Glaucoma in and out of Africa

Pieter W.M. Bonnemaijer

Stellingen behorende bij het proefschrift

Glaucoma in and out of Africa

1. Primair open-kamerhoek glaucoom (POAG) in patiënten van sub-Sahara Afrikaanse afkomst presenteert zich in een ernstiger stadium van de ziekte op een jongere leeftijd. Dit suggereert dat de ziekte een progressiever en destructiever beloop kent ten opzichte van Europese glaucoom patiënten, en vergt daarom extra surveillance. *(dit proefschrift)*

2. Hoewel de centrale corneale dikte dunner is in personen van sub-Sahara Afrikaanse afkomst is dit geen directe risicofactor voor POAG in deze populaties. *(dit proefschrift)*

3. Dat Europese en Aziatische genetische risicofactoren voor POAG een kleinere rol spelen in POAG in sub-Sahara Afrikaanse populaties, ondermijnt de "out of Africa theorie" niet. *(dit proefschrift)*

4. Varianten in het *EXOC4* en *APBB2* gen zijn betrokken bij de pathogenese van POAG in populaties van sub Sahara Afrikaanse afkomst. *(dit proefschrift)*

5. Microvasculaire en neurodegeneratieve ziekten verstoren de nauwkeurigheid van glaucoom diagnostiek met de OCT. Deze ziekten zullen daarom moeten worden meegenomen bij de interpretatie van de zenuwvezel laag dikte. *(dit proefschrift)*

6. Consumptie van Garcinia Kola (bitter kola) verbetert niet alleen de potentie maar verlaagt ook de oogdruk. *(naar Alex A. Ilechie, Acta Ophthalmologica 2020)*

7. Laplace heeft nooit bedoeld om het oog te modelleren; we moeten voorzichtig zijn bij het gebruik van zijn beroemde wet bij de studie van de biomechanica van het oog. (*Cheuk W. Chung, 2016 Investigative Ophthalmology and Visual Science*)

8. Het gebruik van genetische risicostratificatie door middel van polygenetische risicoscores kan een additioneel voorspellend vermogen bieden bij POAG bovenop traditionele risicofactoren, waaronder leeftijd, geslacht, familiegeschiedenis en Afrikaanse afkomst. (*Owen Siggs et al., 2021 Jama Ophthalmology*)

9. Eurocentrisme in genetische studies betekent dat vooruitgang in genoomonderzoek slechts enkelen ten goede komt, niet iedereen. (*editorial, 2019 Nature Human Behaviour*)

10. Het testen van significantie heeft geleid tot veel meer misverstanden en verkeerde interpretaties dan duidelijkheid bij het interpreteren van onderzoeksresultaten. (*Kenneth J. Rothman, 2014 Journal of General Internal Medicine*)

11. Een hybride corona is een modern academisch gezelschap dat klimaat en infectiepreventie hoog in het vaandel heeft.

GLAUCOMA IN AND OUT OF AFRICA

Pieter Willem Marie Bonnemaijer

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Copromotor:	dr. A.A.H.J. Thiadens

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General introduction and aims of this thesis

GENERAL INTRODUCTION

The disease

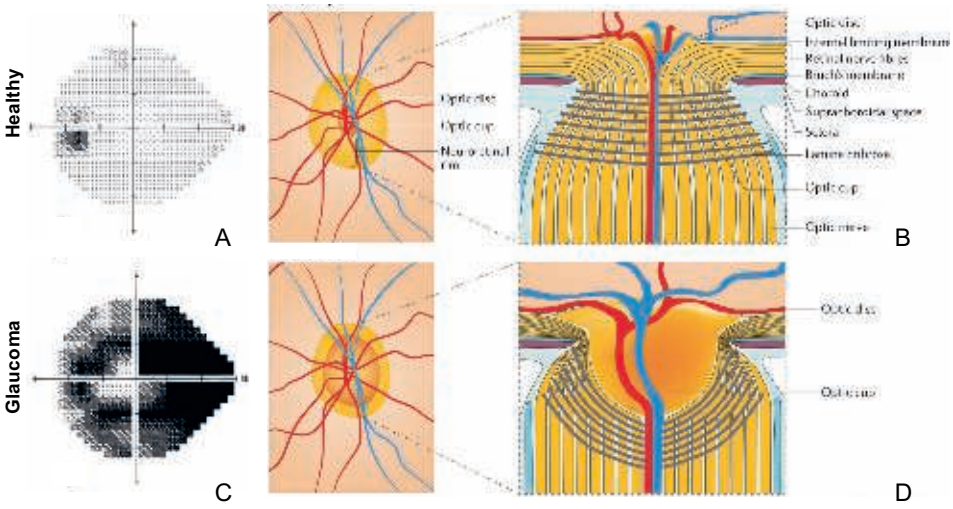
Glaucoma is a heterogeneous group of disorders leading to neurodegeneration of the optic nerve. It is the commonest cause of irreversible blindness in the world affecting over 70 million people[1,2]. The term glaucoma is derived from the Greek word γλαυκωμα (glaucosis) meaning green hue. The Ancient Greeks at the time of Hippocrates (c. 460-c. 370 BC) probably used this term, except for describing iris color, to define a pathologic state of the eye where the pupil was not black. It has been speculated that this condition may be similar to what is now known as acute angle closure glaucoma. The understanding of glaucoma has slowly developed until, in 1862, the Dutch ophthalmologist and pioneer Donders published his paper on glaucoma simplex. He was the first to combine the observations of high eye pressure with deterioration of the visual field and optic nerve head (ONH) changes. Now, glaucoma is characterized by gradual loss of retinal ganglion cells and retinal nerve fibers causing narrowing of the neuroretinal rim at the optic nerve head (ONH) leading to a typical glaucomatous excavation (Fig. 1D). In its early phase, functional consequences are subtle peripheral visual field defects, which are often asymptomatic and undiagnosed. Later in the disease process, the typical arcuate visual field defects may extend and involve central vision leading to blindness (Fig. 1C). This gradual loss of peripheral vision has nicknamed this disorder “the silent thief of sight”.

In the process of retinal ganglion cell and axonal damage intraocular pressure (IOP) plays an important role. IOP is a resultant form the balance between secretion of aqueous humor by the ciliary body and drainage through the trabecular meshwork and uveoscleral outflow route. Two different types of glaucoma can be distinguished by distortion of this outflow pathway. In open-angle glaucoma the aqueous outflow pathway unto the trabecular meshwork is visibly unobstructed, hence aqueous humor can reach the eye’s main drainage system without hinderance. The reduced outflow leading to elevated IOP in this type of glaucoma is presumed to be caused by increased outflow resistance at the level of the trabecular meshwork. When the aqueous outflow pathway is physically blocked by the iris, the drainage angle is inaccessible leading to so called angle-closure. Glaucoma can also be classified as primary, when the cause of elevated IOP is unknown, or secondary when the cause can be identified by clinical examination, for example by pigment blockage. Primary open-angle glaucoma (POAG) is the predominant subtype of glaucoma and the focus of this thesis. POAG is estimated to affect between 53,5 million and 65 million people in 2020 worldwide[1,3]. And its incidence is highly correlated with increasing age[4]. The exact cause of POAG is unknown and its pathophysiology is far from understood. Two major hypotheses have been proposed to account for the observed damage of the ONH. The first emphases on reductions in vascular perfusion of the optic nerve and nerve fiber layer; the second centers on mechanical damage occurring at the level of the lamina cribrosa[5]. For both, the

damage to the optic nerve is thought to be mediated through a combination of vulnerability of the optic nerve head and elevation in IOP beyond what the susceptible eye can tolerate. Other established risk factors are age, African ancestry, family history, and myopia[6-15].

Currently, the only available treatment is lowering of the IOP. Many studies have shown that this slows down disease progression in POAG[16]. Nevertheless, even when IOP is treated, retrospective clinical studies found that 15% to 20% of patients become blind in at least one eye in 15 to 20 years[17]. And before death ~40% of patients suffer from blindness in one eye [18]. Thus, there is a urgent need for effective treatment regimens that will benefit all POAG patients.

Figure 1. Pathophysiology of glaucoma



(A) Normal visual field test showing the blind spot located 15 temporally from fixation (all from left eyes). (B) Normal human optic nerve head. (C) Visual field showing central and temporal islands of vision in an eye that is severely affected by glaucoma. (D) Glaucomatous optic disc of a human eye showing advanced thinning of the neuroretinal rim. Edited from Weinreb, R. N. et al. (2016) Primary open-angle glaucoma Nat. Rev. Dis. Primers doi:10.1038/nrdp.2016.67[5]

Genetics of POAG

Many POAG patients have a positive family history[7]. Family studies showed that first-degree relatives of POAG patients have a 22% risk of developing POAG compared to 2,3% for first-degree relatives of unaffected individuals, almost 10 times higher[9]. This familial nature of POAG strongly suggests that genetic factors contribute to the pathogenesis of POAG. During the past decades, a substantial part of POAG-research has focused on elucidating these genetic factors. The first genetic studies were based on linkage analysis in relatives of families with familial form of POAG that show Mendelian inheritance and resulted in the

identification of rare variants in myocylin (*MYOC* in GLC1A locus*)* and optineurin *(OPTN* in GLC1E locus*)*. These variants display a Mendelian inheritance pattern (monogenic POAG), and although they are highly pathogenic, they only explain approximately 4-6% of the variation in disease risk[19]. In most patients POAG is not inherited as a Mendelian trait but rather constitutes a complex trait; where the disease results from the interaction of multiple genetic factors with small effects and environmental factors. Genome-wide association studies (GWAS) have accelerated gene finding in POAG. Currently, over 100 loci have been identified to be associated with this disease[20]. These results have led to novel insights in disease pathways. In particular extracellular matrix organization, TGF β signaling which features the roles of inflammation and senescence, sex hormones metabolism, and vascular tone homeostasis have all been implicated in POAG pathogenesis through GWAS findings[21-24]. Despite the high scientific impact, these genome-wide significant variants only explain ~3% of the genetic contribution to glaucoma susceptibility, suggesting that additional variants remain yet to be discovered.

Alternative approaches in GWAS have also been employed. In particular, the use of heritable quantitative disease characteristics, so called endophenotypes, has proven to be successful. Studies performed in the Rotterdam Study have focused on investigating glaucoma endophenotypes i.e. IOP and parameters defining the morphology of the ONH have further helped in elucidating missing heritability of the disease trait[25]. This work has been based on defining distinct endophenotypes, i.e., IOP and ONH parameters (vertical cup disc ratio and cup area), which in the disease co-occur. A new avenue of endophenotype research is to study multi-traits. Due to the successes of GWAS, in the past decade the potential of epidemiological research has been overshadowed. From a genetic and epidemiological perspective, there has been paucity of data on ethnicity. The Rotterdam Study consist predominantly of participants of European descent and does not include those of Asian and African origin. Only very recently, progress has been made in the identification of genetic variants associated with POAG in populations with high risk of POAG[26-28].

POAG in Africa

The prevalence of POAG varies widely across the various ethnic groups. Prevalence surveys conducted in primarily African ancestral populations have shown higher rates of POAG than those using similar methods in European populations (Fig. 2). The Baltimore Eye Survey in 1991 was the first study that directly compared POAG prevalence between African Americans and non-Hispanic whites. They observed a 4.3 fold excess prevalence in the age-adjusted prevalence in the African American group[29]. The Tema eye study from Ghana carried out between 2006-and 2009 showed POAG prevalence as high as 6.8%, two times higher than Ghana's HIV prevalence (3.2%) at that time [30]. In addition to the fact that people from SSA descent are disproportional affected, the disease is also much more progressive and has a younger age of onset leading to visual disability and blindness at younger age compared to

European populations. This ethnic predilection may indicate a stronger genetic influence. Several studies have made a first attempt to investigate genetic factors in African populations by targeting known mutations in single genes (monogenic POAG). Studies performed in Ghana and South Africa found that mutations in myocilin gene *MYOC* and optineurin gene *OPTN* play a limited role in the pathogenesis of POAG in Africa[31-34]; another study failed to replicate any of the known loci[35]. This suggests that other genetic risk factors may increase the risk of POAG in Africa. Conducting genetic studies in Africa may also have a particular advantage. As African populations are genetically more diverse than European and Asian populations, as a result of the *out of Africa* migration. The haplotypic diversity with concurrent increase in linkage disequilibrium might help to overcome one of the major barriers in GWAS: the fine mapping of the causal variant[36,37].

Figure 2. Global prevalence of primary open-angle glaucoma

Source Weinreb, R. N. et al. (2016) Primary open-angle glaucoma Nat. Rev. Dis. Primers doi:10.1038/nrdp.2016.67[5]

There has been little progress in the identification of genetic variants associated with POAG in those having the highest population risk; Africans. A study of POAG genetics in African populations is not only highly relevant for understanding the genetics of glaucoma in this particular population, it will also benefit understanding of the pathogenesis of POAG in general. A disease which occurs globally is likely to have several distinct pathways irrespective of ethnicity. A population in which the disease is frequent and severe, such as Africans and POAG, potentially be most efficient to study the disease pathogenesis.

AIMS OF THIS THESIS

This thesis describes epidemiologic and genetic studies on primary open-angle glaucoma in primarily sub-Saharan African, European and Asian populations. In the thesis, I aimed on the one hand to focus on sub-Saharan populations which are at high risk but underrepresented in clinical, epidemiological and genetic research. On the other hand, I aimed to explore novel approaches to study glaucoma endophenotypes. The major goals of our studies were

Chapter 2: to address differences in POAG phenotype in sub-Saharan African populations compared to European populations

Chapter 3: to identify genetic variants associated with POAG in sub-Saharan African populations

Chapter 4: to explore novel approaches to study genetic and phenotypic associations with glaucoma endophenotypes.

Study populations on which this thesis is based

We addressed these aims in various study populations. We joined studies to enlarge sample size in order to improve statistical power and ethnic diversity. A short description of these studies and consortia is listed below.

Genetics in Glaucoma patients of African descent (GIGA) study[38] - This study is an international collaboration including clinicians and researchers from Tanzania (Anna Sanyiwa, Hassan G. Hassan), South-Africa (Colin Cook), Ghana (Angelina Ampong) and the Netherlands (Caroline Klaver, Alberta Thiadens, Cornelia van Duijn and Pieter Bonnemaijer) and is the basis of this thesis. The GIGA study is a case-control study with the aim to find genetic risk factors and describe the clinical characteristics of POAG in sub-Saharan African populations. From 2012 till 2015, POAG patients and normal controls were recruited from hospitals in Tanzania (Muhimbili National Hospital and the Comprehensive Community Based Rehabilitation in Tanzania (CCBRT)) and the Groote Schuur Hospital in Cape Town, South Africa. From 2017 onwards, participants are recruited from the Komfo Anokye Teaching Hospital (KATH), Kumasi in Ghana. All together DNA samples and clinical information (visual fields, fundus photos, OCT's, corneal topography) of >2000 participants have been collected and stored in the joint biobank of The Rotterdam Eye Hospital and Erasmus MC (CORRBI).

Groningen Longitudinal Glaucoma Study[39] – is a prospective, observational cohort study conducted in the clinical setting of the University Medical Center Groningen, comprising both glaucoma patients and glaucoma suspects from predominantly European ancestry. (Chapter 2.1)

Charles Bronfman Institute for Personalized Medicine BioMe BioBank (https://icahn.mssm.edu/research/ipm/programs/biome-biobank) - an electronic medical record-linked biobank of the Mount Sinai Medical Center, New York, USA that is fully integrated in the clinical care processes. The biobank populations include 28% African American, 38% Hispanic Latino predominantly of Caribbean origin, 23% Caucasian/White. The African American population of BioMe was used in Chapter 3 of this thesis.

The Rotterdam Study (RS)[40] – is a population-based study consisting of four cohorts that started in 1990, 2000, 2006 and 2016. By 2020 nearly 18.000 participants over the age of 40 were recruited in the neighborhood of Ommoord, a suburb of Rotterdam in the Netherlands, and re-examined every three to six years. Data from cohort RSI, RSII and RSIII, were used in the analyses of Chapter 4.

Glaucoma in People of African Descent (GGLAD) consortium[41] – Is an occasional consortium encompasses genetic studies from people of SSA heritage from USA, Ghana, Nigeria, Tanzania, South Africa and the UK. The consortium includes studies from the Eyes of Africa genetic consortium, Women's Health Initiative, Genetic Epidemiology Research on Aging (GERA) cohort, the African Descent and Glaucoma Evaluation Study, South London POAG case–control cohort, BioMe and the GIGA study (Chapter 3.2)

International Glaucoma Genetics Consortium – this consortium includes over 25 studies with data of genotypes, POAG endophenotypes and POAG case-control studies from different continents across the world. (Chapter 4.2)

European Eye Epidemiology (E^3) consortium[42] - a collaborative network of population-based studies across Europe, providing ophthalmic data on 170.000 European participants. The aim of this consortium is to increase understanding of eye disease and vision loss in Europe by developing and analyzing large pooled datasets. (Chapter 4.1)

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Chapter 2

Phenotypic variation of POAG in populations from sub-Saharan ancestry and European ancestry

Chapter 2.1

Differences in clinical presentation of primary open-angle glaucoma between African and European populations

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ABSTRACT

Purpose

Primary open-angle glaucoma (POAG) has been reported to occur more frequently in Africans, and to follow a more severe course compared to Europeans. We aimed to describe characteristics of POAG presentation and treatment across three ethnic groups from Africa and one from Europe.

Methods

We ascertained 151 POAG patients from South African Coloured (SAC) and 94 South African Black (SAB) ethnicity from a university hospital in South Africa. In Tanzania, 310 patients were recruited from a university hospital and a referral hospital. In the Netherlands, 241 patients of European ancestry were included. All patients were over 35 years old and had undergone an extensive ophthalmic examination. Patients were diagnosed according to the ISGEO-criteria. A biogeographic ancestry analysis was performed to estimate the proportion of genetic African ancestry (GAA).

Results

The biogeographic ancestry analysis showed that the median proportion of GAA was 97.6% in Tanzanian, 100% in SAB, 34.2% in SAC and 1.5% in Dutch participants. Clinical characteristics at presentation for Tanzanians, SAB, SAC, and Dutch participants, respectively: mean age: 63, 57, 66, 70 years ($P < 0.001$); visual acuity in the worse eye: 1.78, 1.78, 0.3, 0.3 LogMAR ($P < 0.001$); maximum intraocular pressure of both eyes: 36, 34, 29, 29 mmHg ($P_{ANOVA} < 0.001$); maximum vertical cup to disc ratio (VCDR) of both eyes: 0.90, 0.90, 0.84, 0.83 ($P < 0.001$); mean central corneal thickness: 506, 487, 511, 528 µm ($P < 0.001$). Fourteen percent of Tanzanian patients presented with blindness (<3/60 Snellen) in the better eye in contrast to only 1% in the Dutch.

Conclusion

In this multi-ethnic comparative study, sub-Saharan Africans present at a younger age with lower visual acuity, higher IOP, larger VCDR, than SAC and Dutch participants. This indicates the more progressive and destructive course in sub-Saharan Africans.

INTRODUCTION

Glaucoma, a heterogeneous group of eye disorders leading to neurodegeneration of the optic nerve, is a leading cause of irreversible blindness in the world. Various epidemiologic studies have demonstrated that primary open-angle glaucoma (POAG) prevalence is the highest in persons from sub-Saharan African (SSA) ancestry. In 2015, there were an estimated 10 million African people suffering from POAG, compared to 58 million people worldwide[1]. People from SSA ancestry are three times more likely to develop POAG than Europeans[2]. The prevalence of POAG is not only higher in the Africa-derived population, blindness is also four times more frequent in African glaucoma patients compared to Western Europeans[3]. Known risk factors to explain the poor visual prognosis in these patients are: little disease awareness, poor adherence to treatment, and reluctance to the acceptance of surgery. Factors causing a delay in adequate glaucoma treatment are limited affordability, accessibility, and availability of eye care and medication. These socio-economic and socio-geographic conditions may explain a part of the poor visual prognosis in people from SSA ancestry, but an earlier onset and more severe disease course have also been implicated as possible risk factors in POAG[4-6].

In this study, we evaluated the ethnic risk factor for POAG and the influence of a poor healthcare system in developing countries on the visual prognosis in POAG. We compared the clinical characteristics of POAG presentation across three ethnic groups from SSA (one from Tanzania and two from South Africa) ancestry and one European population (the Netherlands).

METHODS

Study population

Genetics in Glaucoma patients of African descent (GIGA) study

The GIGA study is a case-control study comprising open-angle glaucoma patients and healthy subjects from South Africa and Tanzania. The study was conducted in a clinical setting and participants were ascertained at the ophthalmology clinics of the Groote Schuur Hospital in Cape Town, South Africa and from two hospitals in Tanzania: Muhimbili National Hospital and CCBRT Disability Hospital in Dar es Salaam. In all hospitals, the study was incorporated in the daily clinical routine and participants were recruited consecutively from the outpatient department (general and glaucoma clinics). Participants were examined by a local glaucoma specialist for eligibility. In total, 697 patients met the inclusion criteria of the study. Inclusion criteria were as follows: participants of South African Black (SAB), admixed South African Coloured (SAC;[7]) and Tanzanian descent, over 35 years of age, and diagnosed with POAG

according to the International Society of Geographical and Epidemiological Ophthalmology (ISGEO) classification[8]. All other types of glaucoma, including secondary causes or narrow/closed angle glaucoma were excluded. Control subjects that were simultaneously recruited have not been analysed in the current study.

All participants provided written informed consent in accordance with the ethical standards as stated in the Declaration of Helsinki. The study protocol for the GIGA study was approved by the Institutional review boards of the Erasmus MC, Muhimbili University of Health and Allied Sciences, and the University of Cape Town. Additionally, the Medical Research Coordination Committee of the Tanzania National Institute for Medical Research (NIMR) accepted the study protocol.

Groningen Longitudinal Glaucoma Study (GLGS)

A sample of 241 white European ancestry POAG cases was drawn from the Groningen Longitudinal Glaucoma Study (GLGS), a prospective cohort study performed in a clinical setting. The objectives, methods, rationale, and study design have been described earlier[9]. Briefly, the GLGS is an institutional review board-approved observational prospective follow-up study of 875 patients with established or suspect glaucoma who visited the glaucoma outpatient service of the University Medical Center Groningen between July 1, 2000 and June 30, 2001 and who provided informed consent, in accordance with the declaration of Helsinki. The study included conventional perimetry, frequency doubling technique perimetry (FDT; Carl Zeiss Meditec AG, Jena, Germany), and laser polarimetry (GDx; Laser Diagnostic Technologies, San Diego, CA). Out of the initial 875 patients, 452 were classified as having glaucoma (including primary and secondary open angle and angle closure glaucoma). Of these 452 patients with glaucoma, the disease in 372 of them was classified by using standard automated perimetry [Humphrey Field Analyzer (HFA); Carl Zeiss Meditec Inc., Dublin, CA, USA]. The Goldmann perimeter (Haag Streit AG, Bern, Switzerland) was used in 80 patients, who were excluded from this analysis. For the aim of the current study, only European ancestry POAG cases diagnosed according to ISGEO Category 1 diagnosis were included in the analysis, leaving 241 patients.

Estimation of genetic ancestry

To determine the proportion of genetic African ancestry in the GIGA study participants, all POAG patient were genotyped using either Illumina HumanOmniExpressExome or the Illumina HumanOmni2.5Exome BeadChip. The full procedures of this analysis have been described earlier [10]. In brief, the genotypes of the GIGA samples were merged with reference populations from the 1000 Genomes project phase 3. Unlinked single nucleotide polymorphisms were selected and used for biographic ancestry estimation using the program ADMIXTURE (v 1.23) for K=3 putative ancestral populations (African, European and Asian).

For the GLGS cohort, ADMIXTURE (v 1.3) was used to estimate the ancestry components. This cohort was genotyped using Global Screening Array BeadChip and its samples were merged with reference populations from the 1000 Genomes project phase 3. The procedure to calculate the biographic ancestry was similar to the methods applied in the GIGA study, as described above.

Ophthalmic examination in the GIGA study

The complete eye examination in GIGA included visual acuity (VA) by Snellen or Tumbling E chart at 6 meters with and without refractive correction, intraocular pressure (IOP) measurement with Goldmann applanation tonometry, slit-lamp examination including peripheral anterior chamber depth assessment by the Van Herick method, indirect gonioscopy, fundoscopy for optic nerve head examination, and digital fundus photography centred on the optic nerve by means of a Canon CF-60DSi (Canon Inc, Tokio, Japan) fundus camera (South Africa) or Optomed Smartscope® M5 EY3(Optomed Ltd, Oulu, Finland) handheld fundus camera (Tanzania).

Central corneal thickness (CCT) was measured after topical instillation of anaesthesia with an ultrasound pachymeter. In South Africa, 10 readings were automatically captured in both eyes with an A-scan/pachymeter OcuScan RxP (Alcon Laboratories, Inc., Ft. Worth, TX, USA). In Tanzania, the handheld ultrasonic pachymeter Palmscan® P2000FP (Micro Medical Devices Inc, Calabasas, CA, USA) was used and five readings were taken manually in both eyes. As the intra-individual variance was small, and to conform with the CCT measurement methods applied in GLGS these values were averaged.

Visual field testing was performed, VA permitting, with either the Sita Fast strategy (Humphrey Field Analyzer (HFA) 24-2, Carl Zeiss Meditec, Inc., Dublin, CA, USA) or fast threshold strategy (Medmont M700 perimeter, Medmont, Camberwell, Victoria, Australia and Optopol PTS 1000, Optopol Technology, Zawiercie, Poland). A definite visual field defect consistent with glaucoma was defined if the glaucoma hemifield test graded "outside normal limits" or if a cluster of 3 contiguous points was observed at the 5% level of the pattern deviation plot, including at least one of these points <1%. Visual field defects were not attributed to glaucoma in the presence of media opacities or non-glaucomatous disease that could explain the visual field abnormality.

All relevant clinical data relating to the ophthalmic history, course of POAG, presence of other types of glaucoma, treatment, and any eye operations were collected and recorded from the medical charts. Lastly, a questionnaire was filled out by an English/Kiswahili speaking interviewer covering demographic data, self-reported ethnicity, medication, family history, and medical history.

Ophthalmic examination in GLGS

The complete eye examination in GLGS included a VA by Snellen with and without refractive correction, IOP measurement with Goldmann applanation tonometry, slit-lamp examination including gonioscopy, and fundoscopy for optic nerve head (ONH) examination. The ONH was described by using the VCDR as long as the rim was uninterrupted; if interrupted the ONH classified as having a notch (interrupted either inferiorly or superiorly) or (sub)total excavation (interrupted both inferiorly or superiorly). The CCT was measured with an ultrasound pachymeter (Tomey SP-3000; Tomey Ltd, Nagoya, Japan) after the topical instillation of an anaesthetic eye drop. The standard white on white HFA 30–2 Sita Fast algorithm was used for perimetric testing (Carl Zeiss Meditec Inc.).

Classification of a definite visual field defect consistent with glaucoma was defined as in the GIGA study (see aforementioned criteria). Patients with a reproducible visual field defect in at least one eye were classified as glaucoma patients. The first test, however, was left out of consideration because of any learning effects. Therefore, at least three visual fields were required for the diagnosis of glaucoma.

Inclusion criteria

All patients (GIGA + GLGS), were categorized as glaucomatous according to the ISGEO classification for open-angle glaucoma[8]. In GIGA, detailed grading of the ONH was performed by one general ophthalmologist (AAT) and one glaucoma specialist (HGL). They independently interpreted fundus images and visual field results while being masked to other clinical information. In case of any discrepancy between the two graders, adjudication was solved by consensus. If no consensus was reached, participants were excluded. In GLGS, all visual fields were assessed by two independent graders, being a general ophthalmologist (GPH) and a glaucoma specialist (NMJ). Any discrepancies were discussed and solved by consensus. The ONH was assessed clinically by one of three glaucoma experts (NMJ, LJB, PH; for further details, see Heeg et al 2005). The original visual field and ONH classifications were integrated into ISGEO classification for the current study.

Category 1 or 2 ISGEO criteria were required for the diagnosis of glaucoma. The highest level of evidence (Category 1 diagnosis) requires a definite visual field defect, as mentioned above, and loss of the neuroretinal rim with a VCDR ≥ 0.7, or VCDR asymmetry ≥ 0.2 (both values represented the 97.5th percentile for the normal population[11-13]. Category 2 diagnosis requires a severely damaged ONH in the absence of a visual field test, that is, a VCDR ≥ 0.8 or VCDR asymmetry ≥ 0.3 (both values determined by the 99.5th percentile for the normal population[11-13]. In addition, patients with POAG demonstrated an open angle on gonioscopy.

Data analysis

Two time points play a role in our study: the time at which the patient presented with glaucoma at one of the recruitment hospitals, further referred to as 'presentation' and the time at which the patient was included in the study further referred to as "inclusion". To describe the mode of presentation, data on age at presentation, IOP, VCDR, and visual acuity were collected from the medical charts going back to the timepoint the patient was newly diagnosed with POAG. At inclusion additional information was recorded, that is age at inclusion, CCT, family history, medical history (Diabetes mellitus, cardiovascular disease) and the medical file was reviewed to collect information on glaucoma surgeries and treatment that has taken place in the time interval from presentation to inclusion. In some cases, presentation and inclusion coincided. In the GIGA study 298 (53.7%) patients and in the GLGS 67 (27.8%) patients were included in the study within one year from presentation. For IOP and VCDR the maximum value of both eyes was used in the analysis. For CCT the average of both eyes was taken.

Statistical analysis

Quantitative differences measured on a continuous scale between ethnic groups were analysed by one-way analysis of variance (ANOVA) and when reported statistically significant followed up with a Tukey's D post hoc test. For variables in which the homogeneity of variance assumption between the groups was violated, a Welch's ANOVA test was conducted. Statistically significant Welch ANOVA results were followed up by a Games-Howell post-hoc test. Variables that did not follow a parametric distribution were analysed by Kruskal-Wallis H test. The medians of the Kruskal-Wallis H test were interpreted when the distributions were similar for all ethnic groups. Interquartile ranges are reported as first to third quartile defined by the Tukey hinge method. Statistical analysis was performed with SPSS Statistics 24 (SPSS, Chicago, IL,USA) and RSTUDIO (RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/).

RESULTS

Of the 743 glaucoma patients enrolled in the GIGA study, 555 patients met the ISGEO criteria (Category 1 diagnosis: 230 patients; Category 2 diagnosis: 325 patients) after review of fundus photos and visual field results, and these 555 were included in the current analysis. They comprised of 310 Tanzanian, 94 South African black (SAB), and 151 South African Coloured (SAC) POAG patients. For the GLGS, all 241 included subjects had an ISGEO Category 1 diagnosis. We assessed the proportion of genetic African ancestry among the GIGA patients. The median proportion of genetic African ancestry was similar in the Tanzanian and SAB groups (97.6% and 100% respectively) but was greatly reduced in the

SAC group (34.2%), as was to be expected from an admixed population. In the Dutch patients from GLGS, the median proportion of genetic European ancestry was 97.4% and the African ancestry was 1.5%.

Clinical characteristics of POAG presentation

The clinical characteristics of POAG presentation (i.e. the time the patient presented at the hospital and was diagnosed with POAG) in Tanzanians, SAB, SAC, and the Dutch are presented in Table 1. The mean age was statically significant different between the four ethnic groups ($P < 0.001$) and ranged from 57.0 years in SAB patients to 69.7 years in the Dutch (Fig. 1A). In particular Tanzanian and SAB patients presented at younger age compared to SAC and Dutch patients. The maximal IOP of both eyes was the highest in the Tanzanian group (36.2 mm Hg) and the IOP was statically significantly higher in Tanzanian and SAB compared to SAC and the Dutch (Fig. 1B). The maximal VCDR of both eyes was statistically significant different between the four ethnic groups ($P < 0.001$) and ranged from 0.80 in SAC and Dutch patients to 0.90 in Tanzanian and SAB patients (Fig. 1C). Tanzanians (1.78 LogMar) and SAB (1.78 LogMar) patients had a statistically significantly worse visual acuity in the worse-seeing eye than the SAC (0.30 LogMar) and Dutch (0.30 LogMar) patients (Fig.1D). The visual acuity in the better-seeing eye was statically significant higher in the Dutch (0.00 LogMar) than in the three African ancestral groups (Fig. 1E). To eliminate bias due to other causes of reduced vision we executed a sensitivity analysis by excluding all subjects with an additional diagnosis that might have affected their visual acuity (e.g., cataract) or had a cataract operation between presentation and inclusion in the study. For Tanzanian and SAC patients, the median visual acuity in the worse-seeing eye did not change after excluding probable other causes of vision loss (being 1.78 LogMar (IQR 0.5-2.5) and 0.3 LogMar (IQR 0.2-0.6), respectively). After excluding other probable causes of vision loss in the SAB and Dutch group, the median visual acuity in the worse-seeing eye at presentation improved to 0.89 LogMar (IQR 0.3-2.5; not significant) and 0.2 LogMar (IQR 0.0-0.3; $P < 0.05$), respectively. The mean visual acuity in the better-seeing eye did not change after excluding other causes of vision loss. Binocular blindness at presentation, defined by WHO criteria as a VA < 3/60 in the better eye, was reported in 13.5% of Tanzanian, 18.1 % in SAB, 4.1% in SAC, and 1.7% in Dutch patients. The proportion of binocular blindness at presentation was distributed equally among male and female patients in all ethnic groups ($P > 0.05$). Older age at presentation was associated with higher odds of binocular blindness in Tanzanian (OR = 1.45/10 year increase in age, 95%CI = 1.06-1.98, $P = 0.02$) and SAB (OR = 1.80/10 year increase in age, 95%CI = 1.13-2.89, $P = 0.02$). Monocular blindness at presentation was reported in 51.6% of Tanzanian, 48.9% in SAB, 24.5% in SAC, and 16.2% in Dutch patients. The central corneal thickness was statically significantly thinner in the African groups than in the Dutch ($P \leq 0.001$; Fig. 1F) and thinnest in the SAB (487µm; Table 1).

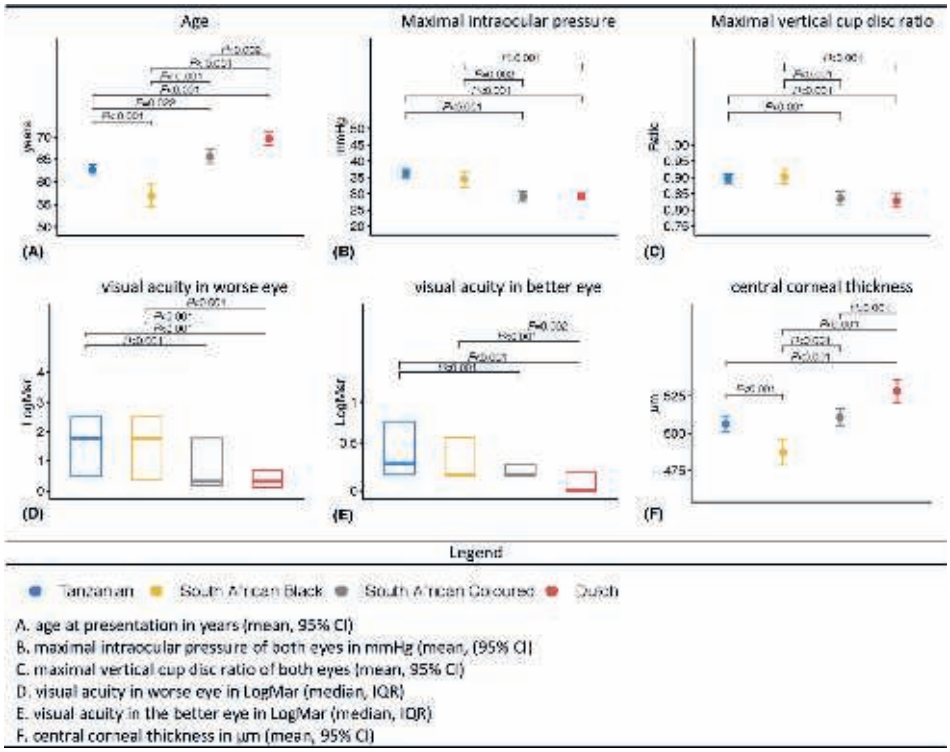
Table 1. Clinical characteristics of the study populations

Clinical and demographic characteristics	Tanzanian N=310	South African Black N=94	South African Coloured N=151	Dutch N=241	*P*-value
Year of presentation, median (IQR)	2013 (2012 - 2014)	2010.50 (2006 - 2013)	2011 (2005- 2013)	1999 (1998 -2001)	
Age at presentation, mean (years) ± SD	62.7 ± 11.0	57.0 ± 12.0	65.7 ± 10.0	69.7 ± 11.7	$F^{\#}(3, 312.155) = 31.255$ $P < 0.001$
Female, % (n)	36.8 (114)	51.1 (48)	55.6 (84)	44.8 (108)	*P*<0.001*
IOP at presentation (mm Hg), maximal OU ± SD	36.2 ± 11.1	34.4 ±11.6	29.1 ±10.0	29.3 ± 8.4	$F^{\#}(3, 303.081) = 29.185$ $P < 0.001$
VCDR at presentation, maximal OU ± SD	0.90 ± 0.12	0.90 ± 0.11	0.84 ± 0.13	0.83 ± 0.16	$F^{\#}(3, 304.748) = 15.327$ $P < 0.001$
Visual acuity (LogMar) at presentation in worse eye, median (IQR)	1.8 (0.5-2.5)	1.8 (0.3-2.5)	0.3 (0.2-1.8)	0.3 (0.1-0.7)	$\chi^{2\dagger}(3) = 115.016$ $P < 0.001$
Visual acuity (LogMar) at presentation in better eye, median (IQR)	0.3 (0.2-0.8)	0.2 (0.2-0.6)	0.2 (0.2-0.3)	0.0 (0.0-0.2)	$\chi^{2\dagger}(3) = 95.190$ $P < 0.001$
CCT, mean(µm) OU ± SD	506.2 ± 33.8	487.1 ± 38.4	510.7 ± 34.7	528.1 ± 40.0	$F^{\ddagger}(3, 509) = 20.171$ *P*<0.001
Family history of glaucoma, % (n)[1]	28.4 (88)	17.0 (16)	17.2 (26)	20.7 (43)	0.015*
Diabetes mellitus, % (n)[1]	11.6 (36)	18.1 (17)	33.1 (50)	13.7(33)	<0.001*
Cardiovascular disease, % (n)[1]	31.3 (97)	62.8 (59)	64.9 (98)	53.3 (126)	<0.001*

P-value obtained from a one-way Welch ANOVA; **P-value* obtained from a *chi*-square test; †*P-value* obtained from a Kruskall Wallis H test; ‡ *P-value* obtained from a one-way ANOVA; IQR= interquartile range; OU= both eyes; SD= standard deviation

[1] parameters were assessed at inclusion in the study, the mean age (± SD) at inclusion was: Tanzanian 63.5(10.8); South African Black 61.5 (11.2); South African Coloured 70.5 (10.3) and Dutch 71.6 (11.4) years

Figure 1. Clinical characteristics at presentation



(A) age at presentation in years (mean, 95% CI). (B) maximal intraocular pressure of both eyes in mm Hg (mean, (95% CI). (C) maximal vertical cup disc ratio of both eyes (mean, 95% CI). (D) visual acuity in worse eye in LogMar (median, IQR). € visual acuity in the better eye in LogMar (median, IQR). (F) central corneal thickness in µm (mean, 95% CI).

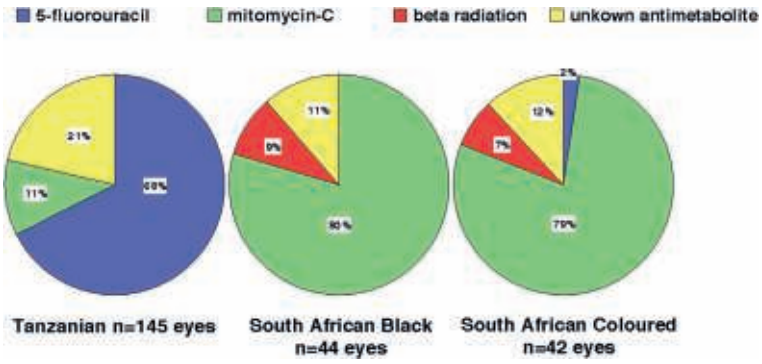
A questionnaire assessment was performed at the inclusion in the studies to evaluate demographic characteristics and heritable risk of glaucoma (Table 1). Reported first-degree family history of glaucoma ranged from 17% in the SAB to 28% in the Tanzanian group ($P <$ 0.05). The self-reported prevalence of diabetes mellitus was the highest in SAC (33.1%). Adjustments for potential confounding by age and sex showed that the difference in prevalence of diabetes mellitus between SAC and Tanzanian and between SAC and the Dutch was statistically significant after multiple testing correction ($OR_{SAC\ vs.\ TZ}$ = 3.98, 95%CI = 2.40-6.62, P <0.001; $OR_{SAC\ vs.\ DUT}$ = 3.27, 95%CI = 1.93-5.43, P <0.001). Cardiovascular disease prevalence was also the highest in SAC patients followed by SAB and the Dutch. Tanzanians had a significantly lower prevalence compared to the other groups when adjusting for age, sex and multiple testing ($OR_{TZ\ vs.\ SAB}$ =0.25, 95%CI = 0.15-0.41, $P < 0.001$; $OR_{TZ\ vs.\ SAC}$ = 0.32, 95%CI = 0.21-0.50,$P < 0.001$; $OR_{TZ\ vs.\ DUT}$ = 0.56, (95%CI = 0.39-0.81, $P = 0.003$). Adjustments for age and sex also indicated a statistically significant higher cardiovascular disease prevalence in SAB compared to the Dutch (OR = 2.30, 95%CI = 1.36-3.90, $P = 0.02$).

