye Study. Ophthalmology. 2014;121(8):1501–7.

147. Müskens RPHM, de Voogd S, Wolfs RCW, Witteman JCM, Hofman A, de Jong PTVM, et al. Systemic antihypertensive medication and incident open-angle glaucoma. Ophthalmology. 2007;114(12):2221–6.

148. Luksch A, Rainer G, Koyuncu D, Ehrlich P, Maca T, Gschwandtner ME, et al. Effect of nimodipine on ocular blood flow and colour contrast sensitivity in patients with normal tension glaucoma. Br J Ophthalmol. 2005;89(1):21–5.

149. Koseki N, Araie M, Yamagami J, Shirato S, Yamamoto S. Effects of oral brovincamine on visual field damage in patients with normal-tension glaucoma with low-normal intraocular pressure. J Glaucoma. 1999;8(2):117–23.

150. Koseki N, Araie M, Tomidokoro A, Nagahara M, Hasegawa T, Tamaki Y, et al. A placebo-controlled 3-year study of a calcium blocker on visual field and ocular circulation in glaucoma with low-normal pressure. Ophthalmology. 2008;115(11):2049–57.

151. Zheng W, Dryja TP, Wei Z, Song D, Tian H, Kahler KH, et al. Systemic Medication Associations with Presumed Advanced or Uncontrolled Primary Open-Angle Glaucoma. Ophthalmology. 2018;125(7):984–93.

152. Langman MJS, Lancashire RJ, Cheng KK, Stewart PM. Systemic hypertension and glaucoma: mechanisms in common and co-occurrence. Br J Ophthalmol. 2005;89(8):960–3.

153. Asefa NG, Neustaeter A, Jansonius NM, Snieder H. Autonomic Dysfunction and Blood Pressure in Glaucoma Patients: The Lifelines Cohort Study. Invest Ophthalmol Vis Sci. 2020;61(11):25.

154. Vergroesen JE, Schuster AK, Stuart K V, Asefa NG, Cougnard-Grégoire A, Delcourt C, et al. Association of Systemic Medication Use with Glaucoma and Intraocular Pressure: The European Eye Epidemiology Consortium. Ophthalmology. 2023;130(9):893–906.

155. Manolio TA, Goodhand P, Ginsburg G. The International Hundred Thousand Plus Cohort Consortium: integrating large-scale cohorts to address global scientific challenges. Lancet Digit Heal. 2020;2(11):e567–8.

156. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 2015;12(3):e1001779.

157. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, et al. The UK Biobank resource with deep phenotyping and genomic data. Nature. 2018;562(7726):203–9.

158. Chua SYL, Thomas D, Allen N, Lotery A, Desai P, Patel P, et al. Cohort profile: design and methods in the eye and vision consortium of UK Biobank. BMJ Open. 2019;9(2):e025077.

159. Khera A V, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. Nat Genet. 2018;50(9):1219–24.

160. Qassim A, Souzeau E, Hollitt G, Hassall MM, Siggs OM, Craig JE. Risk Stratification and Clinical Utility of Polygenic Risk Scores in Ophthalmology. Transl Vis Sci Technol. 2021;10(6):14.

161. Chatterjee N, Shi J, García-Closas M. Developing and evaluating polygenic risk prediction models for stratified disease prevention. Nat Rev Genet. 2016;17(7):392–406.

162. Torkamani A, Wineinger NE, Topol EJ. The personal and clinical utility of polygenic risk scores. Nat Rev Genet. 2018;19(9):581–90.

163. Mabuchi F, Mabuchi N, Sakurada Y, Yoneyama S, Kashiwagi K, Iijima H, et al. Additive effects of genetic variants associated with intraocular pressure in primary open-angle glaucoma. PLoS One. 2017;12(8):e0183709.

164. Tham Y-C, Liao J, Vithana EN, Khor C-C, Teo Y-Y, Tai E-S, et al. Aggregate Effects of Intraocular Pressure and Cup-to-Disc Ratio Genetic Variants on Glaucoma in a Multiethnic Asian Population. Ophthalmology. 2015;122(6):1149–57.

165. Zanon-Moreno V, Ortega-Azorin C, Asensio-Marquez EM, Garcia-Medina JJ, Pinazo-Duran MD, Coltell O, et al. A Multi-Locus Genetic Risk Score for Primary Open-Angle Glaucoma (POAG) Variants Is Associated with POAG Risk in a Mediterranean Population: Inverse Correlations with Plasma Vitamin C and E Concentrations. Int J Mol Sci. 2017;18(11).

166. Qassim A, Souzeau E, Siggs OM, Hassall MM, Han X, Griffiths HL, et al. An Intraocular Pressure Polygenic Risk Score Stratifies Multiple Primary Open-Angle Glaucoma Parameters Including Treatment Intensity. Ophthalmology. 2020;127(7):901–7.

167. Siggs OM, Qassim A, Han X, Marshall HN, Mullany S, He W, et al. Association of High Polygenic Risk With Visual Field Worsening Despite Treatment in Early Primary Open-Angle Glaucoma. JAMA Ophthalmol. 2022;141(1):73–7.

168. Mandrekar JN. Receiver operating characteristic curve in diagnostic test assessment. J Thorac Oncol. 2010;5(9):1315–6.

169. Hunter DJ. Gene-environment interactions in human diseases. Nat Rev Genet. 2005;6(4):287–98.

170. Edenberg HJ. The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res Heal. 2007;30(1):5–13.

171. Lockett AD, Van Demark M, Gu Y, Schweitzer KS, Sigua N, Kamocki K, et al. Effect of Cigarette Smoke Exposure and Structural Modifications on the α-1 Antitrypsin Interaction with Caspases. Mol Med. 2012;18(1):445–54.

172. Manolio TA, Bailey-Wilson JE, Collins FS. Genes, environment and the value of prospective cohort studies. Nat Rev Genet. 2006;7(10):812–20.

173. Pollock JS, Förstermann U, Mitchell JA, Warner TD, Schmidt HH, Nakane M, et al. Purification and characterization of particulate endothelium-derived relaxing factor synthase from cultured and native bovine aortic endothelial cells. Proc Natl Acad Sci U S A. 1991;88(23):10480–4.

174. Nathanson JA, McKee M. Identification of an extensive system of nitric oxide-producing cells in the ciliary muscle and outflow pathway of the human eye. Invest Ophthalmol Vis Sci. 1995;36(9):1765–73.

175. Garthwaite G, Bartus K, Malcolm D, Goodwin D, Kollb-Sielecka M, Dooldeniya C, et al. Signaling from blood vessels to CNS axons through nitric oxide. J Neurosci. 2006;26(29):7730–40.

176. Kang JH, Wiggs JL, Rosner BA, Hankinson SE, Abdrabou W, Fan BJ, et al. Endothelial Nitric Oxide Synthase Gene Variants and Primary Open-Angle Glaucoma: Interactions with Sex and Postmenopausal Hormone Use. Investig Opthalmology Vis Sci. 2010;51(2):971.

177. Madjedi KM, Stuart KV, Chua SYL, Foster PJ, Strouthidis NG, Luben RN, et al. The Association of Female Reproductive Factors with Glaucoma and Related Traits: A Systematic Review. Ophthalmol Glaucoma. 2022;5(6):628–47.

178. Kang JH, Wiggs JL, Rosner BA, Haines J, Abdrabou W, Pasquale LR. Endothelial nitric oxide synthase gene variants and primary open-angle glaucoma: interactions with hypertension, alcohol intake, and cigarette smoking. Arch Ophthalmol. 2011;129(6):773–80.

179. Maas P, Barrdahl M, Joshi AD, Auer PL, Gaudet MM, Milne RL, et al. Breast Cancer Risk From Modifiable and Nonmodifiable Risk Factors Among White Women in the United States. JAMA Oncol. 2016;2(10):1295–302.

