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Glaucoma

Genetic Associations Between Smoking- and Glaucoma-Related Traits

Jessica H. Tran[1], Kelsey V. Stuart[2], Victor de Vries[3], Joëlle E. Vergroesen[3], Clara C. Cousins[4], Pirro G. Hysi[5,6], Ron Do[7], Ghislain Rocheleau[7], Jae H. Kang[8], Janey L. Wiggs[4], Stuart MacGregor[9], Anthony P. Khawaja[2], David A. Mackey[10], Caroline C. W. Klaver[3,11,12], Wishal D. Ramdas[3], and Louis R. Pasquale[1], for the UK Biobank Eye and Vision Consortium, and for the International Glaucoma Genetics Consortium

[1] Department of Ophthalmology, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[2] NIHR Biomedical Research Centre, Moorfields Eye Hospital NHS Foundation Trust & UCL Institute of Ophthalmology, London, UK
[3] Departments of Ophthalmology and Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands
[4] Department of Ophthalmology, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, USA
[5] Department of Ophthalmology, King's College London, St. Thomas' Hospital, London, UK
[6] Department of Twin Research & Genetic Epidemiology, King's College London, St. Thomas' Hospital, London, UK
[7] Charles Bronfman Institute for Personalized Medicine, Department of Genetics and Genomics, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[8] Channing Division of Network Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[9] Department of Statistical Genetics, QIMR Bergohofer Medical Research Institute, Brisbane, Australia
[10] Centre for Ophthalmology and Visual Science, Lions Eye Institute, University of Western Australia, Western Australia, Australia
[11] Department of Ophthalmology, Radboudumc, Radboud University Medical Center, Nijmegen, The Netherlands
[12] Institute of Molecular and Clinical Ophthalmology, University of Basel, Basel, Switzerland

Correspondence: Louis R. Pasquale, One Gustave L. Levy Place, Box 1183, New York, NY 10029, USA.
e-mail: louis.pasquale@mssm.edu

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Purpose: The purpose of this study was to describe the genetic relationship between smoking and glaucoma.

Methods: We used summary-level genetic data for smoking initiation, smoking intensity (cigarettes per day [CPD]), intraocular pressure (IOP), vertical cup-disc ratio, and open-angle glaucoma (OAG) to estimate global genetic correlations (r_g) and perform two-sample Mendelian randomization (MR) experiments that explored relations between traits. Finally, we examined associations between smoking genetic risk scores (GRS) and smoking traits with measured IOP and OAG in Rotterdam Study participants.

Results: We identified weak inverse r_g between smoking- and glaucoma-related traits that were insignificant after Bonferroni correction. However, MR analysis revealed that genetically predicted smoking initiation was associated with lower IOP (−0.18 mm Hg per SD, 95% confidence interval [CI] = −0.30 to −0.06, $P = 0.003$). Furthermore, genetically predicted smoking intensity was associated with decreased OAG risk (odds ratio [OR] = 0.74 per SD, 95% CI = 0.61 to 0.90, $P = 0.002$). In the Rotterdam Study, the smoking initiation GRS was associated with lower IOP (−0.09 mm Hg per SD, 95% CI = −0.17 to −0.01, $P = 0.04$) and lower odds of OAG (OR = 0.84 per SD, 95% CI = 0.73 to 0.98, $P = 0.02$) in multivariable-adjusted analyses. In contrast, neither smoking history nor CPD was associated with IOP ($P \geq 0.38$) or OAG ($P \geq 0.54$). Associations between the smoking intensity GRS and glaucoma traits were null ($P \geq 0.13$).

Conclusions: MR experiments and GRS generated from Rotterdam Study participants support an inverse relationship between smoking and glaucoma.

Translational Relevance: Understanding the genetic drivers of the inverse relationship between smoking and glaucoma could yield new insights into glaucoma pathophysiology.

