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Heritability of glaucoma and glaucoma-related endophenotypes: Systematic review and meta-analysis protocol

Nigus Gebrmedhin Asefa[1,a,*,‡], Anna Neustaeter[2,b,‡], Nomdo Jansonius[2,†], Harold Snieder[1,††]

[1]Department of Epidemiology, University of Groningen, University Medical Center Groningen, The Netherlands

[2]Department of Ophthalmology, University of Groningen, University Medical Center Groningen, The Netherlands

[†]Professor of Ophthalmology, [††]Professor of Genetic Epidemiology

[a]MSc in Public Health, [b]MSc in Quantitative Genomics

[‡]Both authors contributed equally to this work.

Email address:

Nigus Gebrmedhin Asefa- n.g.asefa@umcg.nl, alternative: niguurayu2003@gmail.com

Anna Neustaeter- a.neustaeter@umcg.nl

Nomdo Jansonius- n.m.jansonius@umcg.nl

Harold Snieder- h.snieder@umcg.nl

***Corresponding author:** n.g.asefa@umcg.nl

Alternative email: niguurayu2003@gmail.com

Department of Epidemiology, University of Groningen, University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands

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Abstract

Introduction: Glaucoma is the second-leading cause of age-related vision loss worldwide; it is an umbrella term that is used to describe a set of complex ocular disorders with multifactorial etiology. Both genetic and lifestyle risk factors for glaucoma are well established. Thus far, however, systematic reviews on the heritability of glaucoma have focused on the heritability of primary open-angle glaucoma only. No systematic review has comprehensively reviewed or meta-analyzed the heritability of other types of glaucoma, including glaucoma-related endophenotypes. The aim of this study will be to identify relevant scientific literature regarding the heritability of both glaucoma and glaucoma-related endophenotypes, and summarize the evidence by performing a systematic review and meta-analysis.

Methods and analysis: This systematic review will follow the Meta-analyses of Observational Studies in Epidemiology (MOOSE) criteria checklist, which provides a standardized approach for carrying out systematic reviews, including assessing for bias and heterogeneity. To capture as much literature as possible, a comprehensive step-by-step systematic search will be undertaken in MEDLINE (PubMed), EMBASE, Web of Science, and ScienceDirect. Two reviewers will independently search the articles for eligibility according to predefined selection criteria. A database will be used for screening of eligible articles. The quality of the included studies will be rated independently by two reviewers, using the National Health Institute Quality Assessment tool for Observational Cohort and Cross-Sectional Studies. A random effect model will be used for the meta-analysis. This systematic review is registered with the International Prospective Register of Systematic Reviews (PROSPERO) with a registration number: CRD42017064504.

Ethics and dissemination: We will use secondary data from peer-reviewed published articles, and hence there is no requirement for ethics approval. The results of this systematic review will be disseminated through publication in a peer-reviewed scientific journal.

Strengths and limitations of this study

- This systematic review will not only report meta-analyses of heritability estimates for primary open angle glaucoma, but also for primary angle closure glaucoma, other forms of glaucoma and glaucoma-related endophenotypes.
- The study will cover heritability reports from different study designs (for example, twin vs family vs genome-wide association study) and statistical estimation methods (correlations vs maximum likelihood estimation); as well present a detailed discussion on the possible factors that might explain the variation.
- The absence of a standard list of glaucoma-related endophenotypes may lead to inadvertent exclusion of potentially appropriate traits.
- Heritability depends on varying environmental circumstances in the populations studied; consequently, heritability estimates may be different between populations. This may limit the interpretation of the weighted heritability estimates based on the meta-analyses.

Introduction

The eye is one of the most important sense organs, and vision loss may generate various degrees of psychological suffering, greater than the distress resulting from other forms of sensory impairment.[1] According to a 2010 World Health Organization (WHO) estimate, there are 285 million people visually impaired, of which 39 million are blind.[2] The prevalence of infection-related blindness is decreasing globally, however, age-related blindness is increasing throughout the world; this could be due to an increasingly aged population, or technological advancements in screening for blindness.[3]

Some diseases of the eye are more likely to occur in old-age. Cataract, glaucoma, diabetic retinopathy and macular degeneration are the most common age-related eye diseases.[4–7] The prevalence of these disorders is distributed differently across different ethnicities and socioeconomic backgrounds.[6–8] Visual functions such as visual acuity, visual field, and night vision deteriorate as these eye disorders progress. But age is not the only risk factor; many of these ocular disorders have a genetic component as well.[9–12]

Among the age-related ocular disorders, "Glaucoma" is an umbrella term used to describe a group of multifactorial complex diseases, and disparities exist in its classification.[13] The International Society for Geographical and Epidemiological Ophthalmology (ISGEO) has developed a robust definition of glaucoma for epidemiological purposes by including several empirical factors, such as optic nerve head findings and visual field defects.[14] Traditionally, glaucoma can be defined as, "a multifactorial optic neuropathy associated with characteristic structural changes to the optic nerve and visual function".[13,14] It is asymptomatic until it is severe, thus many patients have a delay in diagnosis, or are examined only after the advanced visual field loss has occurred.[13,15]

Glaucoma is classified into primary and secondary categories. Important risk factors for primary glaucoma are intraocular pressure, age, and family history; however, the biomolecular mechanisms are still poorly defined.[16–20] Secondary glaucoma is a heterogeneous group of diseases resulting from other eye diseases, trauma, use of corticosteroids, or conditions such as pigment dispersion or pseudoexfoliation.[21,22]

Primary glaucoma may be subdivided in primary open-angle glaucoma (POAG) and primary angle closure glaucoma (PACG). Although POAG is the most common type of glaucoma, PACG tends to be relatively more common in certain ethnic groups.[13,20,23] In a 2014 systematic-review, the global prevalence of POAG and PACG combined was estimated to be approximately 4% within a population aged 40-80 years.[23] The prevalence of visual impairment due to glaucoma appears to be age-related, and within a randomly selected sample of 5,147 Australians, the prevalence was found to be very low in 60-year-olds (~1%), compared to those older than 90 years (4%).[4] A systematic review of 50 population-based studies reported that the prevalence of POAG is relatively high in African populations aged ≥40, with an approximate prevalence of 2-4% (23, 24)[23,24]. On the other hand, the prevalence of PACG is found to be relatively higher (~1%) in adult Asian populations.[20,23]

Studies have demonstrated a strong association between the development of POAG and a positive family history.[10,24–28] In a longitudinal study of 224 siblings, from 156 probands (who were clinically confirmed POAG cases), there was a significantly increasing trend in both prevalence and incidence of the disease, with age and a lifetime risk estimated of approximately 20% by age 70.[26] Other studies also suggested that the risk of POAG is higher in siblings of glaucoma cases than their parents or children.[25,27,28] In a population based study in the USA, the risk of open angle glaucoma among siblings of POAG cases was about 10% (with an odds ratio [OR] of 3.69), which is greater than in parents (~6%; OR 2.17), and in children (~1%; OR 1.12) (27). Studies from the Netherlands and India reported similar findings, with a higher prevalence of POAG in siblings (~10% to 15%) than in parent-offspring family connections (~1% to 4%), respectively.[25,28]

Historically, the effect of specific genes in the development of glaucoma has been largely unknown. In the 1960s, Becker et al. studied patients with POAG glaucoma as well as their relatives, and proposed that open-angle glaucoma was a genetically determined disease, where the recessive homozygous ‘gg’ genotype represents glaucoma, and the alternative homozygous ‘nn’ and the heterozygous ‘ng’ genotypes represent non-glaucoma.[29] More recently however, researchers have elucidated both causative and associative genes for glaucoma risk.[18,30–32]

The source of phenotypic variation among individuals in a population originates from both environmental and genetic factors, as well as the various interactions between them.[30]

Heritability (h^2) can be defined as, "the proportion of variance in a particular trait due to variation in genetic factors among individuals in that population".[33–35] The total variation (variance) in a phenotype (V_P) can then be broken down into two parts: the genotypic variance (V_G) and the remaining variance (V_E), due to the environment.[34,35]