180. Kim J, Aschard H, Kang JH, Lentjes MAH, Do R, Wiggs JL, et al. Intraocular Pressure, Glaucoma, and Dietary Caffeine Consumption: A Gene-Diet Interaction Study from the UK Biobank. Ophthalmology. 2021;128(6):866–76.

181. Elliott P, Peakman TC, UK Biobank. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. Int J Epidemiol. 2008;37(2):234–44.

182. Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol. 1997;26(Suppl 1):S6-14.

183. Day N, Oakes S, Luben R, Khaw KT, Bingham S, Welch A, et al. EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. Br J Cancer. 1999;80(Suppl 1):95–103.

184. Khawaja AP, Chan MPY, Hayat S, Broadway DC, Luben R, Garway-Heath DF, et al. The EPIC-Norfolk Eye Study: rationale, methods and a cross-sectional analysis of visual impairment in a population-based cohort. BMJ Open. 2013;3(3):e002684.

185. Raina P, Wolfson C, Kirkland S, Griffith LE, Balion C, Cossette B, et al. Cohort

Profile: The Canadian Longitudinal Study on Aging (CLSA). Int J Epidemiol. 2019;48(6):1752–3.

186. Delcourt C, Korobelnik J-F, Buitendijk GHS, Foster PJ, Hammond CJ, Piermarocchi S, et al. Ophthalmic epidemiology in Europe: the “European Eye Epidemiology” (E3) consortium. Eur J Epidemiol. 2016;31(2):197–210.

187. Neville C, Burns F, Cruise S, Scott A, O’Reilly D, Kee F, et al. Cohort Profile: The Northern Ireland Cohort for the Longitudinal Study of Ageing (NICOLA). Int J Epidemiol. 2023;52(4):e211–21.

188. Bikbov M, Fayzrakhmanov RR, Kazakbaeva G, Jonas JB. Ural Eye and Medical Study: description of study design and methodology. Ophthalmic Epidemiol. 2018;25(3):187–98.

189. Topouzis F, Wilson MR, Harris A, Anastasopoulos E, Yu F, Mavroudis L, et al. Prevalence of open-angle glaucoma in Greece: the Thessaloniki Eye Study. Am J Ophthalmol. 2007;144(4):511–9.

190. Cachulo M da L, Lobo C, Figueira J, Ribeiro L, Laíns I, Vieira A, et al. Prevalence of Age-Related Macular Degeneration in Portugal: The Coimbra Eye Study - Report 1. Ophthalmologica. 2015;233(3–4):119–27.

191. Hofman A, Breteler MMB, van Duijn CM, Krestin GP, Pols HA, Stricker BHC, et al. The Rotterdam Study: objectives and design update. Eur J Epidemiol. 2007;22(11):819–29.

192. Delcourt C, Korobelnik J-F, Barberger-Gateau P, Delyfer M-N, Rougier M-B, Le Goff M, et al. Nutrition and age-related eye diseases: the Alienor (Antioxydants, Lipides Essentiels, Nutrition et maladies OculaiRes) Study. J Nutr Health Aging. 2010;14(10):854–61.

193. Mirshahi A, Ponto KA, Höhn R, Wild PS, Pfeiffer N. Ophthalmological aspects of the Gutenberg Health Study (GHS): an interdisciplinary prospective population-based cohort study. Ophthalmologe. 2013;110(3):210–7.

194. Creuzot-Garcher C, Binquet C, Daniel S, Bretillon L, Acar N, de Lazzer A, et al. The Montrachet Study: study design, methodology and analysis of visual acuity and refractive errors in an elderly population. Acta Ophthalmol. 2016;94(2):e90-7.

195. Schram MT, Sep SJS, van der Kallen CJ, Dagnelie PC, Koster A, Schaper N, et al. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. Eur J Epidemiol. 2014;29(6):439–51.

196. Stark K, Olden M, Brandl C, Dietl A, Zimmermann ME, Schelter SC, et al. The German AugUR study: study protocol of a prospective study to investigate chronic diseases in the elderly. BMC Geriatr. 2015;15:130.

197. Liu M, Jiang Y, Wedow R, Li Y, Brazel DM, Chen F, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. Nat Genet. 2019;51(2):237–44.

198. Coffee and Caffeine Genetics Consortium, Cornelis MC, Byrne EM, Esko T, Nalls MA, Ganna A, et al. Genome-wide meta-analysis identifies six novel loci

associated with habitual coffee consumption. Mol Psychiatry. 2015;20(5):647–56.

199. Wang Z, Emmerich A, Pillon NJ, Moore T, Hemerich D, Cornelis MC, et al. Genome-wide association analyses of physical activity and sedentary behavior provide insights into underlying mechanisms and roles in disease prevention. Nat Genet. 2022;54(9):1332–44.

200. Currant H, Hysi P, Fitzgerald TW, Gharahkhani P, Bonnemaijer PWM, Senabouth A, et al. Genetic variation affects morphological retinal phenotypes extracted from UK Biobank optical coherence tomography images. PLoS Genet. 2021;17(5):e1009497.

201. Springelkamp H, Iglesias AI, Mishra A, Höhn R, Wojciechowski R, Khawaja AP, et al. New insights into the genetics of primary open-angle glaucoma based on meta-analyses of intraocular pressure and optic disc characteristics. Hum Mol Genet. 2017;26(2):438–53.

202. Simcoe MJ, Khawaja AP, Hysi PG, Hammond CJ, UK Biobank Eye and Vision Consortium. Genome-wide association study of corneal biomechanical properties identifies over 200 loci providing insight into the genetic etiology of ocular diseases. Hum Mol Genet. 2020;29(18):3154–64.

203. Luce DA. Determining in vivo biomechanical properties of the cornea with an ocular response analyzer. J Cataract Refract Surg. 2005;31(1):156–62.

204. Chan MPY, Broadway DC, Khawaja AP, Yip JLY, Garway-Heath DF, Burr JM, et al. Glaucoma and intraocular pressure in EPIC-Norfolk Eye Study: cross sectional study. BMJ. 2017;358:j3889.

205. Wain L V, Shrine N, Miller S, Jackson VE, Ntalla I, Soler Artigas M, et al. Novel insights into the genetics of smoking behaviour, lung function, and chronic obstructive pulmonary disease (UK BiLEVE): a genetic association study in UK Biobank. Lancet Respir Med. 2015;3(10):769–81.

206. Uman LS. Systematic reviews and meta-analyses. J Can Acad Child Adolesc Psychiatry. 2011;20(1):57–9.

207. Gopalakrishnan S, Ganeshkumar P. Systematic Reviews and Meta-analysis: Understanding the Best Evidence in Primary Healthcare. J Fam Med Prim care. 2013;2(1):9–14.

208. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.

209. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Drummond R, et al. Meta-analysis of Observational Studies in Epidemiology: A Proposal for Reporting. JAMA. 2000;283(15):2008.

210. Deeks JJ, Higgins JPT, Altman DG (editors). Chapter 10: Analysing data and undertaking meta-analyses. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.2 (updated February 2021). Cochrane; 2021.

211. Wang X, Cheng Z. Cross-Sectional Studies: Strengths, Weaknesses, and Recommendations. Chest. 2020;158(1S):S65–71.

212. Grimes DA, Schulz KF. Bias and causal associations in observational research. Lancet. 2002;359(9302):248–52.

213. Hamid S, Desai P, Hysi P, Burr JM, Khawaja AP. Population screening for glaucoma in UK: current recommendations and future directions. Eye. 2022;36(3):504–9.

214. Musa I, Bansal S, Kaleem MA. Barriers to Care in the Treatment of Glaucoma: Socioeconomic Elements That Impact the Diagnosis, Treatment, and Outcomes in Glaucoma Patients. Curr Ophthalmol Rep. 2022;10(3):85–90.

215. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol. 2008;61(4):344–9.