Introduction

Tobacco smoking is an important risk factor for age-related cataract formation, thyroid eye disease, and age-related macular degeneration.[1–3] Prior observational studies provide conflicting evidence for the relationship between smoking and open-angle glaucoma (OAG).[4–9] For example, an inverse trend, approaching statistical significance, between increased pack years of smoking and incident OAG was reported among health professionals.[6] However, findings from the American Academy of Ophthalmology Intelligent Research in Sight Registry suggested that smokers had higher intraocular pressure (IOP) compared to individuals who had never smoked, independent of glaucoma status.[8] The inconsistent evidence may be due to inherent limitations of these epidemiological studies, such as residual confounding, measurement error, and reverse causation.[10]

Genetic approaches using genome-wide association studies (GWAS) summary statistics can provide alternative estimates of a relationship.[10–12] Global genetic correlations provide a measure of the average correlation of allele effects across the genome between two traits, reflecting their shared heritability.[10,11] Mendelian randomization (MR) is based on the principle that genes are randomly passed on from parents to offspring. In MR, genetic variants serve as a proxy for an exposure (or risk factor), allowing for the estimation of a potential causal link between two traits.[12] As genetic correlations can occur due to different types of pleiotropy, MR analyses can clarify the relation by providing evidence for vertical pleiotropy, in which genetic variants are related to the outcome trait through the exposure.[12,13]

With glaucoma projected to affect more than 110 million individuals by 2040, it is essential to improve our understanding of smoking's contribution to glaucoma.[14,15] Smoking, although largely considered a lifestyle behavior, is a complex trait with a hereditary component.[16–19] Interestingly, a locus on chromosome 15 (rs16969968) encodes a nicotinic acetylcholine receptor and has been strongly associated with heavy smoking and nicotine dependence.[17] Cigarette smoke contains over 4000 chemicals,[20] and studying genetic proxies for smoking traits may circumvent the competing effects of cigarette smoke components. Coincidentally, the first drug to treat glaucoma was an acetylcholine agonist (pilocarpine).[21] Whereas smoking cessation is a fundamental public health priority, we seek an alternative investigation—one focused on how genetic susceptibility to initiation and intensity of smoking is related to various glaucoma traits. We posit this genetic exploration may yield insights regarding drug targets for glaucoma. In this study, we use global genetic correlations and MR experiments to elucidate the shared genetic architecture among smoking- and glaucoma-related traits. We then perform additional analyses using individual-level genetic data on smoking in relation to measured glaucoma traits from the Rotterdam Study to validate our findings.

Methods

The analyses of summary-level data are exempt from institutional review board (IRB) assessment. The Medical Ethics Committee of Erasmus MC (registration number MEC 02.1015) and the Dutch Ministry of Health, Welfare, and Sport (Population Screening Act WBO, license number 1071272-159521-PG) approved the use of the individual-level data in Rotterdam Study participants. The Rotterdam Study is entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and the World Health Organization International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalog number NTR6831. All participants provided written informed consent following the Declaration of Helsinki to participate in the study and to have their information obtained from their treating physicians.

Data Sources

We assembled eight studies with GWAS summary statistics – two focused on smoking-related traits and six focused on glaucoma-related traits – all from European-derived participants (Table 1). For glaucoma-related phenotypes, we included summary statistics from recent GWAS for OAG,[22] IOP,[23] and vertical cup-disc ratio (vCDR),[24] including artificial intelligence-determined vertical cup-disc ratio (AI-vCDR) adjusted for disc diameter.[25] We also used GWAS summary-level data for macular retinal nerve fiber layer (mRNFL) thickness,[26] and macular ganglion cell-inner plexiform layer (mGCIPL) thickness.[26] For smoking-related traits, we used results for smoking initiation and smoking intensity from the GWAS and Sequencing Consortium of Alcohol and Nicotine use (GSCAN).[16] Smoking initiation was defined as a binary phenotype based on any history of smoking. Smoking intensity was a continuous phenotype characterized by the average number of cigarettes smoked per day (CPD) among smokers.

Table 1. Summary of Genome-Wide Association Data Used for Global Genetic Correlations and Mendelian Randomization Studies

Trait	Source [Reference]	Total Sample Size	Heritability or Heritability Range Explained by Classic Twin or Family Studies (PMIDs)	Heritability Explained by GWAS Data (SE)*	SNPs Included in the Instrumental Variable for MR Experiments
Open-angle glaucoma	IGGC [22]	216,257	0.70 (28783162)	0.15 (0.01)	–
Intraocular pressure	UKBB, Epic-Norfolk, IGGC [23]	139,555	0.55 (20851442)	0.16 (0.01)	–
Vertical cup-disc ratio	IGGC [24]	23,899	0.48 (14691154)	0.22–0.31 (0.04)†	–
Artificial intelligence-determined vertical cup-disc ratio	UKBB, CLSA, IGGC [25]	111,724	0.48 (14691154)	0.31 (0.02)	–
Macular retinal nerve fiber layer thickness	UKBB [26]	31,434	0.48–0.82 (17652737, 12824246, 27677702)	0.24 (0.03)	–
Macular ganglion cell-internal plexiform layer thickness	UKBB [26]	31,434	0.82 (32788326)	0.25 (0.02)	–
Smoking initiation‡	GSCAN [16]	632,802	0.51–0.64 (10986552, 21569578, 7198252)	0.08 (0.003)	341
Smoking intensity‡	GSCAN [16]	263,954	0.49–0.51 (2392895, 9065896, 15170444)	0.07 (0.007)	46

*Heritability estimates were calculated on the observed scale except for open-angle glaucoma and smoking initiation, which were calculated using the liability scale.