Within the last thirty years, classical twin studies have been conducted to establish the relative importance of genes and environment in glaucoma risk. Twin studies are an excellent source of information to disentangle and quantify the contributions of genes, the shared environment, the unique environment, and their interactions with respect to complex traits.[36,37]

Population-based genetic studies continue to confirm that many ocular traits have a genetic component.[9] These traits show substantial variation in human populations and are highly heritable, and thus likely to be influenced at least in part by genes.[9,24,29,30,38–41] Glaucoma related endophenotypes, sometimes called intermediate phenotypes, are powerful tools in the identification of genes contributing to glaucoma as they are more likely to be directly influenced by the genes than the resulting disease itself.[42–44] Traits such as central corneal thickness, optic cup area, optic disc area, vertical cup-to-disk ratio, and intraocular pressure are some well-established endophenotypes for the disease.[42]

Heritability estimates for glaucoma endophenotypes differ between studies. For example, for intraocular pressure, heritability estimates range from h^2=0.35, in a total of 2,620 subjects from extended pedigrees from The Netherlands,[38] to h^2=0.5 in 133 subjects from nuclear family groups in the USA.[39] Similarly, the heritability estimates for optic disc parameters range from 0.66 to 0.77 for optic cup area, 0.52 to 0.83 for disc area, and 0.48 to 0.66 for vertical cup-to-disk ratio.[9]

So far, systematic reviews on the heritability of glaucoma have focused on the heritability of primary open-angle glaucoma only.[9,24] Indeed, no systematic review has comprehensively reviewed nor meta-analyzed the heritability of other types of glaucoma, or glaucoma-related endophenotypes. Accurate estimates of genetic risk (i.e., heritability) are imperative when studying diseases with differing prevalences in different ethnicities, it will be an important factor in the near future when patients' genotypes may be used for personalized estimates of disease risk, and it is also a prerequisite for further gene finding studies. Heritability estimates are to be specific to the disease being studied (for example POAG vs PACG), the populations studied (for

example Caucasians vs Asians), and the particular circumstances from which they were derived.[9,40] A systematic review and meta-analysis of the genetic contribution to glaucoma and glaucoma-related endophenotypes will thus provide important insights and assist researchers in designing gene finding studies in the future.

The objective of this systematic review will be to identify relevant studies regarding the heritability of glaucoma and glaucoma-related endophenotypes, and summarize the evidence through meta-analysis. Heritability estimation, commonly reported in %, is the outcome measurement that we will synthesize and report from several studies.

The current study will address the following research questions. How much of the variance in glaucoma and glaucoma-related endophenotypes is due to genetic factors (1)? What is the proportion of variance accounted for by additive genetic influences (A), common environment (C), and unique environment (E) (2)? Do heritability estimates vary between different populations and study designs (3)?

Methods and analysis

This systematic review was initiated in March 2017 and is registered with the International Prospective Register of Systematic Reviews (PROSPERO) with a registration number: CRD42017064504, available at http://www.crd.york.ac.uk/PROSPERO/. This systematic review will follow the Meta-analyses of Observational Studies in Epidemiology (MOOSE) criteria checklist, which consists of a list of 35 items that provide a standardized guide for carrying out systematic reviews, including construction of a protocol, testing for bias and heterogeneity, and other aspects of the review process.[45] Similarly, the quality of the individual studies included in the systematic review will be rated independently by two reviewers using the National Health Institute Quality Assessment tool for Observational Cohort and Cross-Sectional Studies.[46]

Inclusion criteria

Articles describing heritability results based on (1) family (2) twin (3) adoption and (4) GWAS study designs will be included. The search will be restricted to articles describing studies in human subjects written in English language. However, papers written in other languages with at least an English abstract will also be considered. All heritability studies from peer-reviewed journals, published till 30 September 2017, will be included.

Exclusion criteria

Papers that did not estimate heritability or specify ethnicity will be excluded.

Search strategy

To capture as much literature as possible, an initial limited search of MEDLINE will be performed using an initial set of search terms. This will be followed by the identification of additional search terms from the titles and abstracts, and from the Medical Subject Heading (MeSH) index terms used to describe the initially identified articles (Table 1). Second, using all identified keywords and index terms, a comprehensive, step-by-step systematic search will be undertaken in MEDLINE (PubMed), EMBASE, Web of Science, and ScienceDirect. In addition, Google scholar will be used as a supplementary search database. Third, relevant papers from the reference lists of those articles captured in step two will be manually searched for additional input. References will be exported to RefWorks citation management software and duplicates will be removed. Full text as well as relevant data of all selected papers will be retrieved, and authors of the original articles will be contacted by email if additional information is required.

Two reviewers will independently evaluate the abstracts for eligibility, according to predefined selection criteria. A database will be used for screening of eligible articles. Any disagreements will be resolved through discussion between the two evaluators, but if consensus cannot be reached, a third person will be consulted. Finally, selected publications will be approved by a senior investigator.

Table 1 Search string and number of articles found from a preliminary PubMed search

Step	Searching terms	# of articles found[1]
#1	"Quantitative Trait, Heritable"[MeSH][2] OR "Endophenotypes"[MeSH] OR Heritab*[tiab]	35,050
#2	Glaucoma[MeSH] OR Glaucoma*[tiab][3]	61,470
#3	Normal tension glaucoma[MeSH] OR Low tension glaucoma[MeSH] OR Exfoliation Glaucoma[MeSH] OR pseudoexfoliation glaucoma[MeSH] OR Exfoliation Syndrome[MeSH] OR Pigment dispersion syndrome[MeSH] OR Pigment dispersion syndrome[tiab] OR pds[tiab] OR Congenital glaucoma [MeSH] OR Buphthalmos[tiab] OR Buphthalmus[tiab] OR "Juvenile glaucoma"[tiab]	18,799
#4	"Intraocular pressure"[tiab] OR "Ocular pressure"[tiab] OR iop[tiab] OR "Ocular hypertension"[tiab] OR "ocular biometric"[tiab] OR "Central corneal thickness"[tiab] OR "Corneal shape"[tiab] OR "Axial length"[tiab] OR Linear cup-disk ratio OR lcdr OR "Vertical cup to disc ratio"[tiab] OR Vertical cup-to-disc ratio[tiab] OR vcdr[tiab] OR vertical cup:disc ratio[tiab] OR vertical cup-disc ratio[tiab] OR "Corneal hysteresis"[tiab] OR "Anterior chamber depth"[tiab] OR "Anterior chamber angle" OR "Narrow anterior chamber" OR "Optic disc diameter"[tiab] OR "Cup area"[tiab] OR "Disc area"[tiab] OR "Rim area"[tiab] OR "Retinal nerve fiber layer"[tiab] OR "Cilioretinal arteries"[tiab] OR "Retinal ganglion cell layer" OR Horizontal cup:disc ratio[tiab] OR "Disc diameter"[tiab] OR "Cup disc ratio"[tiab] OR "Ganglion cell complex thickness"[tiab] OR "Shallow anterior chamber"[tiab] OR "Iris thickness"[tiab] OR "Iris area"[tiab] OR "Plateau iris" OR "Pupil diameter"[tiab] OR "Pupil size"[tiab] OR "Iridotrabecular angle width"[tiab] OR "Bruch's membrane opening" OR "Neuroretinal rim" OR Excavation[tiab] OR Cupping[tiab] OR "Inner plexiform layer thickness"[tiab]	50,571
#5	#2 OR #3 OR #4	94,270
#6	#5 AND #1	176

[1] Preliminary search conducted on June 21, 2017.
[2] Medical subject heading
[3] Title abstract text word

Quality control and data extraction

For assessing the quality of individual articles in a systematic review, there are a variety of standard tools currently in use. However, most of these tools failed to include critical assessment elements relevant to heritability and genetic studies.[47]

We found that National Health Institute Quality Assessment tool for Observational Cohort and Cross-Sectional Studies[46] is relatively relevant to assess the quality of selected articles in this current study, and will therefore be rated using this tool. The heterogeneity of heritability estimation between articles will also be reported using Cochrane's Q test and I^2-statistic. These tests assess whether there are genuine differences underlying the results of the studies, or the variation in findings is through chance alone. The presence of any potential publication bias will be graphically visualized on a funnel plot, and any asymmetry of the funnel plot will be reported with an Egger's test. If a substantially higher percentage of heterogeneity is observed among studies, I^2-statistic >75%, subgroup meta-analysis will be carried out.