216. Ottman R. Gene-environment interaction: definitions and study designs. Prev Med (Baltim). 1996;25(6):764–70.

217. Davies NM, Holmes MVM V, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ. 2018;362:k601.

218. C Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC), Wensley F, Gao P, Burgess S, Kaptoge S, Di Angelantonio E, et al. Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. BMJ. 2011;342:d548.

219. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol. 2015;44(2):512–25.

220. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. Int J Epidemiol. 2017;46(6):1985–98.

221. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genet Epidemiol. 2016;40(4):304–14.

222. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, et al. Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization: The STROBE-MR Statement. JAMA. 2021;326(16):1614–21.

223. GBD 2019 Blindness and Vision Impairment Collaborators, Vision Loss Expert Group of the Global Burden of Disease Study. Causes of blindness and vision impairment in 2020 and trends over 30 years, and prevalence of avoidable blindness in relation to VISION 2020: the Right to Sight: an analysis for the Global Burden of Disease Study. Lancet Glob Heal. 2021;9(2):e144–60.

224. Bourne RRA, Jonas JB, Bron AM, Cicinelli MV, Das A, Flaxman SR, et al.

Prevalence and causes of vision loss in high-income countries and in Eastern and Central Europe in 2015: magnitude, temporal trends and projections. Br J Ophthalmol. 2018;102(5):575–85.

225. Zhang N, Wang J, Li Y, Jiang B. Prevalence of primary open angle glaucoma in the last 20 years: a meta-analysis and systematic review. Sci Rep. 2021;11(1):13762.

226. Marois G, Bélanger A, Lutz W. Population aging, migration, and productivity in Europe. Proc Natl Acad Sci U S A. 2020;117(14):7690–5.

227. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol. 2006;90(3):262–7.

228. Gallo Afflitto G, Aiello F, Cesareo M, Nucci C. Primary Open Angle Glaucoma Prevalence in Europe: A Systematic Review and Meta-Analysis. J Glaucoma. 2022;31(10):783–8.

229. McCann P, Hogg R, Wright DM, Pose-Bazarra S, Chakravarthy U, Peto T, et al. Glaucoma in the Northern Ireland Cohort for the Longitudinal Study of Ageing (NICOLA): cohort profile, prevalence, awareness and associations. Br J Ophthalmol. 2020;104(11):1492–9.

230. Springelkamp H, Wolfs RC, Ramdas WD, Hofman A, Vingerling JR, Klaver CC, et al. Incidence of glaucomatous visual field loss after two decades of follow-up: the Rotterdam Study. Eur J Epidemiol. 2017;32(8):691–9.

231. Höhn R, Nickels S, Schuster AK, Wild PS, Münzel T, Lackner KJ, et al. Prevalence of glaucoma in Germany: results from the Gutenberg Health Study. Graefes Arch Clin Exp Ophthalmol. 2018;256(9):1695–702.

232. Arnould L, De Lazzer A, Seydou A, Binquet C, Bron AM, Creuzot-Garcher C. Diagnostic ability of spectral-domain optical coherence tomography peripapillary retinal nerve fiber layer thickness to discriminate glaucoma patients from controls in an elderly population (The MONTRACHET study). Acta Ophthalmol. 2020;98(8):e1009–16.

233. United Nations, Department of Economic and Social Affairs, Statistics Division. Standard country or area codes for statistical use (M49) [Internet]. 2024. Available from: https://unstats.un.org/unsd/methodology/m49

234. Barendregt JJ, Doi SA, Lee YY, Norman RE, Vos T. Meta-analysis of prevalence. J Epidemiol Community Health. 2013;67(11):974–8.

235. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med. 1998;17(8):857–72.

236. Eurostat. Revision of the European Standard Population: Report of Eurostat's task force [Internet]. European Union; 2013. Available from: https://ec.europa.eu/eurostat/

237. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539–58.

238. Thompson SG, Higgins JPT. How should meta-regression analyses be undertaken and interpreted? Stat Med. 2002;21(11):1559–73.

239. United Nations, Department of Economic and Social Affairs, Population Division. World Population Prospects 2022 [Internet]. United Nations; 2022. Available from: https://population.un.org/wpp/

240. Kapetanakis VV, Chan MPY, Foster PJ, Cook DG, Owen CG, Rudnicka AR. Global variations and time trends in the prevalence of primary open angle glaucoma (POAG): a systematic review and meta-analysis. Br J Ophthalmol. 2016;100(1):86–93.

241. Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. Arch Public Heal. 2014;72(1):39.

242. Chan EW, Li X, Tham Y-C, Liao J, Wong TY, Aung T, et al. Glaucoma in Asia: regional prevalence variations and future projections. Br J Ophthalmol. 2016;100(1):78–85.

243. Day AC, Baio G, Gazzard G, Bunce C, Azuara-Blanco A, Munoz B, et al. The prevalence of primary angle closure glaucoma in European derived populations: a systematic review. Br J Ophthalmol. 2012;96(9):1162–7.

244. Lundström M, Dickman M, Henry Y, Manning S, Rosen P, Tassignon M-J, et al. Changing practice patterns in European cataract surgery as reflected in the European Registry of Quality Outcomes for Cataract and Refractive Surgery 2008 to 2017. J Cataract Refract Surg. 2021;47(3):373–8.

245. Azuara-Blanco A, Burr J, Ramsay C, Cooper D, Foster PJ, Friedman DS, et al. Effectiveness of early lens extraction for the treatment of primary angle-closure glaucoma (EAGLE): a randomised controlled trial. Lancet. 2016;388(10052):1389–97.

246. Shield KD, Parry C, Rehm J. Chronic Diseases and Conditions Related to Alcohol Use. Alcohol Res. 2013;35(2):155–73.

247. Dguzeh U, Haddad N, Smith K, Johnson J, Doye A, Gwathmey J, et al. Alcoholism: A Multi-Systemic Cellular Insult to Organs. Int J Environ Res Public Health. 2018;15(6):1083.

248. Griswold MG, Fullman N, Hawley C, Arian N, Zimsen SRM, Tymeson HD, et al. Alcohol use and burden for 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2018;392(10152):1015–35.

249. Hiratsuka Y, Li G. Alcohol and Eye Diseases: A Review of Epidemiologic Studies. J Stud Alcohol. 2001;62(3):397–402.

250. Kim YW, Park KH. Exogenous influences on intraocular pressure. Br J Ophthalmol. 2019;103(9):1209–16.

251. Perez CI, Singh K, Lin S. Relationship of lifestyle, exercise, and nutrition with glaucoma. Curr Opin Ophthalmol. 2019;30(2):82–8.

252. Stewart WC. The effect of lifestyle on the relative risk to develop open-angle glaucoma. Curr Opin Ophthalmol. 1995;6(2):3–9.

253. Wang S, Wang JJ, Wong TY. Alcohol and Eye Diseases. Surv Ophthalmol. 2008;53(5):512–25.

254. Stuart KV, Madjedi K, Luben R, Chua S, Warwick A, Khawaja AP, et al. Alcohol, intra-ocular pressure and open angle glaucoma: A systematic review and meta-analysis (protocol). Open Science Framework Registries. 2021.

255. Morgan RL, Thayer KA, Santesso N, Holloway AC, Blain R, Eftim SE, et al. A risk of bias instrument for non-randomized studies of exposures: A users' guide to its application in the context of GRADE. Environ Int. 2019;122:168–84.

256. Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ. 2016;i4919.

257. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7(3):177–88.

258. Grant RL. Converting an odds ratio to a range of plausible relative risks for better communication of research findings. BMJ. 2014;348:f7450.

259. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629–34.

260. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994;50(4):1088–101.

261. Duval S, Tweedie R. Trim and Fill: A Simple Funnel-Plot-Based Method of Testing and Adjusting for Publication Bias in Meta-Analysis. Biometrics. 2000;56(2):455–63.

262. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. 2008;336(7650):924–6.

263. Sun J, Zhou X, Kang Y, Yan L, Sun X, Sui H, et al. Prevalence and risk factors for primary open-angle glaucoma in a rural northeast China population: a population-based survey in Bin County, Harbin. Eye. 2012;26(2):283–91.

264. Lee S, Kim JS, Kim SS, Jung JG, Yoon SJ, Seo Y, et al. Relationship between Alcohol Consumption and Ocular Pressure according to Facial Flushing in Korean Men with Obesity. Korean J Fam Med. 2019;40(6):399–405.

265. Liu H, Qi S, He W, Chang C, Chen Y, Yu J. Association of single-nucleotide polymorphisms in TLR4 gene and gene–environment interaction with primary open angle glaucoma in a Chinese northern population. J Gene Med. 2020;22(1):1–6.

266. Lee JY, Kim JM, Lee KY, Kim B, Lee MY, Park KH. Relationships between Obesity, Nutrient Supply and Primary Open Angle Glaucoma in Koreans. Nutrients. 2020;12(3):1–13.

267. Nusinovici S, Zhang L, Chai X, Zhou L, Tham YC, Vasseneix C, et al. Machine learning to determine relative contribution of modifiable and non-modifiable risk factors of major eye diseases. Br J Ophthalmol. 2020;0:1–8.

268. Topouzis F, Wilson MR, Harris A, Founti P, Yu F, Anastasopoulos E, et al. Risk Factors for Primary Open-Angle Glaucoma and Pseudoexfoliative

Glaucoma in the Thessaloniki Eye Study. Am J Ophthalmol. 2011;152(2).

269. Xu L, You QS, Jonas JB. Prevalence of Alcohol Consumption and Risk of Ocular Diseases in a General Population: The Beijing Eye Study. Ophthalmology. 2009;116(10):1872–9.

270. Yavaş GF, Küsbeci T, Şanli M, Toprak D, Ermiş SS, Inan ÜÜ, et al. Risk Factors for Primary Open-Angle Glaucoma in Western Turkey. Turkish J Ophthalmol. 2013;43(2):87–90.

271. Mwanza JC, Tulenko SE, Barton K, Herndon LW, Mathenge E, Hall A, et al. Eight-Year Incidence of Open-Angle Glaucoma in the Tema Eye Survey. Ophthalmology. 2019;126(3):372–80.

272. Charlson ES, Sankar PS, Miller-Ellis E, Regina M, Fertig R, Salinas J, et al. The Primary Open-Angle African American Glaucoma Genetics Study: Baseline Demographics. Ophthalmology. 2015;122(4):711–20.

273. Zangwill LM, Ayyagari R, Liebmann JM, Girkin CA, Feldman R, Dubiner H, et al. The African Descent and Glaucoma Evaluation Study (ADAGES) III: Contribution of Genotype to Glaucoma Phenotype in African Americans: Study Design and Baseline Data. Ophthalmology. 2019;126(1):156–70.

274. Obstbaum SA, Podos SM, Kolker AE. Low-dose oral alcohol and intraocular pressure. Am J Ophthalmol. 1973;76(6):926–8.

275. Zhao D, Cho J, Kim MH, Guallar E. The Association of Blood Pressure and Primary Open-Angle Glaucoma: A Meta-analysis. Am J Ophthalmol. 2014;158(3):615–27.

276. Husain K, Ansari RA, Ferder L. Alcohol-induced hypertension: Mechanism and prevention. World J Cardiol. 2014;6(5):245.

277. Kahn HA, Milton RC. Alternative Definitions of Open-Angle Glaucoma: Effect on Prevalence and Associations in the Framingham Eye Study. Arch Ophthalmol. 1980;98:2172–7.

278. Ponte F, Giuffré G, Giammanco R, Dardanoni G. Risk factors of ocular hypertension and glaucoma: The Casteldaccia Eye Study. Doc Ophthalmol. 1994;85(3):203–10.

279. Weih LM, Nanjan M, McCarty CA, Taylor HR. Prevalence and Predictors of Open-angle Glaucoma. Ophthalmology. 2001;108(11):1966–72.

280. Khu PM, Dorotheo EU, Lat-Luna MML, Sta. Romana AT. Risk factors for primary open-angle glaucoma in Filipinos. Philipp J Ophthalmol. 2005;30:153–60.

281. Liang YB, Friedman DS, Zhou Q, Yang X, Sun LP, Guo LX, et al. Prevalence of Primary Open Angle Glaucoma in a Rural Adult Chinese Population: The Handan Eye Study. Investig Ophthalmol Vis Sci. 2011;52(11):8250–7.

282. Wood AM, Kaptoge S, Butterworth AS, Willeit P, Warnakula S, Bolton T, et al. Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. Lancet. 2018;391(10129):1513–23.

283. Oh SA, Ra H, Jee D. Socioeconomic Status and Glaucoma: Associations in High Levels of Income and Education. Curr Eye Res. 2019;44(4):436–41.

284. Collins SE. Associations Between Socioeconomic Factors and Alcohol Outcomes. Alcohol Res. 2016;38(1):83–94.

285. Chopra K, Tiwari V. Alcoholic neuropathy: possible mechanisms and future treatment possibilities. Br J Clin Pharmacol. 2012;73(3):348–62.

286. World Health Organisation. Global status report on alcohol and health 2018. Geneva: World Health Organisation; 2018.

287. Khawaja AP, Chan MPY, Garway-Heath DF, Broadway DC, Luben R, Sherwin JC, et al. Associations With Retinal Nerve Fiber Layer Measures in the EPIC-Norfolk Eye Study. Investig Ophthalmol Vis Sci. 2013;54(7):5028–34.

288. Chiotoroiu SM, Pop de Popa D, Ştefăniu GI, Secureanu FA, Purcărea VL, Malaxa N, et al. The importance of alcohol abuse and smoking in the evolution of glaucoma disease. J Med Life. 2013;6(2):226–9.

289. Kim YK, Choi HJ, Jeoung JW, Park KH, Kim DM. Five-Year Incidence of Primary Open-Angle Glaucoma and Rate of Progression in Health Center-Based Korean Population: The Gangnam Eye Study. PLoS One. 2014;9(12):1–14.

290. Kooner K, Albdoor M, Cho BJ, Adams-Huet B. Risk factors for progression to blindness in high tension primary open angle glaucoma: Comparison of blind and nonblind subjects. Clin Ophthalmol. 2008;2(4):757.

291. Moreno-Montañés J, Gutierrez-Ruiz I, Gándara E, Moreno-Galarraga L, Santiago S, Ruiz-Canela M, et al. Carbohydrate intake and risk of glaucoma in the SUN cohort. Eur J Ophthalmol. 2022;32(2):999–1008.

292. Waibel S, Thomaschewski G, Herber R, Pillunat LE, Pillunat KR. Comparison of Different Nutritional and Lifestyle Factors between Glaucoma Patients and an Age-Matched Normal Population. Klin Monbl Augenheilkd. 2021;238(12):1328–34.

293. Zhao Y, Wang C. Glu504Lys Single Nucleotide Polymorphism of Aldehyde Dehydrogenase 2 Gene and the Risk of Human Diseases. Biomed Res Int. 2015;174050:1–9.

294. Kokaze A, Yoshida M, Ishikawa M, Matsunaga N, Makita R, Satoh M, et al. Longevity-associated mitochondrial DNA 5178 A/C polymorphism is associated with intraocular pressure in Japanese men. Clin Exp Ophthalmol. 2004;32(2):131–6.

295. Greenfield TK, Kerr WC. Alcohol Measurement Methodology in Epidemiology: Recent Advances and Opportunities. Addiction. 2008;103(7):1082–99.