Glaucoma surgery in sub-Saharan Africa

Next, we examined the frequency of different types of surgical interventions for glaucoma that had been performed between presentation and inclusion among African groups. As the incidence of glaucoma surgery may depends on the follow-up duration, we calculated the time intervals between presentation and inclusion. Median time from presentation to inclusion was 0.3 year (IQR 0.01-1.2) in Tanzanian, 3.1 years (IQR 0.7-8.4) in SAB, 2.9 years (IQR 0.8-8.1)in SAC.

Laser trabeculoplasty was only performed in one SAC patient that had received Argon laser trabeculoplasty. Trabeculectomy (TE) had been more frequently performed in in the Tanzanians (37.1% of patients; n = 145 eyes) followed by SAB (33% of patients, n = 44 eyes) and SAC (21.9% of patients, n = 42 eyes). Taking into account the time interval between presentation and inclusion, the incidence rate of TE was 0.76 per person-year in Tanzanians, 0.11 TE per person-year in SAB and 0.06 per person-year in the SAC. The median time from presentation to TE was 53 days (IQR 18-211) in Tanzanian, 300 days (IQR 105-967) in SAB, and 314 days (IQR 150-984) in SAC patients. TE combined with phacoemulsification, so-called phacotrabeculectomy had also been performed in Tanzanians, as well as in SAC patients. Among the Tanzanian patients that had undergone a TE, 26.1% (n = 33 eyes) had had a combined procedure, whereas in the SAC patients, only 9.1 % (n = 4 eyes) of patients that had undergone a TE had had a combined procedure. Different types of adjunctive antimetabolites had been used in the African groups during the TE procedures. A total of 231 eyes had undergone a TE in the African groups, of which 37 eyes had undergone combined phacotrabeculectomy. 5-fluorouracil had been used in 43% (n = 99) of eyes, whereas mitomycin-C had been used in 36% (n = 84) of eyes and beta-radiation had been applied in 3% (n = 7) of eyes. The differences between the types of antimetabolites that had been used among the African groups have been displayed in Fig. 2.

Figure 2. Differences in clinical presentation of primary open-angle glaucoma between African and European populations



DISCUSSION

This study compared clinical and demographic characteristics of POAG presentation in sub-Saharan African populations with a European population. We found that Tanzanian and SAB patients presented at a younger age than the Dutch (7-13 years earlier), and in a more advanced disease stage (VCDR 0.9) compared to Dutch patients. The IOP was 5-7 mm Hg higher and the CCT was more than 20 µm thinner in the Tanzanian and SAB groups than in the Dutch. The presenting visual acuity in the better-seeing eye was approximately 15 ETDRS letters (0.3 LogMar units) lower in the Tanzanian group than in the Dutch group; 14% of Tanzanian patients presented with blindness in the better seeing eye in contrast to 1% in the Dutch. These data emphasize the severe presentation of POAG in SSA at middle age.

The relatively younger age at presentation in Tanzanian and SAB and the more structural and functional damage of the optic nerve head implicates that the disease has an earlier age of onset and runs a more severe course in African patients. Also, thinner CCT affects Goldmann applanation tonometry readings and leads to an underestimation of IOP. Our data supports other studies, that the CCT in SSA populations is thinner than in Europeans (SAB:487 µm, Dutch: 528 µm, $P < 0.001$). We therefore conclude that the differences in IOP between Tanzanian, SAB and Dutch patients are probably even higher than noted due to differences in CCT.

The higher IOP might have a more destructive effect on the optic nerve head in African populations. Studies have suggested that larger optic disc diameters may be associated with increased vulnerability to pressure-induced deformation[14,15]. Investigation into ethnic differences in the anatomy of the optic nerve head showed larger optic discs in African individuals[16]. In this context, the higher presenting IOP observed in the African populations may have a potentially more damaging effect on the optic nerve head. However, clinical studies could not validate this hypothesis[17,18].

Our study found a higher VCDR at presentation in the African derived populations compared to SAC and Dutch participants, suggesting more advanced glaucomatous damage in the prior. As VCDR measurements are dependent on disc size[19], ethnic differences in disc size should ideally be taken into consideration when interpreting VCDR between ethnic groups. As measurements of the optic disc size were not available, we compared the VCDR to the normal distribution of VCDR in the population using reference distributions of Tanzania[11], South Africa[12] and the Netherlands[13]. VCDR readings exceeding the 97.5th percentile are generally considered abnormal. The mean of the VCDR values recorded at presentation in this study surpassed the 97.5th percentile threshold in the normal population indicating that the values were deviant from the normal population.

Several epidemiological studies have debated that diabetes mellitus (DM) may be a risk factor for POAG[20]. The recent rise in life expectancy and changes in lifestyle have contributed to a shift in disease patterns and increasing burden of non- communicable diseases like DM in SSA. In our study we observed DM prevalence ranging from 12% in Tanzanian POAG patients to 33% in SAC POAG patients. These numbers do certainly not entail a causal relationship. Conflicting evidence exists whether POAG and diabetes are related however a relative recent meta-analysis showed a significant association between POAG and diabetes considering data from different study designs[21]. Given the rising prevalence of DM in people of SSA[22], POAG evaluation should be warranted in DM patients.

In Tanzanian and SAB patients, the majority had already been monocular blind at presentation (~50%) while only 16% of the Dutch presented with monocular blindness. Monocular blindness may seem to be the trigger for seeking care in healthcare-deprived regions, however it is striking that 14% of POAG patients in Tanzania visited the clinic for the first time when already binocularly blind. This delay further supports the burden of poor access to healthcare facilities which includes long travel distances, poor financial status but also lack of awareness of implications of the disease. The latter has been supported by several African studies assessing awareness levels in glaucoma patients[23,24]. In particular studies that examined awareness in the general rural communities of Osun State in Nigeria and a peri-urban community of Abokobi in Ghana found that, despite the high prevalence of the disease in West-Africa, only 16% and 39% of participants respectively had heard of the disease before[25,26]. In contrast, in Australia where glaucoma is less frequent, awareness levels are much higher: 73% of respondents had heard of the disease before[27]. Another factor that should not be omitted in evaluating patient delay is the impact of the traditional healers who can play a pivotal role in the postponement to seek medical care. And above all ophthalmologists, optometrists, and assistant medical officers of ophthalmology (AMOO) are scarce and medical instruments and the access to medication can be challenging. Interestingly, also in the Dutch population, 1% of POAG presented with binocular blindness. This indicates that an asymptomatic chronic disease may lead to patient delay even when the aforementioned healthcare restrictions are not present[28]. Thus, health care deprivation and socio-economic status seem in all populations independent risk factors for late presentation of POAG.

This study also observed differences in surgical treatment strategies among African groups. TE was performed in over one third of Tanzanians and SAB. In particular Tanzanian patients underwent TE more rapidly after initial diagnosis (median 53 days) compared to SAB and SAC. The more limited healthcare settings in Tanzania with less availability, accessibility and affordability of topical medication compared to South Africa may lead to an earlier surgical intervention instead of medical treatment. Also, the lifelong use of topical medication is not

feasible in most developing countries. Another aspect is the treatment delay in Tanzanian patients, who usually seek eye care only when at least one eye has already been blinded. A pragmatic approach by swift surgical intervention may in these cases be a solution to prevent complete blindness. However, studies have shown that acceptance of TE in Tanzania is poor even when provided for free[29]. A possible reason for this is that TE will not help in visual recovery, and therefore compares poorly with cataract surgery in terms of patients' perception[30]. Moreover, TE will inevitably lead to visual loss at the short term due to associated cataract development. Studies from South Africa and Tanzania have suggested that combining TE with cataract surgery so-called phacotrabeculectomy favours TE in practices where post-operative follow-up is inadequate and acceptance of glaucoma surgery is poor[31,32]. Although TE has been advocated as first line treatment in African glaucoma, excessive scarring which may compromise the filter function is frequently seen in people of African origin[33]. Antimetabolites like 5-fluorouacil (5-FU) and Mitomycin-C (MMC) are commonly applied during TE and phacotrabeculectomy to prevent scarring. In our study there were remarkable differences in the frequency of 5-FU and MMC use between Tanzania and South Africa. In Tanzania 68% of procedures used intraoperative 5-FU while in South-Africa 80% of TE's used MMC. MMC may be more effective in lowering IOP compared to 5-FU, but is also associated with higher rates of complications, in particular bleb leaks, cyst formation, and hypotony[34]. Also, MMC is more expensive and needs to be kept refrigerated to maintain efficacy for up to 2 weeks in contrast to 5-FU, which can be stored at room temperature for several months. These latter two reasons may favour 5-FU in low-income countries with unstable electricity supply. Another anti-scarring agent which has been used in glaucoma surgery for many years is beta radiation. This device has a working life over 20 years and involves minimal maintenance making this an attractive choice for healthcare settings with insufficient drugs supply and technical support. In our study, only 8% of TE/PT procedures in South Africa used beta radiation as adjunctive agent. A study from Tanzania investigated the use of beta radiation in PT and found comparable IOP control in the beta radiation group compared to the 5-FU group[35]. Although beta radiation seems a good alternative to 5-FU and MMC, importing radioactive material in African countries may come with additional logistical burden making antimetabolites more easily available. A striking observation was that none of the African patients was offered selective laser trabeculoplasty. A recent trial showed that SLT is an effective first line treatment in lowering IOP for at least 3 years providing superior IOP stability, at lower costs compared to eye drops[36]. Other studies have shown comparable effectiveness of SLT in African derived patients (30-40 % reduction in IOP)[37]. In SSA where many POAG patients are unable to sustain on topical IOP lowering medication, SLT may be a viable and cost-effective solution for treating POAG. However, its therapeutic range in clinic is considered lower compared to TE, as IOP in SSA POAG patients is high, it may be insufficient on its own in achieving IOP at levels to prevent glaucoma progression.

Limitations of the study are the differences in health resource settings between Europe and Africa that may have introduced bias to the stage of POAG presentation. In the Netherlands, opticians and optometrists are trained to early detect risk factors for POAG in particularly high IOP. This may have led to earlier referral of POAG patients, however even with this surveillance taken into account the Dutch POAG patients presented 7-12 years later than SAB and Tanzanians respectively in a less severe stage. Another aspect is that the data presented here is collected in a retrospective manner, data regarding presentation were extracted from the medical charts, hence these data had been recorded by various clinicians. Also only POAG patients that met the ISGEO criteria were included omitting less advanced glaucoma cases, this selection may have biased the results. Moreover in GLGS all patients were visual field confirmed (ISGEO 1) cases while in GIGA visual field testing was unfeasible in the majority of the patients, therefore a more strict structural criterion (ISGEO 2) needed to be fulfilled for inclusion. As structural and functional glaucomatous changes are not interchangeable the heterogeneity in inclusion criteria may have introduced selection bias towards higher VCDR at presentation in the GIGA study. An additional limitation is that the populations were not recruited in the same time frame. The GLGS cohort had been recruited at the turn of the millennium while the African patients had been recruited approximately 1 decade later.

In conclusion, the present study reinforces the notion that POAG patients of African descent have a more severe presentation compared to patients from European descent; South African Coloured patients (African, Asian, European admixed) have a similar mode of presentation as Europeans. Our study shows that aside from health care inequalities, that might mediate the severity at presentation, sub-Saharan African POAG patients present at a younger age in a much more advanced disease stage with a higher IOP. This indicates that the disease is more progressive and destructive in Africans. Awareness of these disease characteristics are important in first- and second-line glaucoma care. Pathophysiologic pathways have yet to be discovered to explain the higher disease load in Africans. Large genetic studies are on their way.

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Chapter 2.2

Genetic African ancestry is associated with central corneal thickness and intraocular pressure in primary open-angle glaucoma

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ABSTRACT

Purpose

To unravel the relationship between African ancestry, central corneal thickness (CCT), and intraocular pressure (IOP) by estimating the genetic African ancestry (GAA) proportion in primary open-angle glaucoma (POAG) patients and controls from an admixed South African Coloured (SAC) and a South African Black (SAB) population.

Methods

In this case-control study, 268 POAG patients and 137 controls were recruited from a university clinic in Cape Town, South Africa. All participants were genotyped on the Illumina HumanOmniExpress BeadChip or HumanOmni2.5Exome BeadChip. ADMIXTURE was used to infer participant's GAA among 86,632 SNPs. Linear and logistic regression models were used to assess the relation between GAA, POAG, CCT, and IOP.

Results

The median proportion of GAA was 60% in the study population. GAA was significantly associated with thinner CCT ($P < 0.001$) and IOP ($P = 0.034$) in POAG patients. The effect of GAA on CCT was marginally different among POAG patients versus controls ($P = 0.066$). In POAG patients, the CCT was significantly thinner compared to controls after adjusting for age and sex ($P = 0.016$). A stratified analysis in participants with >60% GAA, CCT was not associated with POAG ($P = 0.550$).

Conclusion

This study demonstrated that a higher proportion of GAA was associated with a thinner CCT and a higher IOP in POAG patients. Remarkably, at higher proportions of GAA, the difference in CCT between POAG and controls was reduced. This suggests that thinner CCT is not associated with POAG in Africans.

INTRODUCTION

Primary open-angle glaucoma (POAG) is the predominant type of glaucoma worldwide and a leading cause of irreversible blindness[1, 2]. In African populations, the prevalence is approximately three times higher compared to European populations and it runs a more severe course with higher intraocular pressure (IOP)[3-9]. The IOP is a major risk factor for POAG and the only one that can be modified therapeutically to alter the progression rate of the disease. Therefore, it is essential that it is measured accurately. The reliability of IOP measurements, especially Goldmann applanation tonometry (GAT), is confounded by variations in central corneal thickness (CCT), which affects the rigidity of the cornea[10]. Not only does CCT affect the accuracy of GAT, but CCT also is reported to be a strong predictor of the development of POAG in ocular hypertensive patients, even when IOP is corrected for CCT[11]. Moreover, in the Early Manifest Glaucoma Trial, CCT was reported to be an independent predictive factor for longer-term progression of POAG in patients with higher baseline IOP[12].

Nevertheless, whether the effect of CCT on glaucoma is only due to its effects on IOP measurement error or whether an independent relationship between CCT and glaucoma truly exists remains controversial[11-15] . Large population-based studies could not find any association of CCT with POAG. Other studies suggest that CCT is correlated with scleral and lamina cribrosa thickness, which affects the properties and vulnerability of the optic nerve and, therefore, increases the risk of glaucoma. However, histomorphometric studies in humans and monkeys could not confirm this correlation[16, 17]. Other biomechanical characteristics have been suggested to link CCT with POAG, such as the viscoelasticity of the cornea or corneal hysteresis. Lower corneal hysteresis has been associated with an increased risk of glaucoma and glaucoma progression[18-22].

CCT follows a diurnal rhythm and is affected by sex, age and ethnicity[23-27]. The ethnic variation of CCT has been studied widely. A meta-analysis including 53 studies showed that African individuals have a 20 to 30 µm thinner CCT compared to Europeans, Hispanics, and East Asians[28]. However, the possible relationship between a thinner CCT in individuals of African ancestry and their observed increased POAG risk has not been addressed sufficiently.

The purpose of this study was to disentangle the relationships between African ancestry, CCT, and IOP in POAG patients and controls. To overcome inaccuracy due to reporting bias and to study the effect of African ancestry quantitatively, we assessed each participant's biogeographic ancestry by inferring the genetic African Ancestry (GAA) rather than the self-reported ancestry.

METHODS

Study Population

The Genetics in Glaucoma patients of African descent study (GIGA study) is a case-control study comprising open-angle glaucoma patients and healthy controls from South Africa. All participants (n = 405) provided a written informed consent in accordance with the ethical standards as stated in the Declaration of Helsinki. The institutional review boards of the Erasmus MC and the University of Cape Town granted ethical approval. Participants were ascertained at the ophthalmology outpatient department of the Groote Schuur Hospital in Cape Town, South Africa. In total, 268 POAG patients met the inclusion criteria of the study. Inclusion criteria were participants of South African Black (SAB) or admixed South African Coloured (SAC)[29] descent, over 35 years of age, and diagnosed with either POAG or normal tension glaucoma (NTG). All other types of glaucoma, including secondary causes or narrow/closed angle glaucoma, were excluded. Inclusion criteria for controls (n = 137) were persons aged over 55 years, of SAB or SAC descent, without a diagnosis of any form of glaucoma, and without a family history (first degree relatives) of glaucoma. All participants were examined by a local glaucoma specialist.

Ophthalmic Examination

The complete eye examination included visual acuity (VA) by using a Snellen or Tumbling E chart at 6 m with and without correction, refraction, IOP measurement with GAT, slit-lamp examination including peripheral anterior chamber depth assessment by the Van Herick method, indirect gonioscopy, funduscopy for optic nerve head examination, and digital fundus photography centered on the optic nerve by means of a Canon CF-60DSi fundus camera. CCT was measured after topical instillation of lidocaine anesthetics with an ultrasonic A-scan/pachymeter® OcuScan RxP (Alcon Laboratories, Inc., Ft. Worth, TX, USA). Ten readings were automatically captured in both eyes. Visual field testing was performed with the Humphrey Field Analyzer 24-2 Sita Fast (Carl Zeiss Meditec, Inc., Dublin, CA, USA) strategy. A definite visual field defect consistent with glaucoma was defined if the glaucoma hemifield test graded "outside normal limits" and if a cluster of 3 contiguous points was observed at the 5% level of the pattern deviation plot, including at least 1 of these points <1%. Field defects were not attributed to glaucoma in the presence of media opacities or nonglaucomatous optic nerve disease that could explain the visual field abnormality.

Inclusion Criteria

All patients were categorized as glaucomatous according to the ISGEO classification for open-angle glaucoma[30]. After preliminary screening by local glaucoma specialists, and grading of fundus photographs by one senior ophthalmologist and one trained research grader, detailed grading was performed independently by one general ophthalmologist

(AAT) and one glaucoma specialist (HGL). They interpreted fundus images and visual field results independently while being masked for other clinical information. In case of any discrepancy between the two graders, adjudication was solved by consensus. If no consensus was reached, participants were excluded. Category 1 or 2 ISGEO criteria had to be met to diagnose glaucoma. The highest level of evidence (category 1) requires a definite visual field defect, as mentioned above, and loss of the neuroretinal rim with a Vertical Cup Disc Ratio (VCDR) ≥ 0.7, or VCDR asymmetry ≥0.2 (both values represented the ≥97.5th percentile for the normal SAB population[31]). Visual field testing results with less than 8% false-positive and false-negative responses, and less than 10% fixation losses were considered reliable. Category 2 requires a severely damaged optic disc, that is, a VCDR > 0.8 or VCDR asymmetry > 0.2 (both values determined by ≥99.5th percentile for the normal SAB population[31]) in the absence of a satisfactory visual field test. In addition, patients with POAG demonstrated an open angle on gonioscopy. Nonglaucomatous participants were those who met the following criteria in both eyes: IOP ≤21 mm Hg, a nonglaucomatous optic disc with VCDR < 0.5, and an intereye variation in VCDR <0.2.

Estimation of Genetic Ancestry

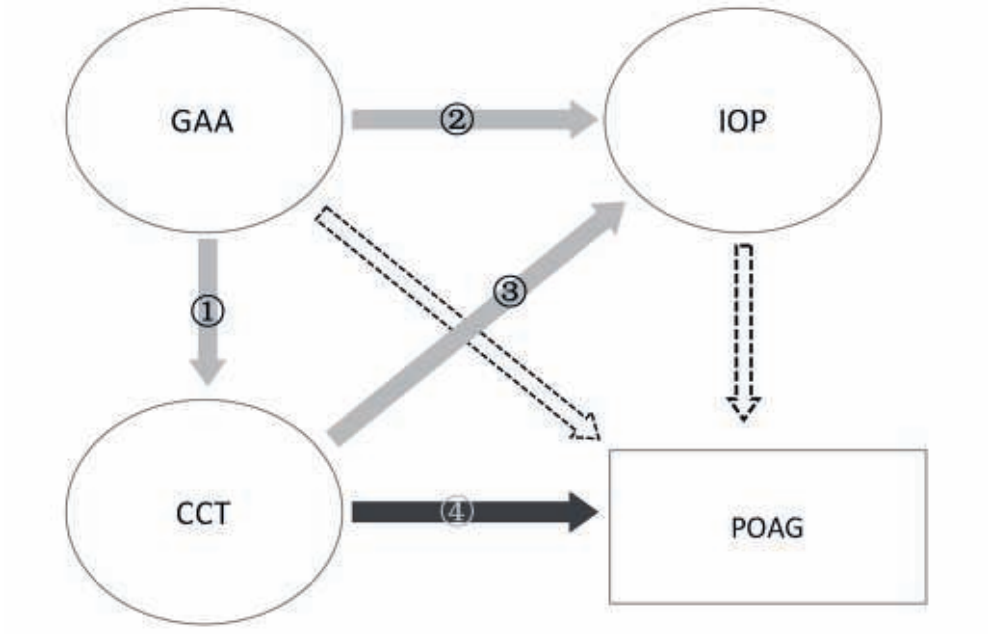
Participants were genotyped on the Illumina HumanOmniExpressExome Beadchip (n = 137) and the Illumina HumanOmni2.5Exome Beadchip (n = 244). Before combining both genotype datasets, PLINK (v1.07) was used to perform extensive quality control checks[32]. No within sample cryptic relatedness was observed during QC. To make inferences based on populations of known ancestry, we merged the combined dataset with three reference populations from Africa (Yoruba in Ibadan, Nigeria and Luhya in Webuye, Kenya), East-Asia (Japanese in Tokyo, Japan, Southern Han Chinese, and Han Chinese in Beijing, China) and Europe (Utah Residents [CEPH] with Northern and Western Ancestry, Tuscany in Italy, Finnish in Finland, British in England and Scotland, and Iberian in Spain) appearing in the 1000 Genomes Project[33]. PLINK then was used to perform linkage disequilibrium pruning on the merged genotype data to produce a reduced set of unlinked single nucleoside polymorphism (SNPs) (–indep-pairwise 50 10 0.1); 86,632 autosomal SNPs with an SNP call rate of 100% were selected from the merged datasets to estimate biogeographic ancestry (BGA). First we examined the genetic clustering by visualizing the principal components calculated in PLINK (Supplementary Fig. S1). The program ADMIXTURE v1.23 then was used to estimate the ancestral fractions of the three putative ancestral populations- African, Asian, and European- among the study samples[34]. ADMIXTURE was run with default settings and $K = 3$ ancestral populations.

Statistical Analysis

Since the mean CCTs of right and left eyes were not statistically different (mean difference 1.1 µm; $P = 0.242$; Pearson correlation = 0.90), we only present the results of the right eye

analyses. If measurements from the right eye were not available, then data from the left eye were used instead. In total, 383 right eyes and 22 left eyes were available for analysis. The average of the first 5 CCT readings was used in the analysis. The independent samples Student's *t*-test was used to compare continuous variables among ethnic and diagnostic groups. We performed χ^2 tests on categorical variables. GAA fractions inferred from SNP data were used instead of self- reported ethnicity to determine any association with African ancestry. We studied four interrelationships among POAG, GAA, CCT, and IOP as depicted in Fig. 1. Univariable and multivariable linear regression models, adjusting for age and sex, were applied to test the association between GAA and CCT, GAA and IOP, and CCT and IOP. Univariable and multivariable logistic regression models, adjusting for age and sex, were used to test the association of CCT with POAG. Also, effect modification of CCT by GAA was tested in the association of CCT with POAG by adding the multiplicative interaction term to the adjusted model. All statistical analyses were performed in SPSS (version 21.0; IBM Corp. Armonk. NY, USA) and R studio (R Core Team [2014]; R Foundation for Statistical Computing, Vienna, Austria).

Figure 1. Directed acyclic graph (DAG) depicting the interrelationships among GAA, CCT, IOP, and POAG



Black arrows represent the relationship between the primary independent variables (i.e., risk factors) and the outcome (i.e., POAG). *Gray arrows* represent the relationships between the independent variables (i.e., risk factors). *Dashed arrows* represent relationships that were not further studied because of the study design.

RESULTS

Demographics and clinical characteristics

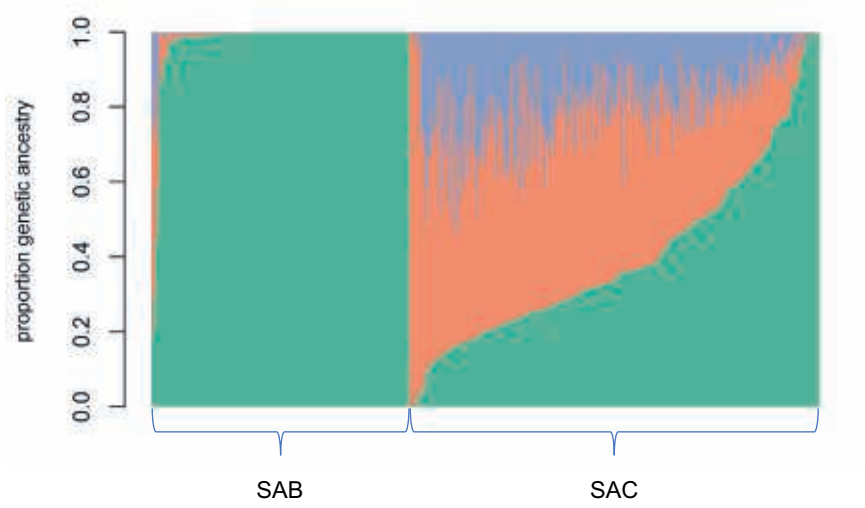
Demographic and clinical characteristics of the 268 POAG patients and 137 controls are given in Table 1. In the control group there were significantly more women, and controls had undergone more ocular surgery (in particular cataract extraction) compared to the POAG patients. In the POAG patients, the untreated IOP was statistically significantly higher ($P < 0.001$) and the CCT was statistically significantly thinner than in control participants ($P = 0.019$). There was no statistically significant difference in mean CCT among participants with and without ocular surgery when adjusted for age, sex, and POAG status (surgery, 507.1 ± 40.5 µm, n = 194; no surgery: 504.5 ± 36.0, n = 211; $P= 0.460$). The CCT did not significantly vary with age (-0.23 µm per year; 95%CI -0.57 – 0.11; $P = 0.188$). All CCT measurements had been performed during daytime (9 AM to 5 PM), and there was no association between CCT and the time of examination. The distribution of self-reported ethnicity/race was similar for POAG patients and controls. This was confirmed by an equal median percentage GAA (Mann- Whitney U Test, $P = 0.750$) for 381 participants that were genotyped successfully. Fig. 2 and Supplementary Table S1 present the distribution of the percentage genetic Asian, African, and European ancestry across SAB and SAC. The median proportion of GAA was significantly different in SAC participants compared to SAB participants (Mann-Whitney U Test, $P < 0.001$). One individual of self-reported SAB descent had less than 25% GAA according to the ADMIXTURE results, while 17 self-reported SAC individuals (7%) had more than 80% GAA; three self-reported SAC individuals (1.2%) did not have any GAA.The results of the single exploratory analysis of the interrelationships between GAA, IOP, CCT and POAG, as graphically depicted in Fig. 1, are presented in Table 2.

Table 1. Demographic and clinical characteristics

Clinical and demographic characteristics	POAG (n= 268)	Controls (n= 137)	*P*-value *
Median age, years (IQR)	68.0 (20)	66.0 (12)	0.828
Female, n (%)	144 (53.7)	91 (66.4)	0.019
Ocular surgery, n (%)	110 (41.0)	84 (61.3)	< 0.001
IOP (untreated), mm Hg ± SD	28.61 ± 9.73	14.20 ± 2.89	< 0.001
CCT, µm ± SD	502.53 ± 37.00	511.92 ± 39.82	0.019
Median VCDR (IQR)	0.90 (0.15)	0.30 (0.20)	< 0.001
Median proportion GAA, % (IQR)	61.24 (71.94)	55.37 (67.66)	0.750

CCT= central corneal thickness; GAA= genetic African ancestry; IOP= intraocular pressure; IQR= interquartile range; POAG= primary open-angle glaucoma; SD=standard deviation; VCDR=vertical cup disc ratio.
**P-value* obtained from a Student's *t*-test for continuous variables and with *chi*-square test for categorical variables; for median age, median VCDR and median proportion genetic African ancestry *P*-values were obtained with Mann-Whitney U Test.

Figure 2. Distribution of African (green), European (orange), and Asian (blue) genetic ancestry per individual



Every individual is represented by a vertical bar which is composed of three colors corresponding to their proportion genetic ancestry from these ancestral populations. The *x*-axis denotes individuals of self-reported SAB descent and SAC descent. The *y*-axis denotes individual proportions of genetic ancestry.

Table 2. Association of genetic African ancestry (GAA), central corneal thickness (CCT), intraocular pressure (IOP) and primary open-angle glaucoma (POAG) as graphically depicted in the directed acyclic graph (Fig. 1)

		Univariable regression model			Multivariable regression model*		
		β	95% CI	P-value	β	95% CI	P-value
1 CCT ~ GAA	All (n=381)	-3.58	-4.65 – -2.51	<0.001	-4.42	-5.38 – -3.14	<0.001
	Control (n=130)	-5.23	-7.10 – -3.36	<0.001	-5.41	-7.36 – -3.45	<0.001
	POAG (n=251)	-2.80	-4.09 – -1.51	<0.001	-3.68	-5.06 – -2.30	<0.001
2 IOP ~ GAA	Control (n=129)	-0.14	-0.29 – 0.01	0.069	-0.12	-0.28 – 0.04	0.131
	POAG (n=200)	0.63	0.24 – 1.02	0.002	0.46	0.05 – 0.87	0.029
3 IOP ~ CCT	Control (n=136)	0.09	-0.04 – 0.21	0.174	0.08	-0.04 – 0.20	0.204
	POAG (n=214)	0.00	-0.35 – 0.36	0.991	-0.05	-0.40 – 0.30	0.767
4 POAG ~ CCT	n=405	1.067#	1.01 – 1.13	0.020	1.07#	1.01 – 1.13	0.019

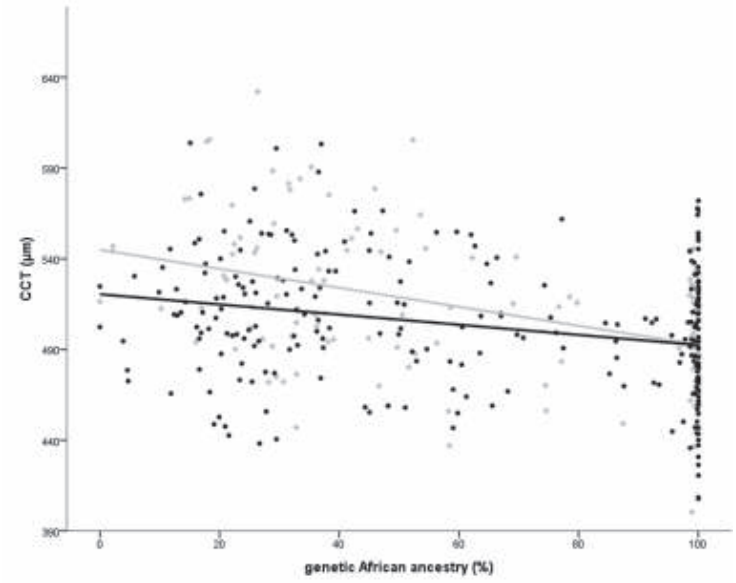
β=effect per 10% increase in GAA or 10 µm decrease in CCT; CI= confidence interval.
Odds Ratio, corresponds to the effect of 10 µm decrease in CCT.
* Multivariable regression model adjusted for age and sex.

1. Relationship of CCT and GAA

For the relationship between CCT and GAA, we found a statistically significant, negative association in the univariable and multivariable regression model for controls and POAG

patients together ($P_{univariable}$ < 0.001, $P_{multivariable}$ < 0.001). For every 10% increase in GAA, CCT showed a mean decrease of 4.4 µm (95%CI, -5.4 to -3.1). The regression lines for CCT as a function of GAA in POAG patients and controls separately are illustrated in Fig. 3. For every 10% increment in GAA, the CCT in the controls decreased by 5.4 µm, on average. In the POAG patients, a 10% increase in GAA was associated with a 3.7 µm decrease in CCT. As an effect of the differences in the slope for controls and POAG patients, the difference in CCT between controls and POAG cases narrowed as the regression lines converged.

Figure 3. Scatter plot depicting the relation between GAA and CCT



Black dots represent POAG patients, every *gray diamond* represents a control. The *black line* is the linear regression adjusted for sex and age for POAG patients (CCT_{POAG} = 563.2–0.37*GAA). The *gray line* is the linear regression adjusted for sex and age for controls ($CCT_{controls}$ = 584.0–0.54*GAA).

2. Relationship of IOP and GAA

In the POAG patients, GAA was significantly associated with IOP (P < 0.029), as shown in the multivariable regression model. As such, for every 10% increase in GAA in POAG patients IOP increased by 0.46 mm Hg. In controls, GAA was not associated with IOP (P = 0.131).

3. Relationship of IOP and CCT

We examined the association between CCT and IOP for POAG patients and controls separately. In none of the groups there was a statistically significant relationship between CCT and IOP in a multivariable regression model adjusting for sex and age (P_{POAG} = 0.767; $P_{control}$ = 0.204).

4. Relationship of POAG and CCT

Logistic regression analysis showed that a thinner CCT was associated with an increased likelihood of POAG ($P_{univariable}$ <0.02, $P_{multivariable}$ < 0.019). A 10 µm decrease in CCT was associated with approximately 7% higher odds of POAG after adjusting for sex (*P* =0.017; odds ratio [OR], 0.59; 95% CI, 0.38 – 0.91), and age (age per year, *P* = 0.694; OR, 1.0; 95% CI, 0.97–1.02). To test if the relationship between CCT and POAG was modified by GAA, we tested for effect modification by adding the multiplicative interaction term between GAA and CCT to the multivariable regression model. In addition, a stratified analysis for median GAA (i.e., below and above the median GAA value of 59.6%) was performed. No statistically significant interaction between GAA and CCT was observed (*P*- interaction = 0.112). When the data were stratified by median GAA, the CCT was associated only with POAG for individuals with a GAA less than 59.6% (*P*= 0.044; Table 3). Since NTG (untreated IOP < 21 mm Hg) is found more commonly in non- Africans and has been associated previously with a thinner CCT, we performed a sensitivity analysis by removing all NTG patients (n = 28) from our analyses. Excluding the NTG patients from the main analysis did not change the association between CCT and POAG (*P* 0.02; OR, 1.07; 95% CI, 1.01–1.13); similarly, the multiplicative interaction between GAA and CCT (*P* = 0.102) did not change.

Table 3. Association of central corneal thickness (CCT) with primary open-angle glaucoma (POAG) stratified by median proportion genetic African ancestry (GAA)

Strata	n_{POAG}; $n_{control}$	OR	95% CI	P-value*
< median GAA (<59.6%)	122 ; 69	1.09	1.002 – 1.18	0.044
≥ median GAA (≥59.6%)	129 ; 61	1.04	0.941 – 1.140	0.477

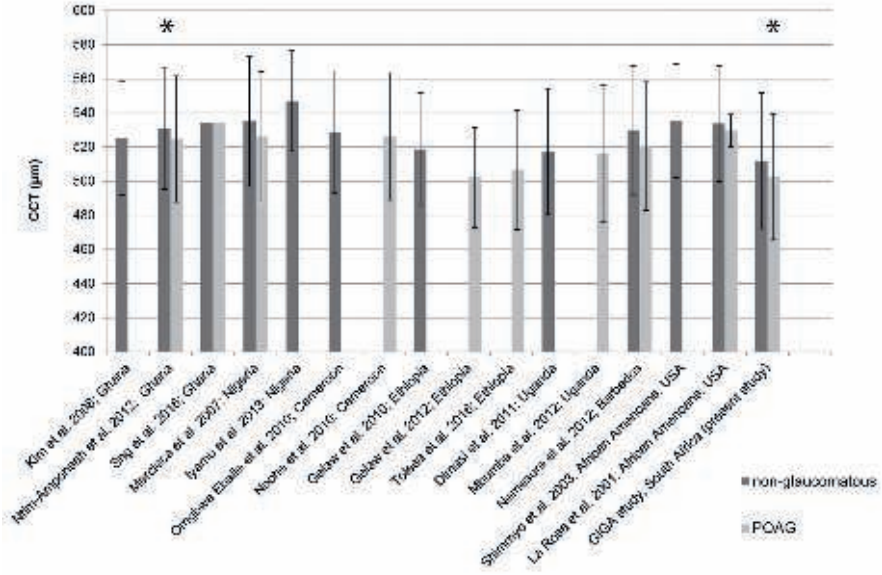
CI= confidence interval; OR= odds ratio per 10 µm decrease in CCT.
* Multivariable regression model adjusted for age and sex.

DISCUSSION

In this study, we found a statistically significant association between GAA, CCT, and IOP in the South African study population. Participants with a higher proportion of GAA had a thinner CCT. African ancestry also was associated with higher IOP in POAG patients. In the total study population, the POAG patients had a significantly thinner CCT, but the association of POAG and CCT was not statistically modified by differences in GAA. However, when stratified by median GAA, only an association between CCT and POAG for individuals with <60% GAA remained statistically significant. This suggests that genetic ancestry may have a role in the association between POAG and CCT.

To our knowledge, this study is the first to investigate the variation in CCT across different ethnic/racial groups in an African population and its association with GAA. Only recently have studies started to investigate the CCT in populations from sub-Saharan Africa, of which most are from West Africa (i.e., Ghana and Nigeria[35-38]). In comparison with other African studies, the mean CCT found for control participants in this South African population was considerably thinner; it was even the thinnest in any African study performed by means of ultrasound pachymetry to date (i.e., 512 ± 39.8; Fig. 4)[28, 35-46]. Associations between GAA and CCT have been studied previously in African Americans and Europeans in the ADAGES study [47]. This study found a similar correlation for CCT and GAA in the entire group. However, a significant association between GAA and CCT in the African American subgroup could not be detected due to a limited degree of admixture in this group.

Figure 4. CCT in African POAG patients and non-glaucomatous African individuals



Error bars: Standard deviation. *Significant difference between POAG patients and controls; Sng et al., no standard deviation available.

The association between IOP and GAA has been studied in Latinos in the LALES study[48]. This population-based study found that IOP increases by 0.38 mm Hg for every 10% increase in GAA. Although West Coast Latinos have a modest contribution of GAA, our study found similar results, that is, a 0.46 mm Hg increase per 10% increase in GAA, for POAG patients. For controls, we did not find any association between GAA and IOP. This could be explained by selection bias that was induced by selecting only controls with an IOP <21 mm Hg.

We did not observe a significant linear correlation between IOP and CCT in either POAG patients or controls. Although most population-based studies find a correlation between IOP and CCT, case control studies could not always detect this association in POAG patients due to selection of severe cases with critical elevated IOP.

Most of the studies investigating the relationship between glaucoma and CCT were based predominantly on European ancestral populations and focused on ocular hypertension and NTG patients. There have been conflicting reports about the CCT of POAG patients versus controls. Several studies did not find any statistically significant differences in CCT between these groups[27, 49-54]. Yet, various other studies have, indeed, reported such differences[26, 55, 56]. A few studies have investigated this relationship in African populations[26, 35, 37, 44, 46]. Only one of these detected a statistically significantly thinner CCT in POAG patients, but this difference was only present in the left eye[35]. Also, the Barbados eye study found a thinner CCT in POAG patients, but this difference was not statistically significant ($P = 0.07$) [44]. Recently, the Tema eye survey, the largest population-based study of CCT on the African continent, could not find an association between CCT and POAG as well[38].

A novel finding of our study was that the difference in CCT between POAG patients and controls attenuated by increasing GAA (Fig. 3). This highlights that in individuals with a high percentage of African ancestry, differences in CCT are little, if at all, associated with POAG. This stresses the importance of anatomic variation between ethnic/racial groups and the possible susceptibility for development of POAG. Investigation into the racial and ethnic differences in the anatomy of the optic nerve head showed, for instance, a thicker retinal nerve fiber layer, and larger optic discs with deeper cups in African individuals[57-61]. In particular, a correlation between thinner CCT and larger optic discs seems to be present in POAG patients[62, 63]. Larger optic disc diameters may be associated with increased vulnerability to pressure-induced deformation. Therefore, eyes with thinner corneas are more susceptible to glaucomatous damage in comparison with those having thicker corneas. This hypothesis may explain why African persons are more vulnerable to glaucomatous optic nerve head damage. Although conflicting evidence exists that CCT is associated with other disc topographic parameters (i.e., rim area, cup area and VCDR)[64-66]. A new property of the cornea, corneal hysteresis, has shown to be a better predictor of glaucomatous damage[15, 21].