The quality of the current study will be ensured by following the MOOSE criteria checklist, which guides the reviewer in planning and carrying out systematic reviews with an observational study design (45)[45] (Supplementary file_1). The full text of the potentially eligible articles will be retrieved and stored in an online citation manager (RefWorks), for easy accessibility and data synthesis.

The data extraction for selected articles will be achieved in a database. In order to ensure all relevant data are collected per study, a standardized form will be utilized (Supplementary file_2). To minimize the risk of transcription errors, data extraction will be conducted independently by two reviewers. The number of articles reviewed, the number of full-text studies retrieved, and the number of studies excluded will be outlined using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart (Supplementary file_3).

Data synthesis

Presuming that heritability estimates are different between populations, we will use a random effect model for meta-analyses. Extracted data will be presented in tables, plots, and graphs, accordingly. Pooled heritability estimates and summary statistics for quantitative data will be presented and described. Quantitative assessment of heterogeneity in findings between studies

and publication bias will be performed and reported. Heritability results across different study designs and statistical methods, as well as the possible factors that might explain the variation in heritability, will be discussed in detail.

Subgroup analysis

Glaucoma is a group of eye diseases characterized by irreversible retinal ganglionic cell death and progressive visual field loss. The current study will report not only the meta-analysis of heritability estimates for glaucoma, but also for glaucoma-related endophenotypes. Consequently, subgroup analysis will be performed on the different types of glaucomas and endophenotypes related to ocular pressure, anterior and posterior eye traits.

Ethical consideration and result dissemination

This systematic review will use secondary data from peer-reviewed published articles, and hence there is no requirement for ethics approval. The results of this systematic review will be disseminated through publication in a relevant, peer-reviewed journal and presented at pertinent conferences.

Authors contribution

Study protocol conception and design: NGA, HS. Search strategy development: NGA, AN, NJ. Protocol quality control design and development: NGA, AN, NJ. Data extraction design: NGA, AN, HS, NJ. Drafting of manuscript: NGA, AN. Critical evaluation and revision of the manuscript: HS, NJ.

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Competing interests

There are no competing interests.

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Meta-analysis Of Observational Studies in Epidemiology (MOOSE) checklist

	Criteria	Yes/No	Page
1. Reporting of background should include			
1.1	Problem definition	Yes	Explained in detail in the introduction, pages 4-5.
1.2	Hypothesis statement	Yes	Included in the introduction, pages 5.
1.3	Description of study outcome(s)	Yes	Included in the outcome measure section, page 7.
1.4	Type of exposure or intervention used	No	NA
1.5	Type of study designs used	Yes	Explained in the inclusion and exclusion criteria, page 7.
1.6	Study population	Yes	Explained in the inclusion and exclusion criteria, page 7.
2. Reporting of search strategy should include			
2.1	Qualifications of searchers (e.g, librarians and investigators)	Yes	Two investigators, NGA and AN will do the searches independently. NGA is a PhD candidate in the department of Genetic Epidemiology, University of Groningen, and holds a Masters Degree in Public Health. AN is MSc holder in Quantitative Genomics and is a PhD candidate in the department of Ophthalmology, University of Groningen. Available on the cover page.
2.2	Search strategy, including time period included in the synthesis and keywords	Yes	Described in page 7.
2.3	Effort to include all available studies, including contact with authors	Yes	Stated in the search strategy, page 7.
2.4	Databases and registries searched	Yes	PubMed, EMBASE, Web of Science and ScienceDirect, (Supplementary file_1). Page 7.
2.5	Search software used, name and version, including special features	No	No search software used.
2.6	Use of hand searching	Yes	Stated in the search strategy, page 7.
2.7	List of citations located and those excluded, including justifications	Yes	We will present the process of search and study selection using PRISMA flow

	Criteria	Yes/No	Page
			process chart. Described on page 9.
2.8	Method of addressing articles published in languages other than English	Yes	Literatures with English language will be included. However, papers written in other languages with at least an English abstract will also be considered, page 7.
2.9	Method of handling abstracts and unpublished studies	Yes	Only published and full text articles will be used. This is stated in the search strategy, page 7.
2.10	Description of any contact with authors	Yes	When it is necessary, authors of original articles will be contacted. Stated in the search strategy, page 7.
3. Reporting of methods should include			
3.1	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Yes	Stated in inclusion and exclusion criteria, page 7.
3.2	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	Yes	Stated in page 7.
3.3	Documentation of how data were classified and coded (eg, multiple raters, blinding, and interrater reliability)	Yes	Two reviewers, NGA and AN, will independently search the articles for eligibility according to predefined selection criteria. Any disagreements will be resolved by discussion between the two reviewers, but if consensus cannot be reached, a third reviewer will be consulted. Page 7-9.
3.4	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	Yes	The presence of any potential publication bias will be reported with an Egger's test. Page 9.
3.5	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Yes	The quality of individual articles will be checked using National Health Institute Quality Assessment tool for Observational Cohort and Cross-Sectional Studies. Two reviewers will independently assess the quality each articles. Quality control and data extraction section states about this (Supplementary information_3), page 7-9.
3.6	Assessment of heterogeneity	Yes	Using Cochrane's Q test and I^2-statistic, the heterogeneity of the articles will be reported. Quality control section, page 9.

	Criteria	Yes/No	Page
3.7	Description of statistical methods(eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	Yes	Random effect model meta-analysis will be used. Data synthesis section described details of statistical methods, page 9-10.
3.8	Provision of appropriate tables and graphics	Yes	The number of articles searched and the number of studies excluded together with the reasons for exclusion will be briefly outlined using a PRISMA flow process chart. Page 9.
4. Reporting of results should include			
4.1	Graph summarizing individual study estimates and overall estimate	Yes	Extracted data will be presented in tables, plots and graphs, accordingly. Pooled heritability estimates (displayed with forest plot) and summary statistics for quantitative data will be presented and described. Stated in page 9.
4.2	Table giving descriptive information for each study included	Yes	Extracted data will be presented in tables and graphs, accordingly. Stated in page 9.
4.3	Results of sensitivity testing (eg, subgroup analysis)	Yes	If higher percentage of heterogeneity is observed among studies, I^2-statistic >75%, subgroup meta-analysis will be carried out. Subgroup analysis will be performed on the different types of glaucomas and endophenotypes related to ocular pressure, anterior and posterior eye traits. Page 9.
4.4	Indication of statistical uncertainty of findings	Yes	Weighted point estimate and its 95% confidence intervals will be reported. Explained in the data synthesis section, page 9.
5. Reporting of discussion should include			
5.1	Quantitative assessment of bias (eg, publication bias)	Yes	The presence of any potential publication bias will be reported with an Egger's test Quality control section, page 9-10.
5.2	Justification for exclusion	Yes	We will present the process of search and study selection using PRISMA flow

	Criteria	Yes/No	Page
			chart. The quality control and data extraction section, described about this issue. Page 9.
5.3	Assessment of quality of included studies	Yes	The quality of individual articles will be checked using National Health Institute Quality Assessment tool for Observational Cohort and Cross-Sectional Studies. Page 9.
6. Reporting of conclusions should include			
6.1	Consideration of alternative explanations for observed results	Yes	We will discuss on the possible factors that might explain the deviation of observed results from what is expected. Page 9-10.
6.2	Generalization of the conclusions	Yes	A conclusion on general interpretation of the findings, in the context of research questions together with implications for future research, will be drawn.
6.3	Guidelines for future research	Yes	Depending on the study findings, we will present clear recommendations and discuss the actions that future researchers should take.
6.4	Disclosure of funding source	Yes	The source of funding is disclosed, page 10.