296. Bero L, Chartres N, Diong J, Fabbri A, Ghersi D, Lam J, et al. The risk of bias in observational studies of exposures (ROBINS-E) tool: concerns arising from application to observational studies of exposures. Syst Rev. 2018;7(1):242.

297. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Performance of the trim and fill method in the presence of publication bias and between-study

heterogeneity. Stat Med. 2007;26(25):4544–62.

298. Kamal H, Tan GC, Ibrahim SF, Shaikh MF, Mohamed IN, Mohamed RMP, et al. Alcohol Use Disorder, Neurodegeneration, Alzheimer's and Parkinson's Disease: Interplay Between Oxidative Stress, Neuroimmune Response and Excitotoxicity. Front Cell Neurosci. 2020;14:282.

299. Liu B, Young H, Crowe FL, Benson VS, Spencer EA, Key TJ, et al. Development and evaluation of the Oxford WebQ, a low-cost, web-based method for assessment of previous 24 h dietary intakes in large-scale prospective studies. Public Health Nutr. 2011;14(11):1998–2005.

300. Galante J, Adamska L, Young A, Young H, Littlejohns TJ, Gallacher J, et al. The acceptability of repeat Internet-based hybrid diet assessment of previous 24-h dietary intake: administration of the Oxford WebQ in UK Biobank. Br J Nutr. 2016;115(4):681–6.

301. Greenwood DC, Hardie LJ, Frost GS, Alwan NA, Bradbury KE, Carter M, et al. Validation of the Oxford WebQ Online 24-Hour Dietary Questionnaire Using Biomarkers. Am J Epidemiol. 2019;188(10):1858–67.

302. Naimi T, Chikritzhs T, Stockwell T. Commentary on Di Castelnuovo et al: Implications of using low volume drinkers instead of never drinkers as the reference group. Addiction. 2022;117(2):327–9.

303. Andréasson S. Alcohol and J-shaped curves. Alcohol Clin Exp Res. 1998;22(7 Suppl):359S-364S.

304. Harrell FE. Regression Modeling Strategies: With Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis. 2nd ed. Springer International Publishing; 2015.

305. Madjedi KM, Stuart KV, Chua SYL, Luben RN, Warwick A, Pasquale LR, et al. The Association between Serum Lipids and Intraocular Pressure in Two Large United Kingdom Cohorts. Ophthalmology. 2022;129(9):986–96.

306. Mancinelli R, Ceccanti M. Biomarkers in alcohol misuse: their role in the prevention and detection of thiamine deficiency. Alcohol Alcohol. 2009;44(2):177–82.

307. UK Department of Health. UK Chief Medical Officers' Low Risk Drinking Guidelines. 2016.

308. U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2020-2025. 9th ed. 2020.

309. Grant A, Roy-Gagnon M-H, Bastasic J, Talekar A, Jessri M, Li G, et al. Alcohol Consumption, Genetic Risk, and Intraocular Pressure and Glaucoma: The Canadian Longitudinal Study on Aging. Invest Ophthalmol Vis Sci. 2023;64(10):3.

310. Ho H, Shi Y, Chua J, Tham Y-C, Lim SH, Aung T, et al. Association of Systemic Medication Use With Intraocular Pressure in a Multiethnic Asian Population: The Singapore Epidemiology of Eye Diseases Study. JAMA Ophthalmol. 2017;135(3):196–202.

311. Daviet R, Aydogan G, Jagannathan K, Spilka N, Koellinger PD, Kranzler HR, et al. Associations between alcohol consumption and gray and white matter volumes in the UK Biobank. Nat Commun. 2022;13(1):1175.

312. Chua SYL, Lascaratos G, Atan D, Zhang B, Reisman C, Khaw PT, et al. Relationships between retinal layer thickness and brain volumes in the UK Biobank cohort. Eur J Neurol. 2021;28(5):1490–8.

313. O'Keefe JH, Bhatti SK, Bajwa A, DiNicolantonio JJ, Lavie CJ. Alcohol and cardiovascular health: the dose makes the poison…or the remedy. Mayo Clin Proc. 2014;89(3):382–93.

314. Wareham LK, Calkins DJ. The Neurovascular Unit in Glaucomatous Neurodegeneration. Front Cell Dev Biol. 2020;8:452.

315. Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, et al. Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants With Those of the General Population. Am J Epidemiol. 2017;186(9):1026–34.

316. Rodgers GP, Collins FS. Precision Nutrition-the Answer to "What to Eat to Stay Healthy." JAMA. 2020;324(8):735–6.

317. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet. 2014;23(R1):R89-98.

318. Macgregor S, Lind PA, Bucholz KK, Hansell NK, Madden PAF, Richter MM, et al. Associations of ADH and ALDH2 gene variation with self report alcohol reactions, consumption and dependence: an integrated analysis. Hum Mol Genet. 2009;18(3):580–93.

319. Auton A, Abecasis GR, Altshuler DM, Durbin RM, Abecasis GR, Bentley DR, et al. A global reference for human genetic variation. Nature. 2015;526(7571):68–74.

320. Polimanti R, Gelernter J. ADH1B: From alcoholism, natural selection, and cancer to the human phenome. Am J Med Genet. 2018;177(2):113–25.

321. Oota H, Pakstis AJ, Bonne-Tamir B, Goldman D, Grigorenko E, Kajuna SLB, et al. The evolution and population genetics of the ALDH2 locus: random genetic drift, selection, and low levels of recombination. Ann Hum Genet. 2004;68(2):93–109.

322. Burgess S, Butterworth A, Thompson SG. Mendelian Randomization Analysis With Multiple Genetic Variants Using Summarized Data. Genet Epidemiol. 2013;37(7):658–65.

323. Burgess S, Bowden J, Fall T, Ingelsson E, Thompson SG. Sensitivity Analyses for Robust Causal Inference from Mendelian Randomization Analyses with Multiple Genetic Variants. Epidemiology. 2017;28(1):30–42.

324. Verbanck M, Chen C-Y, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet. 2018;50(5):693–8.

325. Burgess S, Thompson SG. Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. Am J Epidemiol. 2015;181(4):251–60.

326. Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. Int J Epidemiol. 2016;45(6):1866–86.

327. Burgess S, Thompson SG. Avoiding bias from weak instruments in Mendelian randomization studies. Int J Epidemiol. 2011;40(3):755–64.

328. Bowden J, Del Greco M F, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. Int J Epidemiol. 2016;45(6):1961–74.

329. Heavner W, Pevny L. Eye development and retinogenesis. Cold Spring Harb Perspect Biol. 2012;4(12):a008391.

330. Stuart KV, Madjedi KM, Luben RN, Biradar MI, Wagner SK, Warwick AN, et al. Smoking, Corneal Biomechanics, and Glaucoma: Results From Two Large Population-Based Cohorts. Invest Ophthalmol Vis Sci. 2024;65(1):11.

331. GBD 2019 Tobacco Collaborators. Spatial, temporal, and demographic patterns in prevalence of smoking tobacco use and attributable disease burden in 204 countries and territories, 1990-2019: a systematic analysis from the Global Burden of Disease Study 2019. Lancet. 2021;397(10292):2337–60.

332. Ye J, He J, Wang C, Wu H, Shi X, Zhang H, et al. Smoking and risk of age-related cataract: a meta-analysis. Invest Ophthalmol Vis Sci. 2012;53(7):3885–95.

333. Smith W, Assink J, Klein R, Mitchell P, Klaver CC, Klein BE, et al. Risk factors for age-related macular degeneration: Pooled findings from three continents. Ophthalmology. 2001;108(4):697–704.

334. Thornton J, Kelly SP, Harrison RA, Edwards R. Cigarette smoking and thyroid eye disease: a systematic review. Eye. 2007;21(9):1135–45.

335. Madhukumar E, Vijayammal PL. Influence of cigarette smoke on cross-linking of dermal collagen. Indian J Exp Biol. 1997;35(5):483–6.