Our study has strengths and weaknesses. A strength of our study is that by applying BGA estimation, we were able to objectively measure variation in CCT and IOP related to ethnic/racial differences. Self-reported ethnicity/race frequently is used in epidemiologic studies to assess an individual's background origin. Often participants are asked to specify a single ethnic/racial group based on categories. This method can be unreliable, since these definitions can be imprecise and inconsistent over time[67, 68]. Also, self-reported ethnicity/race can be based on subjective physical characteristics and intrinsic beliefs. Skin color, for

example, often is used as surrogate of race, although visual classification of skin color can be interpreted differently[69-71]. Especially in complex admixed populations, such as SAC, self-reported ethnicity does not reveal the extent of admixture, which is because admixed individuals can have multiple ancestries, and these ancestry proportions can vary greatly per individual. Recent advances in genome-wide genotyping that allow the inference of BGA can set aside the use of proxy methods, such as self-reported ethnicity/race[71]. The high degree of admixture in our study population also is a valuable asset of the study, since it enabled detailed evaluation of the differences in CCT and IOP in relation to African ancestry. A limitation of this study is its relatively small sample size. As a result, this study had limited statistical power to find significant interaction between GAA and CCT when studying the association between CCT and POAG. Also, we performed several numbers of tests. Therefore, chance finding should be considered when interpreting the data. Post hoc power analysis showed that this study was sufficiently powered (power > 80%; $P < 0.013$; considering four tests), for the multivariable associations in the POAG patients. We currently are extending our genotyping efforts in a Tanzanian population. As genotyping progresses, we will have more statistical power to detect any significant differences. Preliminary data from Tanzania strengthens our current findings and confirm that in this African black population CCT is not different among POAG patients and controls. Another limitation of this study includes potential selection bias. For selecting POAG patients based on functional damage (ISGEO category 1), we applied rather strict criteria for assessing the reliability of the visual fields. This might have led to a selection of super-test takers, and, therefore, an underdiagnosis of POAG patients. It turned out, however, that 96% of the probable glaucoma cases that failed our strict reliability criteria were later identified as glaucomatous, based on advanced structural optic nerve head damage (ISGEO category 2). Therefore, the effects of our strict visual field reliability criteria on our results probably were insignificant. In controls, the IOP cut-off for enrolment could have biased the associations in this group. Although high IOP is the main risk factor for POAG, we might have overlooked potentially healthy participants with elevated IOP.

In conclusion, this study shows that in African admixed individuals GAA measurement is an unprejudiced tool to distinguish associations with POAG and their endophenotypes. We found that a higher proportion of GAA is associated with a thinner CCT, and that an increase in GAA in POAG patients is associated with a higher IOP. Interestingly, our current study shows that the difference in CCT between POAG patients and controls is reduced at higher proportions of GAA. This confirms previous studies that did not find significant differences in CCT between POAG patients and controls in Africans[26, 37, 38, 44, 46]. Therefore, some biomechanical properties of the African eye may be different compared to those in other ethnic groups. However, it is not yet clear to what extent they relate to the increased glaucoma susceptibility of Africans.

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Chapter 3

Novel genetic variants associated with POAG in populations from sub-Saharan African ancestry

Chapter 3.1

Genome-wide association study of primary open-angle glaucoma in continental and admixed African populations

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ABSTRACT

Primary open angle glaucoma (POAG) is a complex disease with a major genetic contribution. Its prevalence varies greatly among ethnic groups, and is up to 5 times more frequent in black African populations compared to Europeans. So far, worldwide efforts to elucidate the genetic complexity of POAG in African populations has been limited. We conducted a genome-wide association study (GWAS) in 1113 POAG cases and 1826 controls from Tanzanian, South African and African American study samples. Apart from confirming evidence of association at *TXNRD2* (rs16984299; $OR_{[T]}$= 1.20; *P*=0.003), we found that a genetic risk score combining the effects of the 15 previously reported POAG loci was significantly associated with POAG in our samples (OR= 1.56; 95% CI, 1.26-1.93; *P* = 4.79 x 10-5). By genome-wide association testing we identified a novel candidate locus, rs141186647, harboring *EXOC4* ($OR_{[A]}$= 0.48; *P* = 3.75 x 10-8), a gene transcribing a component of the exocyst complex involved in vesicle transport. The low frequency and high degree of genetic heterogeneity at this region hampered validation of this finding in predominantly West-African replication sets. Our results suggest that established genetic risk factors play a role in African POAG, however, they do not explain the higher disease load. The high heterogeneity within Africans remains a challenge to identify the genetic commonalities for POAG in this ethnicity, and demands studies of extremely large size.

INTRODUCTION

Glaucoma is the leading cause of irreversible blindness worldwide[1]. The disease is an optic neuropathy characterized by loss of retinal ganglion cells resulting in peripheral visual field defects. Later in the disease process, the visual field defects may involve central vision leading to blindness. Primary open-angle glaucoma (POAG) is the commonest subtype of glaucoma. Intraocular pressure (IOP), family history, age, and ancestry are established risk factors. In particular persons of African ancestry have 3-5 x increased risk of POAG, and have a more severe course of disease with a higher risk of blindness[2,3]. This ethnic predilection along with the familial nature strongly suggests that genetic factors contribute to the pathogenesis of POAG.

Recently, progress has been made in the identification of associated variants by using linkage analysis and genome-wide association studies (GWAS). Rare variants with large effects have been identified in *MYOC* and *OPTN*, common variants with smaller effect have been reported in genomic regions that include *CAV1-CAV2*, *CDC7-TGFRB3*, *TMCO1*, *CDKN2B-AS1*, *ABCA1*, *AFAP1*, *GMDS*, *TXNRD2*, *ATXN2*, *FOXC1*, *GAS7*, *ARHGEF12*, *SIX6*, 8q22 and *PMM2*[4-12]. However, these loci explain only 5-10% of cases, leaving the heritability of POAG largely unexplained. Most genetic studies were predominantly conducted in European and Asian populations, leaving African ancestry underrepresented up to now. Recent studies in Africans or in cohorts of African descent (i.e., Ghana, South Africa and in African Americans) could not replicate most of the loci previously identified in GWAS of European and Asian populations[9,13,14].

Gene finding can be more effective in study populations where the disease is more common, of earlier onset and more severe. Therefore, in this study, we conducted a genome-wide meta-analysis using African black and South African Coloured POAG cases and controls, from the Genetics In Glaucoma patients from African descent study (GIGA) recruited at hospitals from South Africa and Tanzania and African Americans enrolled in the BioMe biobank.

RESULTS

The GIGA dataset consisted of 444 participants from South Africa (n_{POAG} = 297; $n_{control}$ = 147) and 695 participants from Tanzania (n_{POAG} = 366; $n_{control}$ = 329). The Tanzanian participants were all from black African origin, 38% of South African participants were also from black African origin while the remaining 62% were self-reported South African Coloured (European, African, Asian admixed). The BioMe dataset consisted of POAG cases (n = 450) and controls (n = 1350) and were all African American. The clinical and demographic characteristics of the GIGA and BioMe participants have been summarized in Table 1.

Table 1. Demographic and clinical characteristics GIGA study and BioMe

Demographic and clinical characteristics	POAG cases	Controls
GIGA	663	476
Median age, years (IQR)	65.0 (18)	65.0 (12)
Female, n (%)	281 (42)	266 (56)
Self-reported ethnicity/race, n (%)		
South African Coloured	179 (27)	96 (20)
African Black	484 (73)	380 (80)
Median proportion GAA, % (IQR)		
South African Coloured	33.86 (37.26)	33.12 (28.18)
African Black	97.90 (7.80)	97.28 (7.81)
BioMe	450	1350
Median age, years (IQR)	64.0 (16)	64.0 (16)
Female, n (%)	290 (64)	870 (64)
Median proportion GAA, % (IQR)	86.84 (12.84)	86.74 (12.99)

GAA: genetic African ancestry; IQR: interquartile range; POAG: primary open-angle glaucoma; SD: standard deviation

Association of previously reported POAG loci in African populations

First, we tested the association of the 15 previously established POAG SNPs identified in GWAS of European and Asian populations in the GIGA and BioMe datasets[4-12]. None of these SNPs replicated at a nominal significance level ($P < 0.05$) in any single ethnicity (Supplementary Table 1), nor in a combined analysis (Table 2 exact replication). Because linkage disequilibrium (LD) patterns may differ between the study populations of the reported GWAS and the current African study participants, we also searched for evidence of transferability of the SNPs. Locuszoom plots were made using the LD pattern of Europeans and Asians (1000 Genomes) to investigate whether SNPs in high LD ($r^2 > 0.8$) with the original lead SNP showed evidence of association in our study (Supplementary Fig. 1). This "local" replication strategy queried a 500 kb window centered on the lead SNP, and yielded a total of 246 SNPs in LD (r^2>0.8) with the 15 lead SNPs. Of these 246 SNPs, three SNPs in the *TXNRD2*, *CDKN2B-AS1,* and *TMCO1* loci were significantly associated with POAG in our study ($P < 0.05$) (Table 2, local replication). rs16984299 in *TXNRD2* had similar effect size as reported by Cook Bailey et al. [4] and survived multiple testing ($OR_{[T]}$,1.20; 95% CI 1.06-1.35; $P_{Bonferonni} = 0.049$) when the association was corrected for the effective number of SNPs (n = 16) in the queried 500 kb window. In addition, we also analyzed three independent POAG variants found in African Americans from the Women Health Initiative[15]. We found rs192917960 at the *RBFOX1* locus associated with POAG in BioMe ($P = 0.02$, Supplementary Table 2), but this association did not withstand correction for multiple testing.

Table 2. Lookup of known POAG SNPs in GIGA BioMe

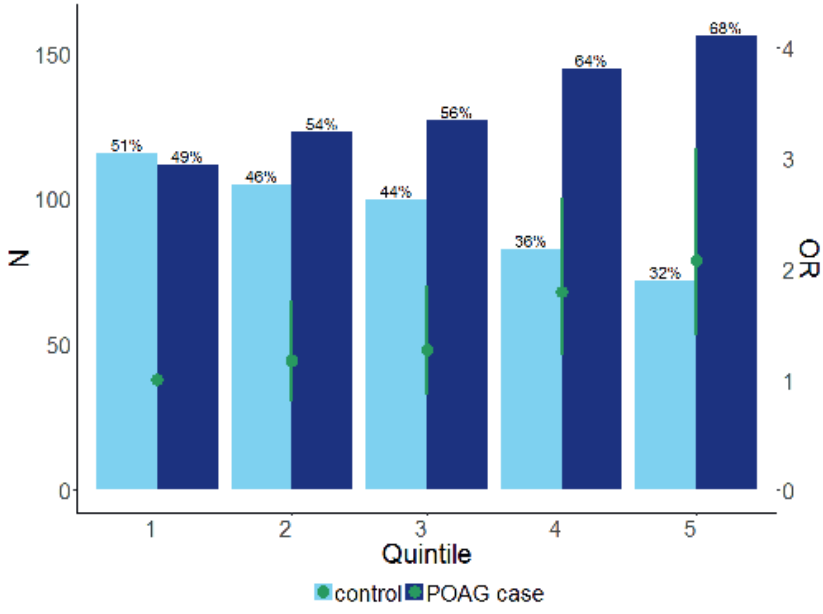
Genomic region	Exact replication				Local replication						
	Lead SNP	A1	OR (95% CI)	*P*-value	EUR Proxy variant	Correlated allele	r^2 between Lead SNP and Proxy	OR	*P*-value	Number of effective SNPs in LD	*P*-adjust
CDC-TGRFB3	rs1192415	G	1.05 (0.88-1.26)	0.5811	.	.	.	.	.	.	.
TMCO1	rs4656461	G	0.98 (0.85-1.12)	0.7305	rs28504591	A	0.99	1.31 (1.07-1.6)	0.0094	8.3	0.07827
AFAP1	rs4619890	G	1.08 (0.93-1.25)	0.3018	rs28605661	G	0.99	1.10 (0.95-1.27)	0.2219	2.98	0.66144
FOXC1	rs2745572	A	0.98 (0.85-1.14)	0.8033	.	.	.	.	.	.	.
GMDS	rs11969985	G	1.05 (0.92-1.21)	0.4471	rs12530211	T	0.98	1.09 (0.94-1.24)	0.2266	10.65	1
CAV1-CAV2	rs4236601	A	1.04 (0.89-1.19)	0.6349	rs10270569	T	0.83	1.08 (0.92-1.28)	0.3411	3.69	1
8q22	rs284489	G	0.99 (0.87-1.12)	0.8401	rs10106029	G	0.97	1.09 (0.97-1.24)	0.1428	4.69	0.66926
CDKN2B-AS1	rs4977756	A	1.06 (0.94-1.19)	0.3473	rs10712703	d	0.84	1.20 (1.04-1.39)	0.0145	6.15	0.08907
ABCA1	rs2472493	G	1.11 (0.98-1.25)	0.09345	rs2437812	C	0.90	1.12 (1-1.26)	0.0560	2.17	0.12131
ARHGEF12	rs58073046	G	1.19 (0.71-2)	0.5113	.	.	.	.	.	.	.
ATXN2	rs7137828	T	0.82 (0.91-1.16)	0.1047	rs7310615	G	0.97	0,79 (0,62-1,01)	0.05864	1.19	0.06968
SIX6	rs10483727	T	1.11 (0.9-1.37)	0.3212	rs6573307	G	0.96	1.10 (0.94-1.28)	0.2355	9.26	1
PMM2	rs3785176	C	1.08 (0.91-1.3)	0.3746	.	.	.	.	.	.	.
GAS7	rs9897123	T	1.04 (0.92-1.16)	0.544	.	.	.	.	.	1	.
TXNRD2	rs35934224	T	1.06 (0.93-1.21)	0.3692	rs16984299	C	0.90	0.83 (0.74-0.94)	0.0032	15.63	0.04984

Lead SNP: SNP reported in European or Asian GWAS associated with primary open-angle glaucoma; A1: effect allele reported in European or Asian GWAS; EUR proxy variant: variant in strong LD (r^2>0.8 EUR 1000 Genomes) with the lead SNP that has a smaller *P*-value in GIGA BioMe than the exact replication of the lead SNP. Cells are left empty (.) when no variant in the queried LD region had a smaller *P*-value; Correlated allele: proxy variant allele correlated with lead SNP A1 allele (LDlink (Machiela and Chanock 2015)); Effective number of SNPs: number of effective SNPs within the queried 500kb region calculated by Genetic Type 1 Error calculator using the 1000 Genomes African samples as a reference (Li et al. 2012); *P*-adjust: corrected *P*-value for number of effective SNPs; d: deletion

Next, we compared effect sizes from the combined analysis of GIGA and BioMe with the effect sizes from published GWAS reports. In total, 12 out of the 15 known lead SNPs had a consistent direction of effect (Supplementary Fig. 2). Allele frequencies for most SNPs were very similar in South African blacks, Tanzanian, and African American datasets, but markedly different compared with the European and Asian studies (Supplementary Fig. 3). In eight of the 15 SNPs, the effect allele had a considerable higher frequency in Africans while five were clearly less frequent compared to Europeans.

To study the contribution of the known SNPs to the risk of POAG in GIGA, we calculated a multilocus Genetic Risk Score (GRS) based on 15 known SNPs. Three known SNPs for *TXNRD2*, *CDKN2B-AS1,* and *TMCO1* were replaced by the proxies that were identified by the local replication approach described above. Scores were weighted based on the effect sizes found in the GWAS meta-analysis of European populations. The GRS, adjusted for age, sex, and first five principal components was associated with POAG in the GIGA sample (OR = 1.56; 95% CI, 1.26-1.93; $P = 4.85 \times 10^{-5}$). We then stratified the GRS in quintiles, and estimated the risk of POAG for each quintile relative to the lowest one (Fig. 1). Trend analysis showed a

Figure 1. Genetic Risk Score in patients and controls



Genetic risk score based on the 15 known POAG-loci identified in Europeans and Asians GWAS (rs1192415, rs28504591, rs4619890, rs2745572, rs11969985, rs4236601, rs284489, rs10712703, rs2472493, rs58073046, rs7137828, rs10483727, rs3785176, rs9897123, rs16984299). Participants were grouped into quintiles of the genetic risk scores. *Green circles* represent the POAG odds ratio (adjusted for age, sex and principal components) when comparing each quintile to the lowest quintile (Q1= reference line). The *green capped lines* represent 95% CI of the POAG odds ratios. *Bars* represent the percentage of POAG cases (*dark blue*) and controls (*light blue*) per quintile.

significant stepwise increase in the risk of POAG per quintile (P_{trend} = 2.81 x 10^{-5}), with a twofold increase in POAG risk for the highest quintile compared to the lowest. The risk attributed to genetics was calculated in reference to the mean genetic risk score in the controls. We found that these 15 known variants taken all together attributed 4% (95% CI, 2%-6%) to the overall POAG risk in this study population when we adjusted for age, sex and principal components.

Discovery (stage 1)

To identify new loci associated with POAG in African populations, we performed GWAS using our African ancestry datasets. The scheme of the study design is depicted in Fig. 2. In the discovery stage, we meta-analyzed GWAS results from the GIGA study (South Africa and Tanzania) and BioMe (African American) including in total 1113 POAG cases and 1826 controls. A total of 13.8 million SNPs were available after applying our QC and filtering criteria (see Methods section). The genomic inflation factor was 0.94 (SE: 1.49 x 10^{-6}) and the quantile-quantile plot did not show any systemic inflation in the association results, suggesting that confounding by cryptic population stratification was unlikely (Supplementary Fig. 4). The discovery association results across the whole genome are shown in Fig. 3. We identified one novel region reaching genome-wide significance (P < 5 x 10^{-8}) in the discovery stage, and two suggestive regions (P < 1 x 10^{-6}) (Table 3). The top newly associated SNPs were rs141186647[A] an intronic variant in *EXOC4* on chromosome 7 (OR = 0.48; P = 3.75 x 10^{-8}), rs9475699[A] downstream of *DST* on chromosome 6 (OR = 1.65; P = 1.25 x 10^{-7}), and rs62023880[A] upstream of *MNS1*-on chromosome 15 (OR = 1.39; P = 5.12 x 10^{-7}). The regional association plots for these three SNPs are shown in Fig. 4. We did not observe any significant heterogeneity for these SNPs in the meta-analysis of GIGA and BioMe. The association results per ethnic group are provided in Supplementary Table 2, showing similar effects in Tanzanians, South Africans, and African Americans. Conditional and joint analyses did not identify any additional independent signals within the set of SNPs reaching P < 1 x 10^{-6}. Additionally we explored if haplotypes encompassing any of the three top SNPs were associated with POAG in GIGA BioMe, the results for this haplotype association analysis are provided in Supplementary Table 3.

Figure 2. Study design

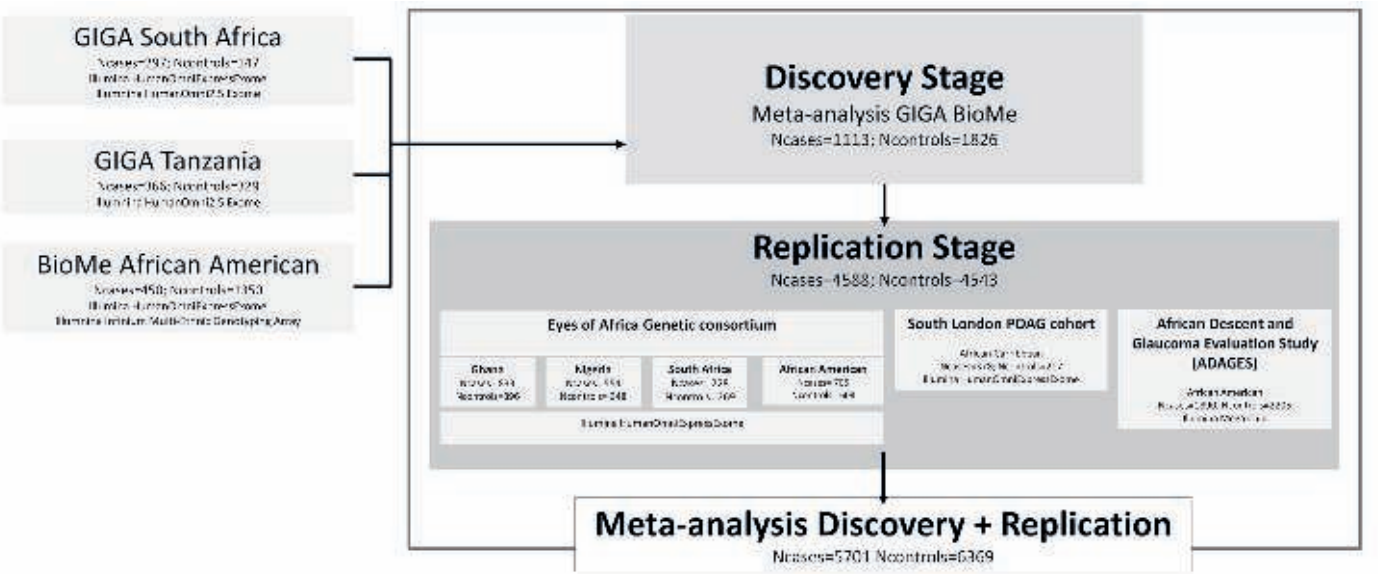
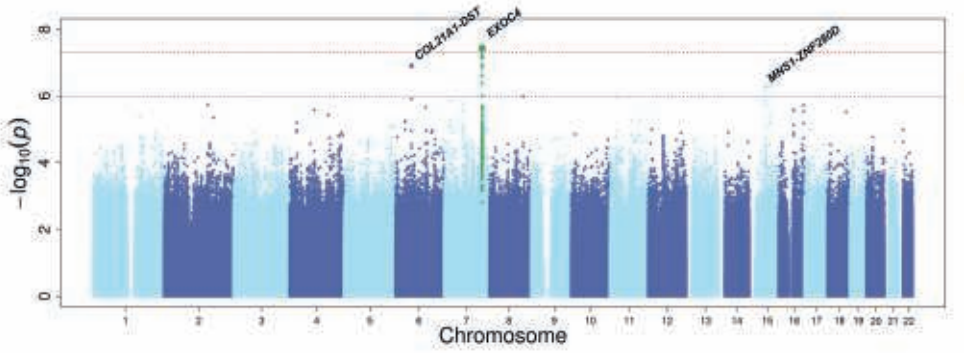


Figure 3. Manhattan plot for association of genome-wide SNPs with primary open-angle glaucoma in GIGA BioMe meta-analysis



Manhattan plot of the GWAS meta-analysis of GIGA and BioMe (N=1113cases/N=1826 controls). The figure shows −log10-transformed *P*-values for all SNPs. The upper dotted horizontal line represents the genome-wide significance threshold of $P < 5.0 \times 10^{-8}$; the lower dotted line indicates a *P*-value of 1×10^{-6}. Green dots represents variants that are in linkage disequilibrium (r^2>0.6 1000 Genomes African ancestry) with the top SNP rs141186647.

Replication of associated variants in African populations (stage 2 and stage 3)

All SNPs reaching $P < 1 \times 10^{-6}$ in stage 1 were followed up in a replication (stage 2) comprising four independent African ancestral studies from South Africa, Ghana, Nigeria and African Americans (Eyes of Africa Genetic Consortium; $n_{cases} = 2320$; $n_{controls} = 2121$), the South London POAG case –control cohort comprising individuals from West African origin ($n_{cases} = 378$; $n_{controls} = 217$) and The African Descent and Glaucoma Evaluation Study (ADAGES) including African Americans ($n_{cases} = 1890$; $n_{controls} = 2205$). In total, 22 SNPs at the three independent loci were brought forward for replication. Variant rs9475699 (downstream of *DST*) reached a nominal level of statistical significance (OR = 1.19, $P = 0.032$) in the Ghanaian study population (Supplementary Table 4). We then performed a meta-analysis of all six replication datasets (stage 2), first using a fixed effects model, and found no statistical significant replication (Table 4). Subsequent meta-analysis by means of the Han and Eskin random-effects model for SNPs with significant ($P < 0.05$) heterogeneity, also did not identify any SNPs with significant association. In stage 3, we performed a meta-analysis of all studies (stage 1 + stage 2), totaling 5701 POAG cases and 6369 controls. Given the high degree of heterogeneity observed in the fixed effect meta-analysis at this stage we performed Han and Eskin random-effects model. Neither fixed effects nor Han and Eskin random-effect meta-analysis resulted in genome-wide significant signals (Table 4, Supplementary Table 4 and Supplementary Fig. 5).

Table 3. Association results for the top SNPs in previously unreported regions with *P*< 1 × 10−6 in the discovery phase (GIGA + BioMe)

						Meta-analysis GIGA BioMe				
SNP	**CHR**	**POS**	**Nearest Gene**	**A1**	**Ncases/ Ncontrols**	**Frequency A1 Cases/ Controls**	**OR (95% CI)**	***P*-value**	**I^2**	***P*Het**
rs9475699	6	56302054	*COL21A1-DST*	A	858/1061	0.22/0.17	1.65 (1.37-1.98)	1.25E-07	41.4	0.1632
rs141186647	7	133634202	*EXOC4*	A	1113/1826	0.04/0.06	0.48 (0.37-0.62)	3.75E-08	29.2	0.2271
rs62023880	15	56770871	*MNS1-ZNF280D*	A	1113/1826	0.30/0.26	1.39 (1.22-1.58)	5.12E-07	30.4	0.2192

SNP (single nucleotide polymorphism): rsID; CHR: chromosome; POS: base pair; Nearest gene (reference NCBI build37) is given as locus label; A1: effect allele; OR: odds ratio on POAG based on allele A1; PHet: *P*-value for heterogeneity

Cross-ethnic validation

We further investigated to what extent loci found in our African ancestry GWAS confer a risk of POAG in Europeans. We investigated the top three ranked loci from the discovery stage in two independent European ancestry studies from the National Eye Institute Glaucoma Human Genetics Collaboration (NEIGHBOR) and the Massachusetts Eye and Ear Infirmary (MEEI) (totaling 2606 POAG cases and 2606 controls) with imputed genotype data using the Haplotype Reference Consortium (HRC)[16]. rs141186647 (*EXOC4*) and rs9475699 (*COL21A1-DST*) are rare in the European cohorts but had excellent imputation scores (MAF 0.00019,r^2 0.987; and MAF 0.0023, r^2 0.963 respectively). However, neither SNP demonstrated significant association in the European datasets (rs141186647 $OR_{[A]}$ = 5.09; P = 0.39; rs9475699 $OR_{[A]}$ = 1.27; P = 0.59). SNP rs62023880 (*MNS1-ZNF280D)* on chromosome 15, which was a common variant in the NEIGHBOR/MEEI sample (MAF = 0.15,) also did not show statistically significant replication (OR = 0.947; P = 0.34).

Bioinformatical lookup of functional and regulatory effects and expression of POAG-associated SNPs

We explored the functional and regulatory annotations of the three lead SNPs found in the discovery stage, including proxy-SNPs within high LD (r^2 > 0.8 in 1000G AFR). The significant top hit rs141186647 at 7q33 represented an intronic variant within the Exocyst Complex Component 4 gene (*EXOC4*). The locus contains a set of SNPs in high LD that reside within the introns and within exon 15 (rs34608222; synonymous) of *EXOC4* of which only rs79198429 (r^2 = 0.92 with rs141186647) is annotated as possibly disrupting; transcription factor binding (RegulomeDB score 3a; Supplementary Table 5). This variant is located inside a region annotated as an enhancer histone mark in multiple tissues by the RoadMap Epigenomics project, which is predicted to bind the transcriptional coactivator protein P300, and to alter

Table 4. Association and meta-analysis of the discovery and replication studies for the top-ranked loci

					GIGA BioMe (discovery)				Meta-analysis Replication Studies						Meta-analysis Discovery and Replication				
SNP	**CHR**	**POS**	**Nearest Gene**	**A1**	**Frequency A1 Cases/ Controls**	**Ncases/ Ncontrols**	**OR**	***P*-value**	**Frequency A1 Cases/ Controls**	**Ncases/ Ncontrols**	**OR**	***P*-value**	**I^2**	**HetP**	**Frequency A1 Cases/ Controls**	**OR#**	***P*-value***	**I^2**	**Het*P***
rs9475699	6	56302054	*COL21A1-DST*	A	0.22/0.17	858/1061	1.65 (1.37-1.99)	1.25E-07	0.22/0.22	2698/2338	0.95# (0.81-1.11)	*0.141**	59.6	0.042	0.22/0.21	1.04 (0.82-1.31)	3.49E-06	85.06	3.03E-06
rs141186647	7	133634202	*EXOC4*	A	0.04/0.06	1113/1826	0.48 (0.37-0.62)	3.75E-08	0.04/0.04	4588/4543	0.97 (0.92-1.02)	0.278	0	0.723	0.04/0.05	0.90 (0.71-1.16)	5.91E-05	79.86	4.30E-05
rs62023880	15	56770871	*MNS1-ZNF280D*	A	0.30/0.26	1113/1826	1.39 (1.22-1.58)	5.12E-07	0.28/0.26	4588/4543	1.02 (0.997-1.04)	0.086	0	0.575	0.28/0.26	1.08 (0.97-1.21)	1.17E-04	76.31	2.97E-04

SNP: rsID; CHR: chromosome; POS: base pair; Nearest gene (reference NCBI build37) is given as locus label; A1: effect allele; OR: odds ratio on POAG based on allele A1; PHet: P-value for heterogeneity;
OR from a random effect meta-analysis; *P-value's in italics represent Han & Eskin's random effect meta-analysis P-values

5 binding motifs including AP-1 transcription factor[17]. None of the explored SNPs in this region were associated with eQTL's.

In silico analyses of SNPs correlated with rs9475699 located 21 kb downstream the *DST* gene and rs62023880 neighboring *MNS1* gene did not identify any markers with evidence for gene regulatory effects.

To assess the expression of the annotated genes in human eye tissues, we examined the online Ocular Tissue Database (https://genome.uiowa.edu/otdb/)[18]. Expression of *EXOC4, DST* and *MNS1* was observed in tissues relevant to POAG, such as the trabecular meshwork, optic nerve head and optic nerve. Supplementary Table 6 depicts the differences in expression levels of these three genes across tissue types. In the optic nerve head, the highest level of expression was found for *DST* gene (PLIER 632.5).

Gene-based tests

We performed gene-based tests using VEGAS2 on the GIGA BioMe meta-analysis results, and first investigated the 15 known POAG genes. None of these were significant at a nominal statistical level, the smallest *P*-value was found for *FOXC1* (P = 0.103, nSNPs = 573) (Supplementary Table 7). We subsequently explored the gene-based test results of a total of 25,590 autosomal genes, using a Bonferroni corrected gene-based significance threshold of $P_{\text{gene-based}} < 1.95 \times 10^{-6}$ (0.05/25590). The *EXOC4* gene ($P = 3.10 \times 10^{-5}$) did not withstand Bonferroni correction.

DISCUSSION

To date, only European and Asian ancestry GWA studies have contributed to the 15 currently known genetic loci for POAG. Although the frequency of POAG in persons from African descent is high compared to those of European or Asian descent, studies of individuals of African descent are missing so far. The current study focuses on filling this gap. In this case-control study consisting of Africans from the African continent as well as of African Americans, we confirmed three POAG loci (*CDKN2B-AS1*, *TMCO1*, *TXNRD2*) at nominal significance that were previously found in Europeans, and report one novel candidate locus (*EXOC4*). A variant (rs1063192) near *CDKNB2-AS1* has previously been shown in the Afro-Caribbean population of Barbados, although this study could not replicate other known putative loci[13]. Another insight gained from the current study was that the "local approach" rather than exact replication yielded these replicable findings in Africans. Interestingly these proxy SNPs in Africans have a very similar effect size compared to the lead SNP in European GWAS.

This study has strengths and limitations. Of particular strength was the Pan-African origin of the study participants. Previous studies from the African continent were smaller and they all focused mainly on West Africans. Our study is the first genetic analysis which included East Africans. A probable disadvantage of applying a Pan-African approach must also be considered. The high genetic diversity present across African populations, even when they are geographically close, may reduce the likelihood of reproducing associations in multi-center studies. Other strengths were the careful diagnosis of cases, the strict criteria for controls, and the application of local replication. Optic discs were graded in an objective manner from fundus photographs by glaucoma experts using internationally accepted standards[19]. Controls underwent the same review process as cases and had to be over 50 years of age to increase diagnostic certainty of non-disease status. The limitations of our study include the relatively low power to detect genome-wide significance for small effect sizes, as reflected by the genomic inflation factor < 1.0, and the lack of a replication set from East Africa.

As the genome of African populations is much older, genetic diversity is increased, and LD across loci is decreased. Rather than focusing only on the lead SNPs from European/Asian GWAS, we considered all variants that were in strong European/Asian LD with the lead SNPs. We analyzed these variants in our African samples, and found evidence for nominal replication of three SNPs in *TMCO1* (rs28504591), *CDKN2B-AS1* (rs10712703), and *TXNRD2* (rs16984299), of which the latter withstood Bonferroni correction for the number of effective SNPs. The most significant SNP identified in GWAS is often not the causal variant (McCarthy and Hirschhorn 2008). We found similar effect sizes compared to the European GWAS for the three SNPs identified by the local replication approach. The overall weaker LD structure in Africans favors proximity of these proxy SNPs to the true causal variant. This makes it more likely that these proxies are functional. We therefore recommend candidate gene studies in African populations that failed to replicate known disease loci found in European or Asian populations to use this local approach.

Although this study found evidence that at least one known POAG gene plays a role in African glaucoma, we could not significantly replicate the remaining 14 associated SNPs even when we applied the local approach. Yet our GRS that was based on known European and Asian POAG SNPs showed a significant trend ($P = 2.81 \times 10^{-5}$) and a twofold increase in POAG risk comparing extreme risk groups. Of note, the allele frequency distributions for these SNPs differed markedly between our African study and the original European/Asian studies. This points towards differences in genetic architecture, and makes it difficult to estimate statistical power.

This study identified a novel candidate variant within the *EXOC4* gene in the meta-analysis of GIGA and BioMe. Recent reports provide evidence that this gene is implicated in cognitive

traits as intelligence and educational attainment, and is also associated with the neurodegenerative Alzheimer's disease[20-22]. The *EXOC4* gene is ubiquitously expressed, and is particularly abundant in the brain. *EXOC4* encodes the SEC-8 protein, a component of a complex which is essential for exocytosis; it directs Golgi-derived secretory vesicles to specific docking sites on the plasma membrane. Exocyst proteins are needed for rapid membrane expansion, which happens during outgrowth of neurons and synaptogenesis. So far, the exocyst complex has not been studied in connection with glaucomatous optic neuropathy, however, it is expressed in the trabecular meshwork. In this tissue, it plays a role in the formation of invadopodia, protrusions that are important for releasing matrix metalloproteinase into the extracellular matrix to decrease trabecular outflow resistance[23]. Strikingly, our African POAG cases had high IOP, and it is intriguing to speculate that *EXOC4* contributes to POAG by interfering with matrix metalloproteinase release and trabecular outflow.

Replication of our genome-wide significant finding from the discovery set in our other African studies was challenging for this relatively rare variant. Meta-analysis of the discovery and replication stage showed considerable variation in effect size and direction of effect between the discovery and the replication set, indicating substantial heterogeneity. This heterogeneity is likely to be caused by differences in genetic ancestry as most of the replication studies were from West-African origin, while the population GIGA BioMe included a substantial proportion of persons from East Africa. Differences in LD pattern between causal variants and identified markers as shown in Supplementary Fig. 7 can cause this directionally inconsistent associations across studies more commonly known as the flip-flop phenomenon[24].

In conclusion, we conducted the first GWAS of POAG comprising continental Africans. We verified the European glaucoma gene *TXNRD2* and identified a novel candidate locus encompassing *EXOC4* that requires further follow up in large African studies. A GRS combining the effects of the known POAG SNPs indicated that these SNPs are implicated to play a role in African POAG. Future studies on POAG in Africa should take the substantial genetic heterogeneity into account by ascertaining large discovery and replication sets from the same geographic area.

METHODS

Study population

The Genetics In Glaucoma patients from African descent study (GIGA) is a multicenter case-control study comprising POAG patients and controls from South Africa and Tanzania.

Participants from Black African and South African Coloured ancestry were ascertained from the ophthalmology outpatient department of the Groote Schuur Hospital in Cape Town, South Africa (n_{cases} = 327; $n_{controls}$ = 194), and from hospitals in Tanzania: Muhimbili National Hospital and CCBRT Disability Hospital in Dar es Salaam (n_{cases} = 395; $n_{controls}$ = 382). The study was conducted according to the guidelines for human research by the National Institute for Medical Research in Tanzania. Ethical approval was obtained from the institutional review boards at each study site, and written informed consent was provided by each participant. The Charles Bronfman Institute for Personalized Medicine BioMe BioBank is an electronic medical record (EMR)-linked clinical care biobank that integrates research data and clinical care information of patients at The Mount Sinai Medical Center New York. This center serves diverse local communities of upper Manhattan with broad health disparities including POAG. The current analysis includes participants who self-reported to be of African ancestry (n_{cases} = 450; $n_{controls}$ = 1350) who were enrolled between September 2007 and October 2014. Ethical approval was obtained from the institutional review boards at Mount Sinai, and written informed consent was obtained from all participants.

Phenotype definition

In GIGA, POAG cases met category 1 or 2 of the ISGEO classification for open-angle glaucoma [19]. In brief, cases had either a definite visual field defect and Vertical Cup Disc Ratio (VCDR) ≥0.7, or VCDR > 0.8 in the absence of a visual field test. Other inclusion criteria were an open angle on gonioscopy and age of onset older than 35 years. Glaucoma patients diagnosed with secondary causes were excluded from this study. Controls were recruited at the same ophthalmology clinics and underwent identical examinations as the POAG cases. Inclusion criteria were: no signs of glaucoma, IOP ≤21 mm Hg, VCDR <0.5, and a VCDR inter-eye asymmetry <0.2, no family history of glaucoma, and age >55 years. Case and control status was adjudicated by two experienced ophthalmologists (AT and HL).

In BioMe information on POAG status, sex, age was derived from patients' EMR. POAG patients were considered cases if they had ≥1 diagnoses for POAG (ICD-9 codes 365.01, 365.05, 365.11, 365.12 or ICD-10 code H40.11). Participants with pre-glaucoma (ICD-9 code 365), ocular hypertension only (ICD-9 code 365.04), unspecified glaucoma (ICD-9 code 365.10) or with secondary glaucoma (Supplementary Table 8) were excluded from the analyses. Details of the ICD-9 or ICD-10 codes used can be found in Supplementary Table 9. Controls were those of African ancestry over 40 years of age not being diagnosed with any type of glaucoma.

Genotyping

In GIGA, 1162 participants were genotyped by using either the Illumina HumanOmni-ExpressExome Beadchip (964193 variants; Illumina, Inc., San Diego, CA, USA; n=999) or the

Illumina HumanOmni2.5Exome Beadchip (2406945 variants; Illumina, Inc., San Diego, CA, USA; n=163). Extensive quality control (QC) was performed on the genotyped data in PLINK v1.07[25]. Variants with a call rate <95%, as well as variants showing an extreme deviation from Hardy-Weinberg equilibrium ($P < 1 \times 10^{-6}$), or MAF <0.01 were excluded. All SNPs were mapped to genome build hg19/GRCh37. Individual level QC was performed by exclusion of individuals with a call rate <95%, disconcordant sex in self-report versus genetically determined sex, excess or reduced heterozygosity, relatedness (PI-HAT > 0.25) or duplicative samples based on identity by descent (IBD) sharing calculations. The final dataset consisted of 663 and 476 successfully genotyped POAG cases and controls, respectively.

Participants from BioMe were genotyped on either Illumina HumanOmniExpressExome-8 v1.0 Beadchip array or the Illumina Multi-Ethnic Genotyping Array (MEGA). As in GIGA, QC was performed following a similar protocol. Exclusion of variants was based on a call rate <95%, extreme deviation from Hardy-Weinberg equilibrium ($P < 1 \times 10^{-5}$), or MAF <0.01. Individual level QC excluded samples with a call rate <95%, gender mismatches, ethnic outliers, excess or reduced heterozygosity and first degree relatives or duplicates.

Imputation

Imputation of unknown genetic variation was performed by means of the "cosmopolitan" approach of using all available populations in a reference panel. The 1000 Genomes Project phase III version 5 was used as an imputation reference panel for GIGA[26]. The pipeline implemented at the Michigan Imputation Server (https://imputationserver.sph.umich.edu) was used for prephasing and imputation (Minimac) of GIGA genotypes[27]. Imputations of BioMe genotypes were carried out with the program IMPUTE by using the 1000 Genomes project phase I version 3 as a reference[28,29].

Population structure

In GIGA, the population structure was examined by principal-components analysis (PCA) in PLINK v1.9[30]; PCA plots for each array and population are displayed in Supplementary Fig. 8. Scree plots were examined to determine the number of principal-components (PC) for adjustment of potential population stratification (shown in Supplementary Fig. 9). The first five PCs were used as covariates for South African samples, the first 4 for Tanzanian samples. In BioMe, population structure (Supplementary Fig. 8) was controlled for by means of genetic matching using the first two PCs. Additional matching was performed based on age and sex.

Replication

The Eyes of Africa Genetic Consortium, the South London POAG case-control cohort and The African Descent and Glaucoma Evaluation Study (ADAGES III) served as replication panels. The Eyes of Africa Genetic consortium is a Pan-African study of genetic determinants

of POAG, and comprises studies recruited from Ghana, Nigeria, South Africa and the USA, totaling a sample size of 2320 POAG cases and 2121 controls. The methods of ascertaining POAG cases has been described in detail elsewhere [9]. In brief, POAG cases met the following inclusion criteria: glaucomatous optic neuropathy (VCDR > 0.7 or notch in the neuroretinal rim), and visual field loss (examined by frequency doubling technology or standard automated perimetry) consistent with optic nerve damage in at least one eye. Controls were participants with no known first-degree relative with glaucoma, IOP less than 21 mm Hg in both eyes without treatment, and no evidence of glaucomatous optic neuropathy in either eye. Genotyping of cases and controls was performed on the Illumina OmniExpressExome array. Genotype QC is described in Appendix 2.

The South London POAG case-control cohort consists of 378 POAG patients and 217 controls of West African ancestry residing in the United Kingdom. Patients were recruited from glaucoma clinics in South London and included if they had visual field loss in at least one eye attributed to glaucoma by a glaucoma specialist, had a VCDR of more than 0.6, were receiving intraocular-lowering medication (or had previous surgery), and had open drainage angles on gonioscopy. Controls were recruited from other eye clinics and were included if the examining ophthalmologist deemed there was no sign of POAG, had healthy optic discs (VCDR <0.6), and normal intraocular pressure without any IOP-lowering therapy (<20 mm Hg). The majority of controls did not have formal visual field testing. Genotyping was performed in a single batch by using Illumina's OmniExpress array. Genotype QC has been described in the Appendix 2.