Heritability of glaucoma and glaucoma-related endophenotypes: Systematic review and meta-analysis, 2017

Data extraction form

Article ID	Publication Year	Volume	Issue
Authors			
Title			
Abstract			

Country of Study	Ethnicity	Study Design	Data Analysis Method	Sample Size	Mean Age	Gender

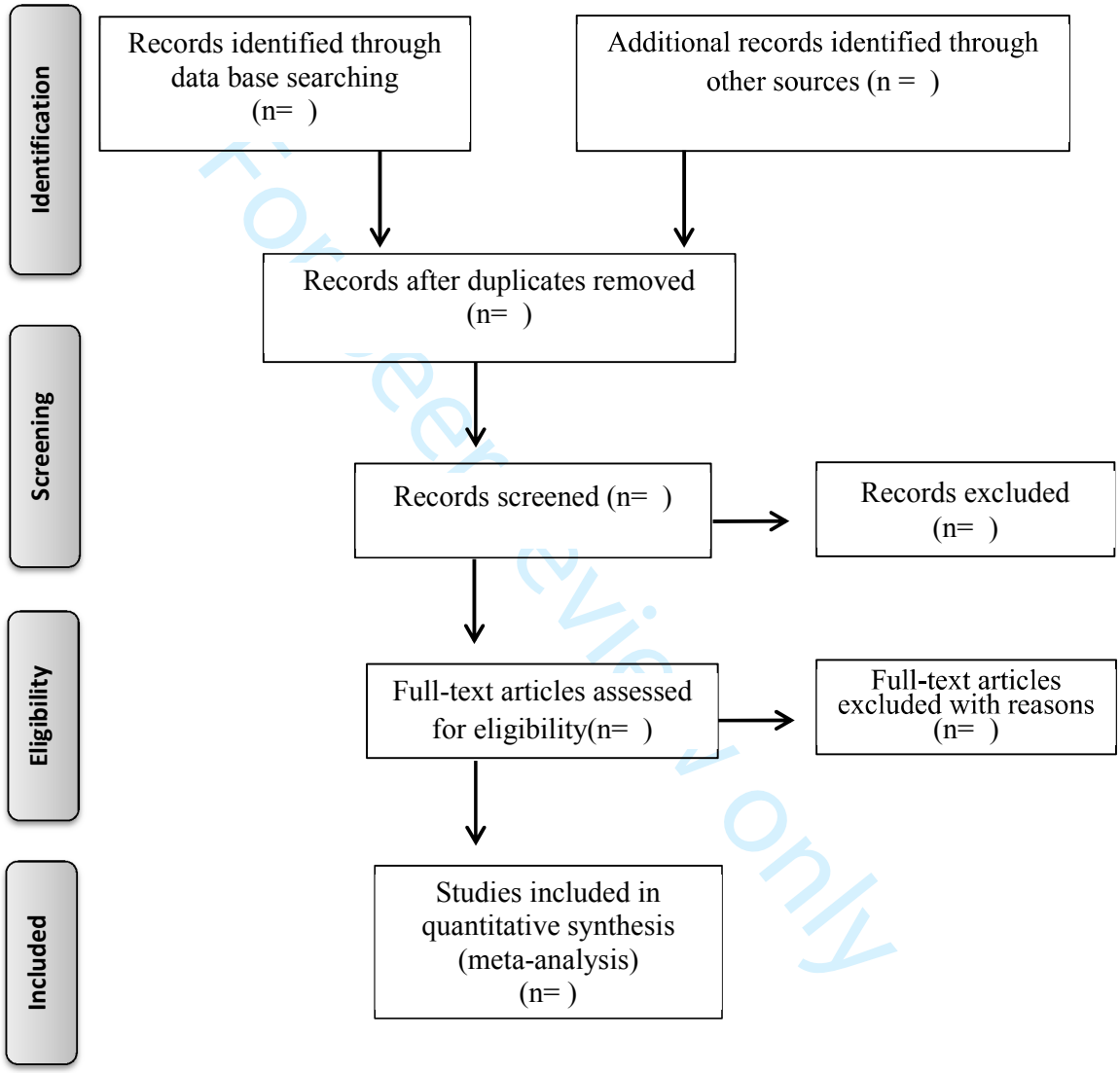
Glaucoma Type	Endophenotype 1	Endophenotype 2	Endophenotype 3
Glaucoma Type-Heritability (h^2)	Heritability (h^2)	Heritability (h^2)	Heritability (h^2)

The Proportion of Variance Accounted by:	Additive Genetic variance (A)	Common Environment (C)	Unique Environment (E)

Additional Comments

Heritability of glaucoma and glaucoma-related endophenotypes: Systematic review and meta-analysis, 2017

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart



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Heritability of glaucoma and glaucoma-related endophenotypes: Systematic review and meta-analysis protocol

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Heritability of glaucoma and glaucoma-related endophenotypes: Systematic review and meta-analysis protocol

Nigus Gebrmedhin Asefa[1,a,*,‡], Anna Neustaeter[2,b,‡], Nomdo Jansonius[2,†], Harold Snieder[1,††]

[1]Department of Epidemiology, University of Groningen, University Medical Center Groningen, The Netherlands

[2]Department of Ophthalmology, University of Groningen, University Medical Center Groningen, The Netherlands

[†]Professor of Ophthalmology, [††]Professor of Genetic Epidemiology

[a]MSc in Public Health, [b]MSc in Quantitative Genomics

[‡]Both authors contributed equally to this work.

Email address:

Nigus Gebrmedhin Asefa- n.g.asefa@umcg.nl, alternative: niguurayu2003@gmail.com

Anna Neustaeter- a.neustaeter@umcg.nl

Nomdo Jansonius- n.m.jansonius@umcg.nl

Harold Snieder- h.snieder@umcg.nl

***Corresponding author:** n.g.asefa@umcg.nl

Alternative email: niguurayu2003@gmail.com

Department of Epidemiology, University of Groningen, University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands

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Abstract

Introduction: Glaucoma is the second-leading cause of age-related vision loss worldwide; it is an umbrella term that is used to describe a set of complex ocular disorders with a multifactorial etiology. Both genetic and lifestyle risk factors for glaucoma are well established. Thus far, however, systematic reviews on the heritability of glaucoma have focused on the heritability of primary open-angle glaucoma only. No systematic review has comprehensively reviewed or meta-analyzed the heritability of other types of glaucoma, including glaucoma-related endophenotypes. The aim of this study will be to identify relevant scientific literature regarding the heritability of both glaucoma and related endophenotypes and summarize the evidence by performing a systematic review and meta-analysis.

Methods and analysis: This systematic review will follow the PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist, which provides a standardized approach for carrying out systematic reviews. To capture as much literature as possible, a comprehensive step-by-step systematic search will be undertaken in MEDLINE (PubMed), EMBASE, Web of Science, and ScienceDirect and studies published until 31st December 2017, will be included. Two reviewers will independently search the articles for eligibility according to predefined selection criteria. A database will be used for screening of eligible articles. The quality of the included studies will be rated independently by two reviewers, using the National Health Institute Quality Assessment tool for Observational Cohort and Cross-Sectional Studies. A random effects model will be used for the meta-analysis. This systematic review is registered with the International Prospective Register of Systematic Reviews (PROSPERO) with a registration number: CRD42017064504.

Ethics and dissemination: We will use secondary data from peer-reviewed published articles, and hence there is no requirement for ethics approval. The results of this systematic review will be disseminated through publication in a peer-reviewed scientific journal.

Strengths and limitations of this study

- The inclusion of endophenotypes, in addition to that of the heritability of glaucoma itself, is a novel approach of our meta-analysis and systematic review providing important information for genetic research of glaucoma.
- Possible heterogeneity in heritability estimates will be explored through conducting subgroup/sensitivity analyses.
- Heritability estimates derived from different data analysis methods may not be directly comparable.
- A straightforward interpretation of weighted heritability estimates in this meta-analyses may be complicated by the variation of heritability estimates between different environments and populations.

Introduction

The eye is one of the most important sense organs, and vision loss may generate various degrees of psychological suffering that can be greater than the distress resulting from other forms of sensory impairment [1]. According to a 2010 World Health Organization (WHO) estimate, there are 285 million people visually impaired, of which 39 million are blind [2]. The prevalence of infection-related blindness is decreasing globally, however, age-related blindness is increasing throughout the world; this could be due to an increasingly aged population, or technological advancements in screening for blindness [3].