336. Hafezi F. Smoking and corneal biomechanics. Ophthalmology. 2009;116(11):2259.

337. Spoerl E, Raiskup-Wolf F, Kuhlisch E, Pillunat LE. Cigarette smoking is negatively associated with keratoconus. J Refract Surg. 2008;24(7):S737-40.

338. McCafferty SJ, Enikov ET, Schwiegerling J, Ashley SM. Goldmann tonometry tear film error and partial correction with a shaped applanation surface. Clin Ophthalmol. 2018;12:71–8.

339. Kohlhaas M, Boehm AG, Spoerl E, Pürsten A, Grein HJ, Pillunat LE. Effect of central corneal thickness, corneal curvature, and axial length on applanation tonometry. Arch Ophthalmol. 2006;124(4):471–6.

340. Bondy SJ, Victor JC, Diemert LM. Origin and use of the 100 cigarette criterion

in tobacco surveys. Tob Control. 2009;18(4):317–23.

341. Luce D. Methodology for Cornea Compensated IOP and Corneal Resistance Factor for the Reichert Ocular Response Analyzer. Investig Opthalmology Vis Sci. 2006;47(13):2266.

342. Zhang B, Shweikh Y, Khawaja AP, Gallacher J, Bauermeister S, Foster PJ, et al. Associations with Corneal Hysteresis in a Population Cohort: Results from 96 010 UK Biobank Participants. Ophthalmology. 2019;126(11):1500–10.

343. McGeoch LJ, Ross S, Massa MS, Lewington S, Clarke R. Cigarette smoking and risk of severe infectious respiratory diseases in UK adults: 12-year follow-up of UK biobank. J Public Heal. 2023;45(4):e621–9.

344. Paik DC, Saito LY, Sugirtharaj DD, Holmes JW. Nitrite-induced cross-linking alters remodeling and mechanical properties of collagenous engineered tissues. Connect Tissue Res. 2006;47(3):163–76.

345. Hafezi F. Tobacco smoking and its impact on corneal biomechanics. Invest Ophthalmol Vis Sci. 2010;51(12):6892.

346. Liu M-X, Li D-L, Yin Z-J, Li Y-Z, Zheng Y-J, Qin Y, et al. Smoking, alcohol consumption and corneal biomechanical parameters among Chinese university students. Eye. 2023;37(13):2723–9.

347. Sady C, Khosrof S, Nagaraj R. Advanced Maillard reaction and crosslinking of corneal collagen in diabetes. Biochem Biophys Res Commun. 1995;214(3):793–7.

348. Nicholl ID, Bucala R. Advanced glycation endproducts and cigarette smoking. Cell Mol Biol. 1998;44(7):1025–33.

349. Tran JH, Stuart KV, de Vries V, Vergroesen JE, Cousins CC, Hysi PG, et al. Genetic Associations Between Smoking- and Glaucoma-Related Traits. Transl Vis Sci Technol. 2023;12(2):20.

350. Schramm S, Carré V, Scheffler J-L, Aubriet F. Active and passive smoking – New insights on the molecular composition of different cigarette smoke aerosols by LDI–FTICRMS. Atmos Environ. 2014;92:411–20.

351. Sit AJ, Chen TC, Takusagawa HL, Rosdahl JA, Hoguet A, Chopra V, et al. Corneal Hysteresis for the Diagnosis of Glaucoma and Assessment of Progression Risk: A Report by the American Academy of Ophthalmology. Ophthalmology. 2023;130(4):433–42.

352. Reitsma MB, Flor LS, Mullany EC, Gupta V, Hay SI, Gakidou E. Spatial, temporal, and demographic patterns in prevalence of smoking tobacco use and initiation among young people in 204 countries and territories, 1990-2019. Lancet Public Heal. 2021;6(7):e472–81.

353. Leite MT, Alencar LM, Gore C, Weinreb RN, Sample PA, Zangwill LM, et al. Comparison of corneal biomechanical properties between healthy blacks and whites using the Ocular Response Analyzer. Am J Ophthalmol. 2010;150(2):163–8.

354. Yang C, Huang X, Li X, Yang C, Zhang T, Wu Q, et al. Wearable and

Implantable Intraocular Pressure Biosensors: Recent Progress and Future Prospects. Adv Sci. 2021;8(6):2002971.

355. Larsson SC, Burgess S. Appraising the causal role of smoking in multiple diseases: A systematic review and meta-analysis of Mendelian randomization studies. EBioMedicine. 2022;82:104154.

356. Kuan V, Warwick A, Hingorani A, Tufail A, Cipriani V, Burgess S, et al. Association of Smoking, Alcohol Consumption, Blood Pressure, Body Mass Index, and Glycemic Risk Factors With Age-Related Macular Degeneration: A Mendelian Randomization Study. JAMA Ophthalmol. 2021;139(12):1299–306.

357. Bjørngaard JH, Nordestgaard AT, Taylor AE, Treur JL, Gabrielsen ME, Munafò MR, et al. Heavier smoking increases coffee consumption: findings from a Mendelian randomization analysis. Int J Epidemiol. 2017;46(6):1958–67.

358. Mahmoudinezhad G, Nishida T, Weinreb RN, Baxter SL, Eslani M, Micheletti E, et al. Impact of Smoking on Visual Field Progression in a Long-term Clinical Follow-up. Ophthalmology. 2022;129(11):1235–44.

359. Stuart KV, Biradar M, Luben R, Dhaun N, Wagner S, Warwick A, et al. Association of urinary sodium excretion with glaucoma and related traits. Investig Ophthalmol Vis Sci. 2023;64(8):2908.

360. Hunter RW, Dhaun N, Bailey MA. The impact of excessive salt intake on human health. Nat Rev Nephrol. 2022;18(5):321–35.

361. Grillo A, Salvi L, Coruzzi P, Salvi P, Parati G. Sodium Intake and Hypertension. Nutrients. 2019;11(9):1970.

362. Freedman LS, Commins JM, Moler JE, Willett W, Tinker LF, Subar AF, et al. Pooled results from 5 validation studies of dietary self-report instruments using recovery biomarkers for potassium and sodium intake. Am J Epidemiol. 2015;181(7):473–87.

363. Park Y, Dodd KW, Kipnis V, Thompson FE, Potischman N, Schoeller DA, et al. Comparison of self-reported dietary intakes from the Automated Self-Administered 24-h recall, 4-d food records, and food-frequency questionnaires against recovery biomarkers. Am J Clin Nutr. 2018;107(1):80–93.

364. Huang L, Crino M, Wu JHY, Woodward M, Barzi F, Land M-A, et al. Mean population salt intake estimated from 24-h urine samples and spot urine samples: a systematic review and meta-analysis. Int J Epidemiol. 2016;45(1):239–50.

365. Mann SJ, Gerber LM. Estimation of 24-hour sodium excretion from spot urine samples. J Clin Hypertens. 2010;12(3):174–80.

366. Perez-Cornago A, Pollard Z, Young H, van Uden M, Andrews C, Piernas C, et al. Description of the updated nutrition calculation of the Oxford WebQ questionnaire and comparison with the previous version among 207,144 participants in UK Biobank. Eur J Nutr. 2021;60(7):4019–30.

367. Bradbury KE, Guo W, Cairns BJ, Armstrong MEG, Key TJ. Association between physical activity and body fat percentage, with adjustment for BMI: a large cross-sectional analysis of UK Biobank. BMJ Open. 2017;7(3):e011843.

368. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New Creatinine- and Cystatin C-Based Equations to Estimate GFR without Race. N Engl J Med. 2021;385(19):1737–49.

369. Faulkner JL, Belin de Chantemèle EJ. Female Sex, a Major Risk Factor for Salt-Sensitive Hypertension. Curr Hypertens Rep. 2020;22(12):99.