The African Descent and Glaucoma Evaluation Study (ADAGESIII) is a large collection of African American POAG patients and healthy controls recruited at five eye centers in the US (La Jolla, California; New York, New York; Birmingham, Alabama; Houston, Texas; Atlanta, Georgia). The methods of recruitment and selection of POAG cases have been described previously[31]. In brief, eligibility for inclusion as a POAG case required glaucomatous visual field damage defined as a pattern standard deviation or glaucoma hemifield test results outside normal limits. If good-quality visual fields were not available glaucomatous optic disc damage defined as evidence of excavation, neuroretinal rim thinning or notching, localized or diffuse retinal nerve fiber layer defect, or an intereye asymmetry of the vertical cup-to-disc ratio of more than 0.2 was required. Controls were ascertained at Wake Forest School of Medicine. Details on genotyping and QC are summarized in the Appendix 2.
Statistical analysis.

We conducted a three stage GWAS. Stage 1 was aimed at discovery and consisted of a meta-analysis on summary data from GIGA and BioMe. Stage 2 included replication of independent and lead SNPs identified at stage 1 reaching a significance level $P < 1x10^{-6}$. Stage 3 combined all results in an overall meta-analysis.

Genome-wide association testing in the GIGA study assumed an additive genetic model adjusting for sex and age and included the aforementioned PCs of the principal-components analysis. Association analyses were carried out in EPACTS (http://www.sph.umich.edu/csg/kang/epacts/index.html) by means of the Firth bias-corrected likelihood-ratio test[32]. In BioMe SNPTEST was applied (https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html) by using the frequentist association tests implemented in the program, based on an additive model[33]. Cases and controls were matched by age, sex and the first two principal components in a 1:2 case/control ratio. In order to control for genotype uncertainty, we used the missing data likelihood score test (the score method).

Stage 1

A centralized filtering was performed on GIGA and BioMe GWAS results prior to the meta-analysis. Summary result files were assessed and filtered for monomorphic SNPs and SNPs with a minor allele frequency < 0.01. SNP's that failed or had low-quality imputation, i.e. Minimac R^2/SNPTEST INFO < 0.5, were also excluded. The cleaned summary statistics of both studies were then meta-analyzed by means of an inverse variance fixed effects meta-analysis implemented in METAL[34]. Summary statistics were corrected by using the 'genomic-control' option in METAL to eliminate any residual bias. Only variants present in GIGA South Africa, GIGA Tanzania, as well as BioMe were taken for further analysis.

We searched for evidence of replication of the 15 known POAG variants found in European and Asian GWA studies by employing two replication strategies. First, we used the "exact" approach that involves only the lead significant markers. *P*-values at each known POAG SNP in our study were examined and a $P < 0.05$ was considered as evidence for statistically significant replication. Next, we analyzed the transferability of SNPs by applying the "local" approach. All SNPs in strong LD (r^2>0.8) with the known POAG SNP in the 1000 Genomes European population were analyzed. For evidence of local transferability, *P*-values were adjusted for the number of effective SNPs within a locus as determined by the Genetic Type I Error Calculator[35].

To identify potential additional independent signals nearby the lead SNP in the meta-analysis of GIGA and BioMe, we conducted a conditional analysis implemented in Genome-wide complex trait analysis (GCTA) software, using the --cojo method, which performs conditional and joint analyses with model selection[36]. The genome-wide meta-analysis summary statistics from the discovery stage were used as the input data. Within the GCTA analysis, MAF was restricted to ≥1% and $P<1\times10^{-6}$. For this analysis, we used the GIGA Tanzania 1000 Genome phase 3 imputed data to calculate LD between pairwise SNPs. SNPs further than 10 Mb apart were assumed to be in LD.

We next applied haplotype association analysis to identify POAG associated haplotypes that harbor the variants identified in the discovery stage with $P < 1\times10^{-6}$. The haplotype association analysis comprised two steps. First, pairwise measures of LD were calculated in Haploview to identify LD blocks (LD)[37]. Second, significant haplotypes were identified using a Chi-squared test implemented in Haploview[38].

Stage 2

SNPs put forward for replication were first assessed in each replication sample. *P*-value thresholds for significance were adjusted for the number of SNPs tested by the Bonferroni method. Results of all 5 replication studies were subsequently combined in an inverse variance meta-analysis.

Stage 3

Finally, results from stage 1 and 2 were combined in a trans ethnic-meta-analysis. SNPs showing evidence of effect heterogeneity between studies (*P*het < 0.05) were analyzed by using the Han Eskin random-effects model[39]. This analysis implemented in METASOFT software increases the power to detect associations under heterogeneity.

Power analysis

Power analysis was performed using the "Power for Genetic Association analyses" (PGA) package[40]. For replication of known POAG SNPs power analysis showed that for α= 0.05/15 tests, (Supplementary Fig. 6 red line) and minor allele frequencies of 0.05, 0.10, 0.25; minimal OR's of 1.5, 1.35 and 1.25 respectively could be detected at statistical significance assuming 80% power. For genome-wide analysis in the discovery stage, we had > 80% power given an alpha of 5×10^{-8} to detect variants with odds ratios of 1.89 and 3.25 for effect allele frequencies of 0.05 and 0.01, respectively (Supplementary Fig. 6 green line). For validation of SNPs in stage 2, we had >80% power at an alpha of 0.05/3 independent SNPs= 0.017 to detect loci with odds ratios of 1.29 and 1.7 for effect allele frequencies of 0.05 and 0.01, respectively (Supplementary Fig. 6 line blue line).

Bioinformatics Analysis

Several bioinformatics tools to assess whether SNPs or their linked genetic variants were associated with a putative function that might affect patient outcomes were consulted. HaploReg v4.1 and the RegulomDB v1.1 that include Genotype-Tissue Expression (GTEx) database from the Encyclopedia of DNA Elements (ENCODE) project were used to identify the regulatory potential on candidate functional variants to examine the particular tracks of interest, such as TF-ChIP signals, DNase peaks, DNase footprints and predicted DNA sequence motifs for transcription factors[41,42]. The GTEx data were used to identify the correlations between SNPs and whole-blood-specific gene expression levels. The Ocular

Tissue Database (IOWA) was checked for expression of associated genes in relevant ocular tissue, in which levels of gene expression are indicated as Affymetrix Probe Logarithmic Intensity Error (PLIER) normalized value (with normalization in PLIER as described in Wagner et al.[18]).

Gene-based tests

We performed a gene-based test in VEGAS2[43] to confirm known POAG genes and to identify additional loci not reaching genome-wide significance in a single marker-based analysis. VEGAS2 examines the association from all SNPs across a gene and corrects for gene size and LD between SNPs. The 1000 Genomes phase 3 African populations were downloaded from the VEGAS website and used as the reference panel for pairwise LD correlations. SNPs were allocated to one or more autosomal genes by using customized gene boundaries of ± 10 kb.

Genetic risk score

To further evaluate to which extent known POAG SNPs confer risk in our study, a genetic risk score (GRS) was calculated in the GIGA dataset. Fifteen well imputed/genotyped (Minimac $R^2 > 0.5$) SNPs that were previously reported in large GWAS were used for constructing the GRS. For each individual, a weighted GRS was computed by multiplying the number of effect alleles with the log (OR) reported in the literature. We assessed the association of the GRS with POAG in a logistic regression model adjusting for sex, age and PCs. An estimation of the attributable genetic risk was calculated using the R package “attribrisk”.

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Chapter 3.2

Association of genetic variants with primary open-angle glaucoma among individuals with African Ancestry

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ABSTRACT

Importance

Primary open-angle glaucoma presents with increased prevalence and a higher degree of clinical severity in populations of African ancestry compared with European or Asian ancestry. Despite this, individuals of African ancestry remain understudied in genomic research for blinding disorders.

Objectives

To perform a genome-wide association study (GWAS) of African ancestry populations and evaluate potential mechanisms of pathogenesis for loci associated with primary open-angle glaucoma.

Design, Settings, and Participants

A 2-stage GWAS with a discovery data set of 2320 individuals with primary open-angle glaucoma and 2121 control individuals without primary open-angle glaucoma. The validation stage included an additional 6937 affected individuals and 14 917 unaffected individuals using multicenter clinic- and population-based participant recruitment approaches. Study participants were recruited from Ghana, Nigeria, South Africa, the United States, Tanzania, Britain, Cameroon, Saudi Arabia, Brazil, the Democratic Republic of the Congo, Morocco, Peru, and Mali from 2003 to 2018. Individuals with primary open-angle glaucoma had open iridocorneal angles and displayed glaucomatous optic neuropathy with visual field defects. Elevated intraocular pressure was not included in the case definition. Control individuals had no elevated intraocular pressure and no signs of glaucoma.

Exposures

Genetic variants associated with primary open-angle glaucoma.

Main Outcomes and Measures

Presence of primary open-angle glaucoma. Genome-wide significance was defined as $P < 5 \times 10^{-8}$ in the discovery stage and in the meta-analysis of combined discovery and validation data.

Results

A total of 2320 individuals with primary open-angle glaucoma (mean [interquartile range] age, 64.6 [56-74] years; 1055 [45.5%] women) and 2121 individuals without primary open-angle glaucoma (mean [interquartile range] age, 63.4 [55-71] years; 1025 [48.3%] women) were included in the discovery GWAS. The GWAS discovery meta-analysis demonstrated association of variants at amyloid-β A4 precursor protein-binding family B member 2 (*APBB2*;

chromosome 4, rs59892895T>C) with primary open-angle glaucoma (odds ratio [OR], 1.32 [95% CI, 1.20-1.46]; $P = 2 \times 10^{-8}$). The association was validated in an analysis of an additional 6937 affected individuals and 14 917 unaffected individuals (OR, 1.15 [95% CI, 1.09-1.21]; $P < .001$). Each copy of the rs59892895*C risk allele was associated with increased risk of primary open-angle glaucoma when all data were included in a meta-analysis (OR, 1.19 [95% CI, 1.14-1.25]; $P = 4 \times 10^{-13}$). The rs59892895*C risk allele was present at appreciable frequency only in African ancestry populations. In contrast, the rs59892895*C risk allele had a frequency of less than 0.1% in individuals of European or Asian ancestry.

Conclusions and Relevance

In this genome-wide association study, variants at the *APBB2* locus demonstrated differential association with primary open-angle glaucoma by ancestry. If validated in additional populations this finding may have implications for risk assessment and therapeutic strategies.

INTRODUCTION

Primary open-angle glaucoma affects millions of people worldwide and is a leading cause of irreversible blindness[1,2]. Genome-wide association studies (GWASs) have identified more than 15 genetic loci associated with increased risk of primary open-angle glaucoma in populations with European or Asian ancestry[3,4], and these results have been supported by analyses of glaucoma-associated quantitative traits, such as intraocular pressure (IOP) and vertical cup-to-disc ratio[5]. In contrast, although GWASs of individuals of African ancestry have been performed[6], no genome-wide significant loci have been identified to date in this disproportionately affected population. Studies have shown that while individuals of European ancestry older than 40 years exhibit a disease prevalence of 1%, the prevalence is markedly higher in individuals with African ancestry older than 40 years (up to 6.8%)[2,7,8]. Primary open-angle glaucoma also has earlier onset and is more severe in individuals of African ancestry[9-11]. A 2018 GWAS examining primary open-angle glaucoma in a multiethnic sample, the Genetic Epidemiology Research on Adult Health and Aging cohort, confirmed that there is a higher prevalence of primary open-angle glaucoma in individuals of African ancestry (16.1%) compared with individuals of East Asian (9.9%) and European (7.4%) ancestry[12].

The objective of this study was to address this disparity in genomic science research by performing a GWAS of primary open-angle glaucoma via a multicenter research partnership to obtain biological insights into disease pathogenesis in individuals with African ancestry.

METHODS

Study Populations

The study populations comprised 2 groups: the GWAS discovery stage followed by a validation stage. The validation stage included 2 separate meta-analyses. All participants were recruited using the same criteria in both stages. Individuals with primary open-angle glaucoma, control individuals without primary open-angle glaucoma, and eye and brain tissue donors (or family members of deceased donors) were enrolled after written informed consent was obtained from each participant, in full adherence to the Declaration of Helsinki. All relevant local and hospital institutional review boards approved the study. Participant ancestry was self-reported.

For the GWAS discovery stage, study participants of African ancestry were recruited from Ghana, Nigeria, South Africa, and Duke University (Durham, NC), where the same phenotype definition was applied to diagnose primary open-angle glaucoma[3,13,14]. Individuals with primary open-angle glaucoma were defined by the presence of glaucomatous optic neuropathy (defined as loss of neuroretinal rim with a vertical cup-to-disc ratio of > 0.7 or an intereye asymmetry > 0.2 and/or notching attributable to glaucoma) with compatible visual field loss, open angles on gonioscopy, and absence of secondary causes of glaucomatous optic neuropathy. Elevation of IOP was not a criterion in inclusion or exclusion of patients. Patients who were unable to give informed consent or those with secondary glaucoma due to trauma, uveitis, neovascularization, exfoliation syndrome, or pigment dispersion were excluded. Control individuals were recruited in a hospital-based or population-based manner. Hospital-based control individuals were older than 40 years and were confirmed to have no sign of glaucoma or other major eye diseases. These participants had an IOP of less than 21 mm Hg with open angles at the time of recruitment, healthy optic nerves, normal visual fields, and no family history of glaucoma. Population-based control individuals were matched by hospital and ancestry and were healthy individuals older than 40 years. The participating institutions are listed in the eAppendix in the Supplement. The sample collections from the discovery GWAS analysis were ascertained or reexamined between January 1, 2012, and December 31, 2017.

The study design and genotyping method for the 7 sample collections analyzed in the first validation meta-analysis have been previously described[6,12,15-18]. The study names of these collections are listed in the eAppendix in the Supplement. The sample collections were ascertained or reexamined between January 1, 2003, and December 31, 2018.

The second validation meta-analysis included individuals with primary open-angle glaucoma and matched control individuals from Mali, Cameroon, Nigeria (Lagos, Kaduna, and Enugu),

Brazil, Saudi Arabia, the Democratic Republic of the Congo, Morocco, and Peru. The participating hospital institutions are listed in the eAppendix in the Supplement. These sample collections were ascertained or reexamined between January 1, 2015, and December 31, 2018.

Genotyping and Quality Control Procedures

For the GWAS discovery stage, genome-wide genotyping was performed using the Illumina OmniExpress beadchip, which directly assessed more than 700 000 single-nucleotide polymorphism (SNP) markers across the human genome. This genotyping array has been successfully used in multiple genome-wide scans[19,20], including scans for primary open-angle glaucoma and other forms of glaucoma[13,21]. Further details on GWAS genotyping and quality control procedures are included in the eAppendix in the Supplement.

The first validation meta-analysis for amyloid-β A4 precursor protein-binding family B member 2 (*APBB2*; RefseqNM_004307) rs59892895 was performed with genome-wide genotyping data from previously described matched primary open-angle glaucoma case-control data sets. The second validation meta-analysis for *APBB2* rs59892895 was performed with the Sequenom MassArray primer extension system, with the genotypes further verified with Applied Biosystems Taqman assays.

Fine-scale imputation analysis using the 1000 Genomes Project cosmopolitan reference panel was performed to increase the density of the discovery GWAS. The IMPUTE version 2 software package was used to perform the imputation[22]. Stringent quality control was applied on the imputed data by only including imputed genotypes with an information score of at least 0.95 and by only including SNPs with minor allele frequency of at least 1%. The imputation accuracy of *APBB2* rs59892895T>C was validated with direct genotyping using the MassArray (Sequenom) and Taqman (Applied Biosystems) systems (with >99% concordance).

Immunohistochemistry and Image Analysis of *APBB2* and β-Amyloid in Donor Retina Tissues

APBB2 was shown to increase β-amyloid flux through both the amyloidogenic and nonamyloidogenic pathways of amyloid precursor protein (APP) processing[23]. Donor eyes were selected from a tissue collection obtained from the Iowa Lions Eye Bank within 8 hours after death (eTable 1 in the Supplement). Retinal tissue sections from the donor eyes were assessed for *APBB2* and β-amyloid expression using immunohistochemistry and image analysis. Further details on the experimental methods are included in the eAppendix in the Supplement.

Immunohistochemistry and Image Analysis of *APBB2* and β-Amyloid in Primary Visual Cortex Tissues

All donor samples (eTable 2 in the Supplement) were obtained from the Duke Kathleen Price Bryan Brain Bank and Biorepository and were matched for age and Alzheimer disease severity stage using the Braak classification[24,25]. Sections from the primary visual cortex of the donor samples were assessed for *APBB2* and β-amyloid expression using immunohistochemistry and image analysis. Further details on the experimental methods are included in the eAppendix in the Supplement.

Statistical Analysis

An analysis of the discovery GWAS and follow-up validation stages were prespecified. The evaluation of previously described loci and their consequences on primary open-angle glaucoma risk in the cohort of participants with African ancestry as well as studies exploring potential pathogenic mechanisms related to *APBB2* were undertaken in an exploratory, post hoc manner.

For the discovery GWAS analysis, the association between individual SNP genotypes and primary open-angle glaucoma risk was modeled additively for each copy of the minor allele using logistic regression adjusted for the top 3 principal components of population stratification using PLINK, version 1.9 (details on principal component analysis are included in the eAppendix in the Supplement)[26]. For imputed genotypes, the information content for allele dosage (range, 0-1; 1 indicates perfect information) were included into the association test model to account for and average across imputation uncertainty. The assumptions of this logistic regression model were that the effective sample size was sufficiently large to allow for χ^2-distributed test statistics (eg, the Wald statistic for logistic regression) to be valid, for the observations to be independent of one another, and for only a low degree of collinearity to exist between the independent variables. The model assumptions were all met.

Genomic inflation (λgc) in the GWAS discovery stage was estimated using the median regression test statistic. The genomic inflation factor is presented for each of the 4 GWAS discovery sites as well as for the GWAS discovery meta-analysis in eFig. 1 in the Supplement. For the GWAS discovery analysis, $P < 5 \times 10^{-8}$ (2-sided test) was considered statistically significant. In the validation stages, the association between *APBB2* rs59892895 and primary open-angle glaucoma risk was measured using logistic regression for an additive model. Because only 1 SNP (*APBB2* rs59892895) was tested in the first and second validation stages, $P < 0.05$ (2-sided test) was considered statistically significant for each validation stage. Meta-analyses were conducted using the inverse-variance fixed-effects method (eAppendix in the Supplement)[27]. Intercohort heterogeneity was assessed for the GWAS discovery and validation stages.

Previous case-control studies of primary open-angle glaucoma in individuals with European and Asian ancestry have robustly implicated at least 26 SNPs mapping to 15 distinct gene loci (*TMCO1*, *FMNL2*, *CADM2*, *AFAP1*, *THSD7A*, *CAV1-CAV2*, *ANGPT1*, *CDKN2B-AS*, *ABCA1*, *LMX1B*, *PLCE1*, *TMTC2*, *SIX6*, *TCF12*, and *GAS7*), accompanied by validation in at least 2 independent studies[3,12,13,28-31]. The association between these 26 SNPs and primary open-angle glaucoma risk were tested using logistic regression in the collections from individuals with African ancestry, which included 5153 affected individuals and 10 014 unaffected individuals with available genotyping data. An inverse-variance, fixed-effects meta-analysis was conducted to summarize the estimates.

A case-only quantitative trait locus analysis was performed between *APBB2* rs59892895T>C and 2 clinical parameters: maximum IOP and vertical cup-to-disc ratio at the time of examination (using the mean ratio if available for both eyes). The association between *APBB2* rs59892895T>C and the clinical parameters was assessed using linear regression with sex and age as covariates. The fluorescence intensity data from the immunohistochemical analysis (eAppendix in the Supplement) of retina and primary visual cortex tissues were analyzed with regards to *APBB2* rs59892895T>C carrier status using a linear mixed model incorporating additional random effect terms for individual (for both retina and visual cortex data) and Braak stage (for visual cortex data only).

RESULTS

A total of 26 295 individuals were included in the study. The number of individuals with primary open-angle glaucoma and control individuals analyzed in the GWAS and validation stages are presented in the Table (quality control results and handling of population stratification can be found in the eAppendix and eFigs 1, 2, and 3 in the Supplement). The discovery meta-analysis identified a genome-wide significant association at 1 locus on chromosome 4, characterized by multiple SNP markers showing high pairwise linkage disequilibrium (Fig. 1; summary statistics for the GWASs are publicly available per the data sharing statement). The association mapped to the *APBB2* rs59892895T>C locus (Fig. 2), whereby the minor C allele was observed to be associated with increased risk of primary open-angle glaucoma (per-allele odds ratio [OR], 1.32 [95% CI, 1.20-1.46]; $P = 2 \times 10^{-8}$).

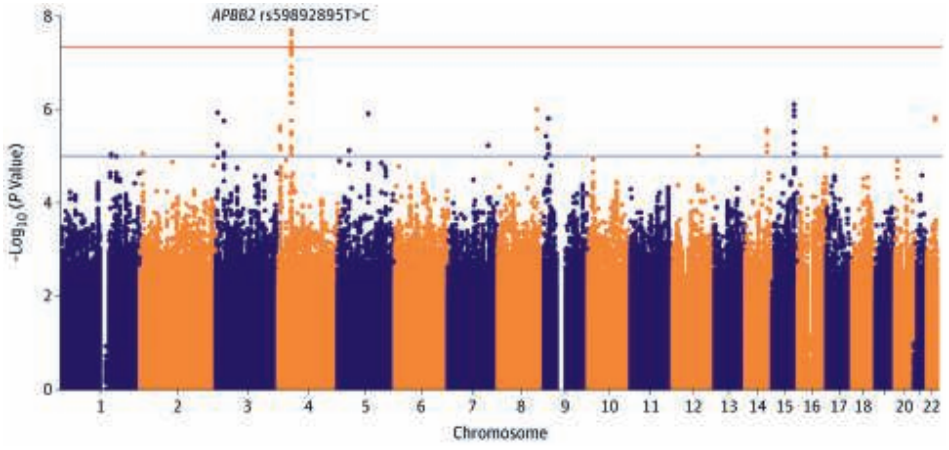
Table. Summary of Case-Control Collections in a Study of the Association of Genetic Variants With Primary Open-Angle Glaucoma Among Individuals With African Ancestry

Sample collection[a]	rs59892895*C Present,%		Sample size, n	
	Individuals with primary open-angle glaucoma	Individuals without primary open-angle glaucoma	Individuals with primary open-angle glaucoma	Individuals without primary open-angle glaucoma
GWAS Discovery				
Ghana	26.9	23.3	833	896
Nigeria	30.4	22.6	554	348
South Africa	31.1	22.9	228	269
United States	26.1	21.7	705	608
Total			2320	2121
First Validation				
Women's Health Initiative	23.1	20.8	1720	6067
Kaiser Permanente GERA	23.3	18.4	300	2700
ADAGES	24.3	21.4	1890	2205
South Africa	13.0	14.4	297	147
Tanzania	30.1	28.3	366	329
United States	23.6	22.6	450	1350
South London	26.6	21.6	378	217
Total			5401	13015
Second Validation				
Cameroon	31.3	29.0	56	57
Nigeria	28.6	26.2	231	61
Nigeria (Kaduna)	29.2	30.2	99	88
Nigeria(African Glaucoma Genetics Project)	32.6	31.0	131	71
Saudi Arabia	5.5	3.1	276	655
Brazil	8.8	4.5	399	460
The Democratic Republic of the Congo	37.7	33.9	124	120
Morocco	6.8	3.5	37	130
Peru	2.0	1.2	51	128
Mali	27.3	23.4	132	132
Total			1536	1902
All validation			6937	14917
Total samples			9257	17038

Abbreviations: ADAGES, African Descent and Glaucoma Evaluation Study; GERA, Genetic Epidemiology Research on Adult Health and Aging; GWAS, genome-wide association study.

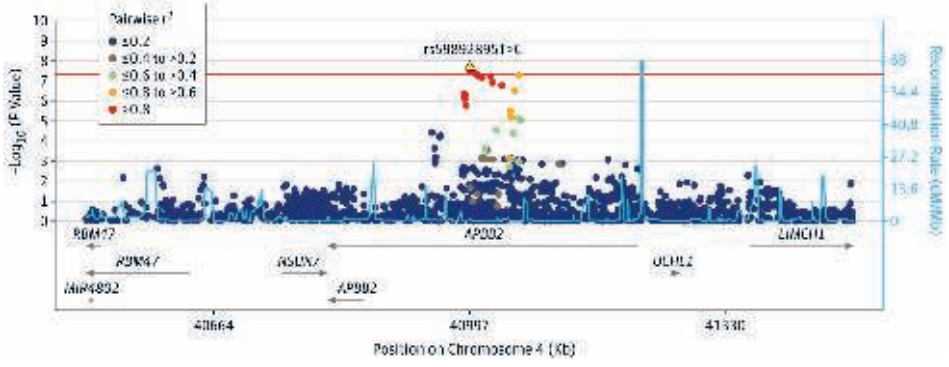
[a] The study site (country) or name of the study that the sample collection was taken from. More information on the sample collections can be found in the eAppendix in the Supplement.

Figure 1. Discovery Analysis of 2320 Individuals With Primary Open-Angle Glaucoma and 2121 Age-Matched Control Individuals



Single-nucleotide polymorphisms (SNPs) were only considered if they were assessed across all 4 genome-wide association discovery study sites. A total of 6 734 161 SNPs are included in this figure. The blue horizontal line indicates a threshold for suggestive statistical significance commonly used in genome-wide association studies ($P = 10^{-5}$) and the red horizontal line indicates the threshold for genome-wide significance ($P = 5 \times 10^{-8}$). Multiple SNPs at the gene encoding for amyloid-β A4 precursor protein-binding family B member 2 (*APBB2*) have a significant association with primary open-angle glaucoma disease risk.

Figure 2. Association at the Amyloid-β A4 Precursor Protein-Binding Family B Member 2 (*APBB2*) Locus on Chromosome 4



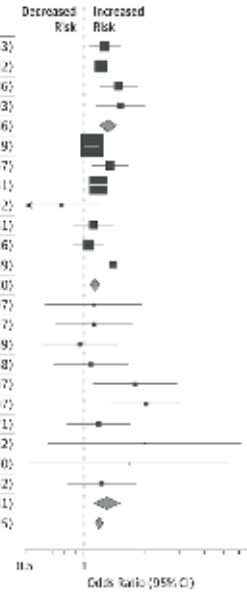
The association between single-nucleotide polymorphisms (SNPs) and primary open-angle glaucoma were plotted by genomic position on chromosome 4 and degree of statistical significance. The horizontal line shows the threshold for genome-wide significance, $P = 5 \times 10^{-8}$. The dots indicate the extent of linkage disequilibrium (LD) between each tested SNP with rs59892895 (based on pairwise r^2 values calculated from the discovery analysis). LD refers to the association between alleles of SNPs located close to one another on the same chromosome; SNPs in strong LD can serve as proxies for one another. Estimated recombination rates were plotted in light blue to reflect the LD structure in individuals with African ancestry. The estimated recombination rate shows the average frequency in which recombination occurs at a particular location. The extent of LD drops with increasing recombination rate. The horizontal lines accompanied by arrows in the lower panel of the plot reflect the genes mapping to the given genomic locations. The arrows indicate the direction of transcription of the genes. The horizontal lines labeled *APBB2* and *RBM47* reflect 2 different gene transcripts of different lengths for both genes.

The *APBB2* rs59892895 association was tested in the first validation meta-analysis comprising 7 independently ascertained sample collections from participants with African ancestry (Table). This first validation data set with prior genome-wide genotyping data available for 5401 individuals with primary open-angle glaucoma and 13,015 individuals without primary open-angle glaucoma had greater than 90% statistical power to validate an SNP with a minor allele frequency as low as 20% and an OR as low as 1.10 at a 2-sided *P* value less than 0.05[32]. The association at *APBB2* rs59892895 was validated, with the risk C allele associated with increased risk of primary open-angle glaucoma (OR, 1.13 [95% CI, 1.07-1.20]; $P < 0.001$; Fig. 3). There was no significant heterogeneity across all sites analyzed (*P*-value for heterogeneity = 0.15).

A second validation meta-analysis for this association was completed with an additional 1536 affected individuals and 1902 unaffected individuals with African ancestry (Table). A significant association between the rs59892895*C allele and increased risk of primary open-

Figure 3. Association Between Amyloid-β A4 Precursor Protein-Binding Family B Member 2 (*APBB2*) rs59892895 and Primary Open-Angle Glaucoma Risk

Collection	No. of Samples Genotyped		APBB2 rs59892895*C Risk Alleles/No. Total Alleles		Odds Ratio (95% CI)	P Value
	Affected Individuals	Unaffected Individuals	Affected Individuals	Unaffected Individuals		
GWAS United States	694	506	362/1388	260/1102	1.27 (1.06-1.53)	
GWAS Ghana	821	884	442/1642	411/1768	1.22 (1.04-1.42)	
GWAS Nigeria	547	345	332/1094	156/690	1.49 (1.20-1.86)	
GWAS South Africa	222	256	138/444	117/512	1.52 (1.14-2.03)	
GWAS discovery summary					1.33 (1.20-1.46)	2.0×10^{-8}
Women's Health Initiative	1720	0067	794/3440	2523/12134	1.09 (1.00-1.19)	
Kaiser Permanente	300	2700	140/600	961/5400	1.35 (1.09-1.67)	
ADAGES	1890	2205	918/3780	943/4410	1.18 (1.06-1.31)	
South Africa	297	146	77/594	42/292	0.77 (0.49-1.22)	
Tanzania	366	329	220/732	186/658	1.11 (0.87-1.41)	
United States	450	1350	212/900	610/2700	1.06 (0.88-1.26)	
South London	376	215	201/756	93/430	1.40 (1.04-1.89)	
First validation meta-analysis					1.13 (1.07-1.20)	<.001
Cameroon	56	57	33/112	33/114	1.12 (0.63-1.97)	
Nigeria	231	61	132/462	32/122	1.12 (0.72-1.77)	
Nigeria (Kaduna)	96	80	56/192	52/172	0.95 (0.61-1.49)	
Nigeria (AGGP)	129	71	84/258	44/142	1.08 (0.70-1.68)	
Saudi Arabia	275	600	80/550	87/1200	1.81 (1.11-2.97)	
Brazil	394	447	207/788	409/894	2.06 (1.58-2.67)	
The Democratic Republic of the Congo	122	118	92/244	80/236	1.18 (0.81-1.71)	
Morocco	37	130	5/74	9/260	2.02 (0.66-6.22)	
Peru	51	128	2/102	3/256	1.69 (0.53-5.40)	
Mali	132	128	72/264	80/256	1.23 (0.82-1.82)	
Second validation meta-analysis					1.30 (1.12-1.51)	<.001
All samples					1.19 (1.14-1.25)	<.001



The oblong data markers represent odds ratios, with the height of the data markers being inversely proportional to the standard error of the odds ratio. The diamonds represent the odds ratios after the meta-analysis, with the width representing the 95% CIs. Collections from the United States were taken from an African American population and the collection from South London was taken from a West African population. ADAGES indicates African Descent and Glaucoma Evaluation Study; AGGP, African Glaucoma Genetics Project; GWAS, genome-wide association study.

angle glaucoma was observed (OR, 1.30 [95% CI, 1.12-1.51];*P* < .001; Fig. 3), with no significant heterogeneity across all 10 sites analyzed (*P*-value for heterogeneity = 0.27). An overall meta-analysis of rs59892895*C involving the 9257 individuals with primary open-angle glaucoma and 17 038 individuals without primary open-angle glaucoma from all cohorts showed consistent association with primary open-angle glaucoma risk (OR, 1.19 [95% CI, 1.14-1.25]; *P* < 0.001) (Fig. 3). The rs59892895*C allele was observed to not be significantly associated with IOP and vertical cup-to-disc ratio (*P* = 0.62) in an analysis of 6179 individuals of African ancestry with available data.

Only individuals of African ancestry or individuals with African ancestry admixture (eg, Brazil, Morocco, Peru, and Saudi Arabia; Table) were polymorphic at *APBB2* rs59892895—the rs59892895*C risk allele was not present in European, South Asian, or East Asian individuals (eFig. 4 in the Supplement). The sources of data accessed to ascertain the frequency of rs59892895 in participants with other ancestries were from the 1000 Genomes Project browser[33] (eFig. 5 in the Supplement), the Genome Aggregation Database (gNOMAD; eTable 3 in the Supplement)[34], as well as previously published GWAS data sets of European[3] (eAppendix in the Supplement) and Asian individuals[14,35]. No SNP within the broad *APBB2* locus was significantly associated with primary open-angle glaucoma in previously studied European or Asian ancestry case-control collections (eFig. 6 in the Supplement). *APBB2* rs59892895T>C was not observed to be in linkage disequilibrium (defined as pairwise $r^2 > 0.8$) with other coding genetic variants or with potentially functional regulatory elements (eFig. 7 in the Supplement).

Post Hoc Evaluation of Previously Described Loci and Their Consequences on Risk in the Cohorts

Analysis of 26 well-validated primary open-angle glaucoma SNPs from studies of individuals of European and Asian ancestry showed that for 23 of the 26 SNPs, the ORs in participants with African ancestry were smaller than in individuals with European ancestry. Heterogeneity tests showed 19 of the SNPs had *P*-values for heterogeneity of less than .05 in support of a smaller OR in participants with African ancestry compared with European individuals (eTable 4 and eFig. 8 in the Supplement). Assessment of the relationship between IOP loci[12,30] and primary open-angle glaucoma risk showed a weak correlation between IOP and primary open-angle glaucoma risk in individuals with African ancestry (eTable 5 and eFig. 9 in theSupplement). Assessment of the relationship between vertical cup-to-disc ratio loci [36] and primary open-angle glaucoma risk showed that all previously reported loci for vertical cup-to-disc ratio were not associated with primary open-angle glaucoma risk in participants with African ancestry (eTable 6 in the Supplement).

Post Hoc Studies Exploring Potential Pathogenic Mechanisms Related to *APBB2*

All donor retina tissues were from individuals of African ancestry. For primary visual cortex tissues, carriers of the *APBB2* rs59892895*C risk allele were of African ancestry, whereas 3 of the 4 individuals with the *APBB2* rs59892895*TT homozygous baseline genotype were of European ancestry to match for the Braak stage of Alzheimer disease diagnosis (eTable 2 in the Supplement). Exploratory analyses using immunohistochemistry on donor retina and primary visual cortex tissues (eTable 1 and eTable 2 in the Supplement) suggested that participants carrying the rs59892895*C risk allele had associated higher *APBB2* expression as well as associated increased staining for β-amyloid compared with participants homozygous for the baseline rs59892895*T nonrisk allele (eFig.s 10, 11, and 12 in the Supplement).

DISCUSSION

This study of 26 295 individuals found a genetic variant in the *APBB2* gene that was associated with a higher risk of primary open-angle glaucoma. The genetic association between primary open-angle glaucoma and *APBB2* was observed only in individuals of African ancestry. The level of genetic diversity in participants of African ancestry was higher than in individuals of European or Asian ancestry, and the functional risk alleles in *APBB2* may not be present in these other populations. The increased risk associated with the *APBB2* allele appeared not to be mediated via increased IOP or optic nerve neuropathy associated with an increasing vertical cup-to-disc ratio, thus suggesting a new insight to primary open-angle glaucoma disease pathogenesis.

Because the odds ratio of the *APBB2* rs59892895*C risk allele on primary open-angle glaucoma appeared to be larger in populations with African ancestry admixture, such as in Saudi Arabia, Brazil, Peru, and Morocco, this raised the possibility that estimates from these 4 populations could have been confounded by population stratification. However, principal component analysis of ancestry-informative markers from Brazil, Peru, and Morocco, which had sufficient DNA to allow assessment of these additional markers, revealed little evidence of population stratification between affected and unaffected individuals (eFig. 13 in the Supplement).

Because *APBB2* was shown to be involved in the amyloidogenic pathway of APP processing, an exploratory analysis of human retinal and primary visual cortex tissues suggested a potential relationship between the *APBB2* rs59892895*C risk allele, increased *APBB2* expression, and associated increased β-amyloid plaque deposition. Primary open-angle

glaucoma neurotoxicity may result from incomplete clearance of amyloid β and other neurotoxins from the interstitial space of the optic nerve[37]. However, there has been no conclusive evidence that these pathways contribute to primary open-angle glaucoma in humans.

The present analysis suggests that the majority of open-angle glaucoma genetic loci described in individuals of European or Asian ancestry have a much more modest effect in individuals of African ancestry. Also, in contrast to studies of European individuals, the present data on individuals of African ancestry showed a much weaker correlation between genetic factors contributing to increased IOP and primary open-angle glaucoma risk. It is possible that these differences in genetic architecture are, at least in part, responsible for the increased prevalence and severity of primary open-angle glaucoma in African ancestry populations.

Limitations

This study has several limitations. First, despite the moderately large sample size of participants of African ancestry, there was only sufficient statistical power to detect associations with common genetic variants. Second, although all study sites used well-established clinical protocols to diagnose primary open-angle glaucoma, the heterogeneity of the primary open-angle glaucoma phenotype may have limited the ability to detect some genetic associations by biasing the effect estimates toward the null. Third, while the association observed between *APBB2* rs59892895 and primary open-angle glaucoma risk was statistically significant, the causal mechanisms of association have yet to be elucidated. Fourth, because the observations from human retinal and visual cortex tissues in this report are based on limited sample sizes, they are interpreted here as exploratory and hypothesis generating, and would require further validation through future research.

CONCLUSIONS

In this GWAS, variants at the *APBB2* locus demonstrated differential association with primary open-angle glaucoma by ancestry. If validated in additional populations this may have implications for risk assessment and therapeutic strategies.

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Chapter 4

Genetic and phenotypic associations with glaucoma endophenotypes

Chapter 4.1

Systemic and ocular determinants of peripapillary retinal nerve fiber layer thickness measurements in the european eye epidemiology (E3) population

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ABSTRACT

Purpose

To investigate systemic and ocular determinants of peripapillary retinal nerve fiber layer thickness (pRNFLT) in the European population.

Design

Cross-sectional meta-analysis.

Participants

A total of 16 084 European adults from 8 cohort studies (mean age range, 56.9 ± 12.3-82.1 ± 4.2 years) of the European Eye Epidemiology (E3) consortium.

Methods

We examined associations with pRNFLT measured by spectral-domain OCT in each study using multivariable linear regression and pooled results using random effects meta-analysis.

Main outcome measures

Determinants of pRNFLT.

Results

Mean pRNFLT ranged from 86.8±21.4 μm in the Rotterdam Study I to 104.7 ± 12.5 μm in the Rotterdam Study III. We found the following factors to be associated with reduced pRNFLT: older age (β = -0.38 μm/year; 95% confidence interval [CI], -0.57 to -0.18), higher intraocular pressure (IOP) (β = -0.36 μm/mm Hg; 95% CI, -0.56 to -0.15), visual impairment (β = -5.50 μm; 95% CI, -9.37 to -1.64), and history of systemic hypertension (β = -0.54 μm; 95% CI, -1.01 to -0.07) and stroke (β = -1.94 μm; 95% CI, -3.17 to -0.72). A suggestive, albeit nonsignificant, association was observed for dementia (β = -3.11 μm; 95% CI, -6.22 to 0.01). Higher pRNFLT was associated with more hyperopic spherical equivalent (β = 1.39 μm/diopter; 95% CI, 1.19-1.59) and smoking (β = 1.53 μm; 95% CI, 1.00-2.06 for current smokers compared with never-smokers).

Conclusions

In addition to previously described determinants such as age and refraction, we found that systemic vascular and neurovascular diseases were associated with reduced pRNFLT. These may be of clinical relevance, especially in glaucoma monitoring of patients with newly occurring vascular comorbidities.

INTRODUCTION

The assessment of peripapillary retinal nerve fiber layer thickness (pRNFLT) with spectral-domain OCT has become of increasing importance in the evaluation of glaucoma and its progression[1,2]. Although debated, pRNFLT measurements hold promise as a biomarker for neurodegenerative diseases such as Alzheimer's disease and multiple sclerosis[3,4].
Although pRNFLT measurements have increased insight into the development of diseases, it has been difficult to evaluate which changes fall within the physiologic range. Most OCT devices compare pRNFLT measurements with reference databases that are built into the machine analysis software. These data are mostly derived from relatively small sample populations. Whether these databases adequately capture normal anatomic variation across a wide age range remains unclear.

Few studies have investigated ocular and systemic determinants of pRNFLT in the general population[5] They reported inconsistent results for many ocular and systemic parameters, including sex and body mass index (BMI)[5,6]. To date, only age[7,8], refraction[9], and axial length (AL)[10] have been consistently associated with measured pRNFLT across studies. In addition, the majority of large-scale studies assessing these associations were performed in young Asian populations[6,11-14]. It is unclear whether these results can be applied to European, that is, mostly white, populations. The purpose of this study was to assess systemic and ocular determinants of pRNFLT using pooled data from 8 European population-based studies.

METHODS

Included Studies

The European Eye Epidemiology (E3) consortium is a collaborative network of population-based studies across Europe with the overarching aim of developing and analyzing large pooled datasets to increase understanding of eye disease and vision loss[15]. For this study, we analyzed data on pRNFLT from 8 different studies. The included data were cross-sectional, and the right eye was chosen to be the study eye. All studies adhered to the tenets of the Declaration of Helsinki and had local ethical committee approval. All participants gave written informed consent.