Some ocular diseases are more likely to occur in old-age. Cataract, glaucoma, diabetic retinopathy and macular degeneration are the most common age-related eye diseases [4–7]. The prevalence of these disorders varies with different ethnicities and socioeconomic backgrounds [6–8]. Ocular-functions such as visual acuity, visual field, and night vision deteriorate as these eye disorders progress. But age is not the only risk factor; many of these disorders have a genetic component as well [9–12].

Among the age-related ocular disorders, glaucoma is the leading cause of irreversible blindness worldwide [13], and disparities exist in its classification [14]. The International Society for Geographical and Epidemiological Ophthalmology (ISGEO) has developed a robust definition of glaucoma for epidemiological purposes by including several empirical factors, such as optic nerve head findings and visual field defects [15]. Glaucoma is an umbrella term that is used to describe a set of complex ocular disorders with multifactorial etiology [14,15]. It can be defined as a progressive loss of retinal ganglion cells associated with characteristic structural changes to the optic nerve and visual function [16]. It is asymptomatic until it is severe, thus many patients have a delay in diagnosis, or are examined only after the advanced visual field loss has occurred [14,17]. Glaucoma is classified into primary and secondary categories. Important risk factors for primary glaucoma are intraocular pressure, age, and family history; however, the biomolecular mechanisms are still poorly defined [18–22]. Secondary glaucoma is a heterogeneous group of diseases resulting from: other eye diseases, trauma, use of corticosteroids, or conditions such as pigment dispersion or pseudoexfoliation [23,24].

Primary glaucoma may be subdivided into primary open-angle glaucoma (POAG) and primary angle closure glaucoma (PACG). Although POAG is the most common type of glaucoma, PACG

tends to be more prevalent in certain ethnic groups [13,22]. In a 2014 systematic review, the global prevalence of POAG and PACG combined was estimated to be approximately 4% in a population aged 40-80 years [13]. The prevalence of visual impairment due to glaucoma appears to be age-related as well, and in a randomly selected sample of 5,147 Australians, the prevalence was found to be low in 60-year-olds (~1%), compared to those older than 90 (4%) [4]. A systematic review of 50 population-based studies reported that the prevalence of POAG is relatively high in African populations aged ≥40, with an estimated prevalence of 2-4% [13,25]. Moreover, the prevalence of PACG is found to be relatively higher (~1%) in adult Asian populations [13,22].

Studies have demonstrated a strong association between the development of POAG and a positive family history [10,25–29]. In a longitudinal study of 224 siblings of 156 clinically confirmed POAG cases, there was a significantly increasing trend in both prevalence and incidence of the disease with age and a lifetime risk estimated of approximately 20% by age 70 [27]. Other studies also suggested that the risk of POAG is higher in siblings of glaucoma cases than in their parents or children [26,28,29]. In a population-based study in Nottingham, the risk of glaucoma among siblings of POAG cases was about 10% (with an odds ratio [OR] of 3.69), which is greater than in parents (~6%; OR 2.17), and in children (~1%; OR 1.12) (27). Studies from the Netherlands and India reported similar findings, with a higher prevalence of POAG in siblings (~10% to 15%) than in parent-offspring family connections (~1% to 4%), respectively [26,29].

Historically, the effect of specific genes in the development of glaucoma has been largely unknown. In the 1960s, Becker et al. studied patients with POAG as well as their relatives, and proposed that POAG was a genetically determined disease, where the recessive homozygous ‘gg’ genotype represents glaucoma, and the alternative homozygous ‘nn’ and the heterozygous ‘ng’ genotypes represent non-glaucoma [30].

More recently, however, researchers have elucidated both causative and associative genes for glaucoma risk [20,31–33]. Family studies have indicated that glaucoma can be inherited as a Mendelian autosomal-dominant or recessive trait, but only 3-5% of adult-onset POAG cases are attributed to single-gene or Mendelian forms of glaucoma [34,35]. The vast majority of cases

have a multifactorial basis and are caused by the combined effects of many genetic and environmental factors [35].

The source of phenotypic variation among individuals in a population originates from both environmental and genetic factors, as well as the various interactions between them [31,36]. Heritability (h^2) can be defined as, "the proportion of variance in a particular trait due to variation in genetic factors among individuals in that population" [37–39]. The total variation (variance) in a phenotype (V_P) can then be broken down into two parts: the genotypic variance (V_G) and the remaining variance (V_E), due to the environment [38].

Within the last thirty years, classical twin studies have been conducted to establish the relative importance of genes and environment in glaucoma risk. Twin studies are an excellent source of information to disentangle and quantify the relative contributions of genes, the shared environment and the unique environment with respect to complex traits [39,40].

Population-based genetic studies continue to confirm that many ocular traits have a genetic component [9]. These traits show substantial variation in human populations and many are highly heritable [9,25,30,31,41–44]. Glaucoma related endophenotypes, sometimes called intermediate phenotypes, are powerful tools in the identification of genes contributing to glaucoma as they are more likely to be directly influenced by the genes than the resulting disease itself [45–47]. An endophenotype is defined as a heritable trait that is associated with a disease and that can be objectively measured, but is not a direct symptom of the disease [47,48]. Traits such as central corneal thickness, optic cup area, optic disc area, vertical cup-to-disk ratio, and intraocular pressure are some well-established endophenotypes for glaucoma [45].

Heritability estimates for glaucoma endophenotypes differ between studies. For example, for intraocular pressure, estimates range from h^2=0.35, in a total of 2,620 subjects from extended pedigrees from The Netherlands [41], to h^2=0.50 in 133 subjects from nuclear family groups in the USA [42]. Similarly, heritability estimates for optic disc parameters range from 0.66 to 0.77 for optic cup area, 0.52 to 0.83 for disc area, and 0.48 to 0.66 for vertical cup-to-disk ratio [9].

So far, systematic reviews on the heritability of glaucoma have only focused on the heritability of POAG [9,25]. Indeed, no systematic review has comprehensively reviewed nor meta-analyzed the heritability of other types of glaucoma, including PACG and congenital glaucoma, or

glaucoma-related endophenotypes. Accurate estimates of genetic risk (i.e., heritability) are imperative when studying diseases with differing prevalences in different ethnicities. It will be an important factor in the near future when patients' genotypes may be used for personalized estimates of disease risk, and it is also a prerequisite for further gene finding studies. Heritability estimates are to be specific to the disease being studied (for example POAG vs PACG), the populations studied (for example Caucasians vs Asians), and the particular circumstances from which they were derived [9,43]. A systematic review and meta-analysis of the genetic contribution to glaucoma and glaucoma-related endophenotypes will thus provide important insights and assist researchers in designing gene finding studies in the future.

The objective of this systematic review will be to identify relevant studies regarding the heritability of glaucoma and related endophenotypes and summarize the evidence through meta-analysis. Heritability estimation, commonly reported in %, is the outcome measurement that we will synthesize and report from several studies.

The current study will address the following research questions: (1) How much of the variance in glaucoma and glaucoma-related endophenotypes is due to genetic factors?; (2) What is the proportion of variance accounted for by additive genetic influences (A), common environment (C), and unique environment (E)?; (3) Do heritability estimates vary between different populations and study designs?

Methods and analysis

This systematic review was initiated in March 2017 and is registered with the International Prospective Register of Systematic Reviews (PROSPERO) with a registration number: CRD42017064504, available at http://www.crd.york.ac.uk/PROSPERO/. This systematic review will follow the PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist, which consists of a list of 17 items that provide a standardized guide for carrying out systematic reviews, including construction of a protocol, testing for bias and heterogeneity, and other aspects of the review process [49]. Similarly, the quality of the individual studies included in the systematic review will be rated independently by two reviewers using the National Health Institute Quality Assessment tool for Observational Cohort and Cross-Sectional Studies [50].