†The UKBB data estimate for clinician-determined vertical cup-disc ratio heritability is 0.22 and the IGGC data estimate is 0.31.

‡The total sample size is lower than the reported sample size in the GSCAN study due to the exclusion of 23andMe participants in the publicly available summary statistics.

CLSA, Canadian Longitudinal Study on Aging; GWAS, genome-wide association study; GSCAN, GWAS and Sequencing Consortium of Alcohol and Nicotine use; EPIC, European Prospective Investigation into Cancer and Nutrition; IGGC, International Glaucoma Genetics Consortium; MR, Mendelian randomization; PMID (PubMed reference number); SE, standard error; SNP, single-nucleotide polymorphism; UKBB, UK Biobank.

Details regarding GWAS summary-level data including sample sizes, participant demographics, genotyping platforms, quality control filters applied, and imputation methods used can be found in the studies listed in Table 1.

The trait heritability from classic twin and family studies versus compiled GWAS data are also included in Table 1. Single nucleotide polymorphism (SNP) heritability using summary-level data was estimated using linkage disequilibrium score regression (LDSC). Of note, the heritability estimates from GWAS are much lower than those from classic twin and family studies, which may overestimate heritability by including shared environmental factors in the estimate.[27]

Global Bivariate Genetic Correlations

We estimated global bivariate genetic correlations (r_g) using cross-trait LDSC. Briefly, LDSC estimates genetic correlations and heritability by regressing GWAS summary statistics on linkage disequilibrium scores against a reference population.[10,11] Because the estimate of the genetic correlation is based on the slope from the regression, it is not biased by sample overlap.[10] LDSC can only be used in homogenous populations. Therefore, our analyses focused on participants of European ancestry with the 1000 Genomes Project European dataset as the reference panel, as this subset provided the highest power in our analyses.[10]

Global genetic correlation estimates range from -1 to $+1$, where traits with $r_g \geq |0.5|$ are regarded as strongly correlated. Traits with values between $|0.2| < r_g < |0.5|$ denote moderate global genetic correlation and weakly correlated traits have $r_g \leq |0.2|$. We estimated heritability and genetic correlations on the observed scale and liability scale for quantitative and binary traits, respectively.[10,14,28] For bivariate genetic correlations between smoking- and glaucoma-related endophenotypes, we established a Bonferroni-corrected statistical significance threshold of

$P < 4.2E-03$, to adjust for comparisons among 2 smoking traits and 6 glaucoma-related traits. Sample sizes for all bivariate genetic correlations were sufficiently powered, as the square root product of trait heritability and their respective sample sizes were >4500, an established cutoff for adequate statistical power (Supplementary Table S1).[29]

Mendelian Randomization

We performed two-sample MR analyses, using summary-level data of genetic variants associated with smoking initiation and smoking intensity to test for causal associations with the summary-level genetic data of six glaucoma-related outcomes. MR is a form of instrumental variable (IV) analysis, analogous to a naturally occurring randomized controlled trial, which allows for an unbiased causal effect estimate of an exposure on an outcome, provided the following three assumptions are met: (1) the IV must be associated with the exposure; (2) the IV must not be associated with any confounder of the exposure-outcome relationship; and (3) the IV must affect the outcome only through the exposure of interest (see Supplementary Methods A for more detail). The main MR analyses were performed using a univariable, inverse-variance weighted (IVW), multiplicative random-effects model. We conducted sensitivity analyses using the weighted median, weighted mode, MR-Egger, and MR pleiotropy residual sum and outlier (MR-PRESSO) and multivariable IVW MR method (see Supplementary Methods A). We included 341 SNPs and 46 SNPs in the smoking initiation and smoking intensity IVs, respectively. SNPs were selected according to the criteria described in Supplementary Methods A and full details of these variants are reported in Supplementary Tables S2 and S3. We calculated relevant test statistics, including measures of instrument strength, heterogeneity, directional pleiotropy, and regression dilution. In instances where global heterogeneity is identified in the IV, estimates can still be valid, provided there is no evidence of directional pleiotropy.[13,30] The strength for MR studies was assessed with the F statistic and all analyses revealed a value >10, the agreed-upon cutoff for adequate instrument strength (Supplementary Tables S4, S5).[31] For the multivariable-adjusted MR, we adjusted for the summary statistics for alcohol (drinks per week)[16] and coffee consumption (cups per day),[32] given their moderate genetic correlations with smoking phenotypes[16,33] (Supplementary Table S6). The Wald test was used to calculate two-tailed P values and we again applied the Bonferroni-corrected significance threshold of 4.2E-03. We applied the STROBE-MR checklist to our MR analyses,[34] which were performed in R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria) using the *TwoSampleMR*, *MendelianRandomization,* and *MRPRESSO* packages.[13,35,36]