Assessments and Data Analyses

Retinal nerve fiber layer thickness was measured as global pRNFLT with different OCT devices, scan modalities (mostly circular scans), and automated segmentation algorithms in the respective studies (Table 1). The pRNFLT outliers were excluded before analyses according to Chauvenet's criterion[16]. Briefly, depending on sample size, we excluded

Table 1. Descriptive data for participating studies

Study	Years	City/Country	pRNFLT Measurements		N	Women (%)	Mean Age in Years ± SD	Mean Global pRNFLT in Microns ± SD
			OCT Device	Details				
Alienor Study	2009–2011	Bordeaux, France	Spectralis OCT, Heidelberg Engineering (Heidelberg, Germany)	12°/3.4-mm diameter ring scan on ONH	529	62%	82.1 ± 4.2	89.2 ± 16.0
Coimbra Eye Study	2016–2017	Coimbra, Portugal			618	54%	71.8 ± 6.2	96.8 ± 12.0
Montrachet Study	2009–2013	Dijon, France			803	60%	82.0 ± 3.7	90.3 ± 13.7
LIFE Study	2011–2014	Leipzig, Germany			8351	52%	56.9 ± 12.3	97.4 ± 10.6
Rotterdam Study I	2009–2011	Rotterdam, The Netherlands	3D OCT 1000, Topcon Medical Systems (Oakland, NJ)	6.0 × 6.0 mm on ONH	1287	57%	79.3 ± 4.6	86.8 ± 21.4
Rotterdam Study II	2011–2012		3D OCT 1000 and 2000, Topcon Medical Systems		1376	55%	72.4 ± 4.9	98.2 ± 17.2
Rotterdam Study III	2012–2013				2267	56%	62.2 ± 5.6	104.7 ± 12.5
Twins UK Study	2014–2016	UK (multiple cities)	iVue, Optovue (Fremont, Calif)	3.45-mm diameter ring scan on ONH	853	98%	61.8 ± 12.2	96.4 ± 9.8

ONH . optic nerve head; pRNFLT . peripapillary retinal nerve fiber layer thickness; SD . standard deviation (See Study Descriptions and pRNFLT Distributions, in the Supplementary).

participants with pRNFLT above or below a certain range of standard deviations from the mean[16]. To investigate the determinants of pRNFLT, multivariable linear regression models including the variables of interest were conducted. Factors to be tested for association with pRNFLT were considered in multiple steps. As the first and most important step, variables were chosen a priori on the basis of literature and availability in the individual studies. Subsequently, we performed univariable linear regression models of potential factors at study level to assess the possible impact on pRNFLT. In the last step, the factors of the multivariable models were decided on as a trade-off between the priority of the respective factors and the maximum possible population size of the model.

The independent variables of the multivariable linear regression model were age, sex, BMI, visual impairment as defined by the World Health Organization (best-corrected visual acuity [BCVA] <0.3 decimal), intraocular pressure (IOP), spherical equivalent (SE), smoking status, and history of systemic hypertension, diabetes, stroke, and dementia. The multivariable regression model was conducted for each individual study, and residuals were then plotted and normal distribution assessed. Because OCT devices were changed within the course of the Rotterdam Study (from 3D-OCT 1000 to 3D-OCT 2000, Topcon Medical Systems, Oakland, NJ), we controlled for the OCT device in the multivariable regression models of the Rotterdam Study II and III. In the TwinsUK Study, we performed a hierarchical multivariable regression model to control for family dependencies between twins (See Study Descriptions and pRNFLT Distributions, available online at www.aaojournal.org).

Subsequently, random-effects meta-analysis was used to combine effect estimates (beta coefficients) of each individual predictor from the multivariable regression models among studies. A random-effects approach was chosen a priori on the basis of the heterogeneity in the data caused by the different OCT devices[17] and the set-up of the studies. Our analyses were conducted twice, with and without patients with known glaucoma.

Not all independent variables of the multivariable regression model were available in every participating study. Therefore, the multivariable regression models in the respective studies were performed without the missing variables, and the study was excluded from the meta-analysis of that respective missing covariate. All analyses were performed with the statistical software RStudio (R version 3.4.1, RStudio Inc., Boston, MA, https://www.rstudio.com/), and statistical significance was set at $P < 0.05$.

RESULTS

A total of 16 084 participants from 8 population-based studies were included; approximately 1% of pRNFLT outliers per study were excluded (Table S1). The mean age of participants ranged from 56.9 ± 12.3 years in the LIFE Study to 82.1 ± 4.2 years in the Alienor Study. Mean global pRNFLT ranged from 86.8 ± 21.4 µm in the Rotterdam Study I to 104.7 ± 12.5 µm in the Rotterdam Study III (Table 1). Further participant characteristics for each study are presented in Table S1. The results of the multivariable regression models for each individual study are reported in Table 2. Data on dementia were only available in the Rotterdam Study cohorts and the Alienor Study. Furthermore, in the TwinsUK Study, no sufficient data were available on visual impairment, glaucoma, hypertension, and smoking status; in the LIFE Study, no data were available on visual impairment, SE, and IOP.

In the meta-analyzed multivariable regression model (Table 3 and Fig. 1A, B), age and IOP were negatively associated with pRNFLT, even after excluding patients with glaucoma. A history of stroke and hypertension were both associated with a reduced pRNFLT. When substituting hypertension with mean systolic blood pressure (in mm Hg), no association was found.

A suggestive, but nonsignificant association with reduced pRNFLT was observed for dementia. Visual impairment as defined by the World Health Organization was associated with reduced pRNFLT in the meta-analysis. We found this association in the Alienor and Rotterdam Study I-III, whereas there was no association in the Montrachet and Coimbra Studies.

Women had a thicker pRNFLT than men did in the meta-analysis. However, when correcting for AL rather than SE in the 5 studies with data on AL, this association disappeared. The SE was positively associated with pRNFLT, even after excluding highly myopic eyes (<−6 diopters) and highly hyperopic eyes (>+4 diopters), as well as eyes with pseudophakia (Fig. S1A, B). Longer AL was associated with reduced pRNFLT in our sensitivity analyses (β = −3.48 µm per mm longer AL, 95% confidence interval [CI], −4.18 to −2.77) (Fig. S1C). Both former and current smoking were associated with thicker pRNFLT, but prevalence and associations differed considerably between studies. To assess the influence of education on smoking, we corrected for education, and the associations persisted. After excluding data from the LIFE Study, which is the largest study with the highest proportion of smokers (data weighted >60% in the meta-analysis), the association remained significant for current but not for former smoking (Fig. S1D–G). For BMI, we found a small but significant association with increased pRNFLT after excluding patients with glaucoma. All associations except for former smoking held true after excluding the 619 patients with known glaucoma (Table 3).

Table 2. Associations with peripapillary retinal nerve fiber layer thickness for each individual study

	Alienor (n=529)		Coimbra (n=618)		Montrachet (n=803)		LIFE (n=8351)	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Age (per year)	−0.22 (−0.55 to 0.11)	0.12	−0.09 (−0.24 to 0.07)	0.28	−0.37 (−0.62 to −0.12)	0.004	−0.10 (−0.12 to −0.08)	<0.001
Female sex	3.98 (0.84–7.11)	0.01	2.78 (0.70–4.85)	0.01	3.85 (1.78–5.92)	<0.001	1.36 (0.90–1.82)	<0.001
BMI (per kg/m²)	0.13 (−0.22 to 0.48)	0.46	−0.10 (−0.30 to 0.11)	0.37	0.33 (0.09–0.57)	0.006	0.08 (0.03–0.13)	0.002
SE (per diopter)	1.22 (0.50–1.94)	<0.001	1.15 (0.60–1.69)	<0.001	1.88 (1.44–2.31)	<0.001	N/A	N/A
IOP (per mmHg)	−0.71 (−1.32 to −0.10)	0.02	−0.52 (−0.84 to −0.19)	0.002	−0.31 (−0.60 to −0.03)	0.03	N/A	N/A
Visual impairmen*	−6.42 (−12.47 to −0.37)	0.04	−0.15 (−4.64 to 4.34)	0.95	−0.49 (−4.46 to 3.48)	0.81	N/A	N/A
Former smoker	−0.47 (−3.74 to 2.81)	0.78	−0.57 (−3.67 to 2.53)	0.72	2.39 (0.25–4.53)	0.03	0.60 (0.06–1.14)	0.03
Current smoker	1.48 (−4.99 to 7.96)	0.65	1.51 (−5.74 to 8.77)	0.68	1.58 (−4.33 to 7.48)	0.60	1.43 (0.85–2.02)	<0.001
Hypertension	1.33 (−1.40 to 4.05)	0.34	−1.08 (−3.07 to 0.91)	0.29	−0.62 (−2.55 to 1.32)	0.53	−0.46 (−0.98 to 0.07)	0.09
Diabetes	2.11 (−2.30 to 6.52)	0.35	1.34 (−0.88 to 3.55)	0.24	−2.08 (−5.29 to 1.13)	0.21	−1.25 (−2.03 to −0.46)	0.002
Stroke	−3.53 (−14.05 to 6.99)	0.51	−2.85 (−7.60 to 1.91)	0.24	0.02 (−5.20 to 5.25)	0.99	−2.96 (4.56 to −1.36)	<0.001
Dementia	−5.11 (−12.06 to 1.84)	0.15	N/A	N/A	N/A	N/A	N/A	N/A

	Rotterdam I (n=1287)		Rotterdam II (n=1376)		Rotterdam III (n=2267)		Twins UK (n=853)	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Age (per year)	−0.61 (−0.87 to −0.36)	<0.001	−0.91 (−1.08 to −0.73)	<0.001	−0.50 (−0.59 to −0.41)	<0.001	−0.24 (−0.29 to −0.18)	<0.001
Female sex	3.70 (1.23–6.16)	0.003	1.36 (−0.31 to 3.03)	0.11	0.49 (−0.50 to 1.48)	0.33	3.58 (−1.01 to 8.16)	0.13
BMI (per kg/m²)	0.11 (−0.18 to 0.40)	0.46	0.00 (−0.002 to 0.002)	1.00	0.12 (0.00–0.24)	0.05	−0.07 (−0.19 to 0.05)	0.27
SE (per diopter)	1.09 (0.58–1.60)	<0.001	1.67 (1.32–2.02)	<0.001	1.29 (1.10–1.49)	<0.001	1.28 (1.02–1.55)	<0.001
IOP (per mmHg)	−0.49 (−0.84 to −0.14)	0.005	−0.65 (−0.91 to −0.39)	<0.001	−0.05 (−0.23 to 0.12)	0.56	−0.05 (−0.26 to 0.16)	0.62
Visual impairment[1]	−11.27 (−17.93 to −4.61)	<0.001	−7.68 (−13.80 to −1.55)	0.01	−9.56 (−14.87 to −4.24)	<0.001	N/A	N/A
Former smoker	2.60 (−0.05 to 5.25)	0.06	0.87 (−0.95 to 2.68)	0.36	−0.13 (−1.20 to 0.95)	0.82	N/A	N/A
Current smoker	2.96 (−1.48 to 7.40)	0.19	3.86 (0.94–6.78)	0.009	1.34 (−0.18 to 2.87)	0.08	N/A	N/A
Hypertension	2.08 (−1.71 to 5.86)	0.28	0.46 (−1.86 to 2.77)	0.70	−1.37 (−2.43 to −0.31)	0.01	N/A	N/A
Diabetes	−0.54 (−3.45 to 2.36)	0.71	−1.72 (−3.92 to 0.48)	0.12	0.40 (−1.30 to 2.10)	0.65	−4.29 (−8.34 to −0.24)	0.04
Stroke	1.25 (−2.83 to 5.32)	0.55	−2.06 (−5.67 to 1.55)	0.26	−1.22 (−4.19 to 1.76)	0.42	−1.51 (−5.49 to 2.48)	0.46
Dementia	−4.17 (−8.82 to 0.48)	0.08	−0.55 (−5.96 to 4.86)	0.84	−1.27 (−24.41 to 21.86)	0.91	N/A	N/A

BMI = body mass index; IOP = intraocular pressure; N/A = not available; SE = spherical equivalent. Results from the multivariable regression models.
* Visual impairment as defined by the World Health Organization (<0.3 decimal BCVA).

Table 3. Meta-analyzed associations with peripapillary retinal nerve fiber layer thickness

	All Participants			Excluding Known Glaucoma		
	β (95% CI)	*P*	I^2	β (95% CI)	*P*	I^2
Age (per year)	−0.38 (−0.57 to −0.18)	<0.001	97%	−0.35 (−0.60 to −0.10)	0.006	97%
Female sex	2.17 (1.15–3.20)	<0.001	69%	1.79 (0.93–2.65)	<0.001	59%
BMI (per kg/m^2)	0.06 (−0.02 to 0.14)	0.15	54%	0.09 (0.00–0.18)	0.05	53%
SE (per diopter)	1.39 (1.19–1.59)	<0.001	49%	1.36 (1.16–1.57)	<0.001	40%
IOP (per mmHg)	−0.36 (−0.56 to −0.15)	<0.001	74%	−0.42 (−0.65 to −0.20)	<0.001	71%
Visual impairmen*	−5.50 (−9.37 to −1.64)	0.005	69%	−4.75 (−9.12 to −0.38)	0.03	77%
Former smoker	0.58 (0.14–1.02)	0.009	0%	0.79 (−0.01 to 1.60)	0.05	43%
Current smoker	1.53 (1.00–2.06)	<0.001	0%	1.49 (0.97–2.02)	<0.001	0%
Hypertension	−0.54 (−1.01 to −0.07)	0.03	4%	−0.62 (−1.11 to −0.13)	0.01	7%
Diabetes	−0.69 (−1.69 to 0.31)	0.18	40%	−0.33 (−1.32 to 0.67)	0.52	40%
Stroke	−1.94 (−3.17 to −0.72)	0.002	7%	−2.06 (−3.27 to −0.84)	<0.001	2%
Dementia	−3.11 (−6.22 to 0.01)	0.05	0%	−2.66 (−5.86 to 0.55)	0.10	0%

BMI = body mass index; CI = confidence interval; IOP = intraocular pressure; SE = spherical equivalent.
All participants: results of the meta-analysis of the multivariable regression models including all participants (n=16 084).
Excluding known glaucoma: results of the meta-analysis of the multivariable regression models excluding the 619 patients with known glaucoma (n=14 695, without TwinsUK Study data, because no data on glaucoma were available); I^2 = heterogeneity of covariate in the meta-analysis.
*Visual impairment as defined by the World Health Organization (<0.3 decimal BCVA).

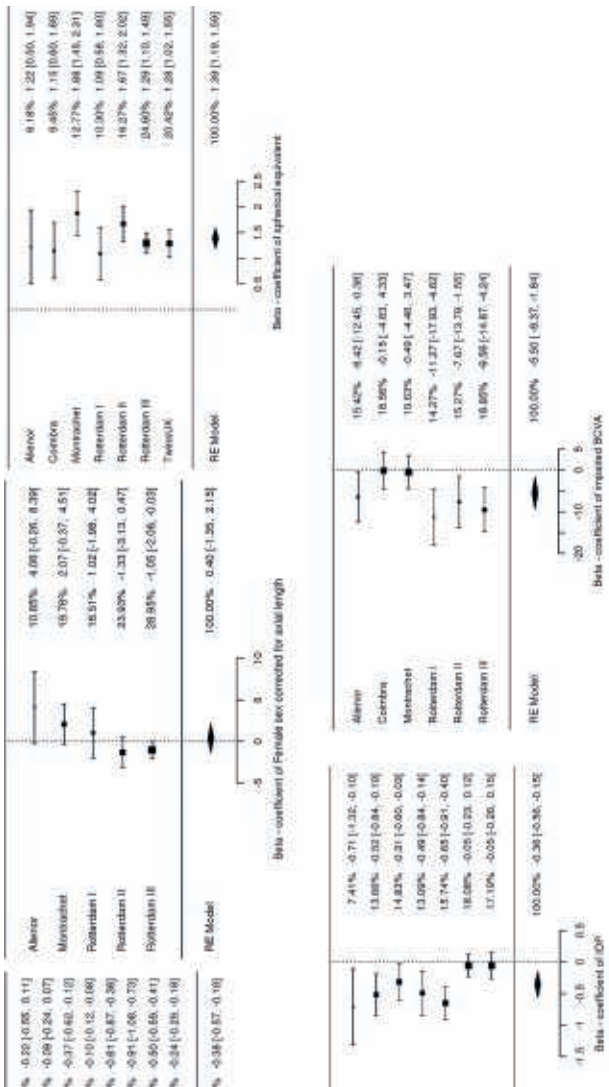
Furthermore, we detected no relevant changes of associations when performing the multivariable regression analyses stratified by sex or when excluding the LIFE study cohort being the largest single study (results not reported).

DISCUSSION

Our study confirms the previously reported associations of age and SE with pRNFLT and identifies several additional factors associated with pRNFLT, namely, IOP (even in individuals without a history of glaucoma), stroke, hypertension, and smoking. Furthermore, we found a trend of reduced pRNFLT in participants with dementia. Our results suggest that a number of ocular and systemic factors need to be considered when assessing pRNFLT. To date, none of this has been implemented as potentially influencing factors in reference databases for OCT devices or any algorithms assessing pRNFLT change.

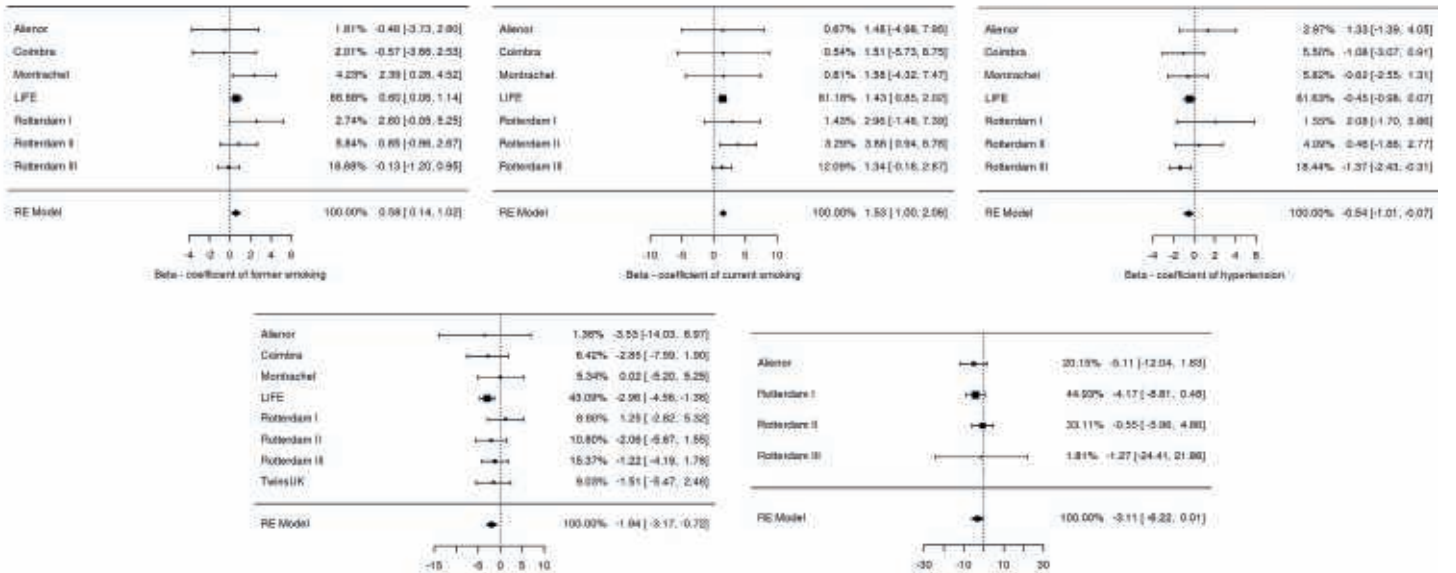
First publications on determinants of OCT-based pRNFLT measurements reported older age and greater AL to be associated with thinner pRNFLT[18,19].Budenz et al.[19] investigated determinants of pRNFLT in 328 normal subjects aged 18 to 85 years using time-domain OCT

Figure 1A.



A. Forest plots of meta-analyzed associations with peripapillary retinal nerve fiber layer thickness (pRNFLT) from multivariable regression models (age, sex, spherical equivalent [SE], intraocular pressure [IOP], and visual impairment).

Figure 1B.



B. Forest plots of meta-analyzed associations with pRNFLT from multivariable regression models (smoking, hypertension, stroke, and dementia). The beta-coefficients (95% CI) show the influence of each parameter on pRNFLT within the respective study, and the percentage represents the mathematically determined weighting of each study within the meta-analysis.

and described a decrease of 2.0 µm pRNFLT per decade and a decrease of 2.2 µm per mm AL[19]. These estimates are smaller but still are comparable to our results (decrease of 3.8 µm pRNFLT on average per decade and 3.48 µm per mm AL). A subsequent study evaluated determinants of pRNFLT in 542 healthy adults aged 40 to 80 years using spectral-domain OCT (Cirrus HD-OCT; Carl Zeiss Meditec, Inc, Dublin, CA) and confirmed the associations of pRNFLT with age and AL[11].

Subsequently, larger population studies mostly from Asia were conducted to investigate further determinants of pRNFLT. We have affirmed results from the Beijing Eye Study in 2548 participants considering the influence of age and refractive error. That study also showed a higher pRNFLT of 2.9 µm in women[14], in keeping with our results of women having a higher pRNFLT of 2.2 µm. Similar to our models, the Beijing Eye Study corrected for refractive error instead of actual AL. Of note, after correcting for AL in our analyses, sex was no longer associated with pRNFLT; thus, we hypothesize that AL, which is on average shorter in women, confounds the effect of sex on pRNFLT. In general, SE is a good proxy for AL, and we found a strong association of higher SE with thicker pRNFLT, even in our sensitivity analysis, which eliminated subjects with high refractive errors. The underlying mechanisms of the association of longer AL and thinner pRNFLT are arguable[20]. Frequently suggested mechanisms are a stretching due to a longer eye bulb or artificially decreased measurements due to magnification[21,22]. However, irrespective of the causal mechanism, the clinical relevance of adjusting for refraction or AL in OCT imaging seems obvious.

Higher IOP was associated with reduced pRNFLT in our analyses even after excluding patients with known glaucoma. However, because glaucoma was self-reported in some of the participating studies, not all patients with actual glaucoma might have been excluded in our analyses. Visual impairment (BCVA <0.3 decimal) as a proxy for any ocular pathology was associated with thinner pRNFLT in the Alienor Study and all of the Rotterdam Studies. The Coimbra and Montrachet Studies were likely underpowered to find an effect, because of few cases with reduced BCVA in these studies.

Previous studies reported contradictory results on the impact of hypertension and blood pressure on pRNFLT[9,23,24]. Our results show reduced pRNFLT in hypertensive patients, but no association of pRNFLT with actual systolic blood pressure. However, blood pressure measurements are known to vary with method, and associations with systolic blood pressure may have been masked by any use of antihypertensive medication. In contrast to hypertension, most studies investigating the effect of diabetes on pRNFLT report diabetic patients to have thinner pRNFLT[25,26]. This is not in agreement with our results that do not show an association of reduced pRNFLT in diabetic patients. Nevertheless, we hypothesize that microvascular pathology and ischemia due to hypertension or diabetes may be a cause for reduced pRNFLT, as has been suggested previously[25].

Both former and current smoking were associated with thicker pRNFLT in our meta-analysis, even in several sensitivity analyses including correction for educational level. This association does not seem biologically plausible given the observed pRNFLT decrease in metabolic diseases. Potential biologic explanations could be reduced axonal flow or axonal swelling in the course of axonal degeneration because of intake of neurotoxins and cytotoxins from cigarette smoke. However, our results are in contrast with findings of earlier studies[27,28], which reported reduced pRNFLT in smokers. Suggested mechanisms leading to decreased pRNFLT were toxic damage through free radicals, increased IOP, and reduced perfusion[27-29]. We controlled for IOP, hypertension, and diabetes, which all may influence perfusion. Therefore, it is unclear what might explain this association. Current smokers were on average younger in our participating studies compared with never- and former smokers. Although we controlled for age in our models, we cannot entirely rule out residual confounding. Also, the European Eye Epidemiology (E3) studies are not representative studies of European populations, and smoking percentages do not reflect actual percentages. There was heterogeneity between studies considering smoking prevalence and oppositional effects of former smoking in some studies. After excluding the LIFE Study, which was dominantly weighted in the smoking meta-analysis, the Rotterdam Study III showed to be weighted strongest for current smoking. When also excluding the Rotterdam Study III, the impact of smoking is weakened but holds true. Still, the associations seem to be particularly driven by the large studies. This is also underlined by increasing heterogeneity for former and current smoking in the meta-analysis after excluding the LIFE Study. Moreover, there is no information on the time interval between cessation of smoking and OCT imaging for the former smokers, which also may have an impact. Further studies are needed to confirm or refute our observation, which may be a chance finding.

Past studies have reported patients with stroke to have thinner pRNFLT, which was hypothesized to be caused by transneuronal retrograde degeneration[30,31]. Our data confirm the association of stroke and decreased pRNFLT. In patients with dementia, we found a trend of reduced pRNFLT. Again, this is in accordance with various previous studies, which report patients with dementia to have reduced pRNFLT[4,32]. Thus far, the underlying mechanisms remain unclear. Loss of peripapillary RNFL is a hallmark of glaucoma, and longitudinal pRNFLT evaluation is a crucial part of glaucoma management. In our meta-analysis, all associations persisted after excluding patients with known glaucoma except for former smoking. This indicates that the detected determinants are independent of the presence of glaucoma.

As described previously, structural decline of pRNFLT occurs before functional loss in perimetry in patients with glaucoma. An earlier study reported the difference in pRNFLT between glaucomatous and healthy eyes 8 years before the onset of visual field impairment

to be approximately 5 μm[33]. This is in the range of some associations found in our study and underlines the potential impact on the interpretation of pRNFLT. Our results have 2 main clinical implications. First, the normative databases built into the devices should reflect our results when presenting normal values for pRNFLT. Also, the presence of vascular disease including a history of stroke should be considered when defining normative datasets or when clinically evaluating pRNFLT. As discussed earlier, the magnitude of impact of the respective determinants may have clinical relevance, especially in the presence of more than 1 factor reducing pRNFLT. Second, in those with glaucoma or other patients followed up with pRNFLT measurements, an incident stroke or dementia may cause a decrease in pRNFLT, which would not primarily be due to glaucoma or other ocular disease progression. For example, this may simulate an aggravation of glaucoma and needs to be considered by the clinician when tailoring the glaucoma management.

The strength of this study consists of the large pooled sample combining data of 8 studies from 5 European countries. To our knowledge, this study represents one of the largest European studies on determinants of pRNFLT thus far. As mentioned, previous population studies reporting data on associations with pRNFLT were conducted in mostly Asian populations, and the results cannot directly be transferred to European individuals. The associations of this study were assessed in meta-analyses of all participating populations; thus, they are not limited to 1 single population only. This reduces the possibility that an association was solely due to chance within 1 population and increases generalizability. However, several limitations of our study need to be considered. The use of different OCT devices between studies may have increased variability and prohibited direct pooling of pRNFLT data. To overcome this lack of direct comparability, we performed the analysis separately within studies and then pooled studies' effect estimates using random-effect meta-analysis. Furthermore, we found no interactions between type of device and any predictor variable in additional sensitivity analyses in the Rotterdam Study II and III, which had a device upgrade within the course of the study. However, residual influence of different OCT devices cannot be entirely excluded. As expected when combining different large-scale population studies, we observed between-study heterogeneity for the independent variables and their influence on pRNFLT. The degree of heterogeneity of the respective covariates was assessed using the I^2 and statistics, and ranged from 0% to 97% (Table 3). As described, this heterogeneity between studies was addressed by using random effect meta-analysis[17]. In accordance with previous literature, the relationship between pRNFLT and age was linear in our sample. Having no data for children and young adults, we do not know whether the relationship between pRNFLT and age is strictly linear throughout life but would assume so on the basis of our data. Thus, we investigated associations using multivariable linear regression modeling, and any nonlinear relationships may have been underrepresented. Quality control was performed within each study differently (Table S2). Some studies

performed manual (re)-segmentation and excluded OCT images below a certain scan quality and scans with artifacts, whereas others included all scans with sufficient quality as evaluated by the performing technician. In the sensitivity analysis, we excluded participants with an image quality value <45 (as recommended by the manufacturer) in the Rotterdam Studies I–III. We found no relevant changes of direction in any association, but the CIs became broader because of a reduced sample size (Table S3). Thus, although the lack of centralized quality control is a limitation to our analyses, the impact of poor-quality scans seems to be low as indicated by our supplemental sensitivity analyses. Within each study, the number of participants in whom OCT imaging could not be performed or in whom the images were of low quality and thus unusable is only a small proportion (Table S2). For example, in the Rotterdam Study I–III the number of participants with no or insufficient OCT data was 10%, 6%, and 15%, respectively. These subjects were older and more likely to have stroke (Rotterdam Study I), dementia (Rotterdam Study II and III), and hypertension (Rotterdam Study III) than the included participants. This indicates that our associations may be underestimations of the true effect. Several independent variables were not available in some studies. Therefore, not all multivariable models could be corrected for all variables. However, no relevant differences of associations were detectable when comparing studies with and without any missing data. Thus, the absence of certain variables in some studies did not relevantly alter the associations of the available data. Methods of assessments varied between our studies. This concerns the BCVA, which was sometimes measured subjectively and sometimes by autorefractor. In addition, information on diseases was assessed differently. Although glaucoma was defined on the basis of optic disc evaluation and perimetry in the Alienor Study and Rotterdam Study I–III, it was self-reported in the LIFE Study. Furthermore, we did not distinguish between the various types of dementia, which may have a different impact on pRNFLT. These differences contribute again to larger heterogeneity, and the relation between self-reported diseases and pRNFLT may have been estimated with less precision. Last, our data were cross-sectional only, and causal deductions from the detected associations are limited and further longitudinal studies are needed.

In conclusion, the current analyses identified important additional determinants of pRNFLT, which should be considered when assessing pRNFLT both clinically and in epidemiologic research. The magnitude of changes in pRNFLT by determinant is likely clinically relevant, and the biology of pRNFLT thinning is complex, with mechanical pressure, microvascular ischemia, and neuronal degeneration being implied. This is reflected in the complexity of factors that influence pRNFLT and thus needs to be considered. In particular, the associations with systemic vascular and neurovascular diseases merit further research.

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Chapter 4.2

Multi-trait genome-wide association study identifies new loci associated with optic disc parameters

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ABSTRACT

A new avenue of mining published genome-wide association studies includes the joint analysis of related traits. The power of this approach depends on the genetic correlation of traits, which reflects the number of pleiotropic loci, i.e. genetic loci influencing multiple traits. Here, we applied new meta-analyses of optic nerve head (ONH) related traits implicated in primary open-angle glaucoma (POAG); intraocular pressure and central corneal thickness using Haplotype reference consortium imputations. We performed a multi-trait analysis of ONH parameters cup area, disc area and vertical cup-disc ratio. We uncover new variants; rs11158547 in *PPP1R36-PLEKHG3* and rs1028727 near *SERPINE3* at genome-wide significance that replicate in independent Asian cohorts imputed to 1000 Genomes. At this point, validation of these variants in POAG cohorts is hampered by the high degree of heterogeneity. Our results show that multi-trait analysis is a valid approach to identify novel pleiotropic variants for ONH.

INTRODUCTION

Glaucoma is the most common cause of irreversible blindness in the world[1]. Primary open-angle glaucoma (POAG) is the most prevalent type of glaucoma accounting for 74% of all glaucoma cases[2,3]. Intraocular pressure (IOP) and the morphology of the optic nerve head (cup area (CA), disc area (DA) and vertical cup-disc ratio (VCDR)) are important features of the glaucomatous process. For each of these traits, twin studies showed a high heritability (h^2_{CA} = 0.75, h^2_{DA} = 0.72, h^2_{IOP} = 0.55 and h^2_{VCDR} = 0.48)[4]. Central corneal thickness (CCT) is also a highly heritable trait (h^2_{CCT} = 0.68-0.95)[5], which is most likely non-physiologically associated with POAG, but rather biases IOP measurement, the major risk factor of POAG [6,7]. CA, DA and VCDR, are significantly correlated both at the genetic level [8]. The high genetic correlation found between the optic nerve head (ONH) traits (R_g = 0.31-0.83) raises the question whether multi-trait analyses will improve the statistical power of the individual GWAS and will find variants with pleiotropic effects[9].

For this study, we generated new data on these five quantitative traits by imputing 12 European ancestry cohorts from the International Glaucoma Genetic Consortium (IGGC) (n_{MAX} = 31 269) to haplotype reference consortium (HRC) release 1 imputation panel, which includes over 39 million variants[10]. A meta-analysis of these 12 European ancestry studies served as a discovery cohort in the analyses. Replication was performed in five Asian ancestry cohorts that were part of the IGGC. The cohorts of Asian descent were imputed to 1000 Genomes as there is little gain in HRC imputation in this ancestry group because there are no additional Asian samples included in HRC (http://www.haplotype-reference-consortium.org/participating-cohorts)[11]. We evaluated the added value of multi-trait analyses using two programs: CPASSOC and multi-trait analysis of GWAS (MTAG). Both use aggregated GWAS results. Whereas CPASSOC performs a meta-analysis assuming homogeneous and heterogeneous effects across traits by applying a inter-trait correlation matrix, MTAG basically increases the power of a single trait analyses by incorporating the GWAS findings of correlated traits.

By multi-trait analysis we identified two novel loci associated with the ONH at rs11158547 in between *PPP1R36* and *PLEKHG3* and at rs1028727 near *SERPINE3* in those of European descent. These loci replicated in the Asian replication sample. Findings for these loci were consistent using a distinct multi-trait approach, MTAG, in both the European and Asian cohorts. This study emphasizes that multi-trait analysis in GWAS pleiotropic traits is an effective approach to identify variants harboring correlated traits

RESULTS

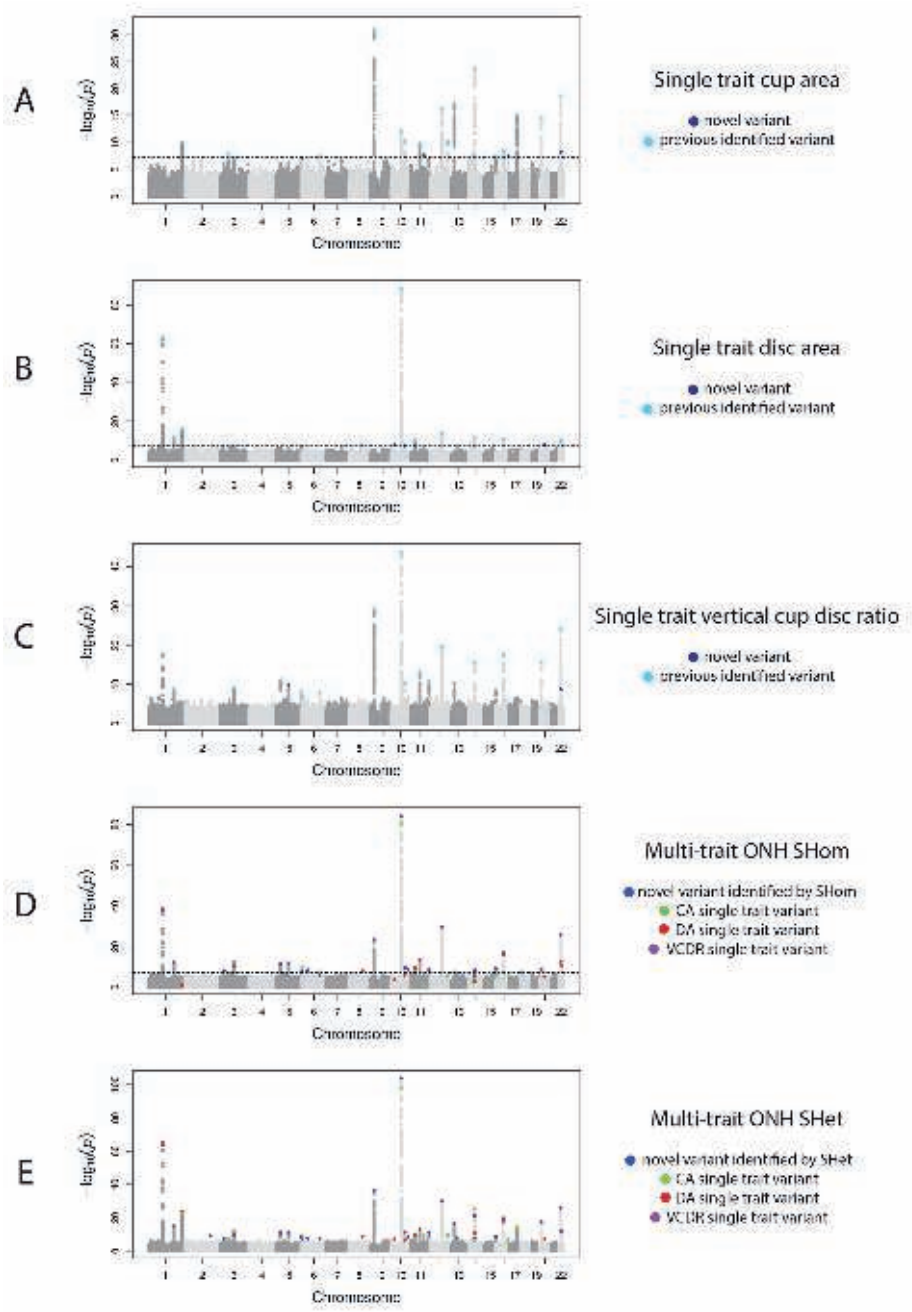
Replication of previous CA, DA, VCDR, IOP, and CCT GWAS results

As a validation we first confirmed previously identified loci for CA, DA, VCDR, IOP, and CCT by Springelkamp et al.[8] (n_{CA} = 22 489; n_{DA}= 22 504; n_{VCDR} = 23 899; n_{IOP} =3 7,930) and Iglesias et al.[12] (n = 17 803) based on 1000 Genomes imputation. Supplementary Fig. 1 and Supplementary Data 1 and 2 show the per trait comparison of our meta-analysis of all European ancestry discovery cohorts using the HRC imputation with the results of the meta-analysis by Springelkamp[8] and Iglesias[12] based on 1000 Genomes. Out of 113 (95%), 107 available variants in HRC replicated at a Bonferroni significance level.

Optic nerve head parameters

In the single trait meta-analyses of ONH traits (CA, DA, and VCDR) in those of European descent (n_{CA} = 24 512, LDSC $intercept_{ca}$ = 1.024 (SE = 0.0083); n_{DA} = 31 269, LDSC $intercept_{DA}$ = 1.041 (SE= 0.0071); n_{VCDR} = 25 180, LDSC $intercept_{VCDR}$ = 1.029 (SE = 0.0076) Supplementary Data 3), 59 loci showed genome-wide significant association with at least one of the traits (Fig. 1 , Supplementary Data 4). The ONH analyses yielded six loci not previously reported (Table 1, Supplementary Data 5), however, none of these novel variants replicated in the Asian replication sample comprising five Asian studies. As the correlation analysis between the ONH traits showed significant correlations at the genetic and phenotype level (Fig. 2), we applied multi-trait analysis to uncover pleiotropic effects. Using multi-trait approach CPASSOC[13], we identified three new loci at $P < 5\times10^{-8}$ by CPASSOC's SHom (*KIF6*, *EPB41L3*,*PPP1R36-PLEKHG3*) (Fig. 1d, Supplementary Data 6). This method assumes that genetic effects are homogenous across traits and cohorts. Two additional new loci were identified by SHet (*ZAK*, *SERPINE3*) (Fig. 1e, Supplementary Data 6), which assumes the genetic effects are heterogenous. Locuszoom plots for these novel variants are depicted in Supplementary Fig. 2. Using an alternative approach (MTAG)[14], the loci emerged consistently as genome-wide significant: rs9471130 near *KIF6* in the DA analysis (P = 2.63 x 10^{-08}), rs11158547 near *PPP1R36-PLEKHG3* in the CA analysis (P = 2.13 x 10^{-08}) and rs1028727 near *SERPINE3* in the DA analysis (P = 4.50 x 10^{-09}) (Supplementary Data 7). rs11158547 (*PPP1R36-PLEKHG3*) and rs1028727 (*SERPINE3*) displayed nominally significant association in the multi-trait analysis (CPASSOC and MTAG) in individuals of Asian ancestry (Supplementary Data 6 and 7). Both variants were not in LD (r^2 < 0.1) with neighboring known variants near *SIX6*, *DDHD1* and *DLCK1*.

Figure 1.