Inclusion criteria

Articles describing heritability results based on (1) family; (2) twin; (3) adoption, and; (4) GWAS study designs or that could be estimated from intraclass correlation or linear regression coefficient will be included. Heritability estimates for any type of glaucoma or endophenotypes related to pressure (intraocular pressure), angle (anterior chamber depth, anterior chamber volume, angle opening distance, angle recess area, trabecular iris space area or Bruch's membrane opening), disk morphology (cup area, cup diameter, disk area, disk diameter, rim area, vertical or horizontal cup-to-disk ratio), ganglion cell complex, retinal nerve fiber layer, or central corneal thickness will be considered. The search will be restricted to articles describing studies in human subjects written in English language. However, papers written in other languages with at least an English abstract will also be considered. All heritability studies from peer-reviewed journals, published until 31st December 2017, will be included.

Exclusion criteria

Papers that did not estimate heritability, did not specify ethnicity or papers that estimate explained genetic variance from only significant SNPs or genetic loci will be excluded.

Search strategy

To capture as much literature as possible, an initial limited search of MEDLINE will be performed using an initial set of search terms. This will be followed by the identification of additional search terms from the titles and abstracts, and from the Medical Subject Heading (MeSH) index terms used to describe the initial identified articles (Table 1). Second, using all identified keywords and index terms, a comprehensive, step-by-step systematic search will be undertaken in MEDLINE (PubMed), EMBASE, Web of Science, and ScienceDirect. In addition, Google Scholar will be used as a supplementary search database. Third, relevant papers from the reference lists of those articles captured in step two will be manually searched for additional input. References will be exported to RefWorks citation management software and duplicates will be removed. Full text, as well as relevant data, of all selected papers will be retrieved, and authors of the original articles will be contacted by email if additional information is required.

Two reviewers will independently evaluate the abstracts for eligibility, according to predefined selection criteria. A database will be used for screening of eligible articles. Any disagreements will be resolved through discussion between the two evaluators, but if consensus cannot be

reached, a third person will be consulted. Finally, selected publications will be approved by a senior investigator.

Table 1 Search string and number of articles found from a preliminary PubMed search

Step	Searching terms	# of articles found[1]
#1	"Quantitative Trait, Heritable"[MeSH][2] OR "Endophenotypes"[MeSH] OR Heritab*[tiab][3]	36,287
#2	Glaucoma[MeSH] OR Glaucoma*[tiab]	62,936
#3	Normal tension glaucoma[MeSH] OR Low tension glaucoma[MeSH] OR Exfoliation Glaucoma[MeSH] OR pseudoexfoliation glaucoma[MeSH] OR Exfoliation Syndrome[MeSH] OR Pigment dispersion syndrome[MeSH] OR Pigment dispersion syndrome[tiab] OR pds[tiab] OR Congenital glaucoma [MeSH] OR Buphthalmos[tiab] OR Buphthalmus[tiab] OR "Juvenile glaucoma"[tiab]	19,552
#4	"Intraocular pressure"[tiab] OR "Ocular pressure"[tiab] OR iop[tiab] OR "Ocular hypertension"[tiab] OR "ocular biometric"[tiab] OR "Central corneal thickness"[tiab] OR "Corneal shape"[tiab] OR "Axial length"[tiab] OR Linear cup-disk ratio OR lcdr OR "Vertical cup to disc ratio"[tiab] OR Vertical cup-to-disc ratio[tiab] OR vcdr[tiab] OR vertical cup:disc ratio[tiab] OR vertical cup-disc ratio[tiab] OR "Corneal hysteresis"[tiab] OR "Anterior chamber depth"[tiab] OR "Anterior chamber angle" OR "Narrow anterior chamber" OR "Optic disc diameter"[tiab] OR "Cup area"[tiab] OR "Disc area"[tiab] OR "Rim area"[tiab] OR "Retinal nerve fiber layer"[tiab] OR "Cilioretinal arteries"[tiab] OR "Retinal ganglion cell layer" OR Horizontal cup:disc ratio[tiab] OR "Disc diameter"[tiab] OR "Cup disc ratio"[tiab] OR "Ganglion cell complex thickness"[tiab] OR "Shallow anterior chamber"[tiab] OR "Iris thickness"[tiab] OR "Iris area"[tiab] OR "Plateau iris" OR "Pupil diameter"[tiab] OR "Pupil size"[tiab] OR "Iridotrabecular angle width"[tiab] OR "Bruch's membrane opening" OR "Neuroretinal rim" OR Excavation[tiab] OR Cupping[tiab] OR "Inner plexiform layer thickness"[tiab] OR "angle opening" distance"[tiab] OR aod[tiab] OR "trabecular iris space area" OR tisa[tiab] OR "angle recess area"[tiab] OR ara [tiab]	62,213
#5	#2 OR #3 OR #4	106,800
#6	#1 AND #5	194

[1] Preliminary search conducted on 14th December, 2017 at 07:20:51.
[2] Medical subject heading
[3] Title abstract text

Quality control and data extraction

For assessing the quality of individual articles in a systematic review, there are a variety of standard tools currently in use. However, most of these tools failed to include critical assessment elements relevant to heritability and genetic studies [51].

We found that the National Health Institute Quality Assessment tool for Observational Cohort and Cross-Sectional Studies [50] is relevant to assess the quality of selected articles in this current study. Quality assessment evaluation includes; whether the research question/objective is clearly stated; if the study population, sample size, randomness of participation and inclusion/exclusion criteria is clearly specified and defined; whether quality of measurement is ensured in the clinical examination of quantitative (endo)phenotypes; if the method of data analysis and outcome measure was clearly defined; and if confounding variables were controlled for their impact on the dependent variable. The heterogeneity of heritability estimation between articles will also be reported using Cochrane's Q test and I^2-statistic. These tests assess whether there are genuine differences underlying the results of the studies, or if the variation in results is through chance alone. The presence of any potential publication bias will be visualized with funnel plots, and any asymmetry of the funnel plots will be statistically tested with an Egger's test.

The quality of this study will be reported according to the PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist, which guides the reviewer in planning and carrying out systematic reviews [49] (Supplementary file_1). The full text of the potentially eligible articles will be retrieved and stored in an online citation manager (RefWorks), for accessibility and data synthesis.

The data extraction for eligible articles will be archived in a database and in order to ensure all relevant data are collected per study, a standardized form will be utilized (Supplementary file_2). To minimize the risk of transcription errors, data extraction will be conducted independently by two reviewers. The number of articles reviewed, the number of full-text studies retrieved, and the number of studies excluded will be reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart (Supplementary file_3).

Data synthesis and statistical analysis

The endophenotypes will be clustered into groups; pressure, angle, cornea, retinal nerve fiber layer, and disk morphology. The different types of glaucoma will also be clustered into primary and secondary: open-angle glaucoma, angle-closure glaucoma or exfoliation, as well as congenital glaucoma. Presuming that heritability estimates are different between populations, we will use a random effects model for meta-analyses. Separate meta-analyses will be performed for each cluster. Pooled heritability estimates, including 95% confidence intervals, and summary statistics for quantitative data will be described and presented in tables and figures. Quantitative assessment of heterogeneity in findings between studies and publication bias will be performed and reported. Heritability estimates from different study designs and statistical methods, as well as the possible factors that might explain the variation in heritability, will be discussed in detail.

Subgroup analysis

For assessing the possible factors that might explain the variation in heritability, we will use a number of approaches. The factors we will explore include ethnicity, study design, data analysis method, number of variables controlled for confounding, mean-age, and methodological quality score. Ethnicity will be classified according to [52], which meta-analyzed the global prevalence of POAG in different ethnicities. Additionally, for IOP, h^2 estimates will be subgrouped based on the device reported in the literature. The potential effect of mean-age, ethnicity, study design, data analysis method, and the number of variables controlled for confounding will also be statistically tested with meta-regression analyses.

Sensitivity analysis

Possible sources of heterogeneity will be determined with the Baujat plot [53]. Following the discovery of outliers, sensitivity analysis will be carried out by excluding the three most heterogenous articles per cluster. To explore the sensitivity of h^2 estimates to mean-age and ethnicity, analyses will be conducted on a series of combinations of these variables.

Ethical consideration and result dissemination

This systematic review will use secondary data from peer-reviewed published articles, and hence there is no requirement for ethics approval. The results of this systematic review will be disseminated through publication in a relevant, peer-reviewed journal and presented at pertinent conferences.