External Validation in the Rotterdam Study

We used individual-level measured glaucoma data from the Rotterdam Study, a prospective population-based cohort study focused on identifying determinants of major diseases, including OAG.[37] Details regarding the study population, genotyping and imputation, and ophthalmic assessment can be found in Supplementary Methods B.

We developed a standardized weighted genetic risk score (GRS) for smoking initiation and smoking intensity using the 341 SNPs and 46 SNPs that comprised their respective IV in the MR experiments (see Supplementary Tables S2, S3). Briefly, a weighted GRS quantifies cumulative genetic susceptibility to a trait by aggregating the effects of its associated SNPs. We estimated the weighted GRS by multiplying the number of risk alleles by their respective effect sizes and then summing the products.[38,39] The GRS was standardized with a mean of zero and standard deviation (SD) of one (see Supplementary Methods B for more detail). We then validated associations between the GRS for smoking initiation and smoking intensity with smoking behaviors (any smoking history and CPD) among the Rotterdam Study participants. Next, multivariable logistic regression analyses were performed for the associations between the smoking initiation GRS, smoking intensity GRS, and smoking behaviors in relation to the odds of OAG. Multivariable linear regression analyses were performed to assess the associations between both GRS and actual smoking exposures in relation to IOP and vCDR. Models were adjusted for age, sex, body mass index (BMI), type 2 diabetes mellitus, chronic obstructive pulmonary disease, hypertension, alcohol consumption, coffee consumption, total caloric intake, anti-asthmatic usage, and systemic corticosteroid usage. Covariate definitions and methods of ascertainment are provided in Supplementary Methods B. As a sensitivity analysis, we also performed a separate logistic regression model adding IOP as a covariate to examine if the associations between smoking exposures and OAG were IOP-independent. These statistical analyses were performed using SPSS version 28.0.1.0 (SPSS Inc., Chicago, IL, USA). A P value of < 0.05 was considered statistically significant.

Results

Global Bivariate Genetic Correlations

We identified weak, nominally significant inverse global genetic correlations between smoking initiation and IOP ($r_g = -0.06$, SE = 0.02, $P = 0.007$), AI-vCDR ($r_g = -0.04$, SE = 0.02, $P = 0.02$), and mGCIPL ($r_g = -0.07$, SE = 0.03, $P = 0.02$). However, the comparisons were null after adjusting for multiple testing (Table 2). There was also a nominal inverse correlation between smoking intensity and AI-vCDR ($r_g = -0.05$, SE = 0.02, $P = 0.04$; see Table 2). Bivariate genetic correlations examining the shared overlap between the two smoking traits and among the glaucoma-related endophenotypes produced mostly expected findings (see Supplementary Tables S6, S7, respectively).