370. Khaw K-T, Bingham S, Welch A, Luben R, O'Brien E, Wareham N, et al. Blood pressure and urinary sodium in men and women: the Norfolk Cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk). Am J Clin Nutr. 2004;80(5):1397–403.

371. Kawasaki T, Itoh K, Uezono K, Sasaki H. A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. Clin Exp Pharmacol Physiol. 1993;20(1):7–14.

372. Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H, et al. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. J Hum Hypertens. 2002;16(2):97–103.

373. Brown IJ, Dyer AR, Chan Q, Cogswell ME, Ueshima H, Stamler J, et al. Estimating 24-hour urinary sodium excretion from casual urinary sodium concentrations in Western populations: the INTERSALT study. Am J Epidemiol. 2013;177(11):1180–92.

374. Charlton KE, Steyn K, Levitt NS, Zulu J V, Jonathan D, Veldman FJ, et al. Ethnic differences in intake and excretion of sodium, potassium, calcium and magnesium in South Africans. Eur J Cardiovasc Prev Rehabil. 2005;12(4):355–62.

375. Richardson SI, Freedman BI, Ellison DH, Rodriguez CJ. Salt sensitivity: a review with a focus on non-Hispanic blacks and Hispanics. J Am Soc Hypertens. 2013;7(2):170–9.

376. Levy J, Tovbin D, Lifshitz T, Zlotnik M, Tessler Z. Intraocular pressure during haemodialysis: a review. Eye. 2005;19(12):1249–56.

377. World Health Organization. Effect of reduced sodium intake on blood pressure, renal function, blood lipids and other potential adverse effects. 2012.

378. Bradbury KE, Young HJ, Guo W, Key TJ. Dietary assessment in UK Biobank: an evaluation of the performance of the touchscreen dietary questionnaire. J Nutr Sci. 2018;7:e6.

379. Tsai M-J, Cheng C-K, Wang Y-C. Association of Body Fluid Expansion With Optical Coherence Tomography Measurements in Diabetic Retinopathy and Diabetic Macular Edema. Invest Ophthalmol Vis Sci. 2019;60(10):3606–12.

380. Zhang Y, Weng H, Li Q, Wang Z. Changes in retina and choroid after haemodialysis assessed using optical coherence tomography angiography. Clin Exp Optom. 2018;101(5):674–9.

381. Tham Y-C, Cheng C-Y. Associations between chronic systemic diseases and primary open angle glaucoma: an epidemiological perspective. Clin Experiment Ophthalmol. 2017;45(1):24–32.

382. World Health Organisation. ATC/DDD Index 2024 [Internet]. World Health Organisation; 2024. Available from: https://atcddd.fhi.no/atc_ddd_index/

383. Phillips CI, Howitt G, Rowlands DJ. Propranolol as ocular hypotensive agent. Br J Ophthalmol. 1967;51(4):222–6.

384. Kastner A, Stuart K V, Montesano G, De Moraes CG, Kang JH, Wiggs JL, et al. Calcium Channel Blocker Use and Associated Glaucoma and Related Traits Among UK Biobank Participants. JAMA Ophthalmol. 2023;141(10):956–64.

385. Kastner A, Montesano G, De Moraes CG, Kang JH, Wiggs J, Pasquale L, et al. Calcium Channel Blocker Use and Risk of Glaucoma in a Large United Kingdom Population. Investig Ophthalmol Vis Sci. 2020;61(7):2739.

386. Shemin DG, Dworkin LD. Chapter 53: Calcium Channel Blockers. In: Wilcox CS, editor. Therapy in Nephrology & Hypertension (Third Edition). Philadelphia: W. B. Saunders; 2008.

387. Audi S, Burrage DR, Lonsdale DO, Pontefract S, Coleman JJ, Hitchings AW, et al. The 'top 100' drugs and classes in England: an updated 'starter formulary' for trainee prescribers. Br J Clin Pharmacol. 2018;84(11):2562–71.

388. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. Nat Rev Nephrol. 2020;16(4):223–37.

389. Salive ME. Multimorbidity in older adults. Epidemiol Rev. 2013;35:75–83.

390. Schnell D. Effect Response of Intraocular Pressure in Normal Subjects and Glaucoma Patients to Single and Repeated Doses of the Coronary Drug Adalat. In: Lochner W, Braasch W, Kroneberg G, editors. 2nd International Adalat® Symposium: New Therapy of Ischemic Heart Disease. Berlin Heidelberg: Springer; 1975. p. 290–302.

391. Monica ML, Hesse RJ, Messerli FH. The Effect of a Calcium-Channel Blocking Agent on Intraocular Pressure. Am J Ophthalmol. 1983;96(6):814.

392. Brewster LM, Seedat YK. Why do hypertensive patients of African ancestry respond better to calcium blockers and diuretics than to ACE inhibitors and β-adrenergic blockers? A systematic review. BMC Med. 2013;11:141.

393. Chong RS, Chee M-L, Tham Y-C, Majithia S, Thakur S, Teo ZL, et al. Association of Antihypertensive Medication with Retinal Nerve Fiber Layer and Ganglion Cell-Inner Plexiform Layer Thickness. Ophthalmology. 2021;128(3):393–400.

394. Kelly SP, Walley TJ. Effect of the calcium antagonist nifedipine on intraocular pressure in normal subjects. Br J Ophthalmol. 1988;72(3):216–8.

395. Araie M, Mayama C. Use of calcium channel blockers for glaucoma. Prog Retin Eye Res. 2011;30(1):54–71.

396. Yamamoto T, Niwa Y, Kawakami H, Kitazawa Y. The effect of nilvadipine, a calcium-channel blocker, on the hemodynamics of retrobulbar vessels in normal-tension glaucoma. J Glaucoma. 1998;7(5):301–5.

397. Tomita G, Niwa Y, Shinohara H, Hayashi N, Yamamoto T, Kitazawa Y. Changes in optic nerve head blood flow and retrobular hemodynamics following calcium-channel blocker treatment of normal-tension glaucoma. Int Ophthalmol. 1999;23(1):3–10.

398. McElnea EM, Quill B, Docherty NG, Irnaten M, Siah WF, Clark AF, et al. Oxidative stress, mitochondrial dysfunction and calcium overload in human lamina cribrosa cells from glaucoma donors. Mol Vis. 2011;17:1182–91.

399. Wojda U, Salinska E, Kuznicki J. Calcium ions in neuronal degeneration. IUBMB Life. 2008;60(9):575–90.

400. Madjedi KM, Stuart KV, Chua SY, Ramulu PY, Warwick A, Luben RN, et al. The Association of Physical Activity with Glaucoma and Related Traits in the UK Biobank. Ophthalmology. 2023;130(10):1024–36.

401. Endres M, Gertz K, Lindauer U, Katchanov J, Schultze J, Schröck H, et al. Mechanisms of stroke protection by physical activity. Ann Neurol. 2003;54(5):582–90.

402. Adamopoulos S, Coats AJ, Brunotte F, Arnolda L, Meyer T, Thompson CH, et al. Physical training improves skeletal muscle metabolism in patients with chronic heart failure. J Am Coll Cardiol. 1993;21(5):1101–6.

403. Lee I-M, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT, et al. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet. 2012;380(9838):219–29.

404. Sofi F, Valecchi D, Bacci D, Abbate R, Gensini GF, Casini A, et al. Physical activity and risk of cognitive decline: a meta-analysis of prospective studies. J Intern Med. 2011;269(1):107–17.

405. Ong SR, Crowston JG, Loprinzi PD, Ramulu PY. Physical activity, visual impairment, and eye disease. Eye. 2018;32(8):1296–303.

406. Wijndaele K, Westgate K, Stephens SK, Blair SN, Bull FC, Chastin SFM, et al. Utilization and Harmonization of Adult Accelerometry Data: Review and Expert Consensus. Med Sci Sports Exerc. 2015;47(10):2129–39.