Manhattan plot of single trait analysis for cup area (A), disc area (B), and vertical cup-disc ratio (C). Manhattan plot for multi-trait analysis of the optic nerve head (ONH), SHom (D), and SHet (E)

Table 1. Genome-wide significant SNPs newly identified for cup area, disc area, vertical cup-disc ratio, intraocular pressure or central corneal thickness in the European HRC discovery

Trait	rsID	Chr:pos	Nearest Gene	EA	N_j	Freq	β	SE	*P*-value	I^2	HetP	β_j	SE_j	*P*-value$_j$
DA	rs4748969	10:25015618	*ARHGAP21*	A	25126	0.277	-0.025	0.004	1.69E-08	0	0.568	-0.025	0.004	1.77E-08
DA	rs10882283	10:95360964	*RBP4*	C	23525	0.377	-0.023	0.004	3.68E-08	0	0.864	-0.023	0.004	3.38E-08
CA	rs7101609	11:92623493	*FAT3*	G	26056	0.354	-0.013	0.002	2.06E-08	0	0.772	-0.013	0.002	1.25E-08
CA	rs1622797	16:86379107	*LINC00917*	T	24593	0.089	0.022	0.004	3.41E-08	45	0.069	0.022	0.004	3.87E-08
DA	rs6119893	20:31142813	*C20orf112*	T	25347	0.327	-0.024	0.004	1.06E-08	0	0.542	-0.024	0.004	9.78E-09
CA[a]	rs2412973	22:30529631	*HORMAD2*	A	26675	0.442	0.013	0.002	5.06E-09	45.9	0.063	0.013	0.002	3.89E-09
VCDR[a]	rs2412973	22:30529631	*HORMAD2*	A	27448	0.442	0.008	0.001	1.97E-09	70.2	0.001	0.008	0.001	1.96E-09
VCDR	rs115456027	5:87919700	*LINC00461*	T	25273	0.078	0.016	0.003	1.66E-10	49.2	0.046	0.016	0.003	2.08E-10
CA[b]	rs17135931	6:625188	*EXOC2*	A	26267	0.189	0.015	0.003	6.25E-08	0	0.537	0.015	0.003	3.86E-08
IOP	rs9853115	3:186131600	*RP11-78H24.1*	A	32544	0.496	-0.158	0.027	2.85E-09	0	0.666	-0.158	0.027	2.91E-09
IOP	rs150202082	18:53027723	*TCF4*	T	30915	0.027	-0.47	0.085	2.97E-08	17.9	0.273	-0.47	0.085	3.06E-08
CCT	rs34869	5:115152694	*CDO1*	C	17810	0.437	2.797	0.397	1.97E-12	0	0.93	2.797	0.398	2.1E-12
CCT	rs1772570	13:81193433	*HNRNPA1P31*	C	18158	0.315	-2.368	0.42	1.74E-08	37.4	0.101	-2.368	0.421	1.79E-08
CCT	rs511651	18:24357736	*AQP4-AS1*	C	18457	0.319	2.452	0.416	3.81E-09	0	0.644	2.452	0.416	3.93E-09

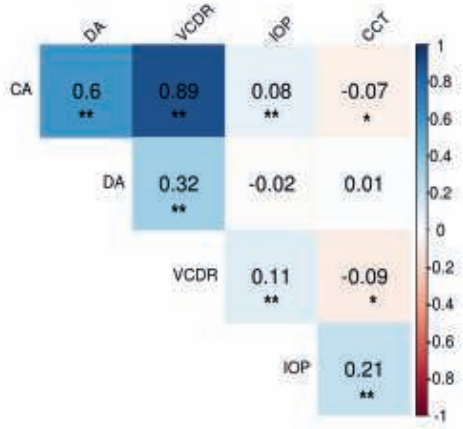
DA disc area, *CA* cup area, *VCDR* vertical cup–disc ratio, *IOP* intraocular pressure, *CCT* central corneal thickness. The position (Chr:pos) of the variant is the position in GRCh37/hg19. The Freq column is the frequency of the effect allele (EA) and the β column is the effect of the effect allele. *N* is the effective sample size and is determined by GCTA. β_j, $SE\beta_j$ and *P*-value$_j$ are the effect size, standard error and *P*-value from a joint analysis of all the selected SNPs, as determined by GCTA. The per cohort statistics can be found in the Supplementary Data 5.

[a]Variant previously identified for DA by Springelkamp et al.[38]

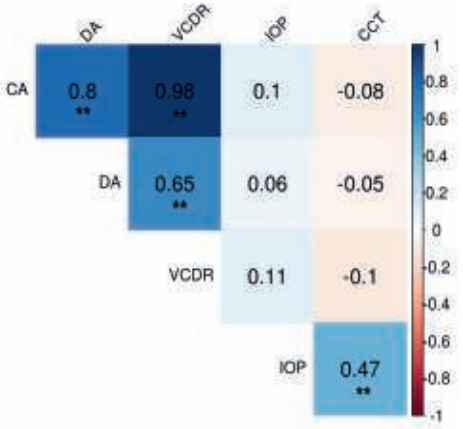
[b]Variant previously identified for VCDR by Springelkamp et al.[39]

Figure 2. Phenotype (A) and genetic (B) correlations between cup area, disc area, vertical cup-disc ratio, intraocular pressure and central corneal thickness

A. Phenotype correlations in Rotterdam Study I



B. Genetic correlation (Rg) in the IGGC HRC meta-analyses



A. Partial pearson correlation coefficient s between cup area (CA), disc area (DA), vertical cup–disc ratio (VCDR), intraocular pressure (IOP), and central corneal thickness (CCT) adjusted for age and sex in the Rotterdam study I. **B**. Genetic correlation coefficient (*R*g) for CA, DA, VCDR, IOP, and CCT calculated by LD score regression; **P* < 0.05, ***P* < 0.0001.

IOP and CCT

Next, we conducted a single trait meta-analysis for IOP and for CCT, the two traits that are not likely physiologically related. For IOP, we meta-analyzed a total of 31 269 participants (LDSC intercept = 1.028; SE = 0.0078, Supplementary Data 4) and identified 9 genome-wide significant regions of which two were novel in the HRC-based imputations and had not been uncovered in the IGGC 1000 Genomes analyses before (Table 1, Supplementary Data 5). The lead single-nucleotide polymorphisms (SNPs) in these genomic regions were a common variant rs9853115 near *DGKG* on 3q27.3 and a rare variant rs150202082[T] (frequency 0.03) near *TCF4* on 18q21.2. rs9853115 failed replication in the Asians (*P = 0.9315*) and rs150202082 could not be examined since this variant was monoallelic in the Asian individuals. A GWAS by Choquet et al.[15] also identified rs9853115 as new variant associated with IOP in multiethnic cohort of predominant (83%) European ancestry. The same study also identified a novel variant near *TCF4*, rs11659764, approximately 300 kb upstream of rs150202082 which was in relatively weak LD (r^2 = 0.4). In a recent study from the UKbiobank by Khawaja et al.[16] the same variant near *DGKG* showed genome-wide significant association with similar effectsize, however, rs150202082 near *TCF4* could not be validated in this study.

In the meta-analysis of CCT, a total of 16 204 participants were included (LDSC intercept = 0.989; SE = 0.0082). We identified 31 independent genome-wide significant signals of which

three were novel (Table 1 and Supplementary Data 5), including a variant, rs34869, near *CDO1*. Again, none of the three new variants replicated at a nominal significance level in the Asian samples. Multi-trait analysis by CPASSOC identified four novel variants (Supplementary Data 8 and 9). In contrast to ONH cross trait analyses, also these could not be replicated in the Asian.

In silico analysis

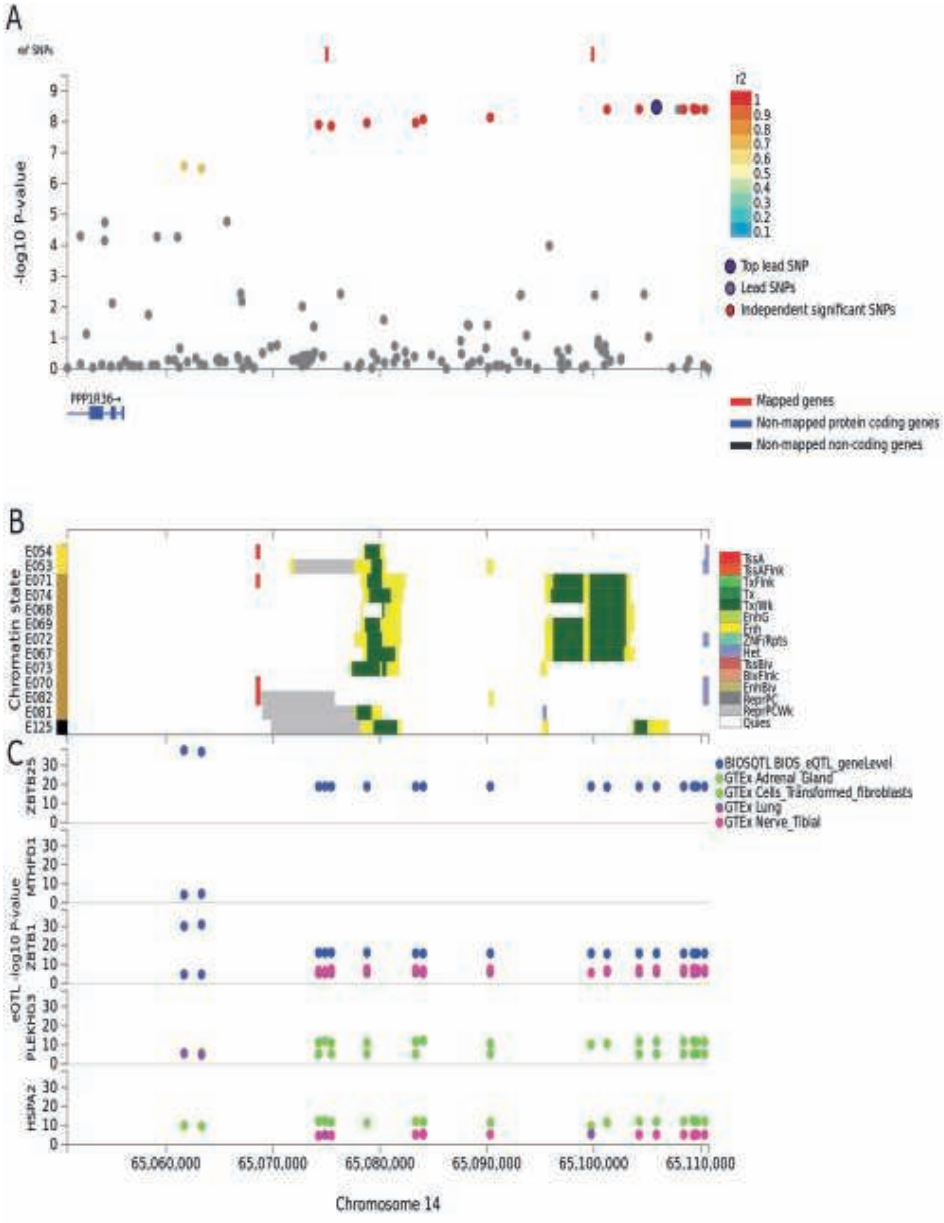
To investigate the functional and regulatory potential, we annotated the variants in linkage disequilibrium (European LD, $r^2 \geq 0.8$) with the lead SNPs at the two new and replicated ONH variants, rs11158547 and rs1028727, using a combination of bioinformatics tools (see Method section). A total of 70 variants in LD with the 2 novel variants were queried. None of the examined variants were predicted to damage protein structure by SIFT, Polyphen, or alternative splicing using Ensembl's Variant Effect Predictor. As all queried variants are noncoding, we reviewed the possible regulatory annotation of these SNPs in experimental epigenetic evidence, including DNAse hypersensitive sites, histone modifications, and transcription factor-binding sites in human cell lines and tissues from the ENCODE[17] and ROADMAP EPIGENOMICS[18] projects, integrated in Haploreg[19]. Annotations of chromatin states indicated that the two novel variants were located in, or in LD with, an active chromatin state region from at least one of the tissues investigated (Supplementary Data 10 and Figs. 3 and 4 for chromatin states in brain tissue). Next, we evaluated the overlaps of *cis*-expression quantitative trait loci (eQTL) in several databases (see Methods). In both novel loci *PPP1R36-PLEKHG3 and SERPINE3*, variants were found to be eQTL's and based on RegulomeDB-scores both ONH loci contained variants that were likely to alter binding (Supplementary Data 10).

Gene prioritization, pathway analysis and gene expression

We explored possible tissue expression and biological functions by pathway analysis for the two novel SNPs. We annotated these SNPs to genes by positional gene mapping, eQTL mapping and chromatin interaction mapping strategies implemented in FUMA[20] (see Method section). For, rs11158547 (*PPP1R36-PLEKHG3*) 9 genes were assigned to this locus and for rs1028727 (*SERPINE3*) 21 genes were mapped to this locus (Supplementary Data 11). Pathway analysis based on enrichment of gene-set terms (MsigDB[21] and Wikipathways[22]) found 5 and 11 Bonferroni significant gene-sets comprising genes mapped to *SERPINE3* locus and *PPP1R36-PLEKHG3* locus respectively. These pathways were particular involved in immune response and cancer development (Supplementary Data 12 highlighted gene-sets).

As expression in eye tissues is not available in GTex[23], we assessed the Ocular Tissue Database[24]. For 26 out of the 30 genes mapped to either rs11158547 or rs1028727 expression data were present in the Ocular Tissue Database (Supplementary Data 11). The

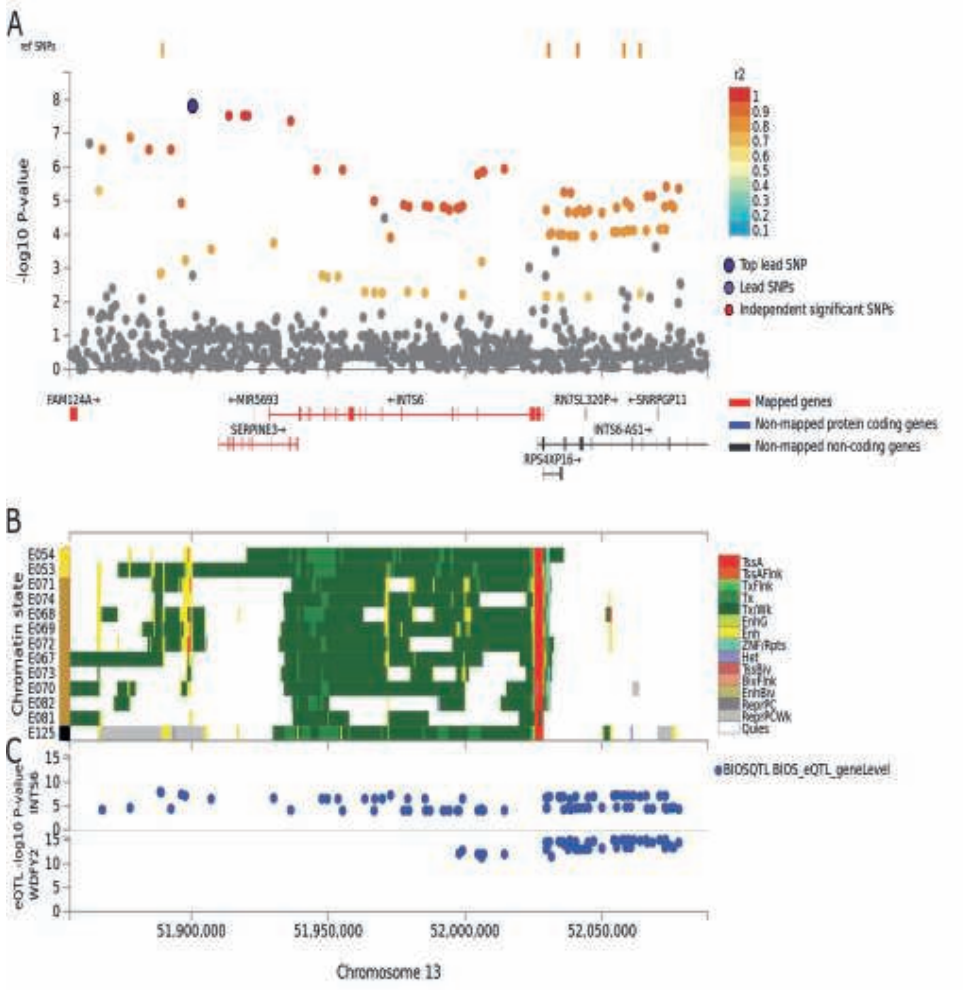
Figure 3. Regional, chromatin state and eQTL plot for rs11158547 (*PPP1R36-PLEKHG3*)



Panel **A**: shows the regional association plots with –log10 *P-value* depicted on the *y*-axis, genes mapped by either position, eQTL or chromatin interaction are depicted in red on the *x*-axis ; Panel **B**: shows 15 core chromatin states of variants plotted in panel **A** for 13 brain tissues from Roadmap Epigenomics Project described on the *y*-axis. E054: Ganglion Eminence derived primary cultured neurospheres; E053: Cortex derived primary cultured neurospheres; E071: Brain Hippocampus Middle; E074: Brain Substantia Nigra; E068: Brain Anterior Caudate; E069: Brain Cingulate Gyrus; E072: Brain Inferior Temporal Lobe; E067: Brain Angular Gyrus; E073: Brain Dorsolateral Prefrontal Cortex; E070: Brain Germinal Matrix; E082: Fetal Brain Female; E081: Fetal Brain Male; E125: NH-A Astrocytes Primary Cells. Panel C depicts variants that overlap eQTLs from selected eQTL databases described in the legend of Panel C.

highest levels of expression in the optic nerve was found for *HSPA2*, a gene associated to rs11158547 via lung, tibial nerve and fibroblasts eQTL's and chromatin interaction mapping (Supplementary Fig. 3).

Figure 4. Regional, chromatin state and eQTL plot for rs1028727 (*SERPINE3*)



Panel **A**: shows the regional association plots with –log10 *P-value* depicted on the *y*-axis, genes mapped by either position, eQTL or chromatin interaction are depicted in red on the *x*-axis ; Panel **B**: shows 15 core chromatin states of variants plotted in panel A for 13 brain tissues from Roadmap Epigenomics Project described on the *y*-axis. E054: Ganglion Eminence derived primary cultured neurospheres; E053: Cortex derived primary cultured neurospheres; E071: Brain Hippocampus Middle; E074: Brain Substantia Nigra; E068: Brain Anterior Caudate; E069: Brain Cingulate Gyrus; E072: Brain Inferior Temporal Lobe; E067: Brain Angular Gyrus; E073: Brain Dorsolateral Prefrontal Cortex; E070: Brain Germinal Matrix; E082: Fetal Brain Female; E081: Fetal Brain Male; E125: NH-A Astrocytes Primary Cells. Panel C depicts variants that overlap eQTLs from selected eQTL databases described in the legend of Panel C.

From endophenotypes to glaucoma

We also investigated the translational potential of these two loci to POAG by carrying out a meta-analysis of three POAG studies; NEIGHBOR, Southampton and UK Biobank Eye and Vision Consortium (n_{case} = 9450; $n_{control}$ = 436,824), from European origin. For rs1028727 a negative effect on the VCDR in the present study predicts a decreased risk of POAG which was seen in NEIGHBOR study and the UKBiobank but not in the Southampton study (Supplementary Data 13). rs11158547 in *PPP1R36-PLEKHG3* is predicted to be associated with increased POAG risk based on the positive effect of VCDR. Indeed in all three POAG studies the effect of the SNP is also positive. Pooling the studies based on a fixed effect analysis yields an OR 1.28 (95% CI: 1.15-1.39; P=4.83 ×10^{-8}) and showed Bonferroni significance (P = 0.025). Given the high degree of heterogeneity of effects at this locus, a random effect meta-analysis was carried out which could not confirm this finding in POAG (Supplementary Data 14). Thus, the findings were partly but not consistently replicated, awaiting larger and more homogeneous data sets for the final replication.

DISCUSSION

Our results implicate two novel loci, one downstream *SERPINE3* and one other in-between *PPP1R36* and *PLEKHG3,* both associated with ONH morphology via CPASSOC multi-trait analysis. *SERPINE3* belongs to the clade E family of extracellular serpins. Family members have been described to play a role in other neurodegenerative diseases such as Alzheimer's disease [25]. Recent studies in glaucomatous human postmortem samples and in rat models identified oxidative inactivation of serpins (neuroserpin) as a molecular mechanism of increased plasmin activity leading to neurodegeneration in high ocular pressure conditions[26]. In the trabecular meshwork, serpins (plasminogen activator inhibitor) may mediate the inhibition of matrix metaloprotienase (MMPs) activity induced by transforming growth factor-beta enhancement[27,28,]. Inactivity of MMPs were found to increase aqueous humor outflow resistance leading to rising IOP[29]. rs11158547 downstream the *PLEKHG3* gene, a pleckstrin homology domain containing protein, is also a relatively unknown gene. It contains a guanide nucleotide exchange factor (GEF) domain which is important for Rho-dependent signal transduction[30]. In mice *PLEKHG3* knockout is associated with an abnormal anterior chamber depth of the eye (IMPC release 3.2 http://www.mousephenotype.org/data/experiments?geneAccession=MGI:2388284). The other gene close to this locus, *PPP1R36*, is less likely a candidate gene for ONH. *PPP1R36*, has been described in connection with autophagy during spermatogenesis. *PPP1R36* encodes a regulatory subunit of protein phosphatase 1 which is involved in multiple cellular functions such as metabolism, immune response, apoptosis, meiosis, mitosis, cytoskeletal reorganization and synthesis. However, its function to the eye has not been characterized.

A general insight from this study was that the strength of genetic correlation is an important condition for investigating the pleiotropic effect of genetic variants on traits. The high genetic and phenotypic correlation observed between the ONH traits enabled multi-trait analysis and yielded plausible and replicable results. We validated these novel variants identified by CPASSOC with another multi-trait analysis method MTAG, which also uses summary statistics as input. The main difference between both methods is that CPASSOC tests whether a SNP is not associated with any of the traits under the null hypothesis. MTAG, on the contrary produces trait-specific effect estimates for each SNP. The variants discovered in the European CPASSOC analysis that replicated in the Asian CPASSOC analysis, also replicated in the MTAG analysis. Sensitivity analysis excluding the Rotterdam studies showed a high correlation of the Som and Set statistic with the SHom/SHet statistic from the full analysis including the Rotterdam studies (r=0.71, $P<2.2x10^{-16}$;r=0.68, $P<2.2x10^{-16}$ respectively). The correlation in MTAG for CA, DA and VCDR was considerably low yet very significant (r_{CA} = 0.31, $P < 2.2x10^{-16}$; r_{DA} = 0.56, $P < 2.2x10^{-16}$,r_{vcdr} = 0.17).

In contrast, the multi-trait analysis between IOP and CCT could not uncover robust new variants. A reason for this observation might be the moderate magnitude of the genetic correlation between IOP and CCT. Also, clinical research has shown that this relation is largely driven by measurement errors in Goldmann applanation tonometry, rather than a pathophysiological process[31,32].

A potential limitation of this study is the application of a different imputation panel for the discovery and replication phase. The European studies were all imputed to the HRC panel which has a beneficial imputation quality compared to 1000 Genomes. By contrast, the Asian replication set was imputed to 1000 Genomes since a recent publication showed that HRC imputations perform less adequate in Asians[11]. Variants associated with cup area and other endophenotypes at genome-wide significance in the European single trait analysis could not be replicated in the Asian replication sample. The use of different imputation panels may be a source bias hampering replication. A theoretical shortcoming, is that the various studies used different methods and equipment to assess ONH parameters among studies. This has most likely reduced the power of the study and has generated most probably false-negative rather than false-positive results. To prevent false-positive findings using novel methods, we aimed to replicate the findings of the primary CPASSOC analyses by another analyses using MTAG. The variants discovered in the CPASSOC analyses could also be replicated in MTAG. As both methods are mathematically distinct we concluded that our results are rather robust and independent of the statistical approach. This underscores the strength of the association as it is consistently found by two independent approaches.

We have no doubt that association of the variants to ONH are of interest to the biology community. To evaluate the implications of our findings in the context of glaucoma we studied three independent POAG studies. Up until now we are not able to link our findings to POAG. Although fixed effect meta-analysis showed Bonferroni significance (P = 0.025) for rs11158547 *in PPP1R36-PLEKHG3*, random effect meta-analysis that takes into account the heterogeneity could not confirm this finding in POAG. The source of the high variability in estimates is unknown and may involve clinical variability and ethnic differences. It is important to realize that the identification of POAG genes is far from complete and a work in progress.

In conclusion, we conducted single and multi-trait meta-analyses of five endophenotypes of glaucoma, based on HRC imputations in European ancestral populations. The HRC single trait analyses in those of European descent did not yield new loci that could be replicated in Asians. We identified two novel loci for ONH in between *PPP1R36-PLEKHG3* at chromosome 14q23.3 and near *SERPINE3* at chromosome 13q14.3 by multi-trait analyses in those of European descent that could be replicated in Asians using CPASSOC. Findings for these loci were consistent using MTAG. The present study underscores that multi-trait analysis in GWAS of true pleiotropic traits in relatively small sample sizes is a powerful approach to identify variants harboring correlated traits. Although these novel loci could not be directly associated with POAG it is likely that the genes in these regions mediate the glaucomatous process by their effect on the optic nerve morphology. For instance, the *PLEKHG3* gene identified in this study is involved in the Rho signaling cascade, this pathway is known to play a crucial role in POAG pathophysiology and is currently targeted for new therapies for POAG[33]. Our bioinformatic analysis suggests that both the *PPP1R36-PLEKHG3* and *SERPINE3* variants are eQTL's opening avenues to counteract the problem by RNA interference. Further research including exome sequencing and functional studies are needed to further define these genes in the mechanism of POAG.

METHODS

Study design

We performed a meta-analysis of European origin GWASs imputed to HRC reference panel release 1. We analyzed five outcomes: CA, DA, VCDR, IOP, and CCT. The CA phenotype was adjusted for DA in all analyses since these phenotypes are clearly correlated (Pearson correlation coefficient 0.6). Subsequently, we performed multi-trait analyses for CA, DA, VCDR, IOP, and CCT. Replication was carried out for the single trait as well as the multi-trait analysis in a meta-analysis of five Asian cohorts imputed to 1000 genomes. We also tested significance of lead SNPs in three independent POAG cohorts.

Study samples, phenotyping, and genotyping

All studies included in this meta-analysis are part of the International Glaucoma Genetics Consortium (IGGC). A description of the details of all cohorts participating in this study can be found in Supplementary Note and Supplementary Tables 1-6. The mean IOP, VCDR, CCT, CA, and DA of both eyes was used for the analyses. In case of missing or unreliable data for one eye, the measurement of the other eye was used instead. For subjects who received IOP-lowering medication, the measured IOP was multiplied by a factor of 1.3. The total number of individuals in the meta-analysis was 24,493 for CA, 24,509 for DA, 25,180 for VCDR, 31,269 for IOP, and 16,204 for CCT. All studies were performed with the approval of the local institutional review board (Supplementary Note) and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Genotyping was performed using commercially available Affymetrix or Illumina genotyping arrays (Supplementary Table7). Quality control was executed independently for each study. To facilitate meta-analysis, each cohort performed genotype imputation using either the Sanger imputation service (https://imputation.sanger.ac.uk) or the Michigan imputation server (https://imputationserver.sph.umich.edu[34]).

Association analysis in discovery cohorts

Within each discovery cohort, each genotyped or imputed variant was tested for association with each of the traits, assuming an additive genetic model. The measurements were adjusted for sex, age, and five principal components in all cohorts and if necessary also for cohort-specific covariates (Supplementary Table 8). Family based studies were adjusted for family structure. Given the clear correlation of CA with DA (Pearson's correlation r= 0.59 in Rotterdam Study I), the CA GWAS was adjusted for DA in all discovery cohorts prior to meta-analysis. Linear regression was employed for studies with unrelated individuals, and linear mixed effects models were used to account for family structure in the family based studies.

Centralized quality control

Before meta-analysis, a centralized quality control procedure implemented in EasyQC was applied to individual study association summary statistics to identify outlying studies[35]. We included variants with imputation quality ≥ 0.3 (e.g., Minimac R^2) and expected minor allele count >6. Additional checks for quality control were applied on the already filtered datasets including review of P-Z-plots, allele frequency plots and calculation of genomic inflation factor λ.

Meta-analysis of discovery cohorts

The association results of all studies were combined in a fixed effect inverse variance meta-analysis in METAL[36], since there was no sample overlap or cryptic relatedness as checked

by LD score regression (see methods genetic overlap). This tool also applies genomic control by correcting the test statistics to account for small amounts of population stratification or unaccounted relatedness. We also assessed heterogeneity by calculating I^2 values and Cochrans Q-test for heterogeneity as implemented in METAL. After meta-analysis of all available variants, we excluded the variants that were not present in at least three studies. This resulted in 11 830 838 variants for CA, 11 764 957 for DA, 11 901 698 variants for VCDR, 12 426 120 for IOP, and 9 249 813 variants for CCT. The remaining variants per trait were used to create Manhattan plots and QQ-plots, see Supplementary Figs. 4 and 5. The meta-analysis resulted in 1918 SNPs with a *P*-value less than 5×10^{-8} for CA, 2029 for DA, 2473 for VCDR, 156 for IOP, and 1288 for CCT. Re-running the meta-analysis excluding TEST-BATS study to show that the significantly younger mean age in this study did not distort our findings, showed nearly perfect correlation between effect estimates from the full analysis and the effect estimates from in the analysis excluding TEST-BATS (CA $r = 0.99$; DA $r = 0.99$; VCDR $r = 0.99$; IOP $r = 0.99$). Furthermore, the mean differences between effect estimates found in the full analysis and the effect estimates found in the analysis excluding TEST-BATS were zero (CA mean difference = 0, SD = 0.00; DA mean difference = 0, SD = 0.00; VCDR mean difference = 0, SD = 0.01; IOP mean difference = 0, SD = 0.06), this also suggests that the younger age in the TEST-BATS study has not biased the results.

Selection of independent variants

We examined whether multiple independent variants at a given locus influenced a trait and if they were independent of previous findings, we used the genome-wide complex trait analysis software (GCTA)[37]. This tool performs a stepwise selection procedure to select multiple associated SNPs by a conditional and joint (--CoJo) analysis approach using summary-level statistics from a meta-analysis and LD corrections between SNPs. The three Rotterdam Study cohorts (n = 5815) , imputed with the HRC reference panel version 1, were used as the reference to calculate the LD, because it represents the largest discovery studies. LD was calculated between pairwise SNPs, but any SNP further than 10 Mb apart were assumed to not be in LD. All autosomal chromosomes were analyzed, with MAF restricted to ≥ 0.01 estimated from the three Rotterdam Study cohorts. The independent variants were annotated by Haploreg[19], see Supplementary Table 9.

Identification of potential novel variants

Previously, Springelkamp et al.[8,38,39], Iglesias et al.[12], Hysi et al.[40] and Lu et al.[41] identified various loci associated with CA, CCT, DA, IOP, and VCDR by GWAS with the HapMap and 1000 Genomes as a reference panel for imputations. To identify new variants, we investigated if any of the independent variants were within 1 Mb of a known loci identified for the same trait by Springelkamp et al.[8,38,39], Iglesias et al.[12], Hysi et al.[40] and Lu et al.[41]. We created locuszoom plots and forest plots of all potential novel variants, see Supplementary Figs. 6

and 7. Variants showing significant association with a trait and that are within 1 Mb of a previous identified locus were annotated to the known variant.

Multi-trait analysis

For multi-trait genome-wide association analysis we applied the CPASSOC package developed by Zhu et al.[13]. We used CPASSOC for two analyses to combine the association results from CA, DA, VCDR, and from IOP and CCT. CPASSOC generates two statistics, SHom and SHet. SHom is similar to the fixed effect meta-analysis method but accounts for the correlation of summary statistics because of the correlated traits. SHom uses the sample size of a trait as a weight instead of variance, so that it is possible to combine traits with different measurement scales. SHet is an extension of SHom, but power can be improved when the genetic effect sizes are different for different traits. To compute statistics SHom and SHet, a correlation matrix is required to account for the correlation among traits or induced by overlapped or related samples from different cohorts. We followed the approach previously described by Park et al.[42], to calculate this correlation matrix. Briefly, we used all independent SNPs ($r^2 < 0.2$) present in datasets that were not associated with any of the traits ($-1.96 > Z\text{-score} < 1.96$), and took the Pearson's correlation of their *Z*-scores[13]. For both tests QQ-plots were created (Supplementary Figure 8). Novel loci identified by CPASSOC ($P < 5 \times 10^{-8}$) that were not implicated in the single-trait analysis were validated using a second multi-trait method, MTAG. Similarly, MTAG also utilizes summary statistics as input, but performs LD score regression to estimate the genotypic and phenotypic variance-covariance matrices. In contrast to CPASSOC, MTAG performs association tests for each individual trait by boosting the power of a signal and providing an estimation of the underlying association via the multi-trait variance-covariance structure. We applied MTAG to SNPs MAF > 0.01 for combining the analysis of CA, DA, and VCDR, and the analysis of IOP and CCT. For the European sample we used the 1000 Genomes European pre-calculated LD scores and for the Asians the 1000 Genomes East-Asian pre-calculated LD scores (https://data.broadinstitute.org/alkesgroup/LDSCORE/). We then validated each of the genome-wide significant signals identified by CPASSOC in the MTAG results.

Replication in Asian cohorts imputed to 1000 Genomes

All independent SNPs identified with $P < 5 \times 10^{-8}$ in the discovery stage (single and multi-trait analysis) were carried forward for replication in Asians. For single-trait analyses, we validated these signals in fixed effect meta-analyses previously reported by Springelkamp et al. (CA, DA, VCDR, and IOP) and Iglesias et al. (CCT). Similar as in the discovery stage, we also performed a multi-trait CPASSOC and MTAG analysis of CA, DA, VCDR, and IOP, CCT in the Asians using the 1000 Genomes summary statistics. Association replication was sought at nominal ($P < 0.05$) levels. A brief description of the cohorts participating in this study can be

found in the Supplementary Note. Descriptive statistics, phenotyping methods, genotype, and 1000 Genomes phase I version 3 (March 2012) imputation quality and control has been described previously in Springelkamp et al.[8] and Iglesias et al.[10].

Validation in POAG case-control studies

To evaluate whether SNPs identified in the European HRC discovery stage ($P < 5 \times 10^{-8}$) that replicated at nominal significance ($P < 0.05$) in Asians 1000 Genomes have a shared component with primary open-angle glaucoma we validated these SNPs in three POAG case-control studies from NEIGHBOR/MEEI, Southampton and UK Biobank Eye and Vision Consortium. Phenotyping and genotyping methods are provided in Supplementary Note 1.3 and Supplementary Table 9. For the queried SNPs summary statistics from NEIGHBOR/MEEI and Southampton were combined in a fixed-effect and random-effect meta-analysis as implemented in Metasoft [43]. Statistical significant level was corrected for the number of queried SNPs by the Bonferroni method.

The genetic overlap between CA, DA, VCDR, IOP and CCT

To further investigate the genetic overlap among CA, DA, VCDR, CCT, and IOP we used the LD Score regression implemented in LDSC[44] to examine the pattern of genetic correlations. The LD score for each SNP measures the amount of pairwise LD (r2) with other SNPs within 1-cM (centimorgan) window based linkage disequilibrium. Bivariate LD score regression can estimate the extent to which two phenotypes share genetic variance.

Summary statistics of the five meta-analyses were formatted to LDSC input files, we followed quality control as implemented by the LDSC software package (https://github.com/bulik/ldsc). We used pre-calculated LD scores provided by the developers for each SNP using individuals of European ancestry from the 1000 Genomes project that are suitable for LD score analysis in European populations. SNP heritability estimates for all traits and genetic correlations were then calculated between the traits, see Supplementary Data 3 and Fig. 2.

Bioinformatical annotation

Using the software HaploReg (version 4.1)[19] and RegulomeDB v1.1[45], we annotated the potential regulatory functions of the replicated GWAS SNPs and their proxies ($r^2 > 0.8$, 1000 genomes CEU) based on epigenetic signatures. We examined whether these variants (GWAS SNPs and variants in LD with the GWAS SNPs) overlapped with regulatory elements including DNAse hypersensitive sites, histone modifications, and transcription factor-binding sites in human cell lines and tissues from the ENCODE Project and the Epigenetic Roadmap Project. We then used the RegulomeDB score to assess their potential functional consequence, as described previously[46].

Pathway analysis

We applied FUMA, which uses a three way gene-mapping strategy, to assign genome-wide significant SNPs to genes of interest. For positional mapping, SNPs in LD with the independent SNPs were mapped to genes using a window of 10 Kb. eQTL mapping was performed by mapping SNPs to genes up to 1 Mb (cis-eQTL). eQTLs from all tissues available in GTEx v6[23], Blood eQTL browser[47], BIOS eQTL browser[48], and BRAINEAC[49] were selected for the mapping. Chromatin interaction was based on GSE87112 (Hi-C) database as implemented in FUMA. We explored possible biological functions by pathway analysis for all variants that reached genome wide significance in the discovery stage and were nominal significant in the Asian replication set. These 55 associated variants (ONH= 32, IOP = 3, CCT = 20) were assigned to genes by FUMA mapping strategies. Prioritized genes for ONH traits were highly overlapping and were combined to form a set of 295 unique genes for further functional annotation in FUMA. For IOP and CCT, 11 and 116 genes were prioritized respectively. We further investigated the FUMA-mapped genes for enrichment using hypergeometric enrichment tests on pre-defined gene-sets derived from MsigDB and WikiPathways. *P*-values were corrected based on Bonferroni method for the number of tested gene-sets.

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Chapter 5

General Discussion

In the thesis, my aims were to: *i*) study the differences in primary open-angle glaucoma (POAG) phenotypes between sub-Saharan Africa (SSA) and European populations **(Chapter 2)**; *ii*) identify genetic variants associated with POAG in sub-Saharan Africa **(Chapter 3)**; and *iii*) use novel approaches to study genetic and phenotypic associations with glaucoma endophenotypes **(Chapter 4)**.

First, I will discuss my findings using novel approaches to study glaucoma endophenotypes **(Chapter 4)**. I will then focus primarily on the question of what factors underlie the increased prevalence of glaucoma in SSA. This remains a difficult question to answer, POAG is likely multifactorial in origin. Nevertheless, socioeconomic and cultural factors likely play a major role, together with ocular and genetic differences, possibly contributing to the observed differences between populations.

5.1 GLAUCOMA ENDOPHENOTYPES

5.1.1 Peripapillary retinal nerve fiber thickness (Chapter 4.1)

Over the past three decades, improvements in imaging technologies have provided a detailed *in vivo* structural assessment of the optic nerve head (ONH), with the aim of better quantifying structural glaucomatous (i.e., glaucoma-related) damage. Confocal scanning laser ophthalmoscopy and scanning laser polarimetry, followed by optical coherence tomography (OCT), have contributed a wealth of information regarding the dimensions and parameters at and around the ONH; however only a few of these measurements have direct clinical relevance with respect to detecting disease and monitoring progression[1,2]. For example, measuring the thickness of the peripapillary retinal nerve fiber layer (pRNFL) has become an essential tool for detecting pre-perimetric glaucomatous damage and monitoring early disease progression[3]. Indeed, many ophthalmologists now use pRNFL thickness in their clinic to assess disease risk, although factors that can affect this measurement are often neglected. An established confounding factor in interpreting global pRNFL thickness is refraction[4]. Myopia is associated with thinning of the entire retina and subsequently a thinner pRNFL. The profile of the pRNFL thickness also changes, with temporal displacement of peak pRNFL thickness, and various optical factors may contribute to this phenomenon[4]. Other factors such as sex and age are also known to influence pRNFL thickness, and most normative databases built into standard OCT devices take factors such as age, sex, and refraction into consideration when interpreting the measurements.

Less obvious determinants such as comorbidity are usually not integrated into the processing of OCT data. For example, in **Chapter 4.1** we showed that vascular and neurological diseases can affect the measurement of pRNFL thickness. Although hypertension may not have a

clinically relevant effect, stroke can affect global pRNFL thickness by as much as 2 µm. In addition, both dementia and diabetic neuropathy have been shown to affect pRNFL thickness[5], even in cases in which no clinical signs of diabetic retinopathy were present. The effect of these comorbid conditions on pRNFL thickness can lead to the misclassification of glaucoma and can obscure the ability to monitor progression on OCT. Even in the absence of these pathologies, defining glaucoma progression on OCT remains a highly debated topic, and precise cutoff values have not been established[6].

Another important caveat when interpreting pRNFL measurements is that most built-in segmentation algorithms are based on a limited number of participants that are designed to represent a normative population. Although these databases do include several ancestral populations, individuals of European descent are often overrepresented. Consequently, data regarding Asian, African, and Latino populations may be less accurate. For example, studies have shown that the effect of age on pRNFL thickness can vary among various ancestral groups[7].

Cross-sectional population-based studies have investigated determinants of pRNFL thickness and found that several comorbid conditions and ancestry can affect pRNFL measurements. This finding is important to the clinician when interpreting OCT data, but is also relevant to epidemiologists, given that some of these conditions such as hypertension, diabetes mellitus, stroke, and dementia have been implicated in the pathogenesis of glaucoma[8]. For example, diabetic retinopathy and glaucoma have several mechanisms in common, including glial dysfunction, cellular apoptosis, and impaired microcirculation. Thus, future longitudinal studies should be designed to investigate whether a temporal relationship exists between glaucoma and comorbidity, lifestyle factors, and/or pRNFL thickness.

5.1.2 New strategies to identify novel genes related to POAG endophenotypes (Chapter 4.2)

Previously, investigators in the Rotterdam Study in collaboration with the International Glaucoma Genetics Consortium showed that using quantitative glaucoma endophenotype data such as intraocular pressure (IOP), vertical cup-disc ratio (VCDR), and cup area representing early pathogenic changes in glaucoma can serve as a powerful approach for studying glaucoma genetics. Given that data regarding these endophenotypes are much more abundant than data regarding POAG in population-based settings, the statistical power to detect novel genetic risk factors is much higher. For example, Springelkamp et al. reported considerable overlap between genetic loci associated with VCDR, cup area, and disc area[9]. Moreover, given that phenotype-genotype correlations have shown that determinants of ONH morphology are highly interrelated, we hypothesized that pleiotropic loci (i.e., genetic loci that influence multiple traits) may play a role.