Mendelian Randomization

MR experiments revealed no significant associations among the smoking initiation IV and OAG, mRNFL, mGCIPL, vCDR, or AI-vCDR ($P \geq 0.14$; Table 3). However, the smoking initiation IV was associated with lower IOP (−0.18 mm Hg per SD, 95% confidence interval [CI] = −0.30 to −0.06, $P = 0.003$) under the IVW method (see Table 3). This was supported by MR-PRESSO (−0.19 mm Hg per SD, 95% CI = −0.30 to −0.08, $P < 0.001$) and the multivariable MR, which adjusted for genetically determined caffeine and alcohol consumption (−0.19 mm Hg per SD, 95% CI = −0.32 to −0.06, $P = 0.003$), but not by the other MR analyses. There was significant global heterogeneity in the smoking initiation IV in relation to IOP under Cochran's Q statistic, Rucker's Q' statistic, and the MR-PRESSO global test ($P < 0.001$ for all; see Supplementary Methods A, Supplementary Table S4), although the MR-Egger intercept test suggested balanced pleiotropy ($P = 0.60$). The smoking intensity IV was significantly associated with decreased glaucoma risk (odds ratio [OR] = 0.74 per SD, 95% CI = 0.61 to 0.90, $P = 0.002$) under the IVW method (see Table 3). The other MR methods yielded similar results; however, the estimate narrowly missed the adjusted significance threshold with the multivariable MR experiment (OR = 0.86 per SD, 95% CI = 0.77 to 0.96, $P = 0.006$). We did not detect any other significant associations between the smoking intensity IV and other glaucoma-related traits after adjusting for multiple comparisons (see Table 3). There was no evidence of pleiotropic effects with the smoking intensity IV in relation to OAG (see Supplementary Methods A, Supplementary Table S5). Full details regarding instrument strength, heterogeneity, directional pleiotropy, and regression dilution are presented in Supplementary Methods A, Supplementary Table S4, and Supplementary Table S5. Scatter plots of the exposure and outcome association estimates for the smoking initiation and smoking intensity instruments can be found in Supplementary Figures S1 and S2.

Assessment of Smoking Genetic Instruments With Glaucoma Traits in the Rotterdam Study

A total of 14,921 participants were included from the Rotterdam Study (Table 4). Most notably, 10,293 (70.2%) participants had a history of smoking with a mean ± SD of 15.1 ± 11.1 CPD. The mean IOP was 14.4 ± 3.5 mm Hg, and 329 (2.5%) participants were diagnosed with OAG.

The GRS for both smoking initiation and smoking intensity were successfully validated for their respective exposures. There was a strong correlation between the smoking initiation GRS and directly assessed history of smoking (OR = 1.20 per SD, 95% CI = 1.15 to 1.25,

Table 2. Genetic Correlations (Standard Error) Between Smoking-Related and Glaucoma-Related Traits*

	OAG†	IOP	vCDR	AI-vCDR	mRNFL	mGCIPL
Smoking initiation†	−0.05 (0.02)	−0.06 (0.02)	−0.05 (0.03)	−0.04 (0.02)	−0.05 (0.03)	−0.07 (0.03)
	$P = 0.06$	$P = 0.007$	$P = 0.12$	$P = 0.02$	$P = 0.09$	$P = 0.02$
Smoking intensity	−0.03 (0.03)	−0.04 (0.03)	−0.06 (0.03)	−0.05 (0.02)	−0.03 (0.04)	−0.06 (0.04)
	$P = 0.32$	$P = 0.12$	$P = 0.07$	$P = 0.04$	$P = 0.42$	$P = 0.10$

*Adjusted P value for multiple comparisons set at $P < 4.2E-3$.

†Genetic correlation was calculated on the liability scale.

AI-vCDR, artificial intelligence-determined vertical cup-disc ratio; IOP, intraocular pressure; mRNFL, macular retinal nerve fiber layer; mGCIPL, macular ganglion cell-internal plexiform layer thickness; OAG, open-angle glaucoma; vCDR, vertical cup-disc ratio.

Table 3. Mendelian Randomization Analyses of Smoking Behaviors on Glaucoma-Related Traits[*,†]