407. Doherty A, Jackson D, Hammerla N, Plötz T, Olivier P, Granat MH, et al. Large Scale Population Assessment of Physical Activity Using Wrist Worn Accelerometers: The UK Biobank Study. PLoS One. 2017;12(2):e0169649.

408. Gleiss AC, Wilson RP, Shepard ELC. Making overall dynamic acceleration work: on the theory of acceleration as a proxy for energy expenditure. Methods Ecol Evol. 2011;2:23–33.

409. White T, Westgate K, Wareham NJ, Brage S. Estimation of Physical Activity Energy Expenditure during Free-Living from Wrist Accelerometry in UK Adults. PLoS One. 2016;11(12):e0167472.

410. Lee MJ, Wang J, Friedman DS, Boland M V, De Moraes CG, Ramulu PY. Greater Physical Activity Is Associated with Slower Visual Field Loss in Glaucoma. Ophthalmology. 2019;126(7):958–64.

411. van Landingham SW, Willis JR, Vitale S, Ramulu PY. Visual field loss and accelerometer-measured physical activity in the United States. Ophthalmology. 2012;119(12):2486–92.

412. E J-Y, Schrack JA, Mihailovic A, Wanigatunga AA, West SK, Friedman DS, et al. Patterns of Daily Physical Activity across the Spectrum of Visual Field Damage in Glaucoma Patients. Ophthalmology. 2021;128(1):70–7.

413. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381–95.

414. Hildebrand M, VAN Hees VT, Hansen BH, Ekelund U. Age group comparability of raw accelerometer output from wrist- and hip-worn monitors. Med Sci Sports Exerc. 2014;46(9):1816–24.

415. Chieffi S, Messina G, Villano I, Messina A, Valenzano A, Moscatelli F, et al. Neuroprotective Effects of Physical Activity: Evidence from Human and Animal Studies. Front Neurol. 2017;8:188.

416. Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, et al. Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. Ann Intern Med. 2006;144(2):73–81.

417. Chrysostomou V, Kezic JM, Trounce IA, Crowston JG. Forced exercise protects the aged optic nerve against intraocular pressure injury. Neurobiol Aging. 2014;35(7):1722–5.

418. He Y-Y, Wang L, Zhang T, Weng S-J, Lu J, Zhong Y-M. Aerobic exercise delays retinal ganglion cell death after optic nerve injury. Exp Eye Res. 2020;200:108240.

419. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample Mendelian randomization. Genet Epidemiol. 2016;40(7):597–608.

420. Mahmoudinezhad G, Nishida T, Weinreb RN, Baxter SL, Chang AC, Nikkhoy N, et al. Associations of smoking and alcohol consumption with the development of open angle glaucoma: a retrospective cohort study. BMJ Open. 2023;13(10):e072163.

421. Fujita A, Hashimoto Y, Matsui H, Yasunaga H, Aihara M. Association between lifestyle habits and glaucoma incidence: a retrospective cohort study. Eye. 2023;37(16):3470–6.

422. Jeong Y, Kim SH, Kang G, Yoon H-J, Kim YK, Ha A. Visual Impairment Risk After Alcohol Abstinence in Patients With Newly Diagnosed Open-Angle Glaucoma. JAMA Netw Open. 2023;6(10):e2338526.

423. Sano K, Terauchi R, Fukai K, Furuya Y, Nakazawa S, Kojimahara N, et al. Association Between Alcohol Consumption Patterns and Glaucoma in Japan. J Glaucoma. 2023;32(11):968–75.

424. 3C Study Group. Vascular factors and risk of dementia: design of the Three-City Study and baseline characteristics of the study population. Neuroepidemiology. 2003;22(6):316–25.

425. Schweitzer C, Korobelnik J-F, Le Goff M, Rahimian O, Malet F, Rougier M-B, et al. Diagnostic Performance of Peripapillary Retinal Nerve Fiber Layer Thickness for Detection of Glaucoma in an Elderly Population: The ALIENOR Study. Invest Ophthalmol Vis Sci. 2016;57(14):5882–91.

426. Brandl C, Zimmermann ME, Günther F, Barth T, Olden M, Schelter SC, et al. On the impact of different approaches to classify age-related macular degeneration: Results from the German AugUR study. Sci Rep. 2018;8(1):8675.

427. Brandl C, Zimmermann ME, Herold JM, Helbig H, Stark KJ, Heid IM. Photostress Recovery Time as a Potential Predictive Biomarker for Age-Related Macular Degeneration. Transl Vis Sci Technol. 2023;12(2):15.

428. Cachulo M da L, Laíns I, Lobo C, Figueira J, Ribeiro L, Marques JP, et al. Age-related macular degeneration in Portugal: prevalence and risk factors in a coastal and an inland town. The Coimbra Eye Study - Report 2. Acta Ophthalmol. 2016;94(6):e442-53.

429. Farinha CVL, Cachulo ML, Alves D, Pires I, Marques JP, Barreto P, et al. Incidence of Age-Related Macular Degeneration in the Central Region of Portugal: The Coimbra Eye Study - Report 5. Ophthalmic Res. 2019;61(4):226–35.

430. Farinha C, Cachulo ML, Coimbra R, Alves D, Nunes S, Pires I, et al. Age-Related Macular Degeneration Staging by Color Fundus Photography vs. Multimodal Imaging-Epidemiological Implications (The Coimbra Eye Study - Report 6). J Clin Med. 2020;9(5):1329.

431. Wild PS, Zeller T, Beutel M, Blettner M, Dugi KA, Lackner KJ, et al. The Gutenberg Health Study. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2012;55(6–7):824–9.

432. Blanc J, Seydou A, Ben Ghezala I, Deschasse C, Meillon C, Bron AM, et al. Vitreomacular Interface Abnormalities and Glaucoma in an Elderly Population (The MONTRACHET Study). Invest Ophthalmol Vis Sci. 2019;60(6):1996–2002.

433. Asman P, Heijl A. Glaucoma Hemifield Test. Automated visual field evaluation. Arch Ophthalmol. 1992;110(6):812–9.

434. Öhnell H, Bengtsson B, Heijl A. Making a Correct Diagnosis of Glaucoma: Data From the EMGT. J Glaucoma. 2019;28(10):859–64.

435. Jonasson F, Damji KF, Arnarsson A, Sverrisson T, Wang L, Sasaki H, et al. Prevalence of open-angle glaucoma in Iceland: Reykjavik Eye Study. Eye. 2003;17(6):747–53.

436. Ikram MA, Brusselle G, Ghanbari M, Goedegebure A, Ikram MK, Kavousi M, et al. Objectives, design and main findings until 2020 from the Rotterdam Study. Eur J Epidemiol. 2020;35(5):483–517.

437. Ramdas WD, Wolfs RCW, Hofman A, de Jong PTVM, Vingerling JR, Jansonius NM. Heidelberg Retina Tomograph (HRT3) in population-based epidemiology: normative values and criteria for glaucomatous optic

neuropathy. Ophthalmic Epidemiol. 2011;18(5):198–210.

438. Skenduli-Bala E, de Voogd S, Wolfs RCW, van Leeuwen R, Ikram MK, Jonas JB, et al. Causes of incident visual field loss in a general elderly population: the Rotterdam study. Arch Ophthalmol. 2005;123(2):233–8.

439. Bikbov MM, Kazakbaeva GM, Zainullin RM, Salavatova VF, Gilmanshin TR, Yakupova DF, et al. Intraocular Pressure and Its Associations in a Russian Population: The Ural Eye and Medical Study. Am J Ophthalmol. 2019;204:130–9.

440. Bikbov MM, Gilmanshin TR, Kazakbaeva GM, Zainullin RM, Rakhimova EM, Rusakova IA, et al. Prevalence of Myopic Maculopathy Among Adults in a Russian Population. JAMA Netw Open. 2020;3(3):e200567.