In **Chapter 4.2**, we used a new approach for mining data from published genome-wide association studies (GWAS); this approach includes a joint analysis of related traits, new meta-analyses of ONH-related traits implicated in primary POAG, and IOP and central corneal thickness based on the latest Haplotype Reference Consortium imputations. An important advantage of this imputation data is that its ability to identify low-frequency variants in European populations is superior to the well-known 1000 Genomes panel[10]; moreover, imputation quality is improved by the curation of this large set of European haplotypes. We performed a multi-trait analysis of ONH parameters such as cup area, disc area, and VCDR, which revealed two new variants—one in the *PPP1R36-PLEKHG3* locus and the other near the *SERPINE3* gene—with genome-wide significance. These novel variants were replicated in independent Asian cohorts imputed to the 1000 Genomes panel. In addition, the variant in the *PPP1R36-PLEKHG3* locus replicated in the recently reported similar multi-trait approach used by Craig et al.[11]. However, the main difference between our method and the method used by Craig et al. is that they combined endophenotypes (i.e., VCDR and IOP) with the disease trait, POAG. Using this approach, Craig et al. identified 49 novel loci associated with POAG, 27 of which were not associated previously with any of the input traits. Similar to our study, these novel loci were not replicated in independent POAG case-control studies; however, Craig et al. showed that the effect sizes of all associated loci were highly concordant with the effect sizes measured in the POAG studies. Thus, the difficulty replicating loci associated with endophenotypes in POAG case-control GWAS seems to be a general problem. In addition, large GWAS measuring IOP—a disease trait that is undoubtedly the strongest risk factor for POAG—failed to replicate most of its findings in POAG case-controls GWAS at statistically significant levels, although similarly to Craig et al.'s study the general direction and magnitude of the effects were consistent[12,13]. In contrast, a polygenetic risk score based on a multi-trait analysis showed good predictive power in POAG case-control studies.

The difficulty replicating significant quantitative disease-related traits can be explained by the much smaller effective sample sizes in these POAG replication cohorts. Moreover, there appears to be heterogeneity with respect in effect size among the various POAG case-control studies. This heterogeneity is likely due to differences in diagnostic accuracy between cases and controls, given that control subjects may not always undergo as extensive an ophthalmic evaluation as the cases. The largest POAG case-control genome-wide meta-analysis was published recently by the International Glaucoma Genetics Consortium and included 34,179 POAG cases and 349,321 controls of European, Asian, and African descent[14]. This large analysis replicated the variant near *SERPINE3*, even when multiple comparisons were taken into account[14]. In conclusion, multi-trait analysis of endophenotypes is a bona fide approach that can improve statistical power by aggregating multiple signals and can improve our understanding of disease etiology by detecting genetic variants with pleiotropic effects[15].

5.2 HIGH PREVALENCE OF GLAUCOMA IN SUB-SAHARAN AFRICA

In this thesis, I focused primarily on characterizing POAG in SSA populations at the phenotypic and genetic levels (**Chapters 2 and 3**) by investigating the clinical presentation of POAG in Africa and identifying new genetic risk loci that are largely exclusive to this population. In this respect, our findings provide new insights into the pathogenesis and clinical course of POAG in Africa. Below, I will discuss the determinants (i.e., ocular, genetic, socioeconomic, and sociocultural factors) that likely contribute to the tremendous burden of glaucoma in Africa and among people of SSA ancestry. Furthermore, I will discuss future avenues of study that will increase our understanding of this disease in African populations, and I will discuss how this information can be exploited to benefit patients worldwide.

5.2.1 Ocular factors

5.2.1.1 Intraocular pressure (IOP)

High IOP is a main risk factor for developing POAG and a major predictor of an aggressive disease course with rapid visual field decline and eventual blindness[16,17]. Several clinical reports of POAG in SSA consistently showed high IOP at presentation[18-22]. Moreover, data from the GIGA study showed IOP values ranging from 34-36 mm Hg among Black South Africans and Tanzanians (**Chapter 2.1**). Finally, The Dutch glaucoma cases in the Groningen Longitudinal Glaucoma Study that we used in our studies as a European group had relatively high IOP (29 mm Hg), but this was still markedly lower than the SSA groups (**Chapter 2.1**).

The striking difference in IOP at presentation between various ancestral groups reported in clinical studies has not been investigated thoroughly in large population-based studies. Indeed, only two such studies—the Baltimore Eye Study and the Barbados Eye Study—compared different ancestral groups within the same study. The Baltimore Eye Study assessed IOP in African-Americans and Americans of European ancestry (non-Latino Whites) with similar socioeconomic backgrounds[23]. The authors found no difference in IOP between groups; interestingly, however, they found that IOP was higher (albeit not significantly) among untreated POAG cases of European descent compared to African-American cases (24.15 ± 5.23 mm Hg vs. 21.48 ± 6.46 mm Hg, respectively)[23]. Moreover, approximately 50% of all POAG patients (irrespective of ancestry) had an IOP value <21 mm Hg. Finally, among the control participants without glaucoma, IOP was slightly—albeit significantly—higher in the Americans of European descent compared to the African-American group (17.17±3.35 mm Hg vs. 16.00 ± 4.18 mm Hg, respectively; $P < 0.001$)[23]. In the baseline visits in the Barbados Eye Study, the mean untreated IOP in African-Caribbean POAG patients was 27.7 mm Hg[24]. In addition, IOP in participants without glaucoma was higher among African-Caribbean participants compared to the participants of European ancestry (18.2 ± 4.3 mm Hg vs. 16.4 ± 3.0 mm Hg, respectively)[25].

Detailed data regarding IOP in population-based studies from Africa are scarce and somewhat contradictory. For example, a study involving Tanzanians with POAG found no difference in IOP compared to European populations[26]. In contrast, however, a study that examined risk factors for advanced glaucoma in Ghana found that 30% of newly diagnosed patients with early glaucoma (i.e., patients with no visual field loss within the central 20°) had high IOP values (>31 mm Hg)[27]. In addition, a population-based study in Nigeria showed that the mean IOP among POAG cases was similar to the mean IOP value measured in the Barbados Eye Study (27 mm Hg)[28]. Moreover, IOP in the Nigerian participants without glaucoma was similar to European control groups[28]. It should be noted that directly comparing these populations is difficult due to differences in study design, glaucoma definitions, and methods used to measure IOP ; nevertheless, these population-based studies indicate that IOP may not differ between SSA populations and Europeans to the extent suggested by clinical studies. Therefore, IOP alone may not be sufficient to explain the higher prevalence of POAG in SSA populations.

Sommer et al. suggested that the optic disc may be particularly sensitive to IOP in individuals of SSA ancestry with POAG, as untreated IOP in these patients was lower than IOP in untreated patients of European descent [23]. Moreover, the authors observed that glaucoma-related optic nerve damage was more prevalent among patients of SSA ancestry, regardless of IOP. The cause of this apparent discrepancy between population-based data and clinical data is currently unclear. Many prevalent and incident POAG cases in population-based studies present with IOP ≤21 mm Hg, regardless of ancestry[29]; however we found that IOP is typically higher in POAG patients of SSA ancestry when they first present to a glaucoma clinic (**Chapter 2.1**). One possible reason for the difference in IOP data between clinical studies and population-based studies in SSA may be related to the relatively late presentation of patients; indeed, most patients in SSA seek care only after their visual field is severely reduced, affecting central vision (**Chapter 2.1**).

High IOP is directly correlated with more aggressive glaucoma[17]; as a result, these patients often present with vision-threatening symptoms at a relatively young age. Given the lower average life expectancy in Africa, patients with lower IOP—and therefore less aggressive glaucoma—might not present to the clinic, as they are not likely to developing severe, sight-threatening glaucoma within their life span. Nevertheless, our study convincingly shows that the proportion of genetic African ancestry was associated with higher IOP—irrespective of age—in POAG patients (**Chapter 2.2**). Similarly, the Los Angeles Latino Eye Study (LALES) found a similar association between genetic African ancestry and IOP[30]. Given the debate with respect to the role of ancestry in IOP, additional longitudinal studies involving mixed-ancestry populations are needed, thus providing important information regarding the extent to which ancestry-related differences in IOP levels contribute to differences in the prevalence of POAG.

5.2.1.2 Corneal thickness and corneal biomechanics

Another factor that has complicated our understanding of differences in IOP between populations of African and European ancestry is the relationship between IOP and central corneal thickness (CCT). Indeed, several studies have shown that the reliability of IOP measurements—particularly using Goldmann applanation tonometry—is confounded by variations in CCT, with a thinner cornea leading to an underestimation of actual IOP[31]. Many studies have shown that people of SSA descent have a thinner CCT compared to European populations (**Chapter 2.2**), and neglecting this finding could lead to a misinterpretation of ancestry-based differences in IOP. For example, the above-mentioned lower IOP values observed in the population of SSA descent in the Baltimore Eye Study mentioned may be attributed—at least in part—to differences in CCT.

In addition to affecting the accuracy of Goldmann applanation tonometry, thinner CCT was found by the Ocular Hypertension Treatment Study (OHTS) and the subsequent European Glaucoma Prevention Study (EGPS) to serve as an independent predictor of incident glaucoma[32,33]. On the other hand, case-control studies in SSA did not find thinner CCT values among POAG patients compared to controls (**Chapter 2.2**), and early reports from the Barbados study and the Tema Eye Survey did not find that CCT was associated with prevalent POAG[34,35]. However, at their 9-year and 8-year follow-up measurements, both the Barbados study and the Tema Eye Survey, respectively, found that thinner CCT was associated with a higher risk of incident POAG[36,37]. Although the cause of this peculiar difference between prevalent POAG and incident POAG, and association with CCT, is unclear, it illustrates the complex relationship between these traits. Based on the findings regarding incident POAG, it may be reasonable to speculate that the thinner CCT among SSA populations may at least partly explain their increased risk of glaucoma. The OHTS found that that African-Americans with ocular hypertension have a higher rate of POAG despite similar baseline IOP, follow-up IOP, and treatment. In their predictive model, the OHTS found that the higher incidence of POAG among African-Americans was accounted for entirely by a larger baseline VCDR and a thinner CCT[16]. Although the OHTS results clarify the role of CCT as a risk factor for developing glaucoma, Medeiros et al. noted that caution should be exercised when attempting to conclude that CCT is an independent risk factor for glaucoma[38]. Moreover, it may not be possible to separate the effect of CCT on IOP in the OHTS, as the measurements obtained using Goldmann applanation tonometry depend on CCT, and the contribution of this relationship can be difficult to disentangle[39]. Thus, it is currently unclear whether the observed increased risk of glaucoma among persons with a thinner CCT (i.e., persons of SSA ancestry) is due to an underestimation of IOP or is due to the cornea's role as a possible proxy of ocular wall integrity, making the eye more vulnerable to developing glaucoma at a given pressure.

Recently, interest has increased regarding the relationship between corneal biomechanics and the risk of glaucoma, particularly with respect to corneal hysteresis, a potential derivative of ocular coat (i.e., the sclera and cornea) compliance. Corneal hysteresis reflects the cornea's viscoelasticity and has been described as the cornea's ability to "absorb" fluctuations in IOP[40]. Thus, eyes with higher corneal hysteresis are less rigid and may tend to have more capacity to cushion increases in IOP, imparting a physiologically protective effect[40].

Studies have shown an inverse relationship between IOP and corneal hysteresis, in which an increase in IOP is associated with a decrease in hysteresis, resulting in stiffening of the ocular coat[41]. These more rigid tissues have a reduced capacity to absorb further increases in IOP-related strain, thus potentially explaining the role of IOP in pathogenesis of glaucoma. The IOP-related strain that is not absorbed by the ocular coat is likely transferred to the weakest parts of the eye, namely the tissues within and around the optic nerve head (ONH). This hypothesis is supported by several studies showing that eyes with POAG have less corneal hysteresis compared with eyes without POAG[42,43]. In addition, reduced corneal hysteresis has been linked to glaucoma progression[44,45] and has been associated with reduced compliance of the optic nerve during changes in IOP[42]. These observations are particularly relevant to SSA populations, as several studies have shown that corneal hysteresis in is generally lower in these populations compared to European populations[46,47]. This difference in corneal biomechanical properties between populations may therefore contribute to the increased susceptibility to glaucoma among SSA populations.

5.2.1.3 Optic nerve head

The ONH is considered the principal area at which damage to the retinal ganglion cell axons occurs, thus leading to glaucomatous optic neuropathy. Anatomical variations in the ONH have been suggested to play a role in the increased susceptibility to glaucomatous damage[48]. In addition, differences in the structure of the ONH have been observed between people of SSA descent and people of European descent. In particular, studies using optic disc photography and confocal laser ophthalmoscopy have consistently shown larger optic disc area in people of SSA ancestry (regardless of disease status) compared to people of European descent. Moreover, the population-based Baltimore Eye Study showed that on average the optic disc is approximately 12% larger in non-glaucomatous African-Americans compared to European-Americans[49]. Differences in other ONH parameters have also been reported. For example, both the Confocal Scanning Laser Ophthalmoscopy Ancillary Study to the OHTS and the African Descent and Glaucoma Evaluation Study (ADAGES) found that in addition to a larger optic disc, African-Americans also had larger cup area and larger neuroretinal rim area compared to other ethnic groups[50,51]. However, after adjusting for optic disc area, these differences between African-Americans and other ethnic groups were no longer statistically significant; consequently, both studies concluded that topographical difference

in the ONH between African-Americans and other ethnic groups could be explained largely by the larger optic disc size among the African-American population. Nevertheless, it should be noted that all of these studies consistently show that neuroretinal rim area increases with increasing disc size, raising the question of whether a relatively large optic disc—as observed in people of SSA descent—contains more nerve fibers and therefore has a higher reserve capacity in optic neuropathies. Several histological studies have attempted to address this hypothesis, with conflicting results[52-55].

The finding of both a larger optic disc and a higher prevalence of glaucoma in SSA populations has led to the theory that a large optic disc may be more prone to glaucomatous damage than a small disc, thus contributing to the higher risk of glaucoma in SSA. For example, in a larger disc the lamina cribrosa (LC) may be less tolerant of mechanical stress and strain exerted at normal IOP levels. For example, the Confocal Scanning Laser Ophthalmoscopy Ancillary Study to the OHTS explored the hypothesis that a large disc may be an important predictor of POAG [56]. In their original report they concluded that disc size was not associated with the development of POAG; interestingly, however, they subsequently compared the rate of change for topographic ONH parameters and found that a larger optic disc area was associated with a faster decrease in neuroretinal rim area in eyes that developed POAG[57]. Paradoxically, one might expect that if a larger disc area was associated with a faster rate of rim area loss, then a larger disc area would be associated with the development of POAG. The authors addressed this inconsistency by proposing that the rate of change in neuroretinal rim area may be easier to detect in larger optic discs. Similarly, another study by Jonas et al. found that in patients of European descent, susceptibility to glaucoma was independent of disc size[58].

Recently, advanced OCT imaging techniques and adaptive optics have been used to perform deep *in vivo* imaging of the ONH, attracting special interest in the role of the LC. The LC is a sieve-like connective tissue that provides support and nutrition to the retinal ganglion cell axons that exit the eye. The LC is the weakest point in the corneal-scleral shell and can be considered a "trampoline" stretched between the fibers of the peripapillary sclera, thus dividing the optic nerve into two pressure compartments, namely a prelaminar compartment in which IOP applies strain to the anterior part of the lamina and a retrolaminar compartment in which intracranial pressure provides counterpressure[59]. The interplay between these two compartments creates a translaminar pressure gradient that causes remodeling of the LC. Thus, the LC provides structural support to the ONH by absorbing translaminar mechanical strain, providing the axons with an open pathway to exit the eye. Furthermore, mechanical strain may affect blood flow and oxygenation of astrocytes[60]. In glaucoma, the LC undergoes characteristic changes by remodeling the extracellular matrix[61]. Although both anterior and posterior displacement of the anterior lamina cribrosa surface depth (ALCSD) have been

associated with glaucoma, patients of SSA descent can present with more significant glaucomatous changes in the ALCSD compared to Europeans[62,63]. In addition, an acute increase in IOP seems to result in more severe posterior bowing of the LC[64]. These dissimilarities suggest possible differences in the LC's load-bearing properties in between different populations, making this structure a promising candidate for further morphological studies in the high-risk SSA population. The scleral canal wall and peripapillary sclera have also been implicated as bearing forces generated by increased IOP[65]. For example, differences in scleral morphology have been observed in SSA populations compared to other populations, including a larger loss of age-related compliance. However, whether these differences contribute to the higher susceptibility of the optic nerve head to glaucoma in Africans is currently unknown[66,67]. In conclusion, the ONH is a relatively weak point in the otherwise robust corneal-scleral shell, with mechanical stress or strain driving remodeling that eventually induce axonal loss. Further research should investigate which aspects of the ONH's neural, vascular, and/or extracellular matrix architecture are affected, as well as determining whether biomechanical properties can be used to predict glaucoma.

5.2.2 Genetic factors

In the past decade, GWAS have rapidly accelerated the discovery of genetic factors related to POAG, with more than 120 loci discovered and confirmed to date[14]. These loci were identified and validated almost entirely by studying European and Asian populations; however, clear link between people of SSA descent and increase risk of POAG is likely to point towards major genetic drivers, and recently studies have begun to unravel the genetic architecture of POAG in SSA populations.

A collaboration between the GIGA study, the BioMe biobank, the Eyes of Africa genetic Consortium, and the South London POAG case-control cohort study led to one of the first GWAS focused solely on people of SSA descent (**Chapter 3.1**). An important finding from this study was that loci that were previously identified in large European and Asian GWAS were not replicated in the SSA population. Similarly, earlier attempts to verify these European/Asian loci in SSA populations by others often failed[68-74]. We therefore hypothesized that a difference in linkage disequilibrium (LD) could be a complicating factor in validating these loci.

Tracing genetic associations across genetically divergent populations requires knowledge regarding historical gene flow patterns. SSA is the ancestral homeland of anatomical modern humans, who evolved from several locations across the African continent ~200,000 years ago and admixed with archaic populations in Africa. The dispersal of modern humans out of Africa some 50,000 to 100,000 years ago was a significant event in human evolutionary history, leaving a strong signature on the genetic variation among all non-African populations. Settlement of modern humans in Eurasia introduced a genetic bottleneck associated with

a founder effect that caused a reduction in the number and diversity of haplotypes and resulted in higher levels of LD in the founder population. This founder population then dispersed to all parts of the world and largely maintained its LD. Another, more recent event that contributed greatly to the genetic diversity in SSA populations is the migration of Bantu -language□speaking populations from their homeland in Cameroon and Nigeria to various parts of SSA over the past 4000 years, followed by their subsequent admixture with—and possible replacement—of indigenous hunter-gatherer populations[75]. This and other migration events shaped the complex genomic landscape of Africa and contributed to the genetic disparity between SSA populations and European populations; thus, SSA populations have the highest degree of genetic diversity among all living populations, as well as an extensive population substructure with smaller degrees of LD[76].

LD is an essential principle when analyzing GWAS data, as the genotyped variant is usually not the causal variant, but rather is in high LD with the variant that does drive the association[77]. As LD blocks became smaller and haplotypes grew more diverse, the tagging capacity of one single nucleotide polymorphism (SNP) declined. Therefore, a SNP association in a European population would theoretically tag several variants spanning a more extended part of the genome compared to a SNP in an SSA population. This varying LD pattern between the causal variant and SNPs that have been genotyped across ethnically divergent populations can have major implications with respect to interpretating genetic analyses. For example, directly replicating variants previously found in European/Asian studies can be particularly difficult in SSA populations, as the strategy is based on the assumption that the previously associated marker and the causal variant have remained in LD across populations[78]. Most previous attempts to directly replicate known POAG variants ultimately failed in SSA populations because the methods did not consider how SNPs are transferred between LD blocks across different ancestries. This can be accomplished by evaluating all variants that are in strong LD with the lead SNPs in the discovery population (i.e., the European or Asian population). Using this so-called "local" strategy in the GIGA-BioMe study (**Chapter 3.1**), we successfully validated known POAG variants (in the *TXNRD2*, *TMCO1*, and *CDKN2B-AS1* loci) discovered in persons of European ancestry in persons of SSA ancestry . A more recent study involving African-Americans (the ADAGES III study) also confirmed associations in the *CDKN2B-AS1*, *FNDC3B*, *8q22*, *AFAP1*, and *TMCO1* loci[79]. Notably, the majority of these validated variants were not in LD with known variants, but were separated by a region with high recombination. Two of the loci confirmed in the ADAGES III study (*CDKN2B-AS1* and *TMCO1*) showed evidence of SNP transferability from European LD to an African LD, similar to our findings in the GIGA-BioMe study (**Chapter 3.1**). Moreover, genetic risk scores combining the effects of previously reported POAG loci revealed significant associations in participants/populations of African descent, but the area under the curve of these scores showed poor discriminating ability **(Chapter 3.1)**[79,80].

Taken together, the results of these studies indicate that certain known European/Asian POAG risk loci can indeed be replicated in persons of SSA descent when considering dissimilarities in LD, and the likelihood of replicating these loci is higher in recently admixed populations such as African-Americans[79]. On the other hand, these genetic risk loci likely play a smaller role in SSA populations than in admixed populations, due to differences in allelic architecture, LD, and/or environmental factors that have historically contributed to unique selection pressures within SSA[81]. It is therefore reasonable to hypothesize that novel loci may be implicated in playing a more prominent role in the pathogenesis of POAG in SSA populations.

The search for novel genetic determinants for POAG in SSA populations is still in its early stages. As of the writing of this thesis, five studies used GWAS to identify novel loci (**Chapters 3.1 and 3.2**)[14,79,82], and the majority of these studies predominantly included African-American populations. Today, variants in the *DNAJC24-ELP4*, *TRIM9-TMX1*, *FAM86A-RBFOX1*, *EXOC4*, *EN04*, *APBB2*, and *IQGAP1* gene have been found to have genome-wide significant associations with POAG, and replication in independent cohorts of SSA ancestry was confirmed for the variants in *TRIM9-TMX1*, *FAM86A-RBFOX1*, and *APBB2*.

The *APBB2* locus was identified and validated in a large consortium that included persons of SSA descent in 14 countries, including populations in the African continent (**Chapter 3.2**). The association is unique to populations of SSA descent, as the locus in monomorphic in Europeans and Asians. The risk allele has a potential effect of increasing the expression of *APBB2* (amyloid-β A4 precursor protein-binding family B member 2), with increased β-amyloid plaque deposition in the retina and visual cortex (**Chapter 3.2**). Importantly, β-amyloid plaque formation is a known mechanism in Alzheimer's disease (AD). Although studies investigating a possible comorbidity between glaucoma and AD based on epidemiological data have been largely contradictory[83,84], persons of African ancestry were largely underrepresented in these studies. Interestingly, however, glaucoma and AD share several neurodegenerative features[85], and β-amyloid deposits have also been reported in a mouse model of glaucoma[86]. Thus, whether glaucoma and AD have a similar etiology and/or pathogenesis remains unclear; for example, the *ABBP2* locus has not been associated with the risk of AD.

Genetic studies of POAG in SSA populations are relatively recent but are an important addition to the field of glaucoma genetics. For example, we found that contrary prior hypotheses, known European/Asian loci play only a limited role in POAG in SSA patients. On the other hand, the novel variants identified in SSA populations are monomorphic in European and Asian populations. Thus, a robust genetic common denominator relevant to all of these populations has not yet been identified.

5.2.2 Socioeconomic and sociocultural factors in SSA

The burden due to glaucoma in SSA is exacerbated by difficulties associated with reduced access to the healthcare system and social and cultural factors. In SSA, patients with POAG typically first present in a relatively late stage of the disease, often with unilateral blindness[21,22,27,87-90] **(Chapter 2.1)**. Indeed, blindness often triggers the patient to seek medical care (**Chapter 2.1**). This delay in seeking medical care can be attributed to decreased awareness, poor access and/or availability of eye care services, the limited availability of resources, medication, equipment, and/or finances. Moreover, poor compliance and acceptance of therapy results in inadequate treatment and reduced follow-up. Indeed, when collecting data for the GIGA study in Tanzania, I often confronted these issues firsthand. Below, I discuss the obstacles that both ophthalmologists and patients in SSA often face when treating glaucoma.

Strikingly, population-based studies conducted in Ghana and South Africa revealed that nearly all participants with glaucoma were unaware of their disease[91,92]. Similarly, community-based cross-sectional research in Ethiopia, Nigeria, and Ghana found relatively low levels of awareness in the general population, with 2.4-39% of participants reporting that they never even heard of the disease[93-95]. Higher levels of awareness were reported in more urbanized regions, likely due to the increased proximity to eye care facilities and improved socioeconomic conditions; thus, low education levels and higher age appear to be associated with a lower level of glaucoma awareness[96]. Illiteracy in SSA remains relatively high (34% of the population was illiterate in 2020[a]), particularly in the older population, reflecting the inability of patients to acquire suitable information. In addition, some studies found that females were generally more aware of glaucoma than males, possibly due to the fact that women are often assigned as caregivers of the sick and disabled[93]. Local religion may also contribute to low disease awareness; for example, a community-based study in the Nigerian state of Anambra found that people generally believe that eye diseases and blindness are caused by evil spirits[97].

Raising awareness regarding the existence of a chronic, often asymptomatic disease may be a key step towards controlling the disease. Unfortunately, limited efforts are being directed at raising the awareness of glaucoma in Africa, and the lack of awareness and knowledge regarding glaucoma leads to a delay in receiving timely eye examinations when no overt symptoms are present. Another factor is that little priority is placed on taking preventive measures for a disease that is asymptomatic in the beginning and develops gradually, particularly given that in 2018, 40% of the population in SSA was below the poverty line of 1.9 USD a day[b] and long-term prospects were uncertain.

a Source: https://data.worldbank.org/indicator/SE.ADT.LITR.ZS?locations=ZG-1W.

b Source: http://iresearch.worldbank.org/PovcalNet/povDuplicateWB.aspx.

Both poor access and low availability of eye care services are additional factors that exacerbate the burden associated with glaucoma. Most eye care facilities are based in urban regions, while the majority of the SSA population (59% in 2020[c]) live in rural communities, with no or extremely limited availability of basic ophthalmic care. In addition, specialized glaucoma care is provided only at tertiary centers in larger cities, requiring a long journey from rural areas. To illustrate this issue, a study investigating barriers to accessing eye care services for glaucoma patients in Tanzania showed that patients have to travel an average distance of 152 km (nearly 100 miles) to reach a specialized care center[98]. Moreover, SSA has one of the lowest number of ophthalmologists per capita, with an estimated 2.5 ophthalmologists for every 1 million inhabitants[99,100]. This scarce number of eye care professionals are concentrated in urban areas, as they often lack the motivation to live and work in rural areas, where basic services such as good schools, clean tap water, and electricity are not steadily available. As a result, patients in rural areas are left at the mercy of local traditional healers who—although respected and trusted—often contribute to delays in receiving a medical diagnosis and modern treatment[101].

Community outreach projects that target remote areas have been applied in SSA, raising awareness with respect to preventing blindness due to cataracts, resulting in increased cataract surgery coverage[102]. Integrating glaucoma detection into existing outreach programs has shown promise in terms of early identification and referral of patients to the tertiary center; however, opportunistic screening for glaucoma in an outreach setting—with minimal equipment and often operated by low-level eye care practitioners—may lead to inappropriate referrals, as these eye care workers are typically trained to identify only advanced glaucoma and/or blindness. This may place an additional workload on already understaffed and scarce eye care facilities, particularly given that detecting glaucoma in the early stages usually requires a comprehensive eye examination by a trained ophthalmologist. Nevertheless, a clear advantage asset of these outreach programs is that they increase community awareness and knowledge regarding glaucoma[103].

Even patients in SSA who do have access to ophthalmic care can experience additional barriers that prevent them from receiving proper glaucoma management. For example, even facilities that offer some level of eye care services often lack basic diagnostic equipment such as a tonometer, visual field analyzer, slit lamp microscope, and diagnostic lenses. In addition, a lack of specialized ophthalmic personnel and/or medications can further complicate the situation.

c Source: https://data.worldbank.org/indicator/SP.RUR.TOTL.ZS?locations=ZG-EU-1W.

In SSA, treating glaucoma using medication is often doomed to fail due to only a limited availability and choice of medicines (e.g., only beta-blockers are available in most countries), and the fact that these medicines are relatively expensive compared to the patient's income. For example, a study published in 2018 examined the cost of a 1-year supply of various generic IOP-lowering medicines in 38 countries and found that a 1-year supply of the generic beta-blocker timolol cost 5% of the median annual household income in Ghana, compared to only 0.06% in the UK[104]. This large disparity was even more striking when comparing the cost of a 1-year supply of the generic prostaglandin analog latanoprost, which cost 16% of the median annual household income in Ghana compared to only 0.09% in the UK[104]. The difference in median annual household income only partially explains these differences, as the price of a given medication is often 2-3 times higher in SSA compared to Europe. In addition, most eye drops need to be kept refrigerated, and counterfeit medicines circulate throughout SSA. Together, these issues understandably affect treatment compliance, making medicine-based therapies a less favorable option for treating glaucoma in SSA.

Given the limitations regarding medicine-based treatment, surgery is often advocated as the treatment of choice in SSA, with trabeculectomy serving as the primary surgical approach due to its markedly low cost and high efficacy in lowering IOP[105-107]. For example, the GIGA study found that trabeculectomy was performed in more than one-third of glaucoma patients in Tanzania, and in many patients it was performed as the initial therapy (**Chapter 2.1**). Despite its efficacy, excessive scarring—which can compromise the eye's filter function—is a common complication in patients of African origin[108,109]. Nevertheless, some key opinion leaders have proposed trabeculectomy as a first-line treatment, even though long-term data to support this proposal are lacking, as the majority of study participants fail to show for their follow-up visits. Thus, in SSA postoperative care is limited and glaucoma surgery carries risks; however, the risk should be weighed against the likelihood of eventual blindness if surgery is not performed at all. Moreover, ophthalmologists in SSA are often hesitant to perform surgery due to the uncertainty regarding the results and low patient motivation[110]. In addition, trabeculectomy does not lead to visual recovery and therefore compares poorly with cataract surgery in terms of patient perception; thus, acceptance of trabeculectomy is relatively low. Another factor is that the majority of patients are already blind in one eye when they present to the hospital; thus, offering no viable treatment for the blind eye and surgery for the "good" eye may instill a fear of total blindness, discouraging the patient. A study by Quigley et al. found that in Tanzania even when trabeculectomy was provided for free and the patients' costs for transport, food, and hospitalization were covered, only 46% of participants agreed to the surgery[111]. Performing phacotrabeculectomy (i.e., combined trabeculectomy and cataract surgery) has been proposed, as it provides tangible visual benefits and may therefore motivate patients to undergo surgery; on the other hand, studies have shown that performing trabeculectomy alone is more effective at

lowering IOP and has a higher success rates compared to phacotrabeculectomy[112,113]. Thus, the presumed benefits of a higher acceptance rate with phacotrabeculectomy can present the ophthalmologist with a dilemma, given that trabeculectomy alone might be more successful but will inevitably lead to subsequent cataract surgery requiring an additional trip, additional costs, and the risk of undoing the effects of the existing trabeculectomy[114].

In summary, the specific combination of socioeconomic and behavioral factors in SSA contribute to the complexity of detecting and managing POAG in these countries. Glaucoma is a major public health problem in many SSA countries, and as the average life expectancy is predicted to increase, its societal impact will only increase. Moreover, population-side screening is not feasible, as a simple, sensitive test with acceptable specificity for detecting early-stage glaucoma is currently not available. Therefore, prioritizing glaucoma in national healthcare policies and investing in awareness campaigns will help promote care-seeking behavior. In addition, investing in access to ophthalmologic care at the community level should be a priority, as more patients will need early and easy access to diagnostics, treatment, and referral centers, particularly as the demand for these services increases. Finally, pharmaceutical companies have a responsibility to provide affordable medications to SSA countries. The Vision 2020 program, which was launched in 1999 by the World Health Organization and the International Agency for the Prevention of Blindness[d], has raised awareness in the global ophthalmic community regarding the high burden of glaucoma in SSA; the next challenge will be raising awareness among sub-Saharan Africans themselves regarding this sight-threatening condition.

5.2.3 Glaucoma research in Africa can provide a clearer understanding of this global sight-threatening disease

A commonly asked question is how glaucoma research conducted in Africa will provide benefits outside of the African continent. Given that generalizability and external validity are important epidemiological values, this question may seem perfect legitimate. On the other hand, this question is rarely asked in connection with glaucoma research conducted in Europe. In an attempt to answer this question, it is necessary to first understand that persons of SSA descent are widely dispersed throughout much of the world's population. For example, in the United States, approximately 10% of the population is of SSA descent[e]; in the UK, Belgium, and the Netherlands, 3-5% of the population is of SSA descent[f]. As persons of SSA descent have a considerably higher *a priori* risk of developing POAG, as well as a higher risk of developing a more aggressive form of POAG, more knowledge regarding

d See https://www.iapb.org/about/history/vision-2020/

e Source: https://www.census.gov/quickfacts/fact/table/US/PST045219

f Source: https://opendata.cbs.nl/statline/#/CBS/en/dataset/37325eng/table?ts=1577855153711

the underlying mechanism and disease course of glaucoma in this particular population is also relevant to the global ophthalmology community. In addition, studying POAG in Africa will improve our understanding of the pathophysiology of POAG in general, as any disease that occurs globally is likely to have several common pathways, regardless of ancestry. Moreover, studying a population in which the disease is relatively common and severe can provide the most efficient means to study the disease's pathogenesis. Another advantage is the unique genetic and evolutionary position of SSA populations. Given that SSA is the cradle of anatomical modern humans (see Section 5.2.2 on genetic factors), it is reasonable to speculate that non-communicable diseases that occur globally but are disproportionally prevalent among persons of SSA descent likely share a common, conserved genetic cause. The high degree of haplotype diversity in SSA populations may also provide a powerful tool for fine mapping the causal variants that underlie disease associations. Finally, gene-environment interactions, as well as epistatic interactions among loci, likely influence disease susceptibility[115]. In summary, studying different populations in different environmental states is a necessary step in order truly unravel the disease pathways, thus benefiting the entire world population.

5.3 METHODOLOGICAL CONSIDERATIONS AND FUTURE DIRECTIONS

The studies described in this thesis provide an important starting point toward understanding the pathological processes that underlie POAG in SSA populations. To date, few studies investigated why persons of African descent have a higher risk of POAG, and these studies contained potential limitations, as discussed below.

5.3.1 Epidemiological limitations

The relevant methodological issues for the studies included in this thesis have been addressed in detail in the discussion sections of each corresponding chapter. Here, I will review some of the more general epidemiological considerations of this thesis. First, the GIGA study was a case-control study with the primary aim of facilitating a rapid yet comprehensive case and control identification for conducting a subsequent GWAS. A major challenge in this study was defining POAG in a resource-poor research setting. Visual field testing—which has been the cornerstone for detecting and defining cases in epidemiologic research—was either unfeasible or unavailable for the majority of eligible cases. Thus, based on this common problem in developing countries, the International Society of Geographical and Epidemiological Ophthalmology introduced a framework for classifying glaucoma cases in cross-sectional studies by considering functional damage (i.e., visual field loss) as well as structural damage (vertical cup-disc ratio). Thus, in absence of a visual field test patients

with a VCDR above the 99.5th percentile of the normal population were classified as having glaucoma. This rather strict inclusion criterion based on VCDR increases the inclusion of advanced glaucoma cases, which has an advantage in GWAS as relatively fewer numbers of advanced cases can reveal more genetic variants. However, for our non-genomic studies (**Chapter 2**) this potentially biased selection of relatively advanced glaucoma cases may have led to an overestimation and/or underestimation of the effect sizes, as patients with mild POAG may not have been included in the study population.

To perform a meta-analysis, we combined datasets from several study groups **(Chapter 3)**. However, a recurring issue with POAG meta-analyses is the heterogeneity of case and/or control definitions among studies; moreover, clinical heterogeneity of POAG may have biased the effect estimates. In addition, differences in age criteria for controls may have affected the analysis, as some studies included relatively young controls who may have been at risk of developing POAG and were therefore false negatives.

5.3.2 Advances in African genetics

Genetic studies regarding human diseases have not yet matched our level of global diversity. For example, in 2018 78% of participants included in GWAS were of European ancestry, compared to only 2% of participants of African descent[116]. In addition, the majority (72-93%) of participants of SSA ancestry in genetic studies are African-American or African-Caribbean, with primarily West African roots[117]. Moreover, several reports have challenged the Euro-centric bias of GWAS[116,118], warning that the resulting underrepresentation of ethnically diverse populations impedes our ability to generalize and translate genetic research into clinical practice or public health policies, as they do not cover population-specific variations[116]. The lack of diversity in genomic research is a particularly large impediment to the genetic analysis of Africans and the ability to identify genes for POAG in SSA populations. Indeed, the majority of human genetic data in the public domain is derived from non-African populations. Most genotyping arrays were developed for populations of non-African descent and therefore do not cover genomic variation specific to African populations, particularly rare variants that are abundant in the genome of SSA populations. For example, a study examining the coverage of several widely used genotype arrays in various populations emphasized that genotype coverage of Africans is highly dependent on array density[119]. Specifically, the authors found a 40% increase in the number of common variants that passed the imputation quality (r^2 threshold of 0.8)when comparing the least dense array with the most dense array; in contrast, in Europeans 75% of the common variants were accurately covered by the least dense genotype array. Given the relatively high degree of genetic diversity in SSA populations—reflected by the small LD blocks—larger numbers of variants need to be genotyped in order to provide the same coverage as other populations. Another aspect is that the majority of available genotype arrays are based on sequencing

projects that predominantly included European samples, thus prioritizing SNPs that are common in all populations, leading to an overrepresentation of high-frequency European SNPs and an underrepresentation of SNPs that are common in Africa but either rare or absent in other populations[120]. In this respect, an important development was the design of multi-ancestry arrays such as the Multi-Ethnic Global Array (MEGA), the Global Screening Array (GSA), and the Human Heredity and Health in Africa (H3Africa) array[121]. Indeed, the H3Africa array seems particularly interesting for use in future GWAS in Africa, as the H3Africa project combines previous 1000 Genomes data from African subjects and data from the African Genome Variation Project, as well as new high-coverage sequence data of 350 samples taken from various African ethno-linguistic groups that were not represented in previous projects. Although these arrays will facilitate future African GWAS, they cannot capture novel, population-specific variants. Thus, an alternative method that recently drawn attention for use in genetically diverse samples is low-depth whole-genome sequencing (WGS)[122]. A major advantage of this method is that unlike arrays, WGS is not biased towards any population and is therefore able to detect novel variants in low LD.

Given that WGS is still a developing field and requires advanced bioinformatics facilities, many genetic studies still draw on the reliability and convenience of imputation panels to increase the number of markers available for association testing. Thus, universally applied imputation reference panels such as HapMap, 1000 Genomes, and the Haplotype Reference Consortium have historically focused on the curation of haplotype data from European and Asian populations and do not represent the full extent of haplotype diversity in SSA populations, as these panels cover only 5 populations from the African continent[123]. Recent interest in African genomic studies has led to the design of imputation panels that provide more thorough coverage of the diversity of African haplotypes. For example, the Trans-Omics for Precision Medicine (TOPMed) consortium released a novel imputation reference panel that significantly improves the imputation quality for use in samples from Africa[124]. Similarly, the H3Africa consortium recently announced a reference panel that provides improved coverage of diverse SSA haplotypes, a welcome addition for facilitating genetic studies in Africa.

Other advances should be directed at improving methods for analyzing data obtained from African GWAS. A major challenge with respect to analyzing GWAS data from multiple SSA populations is the population structure. African populations have the highest level of within-population genetic diversity, and this diversity is closely correlated with the linguistic diversity abundant throughout the African continent (with >2000 distinct languages) and reflects the spread of languages, genes, and cultures[125]. This population substructure can complicate the interpretation of GWAS data when not adequately accounted for; for example, it can lead to false-positive associations that are actually correlated with differences in genetic ancestry rather than reflecting true genetic effects of underlying disease. Statistical

techniques such as genomic control, LD score intercept, and principal components analysis have been introduced for monitoring and adjusting for this bias during the discovery stage of GWAS. In addition, mixed models are also being developed in order to model genetic similarities between individuals to control for population stratification when diverse samples are analyzed together.

The challenges associated with population stratification play an even more important role in the second (replication) stage and the meta-analysis stage of GWAS. In Europe, it is common practice to verify GWAS findings in independent replication studies. This established gold standard works well in European populations, where the LD between causal variants and genotyped SNPs is relatively consistent between studies. In contrast, in multi-center African replication studies differences in the patterns of LD between the causal variant and the genotyped SNP can cause a failure to replicate the variant identified in the discovery stage. Thorough knowledge regarding the LD patterns in SSA populations is therefore essential in order to evaluate the transferability of associated SNPs between diverse ancestral populations. Thus, large LD-mapping projects in Africa should be encouraged.

In meta-analyses, diverging LD patterns can also cause heterogeneity among studies. Therefore, random-effects meta-analysis (**Chapter 3.1**) and Bayesian statistics software have been developed in order to control for this heterogenity[126]. Another consequence of the diverging, small LD among SSA populations is that the power to detect genome-wide significant variants is reduced, causing the need for large sample sizes. We have therefore initiated a study in Ghana in order to increase the number of POAG samples for use in GWAS. Finally, the limited number of researchers who investigate genetic risk factors for POAG in SSA should work together in order to facilitate large meta-analyses.

To address these issues, future research in the field of African genetics should:
1. Use high-density genotype arrays that adequately cover the diversity of African haplotypes;
2. Continue expanding the diversity of imputation panels, thus improve the imputation accuracy in various ancestries;
3. Develop and apply novel methods to control for population stratification; and
4. Use random-effects analyses or modified approaches in meta-analyses to compensate for heterogeneity due to differences in genetic backgrounds.