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Contributor ship statement

NGA and HS conceived and designed the study. NGA, AN, and NJ developed the search strategy. NGA, AN wrote the protocol. HS and NJ evaluated and revised the protocol. All the authors read the protocol and have given the final approval for publication.

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Competing interests

There are no competing interests.

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Meta-analysis Of Observational Studies in Epidemiology (MOOSE) checklist

	Criteria	Yes/No	Page
1. Reporting of background should include			
1.1	Problem definition	Yes	Explained in detail in the introduction, pages 3-6.
1.2	Hypothesis statement	Yes	Included in the introduction, pages 5.
1.3	Description of study outcome(s)	Yes	Included; Heritability estimation, commonly reported in %, is the outcome measurement that we will synthesize and report from several studies. Page 6.
1.4	Type of exposure or intervention used	No	NA
1.5	Type of study designs used	Yes	Explained in the inclusion and exclusion criteria, page 7.
1.6	Study population	Yes	Explained in the inclusion and exclusion criteria, page 7.
2. Reporting of search strategy should include			
2.1	Qualifications of searchers (e.g, librarians and investigators)	Yes	Two investigators, NGA and AN will do the searches independently. NGA is a PhD candidate in the department of Genetic Epidemiology, University of Groningen, and holds a Masters Degree in Public Health. AN is MSc holder in Quantitative Genomics and is a PhD candidate in the department of Ophthalmology, University of Groningen. Available on the cover page.
2.2	Search strategy, including time period included in the synthesis and keywords	Yes	Briefly described on pages 7-9.
2.3	Effort to include all available studies, including contact with authors	Yes	Stated in the search strategy, page 7-8.
2.4	Databases and registries searched	Yes	PubMed, EMBASE, Web of Science and ScienceDirect, (Supplementary file_1). Page 7.
2.5	Search software used, name and version, including special features	No	No search software used.
2.6	Use of hand searching	Yes	Stated in the search strategy, page 7.

	Criteria	Yes/No	Page
2.7	List of citations located and those excluded, including justifications	Yes	We will present the process of search and study selection using PRISMA flow process chart. Described on page 10.
2.8	Method of addressing articles published in languages other than English	Yes	Literatures with the English language will be included. However, papers written in other languages with at least an English abstract will also be considered, page 7.
2.9	Method of handling abstracts and unpublished studies	Yes	Only published and full text articles will be used. This is stated in the search strategy, page 7.
2.10	Description of any contact with authors	Yes	When it is necessary, authors of original articles will be contacted. Stated in the search strategy, page 7.
3. Reporting of methods should include			
3.1	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Yes	Stated in inclusion and exclusion criteria, page 6-7.
3.2	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	Yes	Stated on page 7.
3.3	Documentation of how data were classified and coded (eg, multiple raters, blinding, and interrater reliability)	Yes	Two reviewers, NGA and AN, who will be blinded to each other, will independently search the articles for eligibility according to predefined selection criteria. Any disagreements will be resolved by discussion between the two reviewers, but if consensus cannot be reached, a third reviewer will be consulted. Pages 7-8.
3.4	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	Yes	For assessing the possible factors that might explain the variation in heritability, we will use a number of approaches. The factors we will explore include ethnicity, study design, data analysis method, number of variables controlled for confounding, mean-age, and methodological quality score. Page 11.
3.5	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Yes	The quality of individual articles will be checked using National Health Institute Quality Assessment tool for Observational Cohort and Cross-Sectional Studies. Two reviewers, who are blinded to each other, will

	Criteria	Yes/No	Page
			independently assess the quality each article. Quality control and data extraction section states about this, 10.
3.6	Assessment of heterogeneity	Yes	Using Cochrane's Q test and I^2-statistic, the heterogeneity of the articles will be reported. Quality control section, page 9.
3.7	Description of statistical methods(eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	Yes	Random effect model meta-analysis will be used. Data synthesis section described details of statistical methods, pages 10-11.
3.8	Provision of appropriate tables and graphics	Yes	The number of articles searched and the number of studies excluded together with the reasons for exclusion will be briefly reported using a PRISMA flow process chart. Page 10.
4. Reporting of results should include			
4.1	Graph summarizing individual study estimates and overall estimate	Yes	Extracted data will be presented in tables, plots and graphs, accordingly. Pooled heritability estimates (displayed with forest plot) and summary statistics for quantitative data will be presented and described. Stated on page 11.
4.2	Table giving descriptive information for each study included	Yes	Extracted data will be presented in tables and graphs, accordingly. Stated on page 11.
4.3	Results of sensitivity testing (eg, subgroup analysis)	Yes	For assessing the possible factors that might explain the variation in heritability, subgroup and sensitivity analysis will be performed. The potential effect of mean-age, ethnicity, study design, data analysis method, and the number of variables controlled for confounding will also be statistically tested with meta-regression analyses, page 11.

	Criteria	Yes/No	Page
4.4	Indication of statistical uncertainty of findings	Yes	Weighted point estimate and its 95% confidence intervals will be reported. Explained in the data synthesis section, pages 10-11.
5. Reporting of discussion should include			
5.1	Quantitative assessment of bias (eg, publication bias)	Yes	The presence of any potential publication bias will be visualized with funnel plots, and any asymmetry of the funnel plots will be statistically tested with an Egger's test, page 10.
5.2	Justification for exclusion	Yes	We will present the process of search and study selection using PRISMA flow chart. The quality control and data extraction section, described about this issue. Page 10.
5.3	Assessment of quality of included studies	Yes	The quality of individual articles will be checked using National Health Institute Quality Assessment tool for Observational Cohort and Cross-Sectional Studies. Page 10.
6. Reporting of conclusions should include			
6.1	Consideration of alternative explanations for observed results	Yes	We will discuss on the possible factors that might explain the deviation of observed results from what is expected. Page 11.
6.2	Generalization of the conclusions	Yes	A conclusion on the general interpretation of the findings, in the context of research questions together with implications for future research, will be drawn.
6.3	Guidelines for future research	Yes	Depending on the study findings, we will present clear recommendations and discuss the future actions that researchers should take.
6.4	Disclosure of funding source	Yes	The source of funding is disclosed, page 11.

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Data extraction form

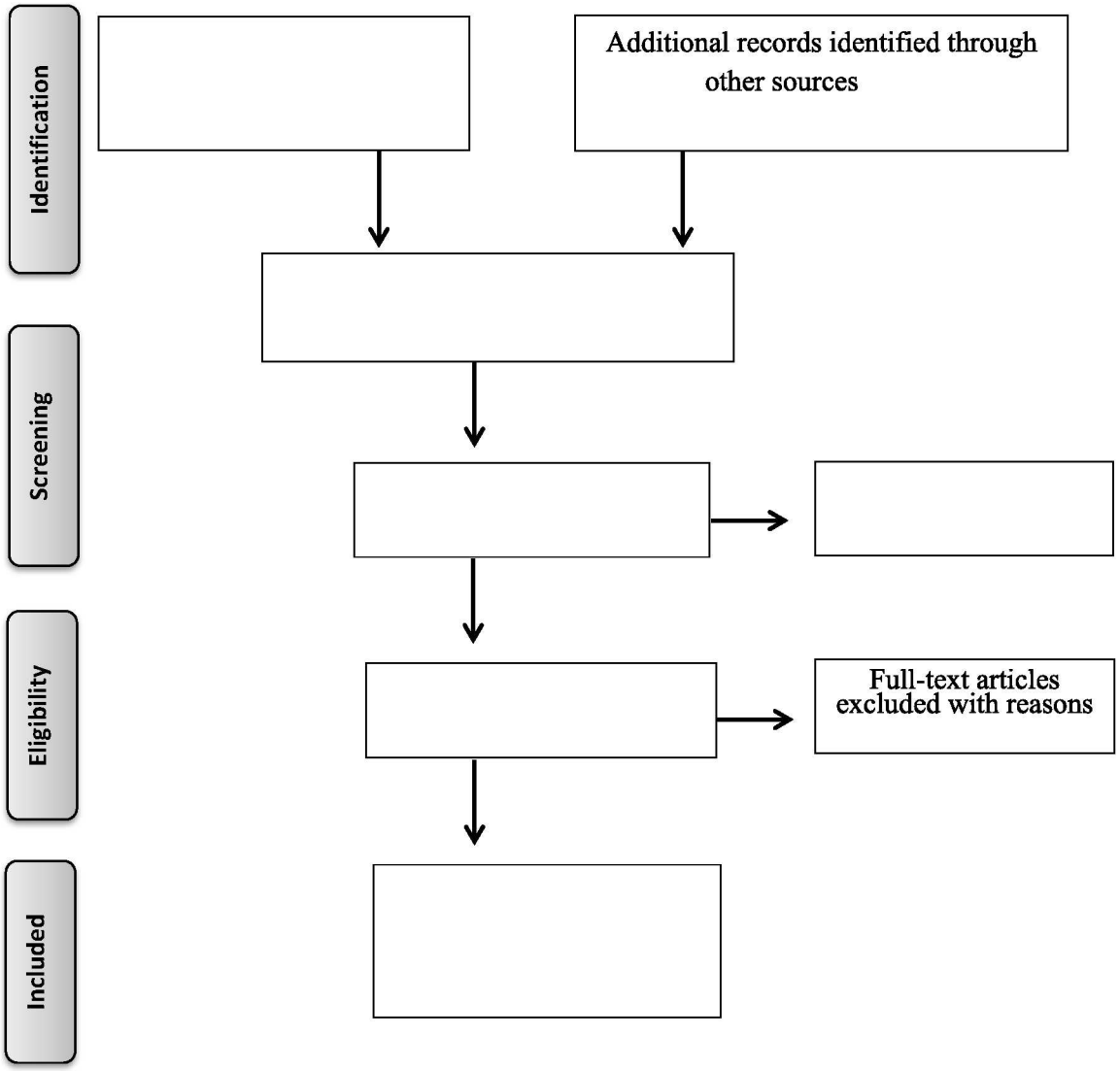
Article ID	Publication Year	Volume	Issue
Authors			
Title			
Abstract			