5.3.3 From genomics, proteomics, and metabolomics to integrated systems biology

The recent “omics” revolution in science has led to major breakthroughs in terms of unraveling disease pathways, identifying treatment targets and biomarkers, and developing risk profiles. With respect to glaucoma, much research has focused on dissecting the

"POAGome" by performing GWAS of quantitative endophenotypes and case-control studies. However, conducting a GWAS is not a goal in its own right, as the principal goal of a GWAS is to identify starting points for pathological processes and ultimately understand the disease pathogenesis, requiring further research in order to validate these genetic associations. This remains extremely challenging, as many of these findings do not point to a single gene, and many are located in intergenic regions. Thus, studying epigenetics and transcriptional regulatory elements is essential in order to put these putative genetic associations in the proper perspective. Large projects such as the Encyclopedia of DNA Elements (ENCODE), the NIH Roadmap EpiGenome Project, and the Functional Annotation of the Mouse/Mammalian Genome (FANTOM) consortium have shed new light on the functional elements contained in the human genome and have helped further annotate GWAS findings *in silico*. Annotated variants can now be used in pathway analyses in order to construct gene sets that can predict biological consequence. On the other hand, a major drawback of these bioinformatics approaches is that they are based on prior knowledge and data. Moreover, expression of genes—and their downstream consequences—can differ between diseased tissues and healthy tissues, as well as between different tissue types and different time points, and these differences are often not considered in most databases[127]. Functional studies performed in targeted tissues both *in vitro* and *in vivo* are therefore preferred; however, classic techniques such as cell lines and animal models are often expensive and time-consuming. Moreover, important differences exist between the rodent retina and the primate retina[128]. The recent development of retinal organoids (i.e., the so-called "retina in a dish") combined with CRISPR-Cas9 gene-editing technologies may provide the opportunity to study ophthalmic genetics *in vitro* in relatively less time and at less cost. With respect to congenital forms of POAG, retinal organoids have already been used to functionally characterization the loss of retinal ganglion cells caused by mutation in the *OPTN* gene[129]. Further development of organoids that also model the trabecular meshwork and optic nerve head will likely facilitate the study of genomic research.

To date, genomics has provided a wealth of information regarding the POAG genotype; however, these studies often fail to translate these observations to phenotypic variations. A relatively recent field of omics research—metabolomics—examines metabolite profiles. Metabolomics reflects the complex interplay between genetics and environmental factors and therefore can serve as a proxy for phenotypic expression. Thanks to the close connection between the metabolome and phenotype, metabolomics may be more directly translatable to clinical practice. The discovery of biomarkers for POAG has attracted considerable attention lately, and to date more than 17 metabolites have been associated with POAG[130]. For example, findings with respect to metabolites in the nitric oxide (NO) synthesis pathway, which regulates ocular vascular tone and aqueous humor outflow, have complemented earlier findings from genomic studies.

Omics (e.g., genomics, transcriptomics, proteomics, and metabolomics) data can be used as a biomarker for diagnostics, prognostics, and predicting disease outcome, as well as to understand the underlying features and complexities associated with the disease. As omics data become more accessible—including in the field of glaucoma—scientists are attempting to integrate these datasets in order to establish links between disease pathways identified using various omics approaches[131]. Integrating data from various omics sources has the potential to reveal novel yet common biomarkers; however, achieving such an integration is challenging due to the heterogeneity, size, and complexity of the data. Machine learning has recently be used to integrate multi-omics data, and showed to be proficient in identifying features in varieties of cancer data [132]. Thus, integrative analyses using GWAS combined with multi-omics data will likely advance our understanding of the molecular phenomena that underlie glaucoma.

5.3.4 Ocular & environmental factors

In addition to omics research, studies should also investigate anatomical (i.e., structural) differences in the eye that might underlie the increased susceptibility of SSA populations to developing POAG. As discussed above, age-related remodeling and biomechanical differences in the ocular shell may explain at least part of the difference in susceptibility to POAG between Africans and Europeans. The recent introduction of spectral domain OCT in Africa will allow for the detailed examination of key ocular structures such as the retinal nerve fiber layer, ganglion cell complex, and disc area, driving the direct *in vivo* study of pathological processes regarding the ONH. In addition, new OCT imaging techniques such as enhanced depth imaging and optimized image-processing algorithms allow visualization of the LC and the peripapillary sclera, structures that are believed to play a key role in the development of axonal injury in glaucoma and considered to have different properties between Africans and Europeans[133-135]. Future research should be performed in order to determine which morphologic characteristics of the ONH are associated with remodeling of the LC and ONH during disease progression, and to determine whether these phenotypic features have an underlying genetic basis. Other ocular biomechanical properties such as corneal hysteresis also should be studied further in African populations. For example, decreased corneal hysteresis appears to be associated with glaucoma progression and may serve as a surrogate biomarker of the viscoelastic properties of the LC, the posterior sclera, and other structures of the ONH[136]. The putative differences between populations with respect to corneal hysteresis, the ONH, and the properties of the load-bearing connective tissues in the LC have yet to be determined.

Interestingly, studies involving other diseases have shown that certain fruits and vegetables can have neuroprotective properties. These foods contain various substances that can regulate several cellular molecular pathways involved in the regulation of inflammation,

oxidative stress, metabolism, and apoptosis. With respect to ocular disease, these so-called "nutraceuticals" have been studied extensively studied in age-related macular degeneration (AMD), in which a Mediterranean diet rich in fruit, vegetables, legumes, and fish can have a protective effect[137]. In the past decade, nutrition has also gained interest as a potential environmentally modifiable risk factor for POAG. For example, consuming NO-rich foods such as green leafy vegetables is reported to be protective against POAG, while consuming foods rich in selenium and/or iron may increase the risk of POAG[138]. The dietary intake of vitamins A and C has also been shown to provide benefits in glaucoma[139]. In this respect, it is important to note that vitamin A deficiency is still quite prevalent in in SSA; indeed, more than 40% of children in SSA have vitamin A deficiency[140]. Moreover, nutrition-associated optic neuropathies are endemic in some SSA countries[141,142]. Nevertheless, the extent to which dietary differences account for difference in the prevalence of POAG is currently not clear. Finally, other environmental factors that were evaluated in European cohorts such as smoking, BMI, alcohol use, and diet should also be studied in SSA.

5.4 CONCLUSIONS

Glaucoma is the leading cause of irreversible blindness in the world, with a particularly high prevalence in sub-Saharan Africa. The GIGA study, which served as the basis of this thesis, is one of the first studies to investigate the genetics of POAG in SSA populations. This study is a unique joint effort combining scientists in Africa, Europe, and the US. Importantly, our study identified novel POAG-associated genetic variants in SSA. We also investigated phenotypic variations among African and European POAG patients and found that SSA patients generally present with a more progressive and more destructive disease course. Therefore, diversity is essential when conducting epidemiological research in order to obtain a more comprehensive understanding of variations among populations and elucidate the factors that underlie complex diseases. Future analyses should include several SSA populations, thus reflecting the genetic diversity among peoples living in this region. Given that the glaucoma burden in SSA is likely to be multifactorial, future studies should focus on genetics, as well as epigenetics, environmental factors, and anatomical variations in SSA populations. In this respect, our new study site in Ghana will be a valuable addition by expanding the collection of genetic data in the GIGA study and providing advanced phenotyping (including OCT data and Scheimpflug imaging of the anterior chamber), dietary intake, and an assessment of putative environmental risk factors, thus helping solve the puzzle of glaucoma not only in Africa, but also out of Africa.

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Chapter 6

Summary/Samenvatting

Chapter 6.1

Summary

SUMMARY

Glaucoma is the most important cause of irreversible blindness worldwide. Primary open-angle glaucoma (POAG) is the most common subtype of glaucoma, and the least understood. Intra-ocular pressure (IOP), family history, and race are well-known risk factors. Persons from African ancestry have a 3-5x increased risk of POAG; they have a more severe course of disease with a higher risk of blindness. Given the familial nature and racial predisposition genome-wide association studies (GWAS) have identified many genetic risk factors for POAG and POAG endophenotypes, but these factors explain only a minor proportion of POAG cases. Strikingly, these studies are predominantly based on study participants of European descent. It is likely that gene finding will be more effective in study populations which have a high frequency of disease and a more severe phenotype. **Chapter 1** gives a general introduction and describes the aims of the thesis.

The aims of this thesis were:

1. to address differences in POAG phenotype in sub-Saharan African populations compared to European populations
2. to identify genetic variants associated with POAG in sub-Saharan African populations
3. to apply novel approaches to study genetic and phenotypic associations with glaucoma endophenotypes.

Phenotypic variation of POAG in sub-Saharan African and European populations

Chapter 2.1 discusses difference in POAG presentation across Tanzanian, South African Black, South African Coloured and a white Dutch population. We showed that Black sub-Saharan Africans (SSA) glaucoma patients presented with a much more severe and aggressive phenotype compared to white Dutch patients. In particular the IOP at presentation was 5-7 mm Hg higher compared to the Dutch and visual acuity was already severely affected at time of presentation. The Cape Coloured population had an intermediate phenotype, but more similar to the Dutch. SSA black patients presented also at much younger age, this indicates that the disease is more progressive and destructive in this population. We therefore emphasize that programs to create disease awareness are of particular importance in Africa.

In **Chapter 2.2** we aimed to further elucidate the relationships between genetic ancestry and central corneal thickness (CCT) and IOP. While some studies have pointed towards CCT as an independent risk factor for POAG, irrespective of its bias on Goldmann IOP measurements. Review of African literature could not validate this concept in case- control studies. In a highly admixed group of Coloured glaucoma patients and controls we were

able to study these relationships in an unbiased approach by measuring the compound of genetic African ancestry (GAA). We found that GAA was associated with thinner CCT and that increase in GAA in POAG patients was associated with higher IOP. A key point was that the difference in CCT between POAG patients and controls was reduced at higher proportions of GAA.

Novel genetic variants associated with POAG in populations from Sub-Sahara African ancestry

In **Chapter 3.1** we showed the results from the first GWAS of POAG comprising participants from continental Africa. We identified a novel candidate locus encompassing *EXOC4* reaching genome-wide significance in discovery stage. *EXOC4* encodes a protein involved in exocytosis, an important process during outgrowth of neurons and synaptogenesis. Replication in various cohorts from SSA heritage failed, likely due to divergent LD patterns that characterize African populations giving rise to low statistical power. A genetic risk score considering previous identified POAG variants in European and Asian studies was statistically significant associated with POAG in our study. Though the individual effect sizes of the studied variants were substantially smaller compared to the European and Asian results. Our results therefore suggest that established genetic risk factors may be implicated in SSA POAG, but probably to a lesser extent. And so do not explain the higher disease load encountered in these populations.

To improve statistical power we joined an international genetic consortium of POAG studies comprising over 26 000 persons from SSA descent or SSA admixed heritage (The Genetics of Glaucoma in People of African Descent (GGLAD) Consortium). In **Chapter 3.2** we present the results from a large multi-stage GWAS performed in this consortium. We found one genomic region reaching genome wide significance in the *APBB2* gene, a gene that is involved in the proteolytic processing of amyloid precursor protein. This association is unique to African diaspora populations. The amyloid precursor protein is required for normal development of the retina. However, proteolytic processing produces amyloid beta peptides, which are toxic and aggregate to form amyloid plaques, a well-known hallmarks of Alzheimer's disease. Explanatory immunohistochemical analysis of human retinal and primary visual cortex tissues from post-mortem African Americans suggested a potential relationship between the *APBB2* risk allele, increased *APBB2* expression in the retina, and associated increased β-amyloid plaque deposition. These proposed pathologic mechanisms require further investigation in larger samples.

Phenotypic and genetic associations with glaucoma endophenotypes

In **Chapter 4.1** we investigated the systemic and ocular determinants of peripapillary retinal nerve fiber layer thickness (pRNFLT) in 8 population based studies of the E3 consortium.

We confirmed previously reported associations of age and spherical equivalent with pRNFLT and identified several additional factors associated with pRNFLT. We found that IOP, stroke and hypertension were associated with thinner pRNFLT. Also, we found a trend in reduced pRNFLT in patients with dementia. Our results indicate that also non-glaucoma traits could affect pRNFLT measurements. Microvascular disease and neuronal degeneration should therefore be taken into account when interpreting the pRNFLT in glaucoma screening and management.

Chapter 4.2 examines novel statistical methods to analyze phenotypically and genetically correlated glaucoma endophenotypes jointly, and to find pleiotropic genetic loci. We performed a multi-trait GWAS of optic nerve head parameters (vertical cup-disc ratio, cup area and disc area), that showed high genetic and phenotypic correlation. We identified two genomic loci in *PPP1R36-PLEKHG3* and near *SERPINE3* at genome-wide significance that replicate in independent Asian cohorts.

Finally, in **Chapter 5** the main findings are placed in the context of my view on the pathophysiology of glaucoma and discusses the challenges encountered when conducting this research and proposes direction for future research.

Chapter 6.2

Samenvatting

SAMENVATTING

Glaucoom is de belangrijkste oorzaak van irreversibele blindheid wereldwijd. De ziekte kenmerkt zich door schade en verlies van zenuwvezels in de oogzenuw, wat leidt tot uitval van het gezichtsveld. De meest voorkomende vorm van glaucoom is primair open-kamerhoek glaucoom (POAG). Hoge oogdruk is de belangrijkste risicofactor voor POAG. Het reguleren van de oogdruk is op dit moment de enige therapie, maar helaas is dit niet altijd effectief. Naast hoge oogdruk zijn er andere risicofactoren voor POAG zoals hoge leeftijd, familiare belasting en sub-Sahara Afrikaanse afkomst. Het familiair en etnisch voorkomen van POAG suggereren een erfelijke component waarbij genetische factoren bijdragen aan de pathogenese. Sinds de introductie van genoom wijde associatie studies (GWAS) is er veel aandacht gekomen en progressie gemaakt met het in kaart brengen van genen geassocieerd met POAG en POAG endofenotypen. Toch verklaren deze genetische varianten samen maar een klein deel van de ziekte. Er moeten dus nog veel meer genetische factoren zijn die vooralsnog niet ontdekt zijn. Een belangrijke tekortkoming van het onderzoek naar de genetica van POAG is dat onderzoek tot op heden alleen is gericht op Europese en Aziatische populaties. Deze populaties representeren niet de patiëntengroepen waar de ziekte het meest in voorkomt en het ernstigste beloop kent. Onderzoek naar een ziekte in een populatie waar deze veel voorkomt en een ernstiger beloop kent heeft een grotere kans van slagen om ziekteoorzaken te vinden, dan in populaties waar de ziekte weinig voorkomt. Een algemene inleiding en de opzet van dit proefschrift zijn beschreven in **Hoofdstuk 1**.

De doelstellingen van dit proefschrift waren:
1. het beschrijven van de verschillen in POAG fenotype tussen sub-Sahara Afrikaanse populaties en Europese populaties.
2. het identificeren van nieuwe genetische varianten geassocieerd met POAG in sub-Sahara Afrikaanse populaties
3. nieuwe strategieën toepassen om genetische en fenotypische associaties met glaucoom-endofenotypen te bestuderen.

Variatie in het fenotype van POAG tussen sub-Sahara Afrikaanse en Europese populaties

Hoofdstuk 2.1 beschrijft de verschillen in ziektepresentatie tussen zwarte Tanzaniaanse, zwarte Zuid-Afrikaanse, Zuid-Afrikanen met gemixte afkomst en witte Nederlandse populaties. Wij laten zien dat zwarte sub-Sahara Afrikaanse (SSA) glaucoom patiënten zich presenteren met een veel ernstiger en agressiever fenotype in vergelijking tot witte Nederlandse glaucoom patiënten. Opvallend is dat de oogdruk bij presentatie gemiddeld 5-7 mm Hg hoger is in vergelijking met de witte Nederlandse populatie. Ook was de gezichtsscherpte al ernstig aangedaan in de zwarte SSA groep ten tijde van presentatie. De

Zuid-Afrikanen met een gemixte afkomst hebben een intermediair fenotype dat enige gelijkenis kent met dat van de witte Nederlanders. De zwarte SSA patiënten presenteerden zich ook op veel jongere leeftijd ten opzichte van de andere groepen. Dit geeft aan dat de ziekte bij deze populatie progressiever en destructiever is. We benadrukken daarom dat programma's om ziektebewustzijn te creëren van bijzonder belang zijn in Afrika.

In **Hoofdstuk 2.2** wilden we de relaties tussen genetische afkomst en centrale corneale dikte (CCT) en oogdruk (IOP) verder ophelderen. Hoewel sommige onderzoeken hebben gewezen op CCT als een onafhankelijke risicofactor voor POAG, ongeacht de bias ervan op Goldmann IOP-metingen, kon beoordeling van Afrikaanse literatuur dit concept niet valideren in case-control studies. In een groep van Zuid-Afrikaanse Cape Coloured glaucoompatiënten en controles die een sterk gemengde afkomst hebben (Afrikaans, Aziatisch, Europees) waren we in staat om deze relaties op een onbevooroordeelde manier te bestuderen door de mate van genetische Afrikaanse afkomst (GAA) te meten. We ontdekten dat GAA geassocieerd was met dunnere CCT en dat toename van GAA bij POAG-patiënten geassocieerd was met hogere IOP. Een belangrijk punt was dat het verschil in CCT tussen POAG-patiënten en controles afnam bij hogere proporties van GAA.

Nieuwe genetische varianten geassocieerd met POAG in populaties van sub-Sahara Afrikaanse afkomst

In **Hoofdstuk 3.1** lieten we de resultaten zien van de eerste GWAS van POAG met deelnemers uit continentaal Afrika. We identificeerden een nieuwe kandidaat-locus in het *EXOC4* gen dat genoomwijde significantie bereikte in de eerste fase van de studie. *EXOC4* codeert voor een eiwit dat betrokken is bij exocytose, een belangrijk proces tijdens de uitgroei van neuronen. Replicatie van onze bevinding in verschillende onafhankelijke cohorten van SSA afkomst mislukte, waarschijnlijk als gevolg van verschillen in linkage disequilibrium-patronen die Afrikaanse populaties karakteriseren en die aanleiding geven tot een lagere statistische power. Een genetische risicoscore, bestaande uit POAG-varianten die eerder waren geïdentificeerd in Europese en Aziatische studies, was statistisch significant geassocieerd met POAG in onze studie. De individuele effectgroottes van de bestudeerde varianten waren daarentegen aanzienlijk kleiner in vergelijking met de Europese en Aziatische resultaten. Onze resultaten suggereren daarom dat gevestigde genetische risicofactoren een rol kunnen spelen bij POAG in SSA populaties, maar waarschijnlijk in mindere mate. De reeds bekende POAG varianten verklaren dus niet de hogere ziektelast die in deze populaties wordt aangetroffen.

Om de statistische power te verbeteren, hebben we ons aangesloten bij een internationaal genetisch consortium van POAG-onderzoeken. Dit consortium omvat meer dan 26 000 personen van SSA-afkomst of SSA-gemixte afkomst (The Genetics of Glaucoma in People

of African Descent (GGLAD) Consortium). In **Hoofdstuk 3.2** presenteren wij de resultaten van een grote GWAS die in dit consortium is uitgevoerd. Eén locus in het *APBB2*-gen bereikte genoomwijde significantie en kon gerepliceerd worden in onafhankelijk studies. Het *APBB2*-gen is betrokken bij de proteolytische verwerking van amyloïde precursoreiwitten. Deze variant is uniek voor Afrikaanse populaties. Het amyloïde precursoreiwit is nodig voor de normale ontwikkeling van de retina. Proteolytische verwerking produceert echter amyloïde beta-peptiden; die giftig zijn en verenigen om amyloïde plaques te vormen, een bekend kenmerk van de ziekte van Alzheimer. Immunohistochemische analyse van de retina en primaire visuele cortexweefsels van post-mortem Afro-Amerikanen, suggereerde een relatie tussen het *APBB2*-risico-allel, verhoogde *APBB2*-expressie in het netvlies en verhoogde amyloïde plaque-afzetting. Deze voorgestelde pathologische mechanismen vereisen echter nog verdere validatie in grotere onderzoeken.

Fenotypische en genetische associaties met glaucoom-endofenotypen

In **Hoofdstuk 4.1** hebben we de systemische en oculaire determinanten van peripapillaire retinale zenuwvezellaagdikte (pRNFLT) onderzocht in 8 populatiestudies van het E3 consortium. We bevestigden de eerder gerapporteerde associaties van leeftijd en sferisch equivalent met pRNFLT en identificeerden verschillende aanvullende factoren die geassocieerd zijn met pRNFLT. We vonden dat IOP, beroerte en hypertensie geassocieerd waren met dunnere pRNFLT. Ook vonden we een trend in verlaagde pRNFLT bij patiënten met dementie. Onze resultaten impliceren dat ook niet direct aan glaucoom gerelateerde ziekten pRNFLT-metingen kunnen beïnvloeden. Microvasculaire aandoeningen en neuronale degeneratie moeten daarom in beschouwing worden genomen bij het interpreteren van de pRNFLT bij screening en behandeling van glaucoom.

Hoofdstuk 4.2 onderzoekt nieuwe statistische methoden om fenotypisch en genetisch gecorreleerde glaucoom endofenotypen gezamenlijk te analyseren, en om pleiotrope genetische loci te vinden. We voerden een multi-trait GWAS uit van anatomische papil parameters (verticale cup-disc ratio, grootte van de neuroretinale rand van de papil en grootte van de papil), die een hoge genetische en fenotypische correlatie vertoonden. We identificeerden twee loci in *PPP1R36-PLEKHG3* en in de buurt van *SERPINE3* die repliceerden in onafhankelijke Aziatische cohorten.

Ten slotte, worden in **Hoofdstuk 5** de belangrijkste bevindingen van dit proefschrift besproken en geplaatst in de context van mijn visie op de pathofysiologie van glaucoom. Ook worden de uitdagingen besproken die we tegenkwamen bij het uitvoeren van dit onderzoek en doe ik suggesties voor toekomstig onderzoek.

Chapter 7

Epilogue

7.1 PHD PORTFOLIO

Summary of PhD training

Name PhD student:	Pieter Willem Marie Bonnemaijer
Erasmus MC Departments:	Ophthalmology and Epidemiology
Research school:	NIHES
PhD-period:	2013-2021
Supervisors:	prof.dr. C.C.W. Klaver, prof.dr.ir C.M. van Duijn, dr. A.A.H.J. Thiadens

	Year	Workload
1. PhD Training		
Courses		
Master of health Sciences, Genetic Epidemiology (NIHES)	2015 -2017	70
Scientific Integrity	2015	0.3
Seminars, symposia and workshops		
Rotterdam glaucoma evening (oral presentation), Rotterdam, The Netherlands	2015	1.0
Workgroup for Tropical Ophthalmology the Netherlands (oral presentation), Utrecht, The Netherlands	2015	1.0
5th European Eye Epidemiology meeting, London, UK	2015	0.3
Refereeravond Oogziekenhuis Rotterdam (oral presentation), Rotterdam, The Netherlands	2016	1.0
Limburgse Oogheelkundige Liga (LOL), Roermond, The Netherlands	2016	1.0
Ingenuity Pathway Analysis (IPA)	2016	0.3
7th European Eye Epidemiology annual meeting, Mainz, Germany	2017	0.6
Weekly seminars, department of Ophthalmology, Erasmus MC	2013 - 2017	2.0
National conferences		
Dutch Ophthalmology PhD Symposium, Nijmegen (oral presentation)	2015	1.0
Nederlands Oogheelkundig Gezelschap annual meeting, Groningen	2015	0.6

Dutch Ophthalmology PhD Symposium, Nijmegen (oral presentation)	2016	1.0
Nederlands Oogheelkundig Gezelschap annual meeting, Maastricht (oral presentation)	2016	1.0
Dutch Ophthalmology PhD Symposium, Nijmegen (oral presentation)	2017	1.0
Nederlands Oogheelkundig Gezelschap annual meeting, Maastricht (oral presentation)	2017	1.0

International conferences

South African Glaucoma Society conference, Stellenbosch, South Africa (oral presentation)	2014	1.0
Association for Research in Vision and Ophthalmology, Seattle WA, USA, (poster presentation)	2016	1.0
Association for Research in Vision and Ophthalmology, Baltimore MD, USA, (oral presentation)	2017	1.0
Association for Research in Vision and Ophthalmology, Honolulu, HI, USA, (poster presentation)	2018	1.0

2. Teaching – Supervising Master's theses

Supervising master research, Suzanne van Schaik (21 weeks)	2013
Supervising master research, Milou van Bruchem (21 weeks)	2013
Supervising master research, Hannah Hardjosantoso (21 weeks)	2014
Supervising master research, Katinka Snoek (21 weeks)	2014
Supervising master research, Chawan Amin (21 weeks)	2015
Supervising master research, Vicky Hokken(21 weeks)	2015
Co-supervisor PhD student, Sjoerd Driessen	2019-

3. Grant applications	**Awarded**	
Combined Ophthalmic research Rotterdam 2014, Rotterdam, the Netherlands;	EUR 101.000	Co-applicant
UitZicht 2014, Utrecht, the Netherlands	EUR 50.000	Co-applicant
Bright Focus Foundation 2015, USA	USD 100.000	Co-applicant
UitZicht 2015, Utrecht, the Netherlands	EUR 35.000	Co-applicant
Rotterdamse Stichting Blindenbelangen 2015, Rotterdam, the Netherlands	EUR 50.000	Co-applicant

Rotterdamse Stichting Blindenbelangen 2017, Rotterdam, the Netherlands	EUR 64.000	Co-applicant
Combined Ophthalmic research Rotterdam 2017, Rotterdam, the Netherlands;	EUR 144.000	Co-applicant
Combined Ophthalmic research Rotterdam 2019, Rotterdam, the Netherlands;	EUR 120.000	Co-applicant
Bright Focus Foundation 2020, USA	USD 150.000	Co-applicant

Other

Reviewer for several international peer-reviewed journals (Brain Research, IOVS, Journal of Ophthalmology)	2014-today
Treasurer Stichting Promeras (Erasmus MC representing body for PhD students)	2015- 2017

7.2 LIST OF PUBLICATIONS

Publications on which this thesis is based:

1. **Bonnemaijer, P.W.M.**, Cook, C., Nag, A., Hammond, C.J., van Duijn, C.M., Lemij, H.G., Klaver, C.C.W. & Thiadens, A. Genetic African Ancestry Is Associated With Central Corneal Thickness and Intraocular Pressure in Primary Open-Angle Glaucoma. Investigative Ophthalmology & Visual Science 58, 3172-3180 (2017).

2. **Bonnemaijer, P.W.M.**, Iglesias, A.I., Nadkarni, G.N., Sanyiwa, A.J., Hassan, H.G., Cook, C., Simcoe, M., Taylor, K.D., Schurmann, C., Belbin, G.M., Kenny, E.E., Bottinger, E.P., van de Laar, S., Wiliams, S.E.I., Akafo, S.K., Ashaye, A.O., Zangwill, L.M., Girkin, C.A., Ng, M.C.Y., Rotter, J.I., Weinreb, R.N., Li, Z., Allingham, R.R., Nag, A., Hysi, P.G., Meester-Smoor, M.A., Wiggs, J.L., Hauser, M.A., Hammond, C.J., Lemij, H.G., Loos, R.J.F., van Duijn, C.M., Thiadens, A., Klaver, C.C.W., Grp, G.S., Eyes Africa Genetics, C. & Consortium, N. Genome-wide association study of primary open-angle glaucoma in continental and admixed African populations. Human Genetics 137, 847-862 (2018).

3. Mauschitz, M.M., **Bonnemaijer, P.W.M.**, Diers, K., Rauscher, F.G., Elze, T., Engel, C., Loeffler, M., Colijn, J.M., Ikram, M.A., Vingerling, J.R., Williams, K.M., Hammond, C.J., Creuzot-Garcher, C., Bron, A.M., Silva, R., Nunes, S., Delcourt, C., Cougnard-Gregoire, A., Holz, F.G., Klaver, C.C.W., Breteler, M.M.B., Finger, R.P. & European Eye Epidemiology, E. Systemic and Ocular Determinants of Peripapillary Retinal Nerve Fiber Layer Thickness Measurements in the European Eye Epidemiology (E3) Population. Ophthalmology 125, 1526-1536 (2018).

4. **Bonnemaijer, P.W.M.**, van Leeuwen, E.M., Iglesias, A.I., Gharahkhani, P., Vitart, V., Khawaja, A.P., Simcoe, M., Hohn, R., Cree, A.J., Igo, R.P., Burdon, K.P., Craig, J.E., Hewitt, A.W., Jonas, J., Khor, C.C., Pasutto, F., Mackey, D.A., Mitchell, P., Mishra, A., Pang, C., Pasquale, L.R., Springelkamp, H., Thorleifsson, G., Thorsteinsdottir, U., Viswanathan, A.C., Wojciechowski, R., Wong, T., Young, T.L., Zeller, T., Atan, D., Aslam, T., Barman, S.A., Barrett, J.H., Bishop, P., Blows, P., Bunce, C., Carare, R.O., Chakravarthy, U., Chan, M., Chua, S.Y.L., Crabb, D.P., Cumberland, P.M., Day, A., Desai, P., Dhillon, B., Dick, A.D., Egan, C., Ennis, S., Foster, P., Fruttiger, M., Gallacher, J.E.J., Garway, D.F., Gibson, J., Gore, D., Guggenheim, J.A., Hardcastle, A., Harding, S.P., Hogg, R.E., Keane, P.A., Khaw, P.T., Lascaratos, G., Macgillivray, T., Mackie, S., Martin, K., McGaughey, M., McGuinness, B., McKay, G.J., McKibbin, M., Mitry, D., Moore, T., Morgan, J.E., Muthy, Z.A., O'Sullivan, E., Owen, C.G., Patel, P., Paterson, E., Peto, T., Petzold, A., Rahi, J.S., Rudnikca, A.R., Self, J., Sivaprasad, S., Steel, D., Stratton, I., Strouthidis, N., Sudlow, C., Thomas, D., Trucco, E.,

Tufail, A., Vernon, S.A., Viswanathan, A.C., Williams, C., Williams, K., Woodside, J.V., Yates, M.M., Yip, J., Zheng, Y., Allingham, R., Budenz, D., Bailey, J.C., Fingert, J., Gaasterland, D., Gaasterland, T., Haines, J.L., Hark, L., Hauser, M., Kang, J.H., Kraft, P., Lee, R., Lichter, P., Liu, Y., Moroi, S., Pasquale, L.R., Pericak, M., Realini, A., Rhee, D., Richards, J.R., Ritch, R., Scott, W.K., Singh, K., Sit, A., Vollrath, D., Weinreb, R., Wollstein, G., Wilmer, D.Z., Gerhold-Ay, A., Nickels, S., Wilson, J.F., Hayward, C., Boutin, T.S., Polasek, O., Aung, T., Khor, C.C., Amin, N., Lotery, A.J., Wiggs, J.L., Cheng, C.Y., Hysi, P.G., Hammond, C.J., Thiadens, A., MacGregor, S., Klaver, C.C.W., van Duijn, C.M., Int Glaucoma Genetics, C., Uk Biobank Eye Vision, C. & Consortium, N. Multi-trait genome-wide association study identifies new loci associated with optic disc parameters. Communications Biology 2(2019).

5. Hauser, M.A., Allingham, R.R., Aung, T., Van Der Heide, C.J., Taylor, K.D., Rotter, J.I., Wang, S.H.J., **Bonnemaijer, P.W.M.**, Williams, S.E., Abdullahi, S.M., Abu-Amero, K.K., Anderson, M.G., Akafo, S., Alhassan, M.B., Asimadu, I., Ayyagari, R., Bakayoko, S., Nyamsi, P.B., Bowden, D.W., Bromley, W.C., Budenz, D.L., Carmichael, T.R., Challa, P., Chen, Y.D.I., Chuka-Okosa, C.M., Bailey, J.N.C., Costa, V.P., Cruz, D.A., DuBiner, H., Ervin, J.F., Feldman, R.M., Flamme-Wiese, M., Gaasterland, D.E., Garnai, S.J., Girkin, C.A., Guirou, N., Guo, X.Q., Haines, J.L., Hammond, C.J., Herndon, L., Hoffmann, T.J., Hulette, C.M., Hydara, A., Igo, R.P., Jorgenson, E., Kabwe, J., Kilangalanga, N.J., Kizor-Akaraiwe, N., Kuchtey, R.W., Lamari, H., Li, Z., Liebmann, J.M., Liu, Y.T., Loos, R.J.F., Melo, M.B., Moroi, S.E., Msosa, J.M., Mullins, R.F., Nadkarni, G., Napo, A., Ng, M.C.Y., Nunes, H.F., Obeng-Nyarkoh, E., Okeke, A., Okeke, S., Olaniyi, O., Olawoye, O., Oliveira, M.B., Pasquale, L.R., Perez-Grossmann, R.A., Pericak-Vance, M.A., Qin, X., Ramsay, M., Resnikoff, S., Richards, J.E., Schimiti, R.B., Sim, K.S., Sponsel, W.E., Svidnicki, P.V., Thiadens, A., Uche, N.J., van Duijn, C.M., de Vasconcellos, J.P.C., Wiggs, J.L., Zangwill, L.M., Risch, N., Milea, D., Ashaye, A., Klaver, C.C.W., Weinreb, R.N., Koch, A.E.A., Fingert, J.H., Khor, C.C. & Genetics Glaucoma People, A. Association of Genetic Variants With Primary Open-Angle Glaucoma Among Individuals With African Ancestry. Jama-Journal of the American Medical Association 322, 1682-1691 (2019).

6. **Bonnemaijer, P.W.M.**, Lo Faro, V., Sanyiwa, A.J., Hassan, H.G., Cook, C., Van de Laar, S., Lemij, H.G., Klaver, C.C.W., Jansonius, N.M., Thiadens, A. & Grp, G.S. Differences in clinical presentation of primary open-angle glaucoma between African and European populations. Acta Ophthalmologica (2020).

Other publications

7. Iglesias, A.I., van der Lee, S.J., **Bonnemaijer, P.W.M.**, Hohn, R., Nag, A., Gharahkhani, P., Khawaja, A.P., Broer, L., Foster, P.J., Hammond, C.J., Hysi, P.G., van Leeuwen, E.M., MacGregor, S., Mackey, D.A., Mazur, J., Nickels, S., Uitterlinden, A.G., Klaver, C.C.W., Amin, N., van Duijn, C.M. & Iggc. Haplotype reference consortium panel: Practical implications of imputations with large reference panels. Human Mutation 38, 1025-1032 (2017).

8. Mutlu, U., **Bonnemaijer, P.W.M.**, Ikram, M.A., Colijn, J.M., Cremers, L.G.M., Buitendijk, G.H.S., Vingerling, J.R., Niessen, W.J., Vernooij, M.W., Klaver, C.C.W. & Ikram, M.K. Retinal neurodegeneration and brain MRI markers: the Rotterdam Study. Neurobiology of Aging 60, 183-191 (2017).

9. Nag, A., Lu, H., Arno, M., Iglesias, A.I., **Bonnemaijer, P**., Broer, L., Uitterlinden, A.G., Klaver, C.C.W., van Duijn, C., Hysi, P.G. & Hammond, C.J. Evaluation of the Myocilin Mutation Gln368Stop Demonstrates Reduced Penetrance for Glaucoma in European Populations. Ophthalmology 124, 547-553 (2017).

10. Iglesias, A.I., Mishra, A., Vitart, V., Bykhovskaya, Y., Hohn, R., Springelkamp, H., Cuellar-Partida, G., Gharahkhani, P., Bailey, J.N.C., Willoughby, C.E., Li, X.H., Yazar, S., Nag, A., Khawaja, A.P., Polasek, O., Siscovick, D., Mitchell, P., Tham, Y.C., Haines, J.L., Kearns, L.S., Hayward, C., Shi, Y., van Leeuwen, E.M., Taylor, K.D., **Bonnemaijer, P**., Rotter, J.I., Martin, N.G., Zeller, T., Mills, R.A., Staffieri, S.E., Jonas, J.B., Schmidtmann, I., Boutin, T., Kang, J.H., Lucas, S.E.M., Wong, T.Y., Beutel, M.E., Wilson, J.F., Uitterlinden, A.G., Vithana, E.N., Foster, P.J., Hysi, P.G., Hewitt, A.W., Khor, C.C., Pasquale, L.R., Montgomery, G.W., Klaver, C.C.W., Aung, T., Pfeiffer, N., Mackey, D.A., Hammond, C.J., Cheng, C.Y., Craig, J.E., Rabinowitz, Y.S., Wiggs, J.L., Burdon, K.P., van Duijn, C.M., MacGregor, S., Blue Mountains Eye Study, G.G., Consortium, N. & Wellcome Trust Case Control, C. Cross-ancestry genome-wide association analysis of corneal thickness strengthens link between complex and Mendelian eye diseases. Nature Communications 9(2018).

11. Mutlu, U., Colijn, J.M., Ikram, M.A., **Bonnemaijer, P.W.M.**, Licher, S., Wolters, F.J., Tiemeier, H., Koudstaal, P.J., Klaver, C.C.W. & Ikram, M.K. Association of Retinal Neurodegeneration on Optical Coherence Tomography With Dementia A Population-Based Study. Jama Neurology 75, 1256-1263 (2018).

12. Mutlu, U., Ikram, M.K., Roshchupkin, G.V., **Bonnemaijer, P.W.M.**, Colijn, J.M., Vingerling, J.R., Niessen, W.J., Ikram, M.A., Klaver, C.C.W. & Vernooij, M.W. Thinner retinal layers are associated with changes in the visual pathway: A population-based study. Human Brain Mapping 39, 4290-4301 (2018).

13. Iglesias, A.I., Ong, J.S., Khawaja, A.P., Gharahkhani, P., Tedja, M.S., Verhoeven, V.J.M., **Bonnemaijer, P.W.M.**, Wolfs, R.C.W., Young, T.L., Jansonius, N.M., Craig, J.E., Stambolian, D., van Duijn, C.M., MacGregor, S., Klaver, C.C.W., Iggc & Cream. Determining Possible Shared Genetic Architecture Between Myopia and Primary Open-Angle Glaucoma. Investigative Ophthalmology & Visual Science 60, 3142-3149 (2019).

14. Yonova-Doing, E., Zhao, W.T., Igo, R.P., Wang, C.L., Sundaresan, P., Lee, K.E., Jun, G.R., Alves, A.C., Chai, X.R., Chan, A.S.Y., Lee, M.C., Fong, A., Tan, A.G., Khor, C.C., Chew, E.Y., Hysi, P.G., Fan, Q., Chua, J., Chung, J., Liao, J.M., Colijn, J.M., Burdon, K.P., Fritsche, L.G., Swift, M.K., Hilmy, M.H., Chee, M.L., Tedja, M., **Bonnemaijer, P.W.M.**, Gupta, P., Tan, Q.N.S., Li, Z., Vithana, E.N., Ravindran, R.D., Chee, S.P., Shi, Y., Liu, W.T., Su, X.Y., Sim, X.L., Shen, Y., Wang, Y.X., Li, H.T., Tham, Y.C., Teo, Y.Y., Aung, T., Small, K.S., Mitchell, P., Jonas, J.B., Wong, T.Y., Fletcher, A.E., Klaver, C.C.W., Klein, B.E.K., Wang, J.J., Iyengar, S.K., Hammond, C.J. & Cheng, C.Y. Common variants in SOX-2 and congenital cataract genes contribute to age-related nuclear cataract. Communications Biology 3(2020).

15. Currant, H., Hysi, P., Fitzgerald, T.W., Gharahkhani, P., **Bonnemaijer, P.W.M.**, Senabouth, A., Hewitt, A.W., Atan, D., Aung, T., Charng, J., Choquet, H., Craig, J., Khaw, P.T., Klaver, C.C.W., Kubo, M., Ong, J.S., Pasquale, L.R., Reisman, C.A., Daniszewski, M., Powell, J.E., Pebay, A., Simcoe, M.J., Thiadens, A., van Duijn, C.M., Yazar, S., Jorgenson, E., MacGregor, S., Hammond, C.J., Mackey, D.A., Wiggs, J.L., Foster, P.J., Patel, P.J., Birney, E., Khawaja, A.P., Consortium, U.K.B.E.V. & Int Glaucoma Genetics, C. Genetic variation affects morphological retinal phenotypes extracted from UK Biobank optical coherence tomography images. Plos Genetics 17(2021).

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7.3 ABOUT THE AUTHOR

Pieter Willem Marie Bonnemaijer was born on the 3rd of March 1987 in Maastricht, the Netherlands. He graduated in 2005 from the Bisschoppelijk College Schöndelen in Roermond and thereafter started his studies in Law at Leiden University. Upon receiving his first year certificate in law he went to study medicine at the Erasmus MC in Rotterdam, the Netherlands. During his elective research program he joined a new research initiative, led by prof. dr. Caroline Klaver and dr. Alberta Thiadens, to study the genetics of primary open-angle glaucoma in African populations. For this elective course he moved to Dar es Salaam, Tanzania, where he started the inclusion of patients in the Genetics in Glaucoma of people from African descent study (GIGA study). This study was conducted at Muhimbili University of Allied Sciences and the Comprehensive Community Based Rehabilitation in Tanzania (CCBRT), under the supervision of dr. Anna Sanyiwa and dr. Alberta Thiadens. At the conclusion of his elective research period in Africa, Pieter graduated from medical school in 2013. The vibrant research setting at the Erasmus MC together with the great experience he got in Tanzania, motivated him to continue this research as PhD candidate. The work from the GIGA study is presented in this thesis. From 2013 – 2014 Pieter moved back to Tanzania where he expanded the GIGA study to other hospitals in Tanzania and started a new research site at the Ophthalmology department in the Groote Schuur Hospital in Cape Town, South Africa, that was headed by prof. dr. Colin Cook. When returning to the Netherlands early 2015 he started a master degree in Genetic Epidemiology at the Netherlands Institute of Health Sciences (NIHES) from which he graduated in 2017. Since December 2017, Pieter is a resident in ophthalmology at the Rotterdam Eye Hospital and currently finalizing his training. In addition he is still involved in the GIGA study, and helped to set up a new research site at the ophthalmology department at Komfo Anokey Teaching Hospital (Kumasi, Ghana) which is in collaboration with glaucoma specialist Angelina Ampong and Doreen Nelson. Currently, Pieter also supervises his successor Sjoerd Driessen in his PhD training.

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Pieter Bonnemaijer
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WODI YA MACHO
TAKA


OPERATION ROOM
INJECTION ROOM
TAKA


DOCTOR ROOM
EXIT
HE'S WALK'
HUJAFA HUJAUMBIKA
USICHEKE'
KARIBUNI SANA CCBRT
HERE