Country of Study	Ethnicity	Study Design	Data Analysis Method	Sample Size	Mean Age	Gender

Glaucoma Type	Endophenotype 1	Endophenotype 2	Endophenotype 3
Glaucoma Type-Heritability (h^2)	Heritability (h^2)	Heritability (h^2)	Heritability (h^2)

The Proportion of Variance Accounted by:	Additive Genetic variance (A)	Common Environment (C)	Unique Environment (E)

Additional Comments


Identification
Additional records identified through other sources
Screening
Eligibility
Full-text articles excluded with reasons
Included

Heritability of glaucoma and glaucoma-related endophenotypes: Systematic review and meta-analysis protocol, 2017

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol

Section and topic	Item No	Checklist item	Yes/No
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	Yes, included under the title: Heritability of glaucoma and glaucoma-related endophenotypes: Systematic review and meta-analysis protocol
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	No, this is a new protocol.
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	Yes, this protocol is registered in PROSPERO, with a registration number: CRD42017064504.
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Yes; stated on the cover page.
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	Yes, page 15.
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	No, this is a new protocol.
Support:			
Sources	5a	Indicate sources of financial or other support for the review	Yes, page 15.

Sponsor	5b	Provide name for the review funder and/or sponsor	Yes, page 15.
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	Yes, as stated on page 15.
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	Yes, briefly stated from page 1 to 5.
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Yes. The study will address the following research questions: 1. How much of the variance in glaucoma and glaucoma-related endophenotypes is due to genetic factors? 2. What is the proportion of variance accounted for by additive genetic influences (A), common environment (C), and unique environment (E)? 3. Do heritability estimates vary between different populations and study designs? Page 6.
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Yes; it is explained under inclusion and exclusion criteria, page 7.
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	Yes. To capture as much literature as possible, systematic search will be undertaken in MEDLINE (PubMed), EMBASE, Web of Science, and ScienceDirect. In addition, Google Scholar will be

			used as a supplementary search database. Only published and full text articles will be used. If additional information is required, authors of the original articles will be contacted by email. Stated on page 7.
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Yes. Search string and number of articles found from a preliminary PubMed search, is presented under table 1, page 9.
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Yes. Eligible articles will be exported to RefWorks citation management software and duplicates will be removed. Full text, as well as relevant data, of all selected papers will be retrieved. Details are presented on page 7.
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	Yes. To minimize errors, abstract and full paper screening and data extraction will be conducted independently by two reviewers. We will present the process of search and study selection using PRISMA flow process chart. Briefly stated on page 10.
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators.	Yes. The data extraction for eligible articles will be archived in a database and in order to ensure all relevant data are collected per study, a standardized form will be utilized (Supplementary file_2). Briefly stated on page 10.

Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	Yes. Heritability estimates for any type of glaucoma or endophenotypes related to pressure (intraocular pressure), angle (anterior chamber depth, anterior chamber volume, angle opening distance, angle recess area, trabecular iris space area or Bruch's membrane opening), disk morphology (cup area, cup diameter, disk area, disk diameter, rim area, vertical or horizontal cup-to-disk ratio), ganglion cell complex, retinal nerve fiber layer, or central corneal thickness will be considered. Page 7.
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Yes. Heritability estimation, commonly reported in %, is the outcome measurement that we will synthesize and report from several studies. Articles describing heritability results based on: 1. Family 2. Twin 3. Adoption, and 4. GWAS study designs will be included; described on page 6 and 7.
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis.	Yes. The methodological quality of selected articles will be assessed and rated using the National Health Institute Quality Assessment tool for Observational Cohort and Cross-Sectional Studies.

			Quality score of individual articles will be used in sub-group analysis for exploring the variation in heritability estimates. Page 6 and 11.
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	No. Presuming that heritability estimates are different between populations, heterogeneity between studies is expected; we didn't put an upper threshold for I^2-statistic. However, original studies conducted on any type of glaucoma and glaucoma-related endophenotypes and those which reported heritability outcome data, or that could be estimated from intraclass correlation or linear regression coefficient will be included for quantitative analysis. Possible heterogeneity in heritability estimates will be explored through conducting subgroup/sensitivity analyses. Papers that didn't estimate heritability or estimated heritability from only significant SNP/s or genetic loci will not be considered for quantitative analysis. Page 7 describes about this.
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of	Yes. Heritability estimates are different between populations, we will use a random effects model for meta-analyses. Pooled

		consistency (such as I^2, Kendall's τ)	heritability estimates, including 95% confidence intervals, and summary statistics for quantitative data will be described and presented in tables and figures. Quantitative assessment of heterogeneity in findings between studies and publication bias will be performed and reported. The heterogeneity of heritability estimation between articles will be reported using Cochrane's Q test and I^2-statistic. The presence of publication bias will be visualized with funnel plots, and statistically tested with an Egger's test. Page 10 and 11.
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	Yes. For assessing the possible factors that might explain the variation in heritability, subgroup analysis will be performed based on; ethnicity, study design, data analysis method, number of variables controlled for confounding, mean-age, and methodological quality score. In addition, sensitivity analysis will be carried out by excluding the three most heterogenous articles per endophenotype. To explore the sensitivity of heritability estimates to mean-age and ethnicity, analyses will be conducted on a series of combinations

			of these variables. Page 11.
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	Yes. If search result does not have sufficient studies per glaucoma or endophenotype, or if studies are not eligible for quantitative analysis, findings will be synthesized and narrated, and summary statistics for quantitative data will be described and presented in tables and figures. Page 7 and 11.
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	Yes. The presence of any potential publication bias will be visualized with funnel plots, and any asymmetry of the funnel plots will be statistically tested with an Egger's test; described on page 10.
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	Yes. Two independent reviewers, who will be blinded to each other, will assess the methodological quality of each study using the National Health Institute Quality Assessment tool for Observational Cohort and Cross-Sectional Studies, which contains 14-yes/no checklists. Quality assessment evaluation includes; whether the research question/objective is clearly stated; if inclusion/exclusion criteria is clearly specified and defined; whether method of data analysis and outcome measure was

	clearly defined; and if confounding variables were controlled for their impact on the dependent variable. Page 10 briefly describes about this issue.

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.