		Outcome Trait											
		OAG		IOP (mmHg)		vCDR		AI-vCDR		mRNFL (μm)		mGCIPL (μm)	
Exposure Trait	MR Method	OR (95% CI)	*P* Value	Estimate (95% CI)	*P* Value	Estimate (95% CI)	*P* Value	Estimate (95% CI)	*P* Value	Estimate (95% CI)	*P* Value	Estimate (95% CI)	*P* Value
Smoking initiation	IVW	0.97 (0.87, 1.08)	0.58	−0.18 (−0.30 to −0.06)	0.003*	0.00 (−0.01 to 0.01)	0.63	0.00 (−0.01 to 0.00)	0.37	−0.05 (−0.32 to 0.23)	0.74	−0.17 (−0.56 to 0.21)	0.38
	Weighted median	0.91 (0.79, 1.04)	0.18	−0.09 (−0.22 to 0.05)	0.21	0.00 (−0.01 to 0.02)	0.70	0.00 (−0.01 to 0.01)	1.00	0.18 (−0.17 to 0.53)	0.32	−0.07 (−0.53 to 0.40)	0.78
	Weighted mode	0.83 (0.59, 1.15)	0.26	0.11 (−0.28 tp 0.51)	0.58	0.00 (−0.03 to 0.04)	0.86	0.00 (−0.01 to 0.00)	0.62	0.69 (−0.38 to 1.77)	0.21	0.50 (−0.79 to 1.79)	0.66
	MR-Egger	0.91 (0.58, 01.45)	0.71	−0.31 (−0.80 to 0.18)	0.22	−0.02 (−0.05 to 0.03)	0.47	0.00 (−0.02 to 0.01)	0.79	0.63 (−0.53 to 1.79)	0.28	0.02 (−1.61 to 1.65)	0.98
	MR-PRESSO	0.96 (0.86, 1.06)	0.41	−0.19 (−0.30 to −0.08)	<0.001	0.00 (−0.01 to 0.01)	0.75	0.00 (−0.01 to 0.00)	0.14	−0.06 (−0.31 to 0.20)	0.67	−0.21 (−0.56 to 0.14)	0.25
	Multivariable MR[‡]	0.98 (0.88, 1.09)	0.77	−0.19 (−0.32 to −0.06)	0.003	0.00 (−0.01 to 0.01)	0.77	0.00 (−0.01 to 0.00)	0.28	−0.01 (−0.28 to 0.27)	0.96	−0.07 (−0.45 to 0.31)	0.72
Smoking intensity	IVW	0.74 (0.61, 0.90)	0.002	−0.08 (−0.34 to 0.18)	0.54	0.00 (−0.02 to 0.02)	0.82	0.00 (−0.01 to 0.01)	0.60	0.10 (−0.45 to 0.66)	0.71	−0.76 (−1.50 to −0.03)	0.04
	Weighted median	0.60 (0.46, 0.78)	<0.001	−0.06 (−0.32 to 0.20)	0.65	−0.01 (−0.04 to 0.02)	0.49	0.00 (−0.01 to 0.01)	0.63	−0.10 (−0.85 to, 0.66)	0.81	−1.08 (−2.07 to −0.09)	0.03
	Weighted mode	0.64 (0.50, 0.84)	0.002	−0.03 (−0.26 to 0.19)	0.76	−0.01 (−0.04 to 0.01)	0.30	0.00 (−0.01 to 0.00)	0.55	0.06 (−0.68 to 0.80)	0.88	−0.95 (−1.86 to −0.04)	0.05
	MR-Egger	0.60 (0.44, 0.83)	0.002	0.10 (−0.35 to 0.54)	0.67	−0.01 (−0.05 to 0.03)	0.57	0.00 (−0.02 to 0.01)	0.74	0.56 (−0.41 to 1.53)	0.26	−0.66 (−1.95 to 0.63)	0.32
	MR-PRESSO[§]	–	–	−0.13 (−0.35 to 0.08)	0.23	–	–	0.00 (−0.01 to 0.00)	0.43	–	–	–	–
	Multivariable MR[‡]	0.86 (0.77, 0.96)	0.006	−0.05 (−0.19 to 0.10)	0.54	0.00 (−0.01 to 0.01)	0.62	0.00 (−0.01 to 0.00)	0.47	0.05 (−0.21 to 0.32)	0.69	−0.36 (−0.70 to −0.02)	0.04

[*]All effect sizes are per standard deviation increase in the exposure trait.

[†]Adjusted *P* value for multiple comparisons set at $P < 4.2E\text{-}3$.

[‡]Multivariable MR adjusted for genetically determined alcohol and coffee consumption.

[§]MR-PRESSO produces estimates after removal of significant outlying variants. If there are no significant outliers, there is no estimate.

AI-vCDR, artificial intelligence-determined vertical cup-disc ratio; CI, confidence interval; IOP, intraocular pressure; IVW, inverse variance weighted; mGCIPL, macular ganglion cell-inner plexiform layer; MR, Mendelian randomization; mRNFL, macular retinal nerve fiber layer; MR-Egger, Mendelian Randomization-Egger; MR-PRESSO, Mendelian Randomization-Pleiotropy Residual Sum and Outlier; OAG, open-angle glaucoma; OR, odds ratio; vCDR, vertical cup-disc ratio.

Table 4. Baseline Characteristics of Participants From the Rotterdam Study

Characteristic	Total N	Mean ± Standard Deviation or n (%)
Age, y	14,921	66.1 ± 10.5
Female sex, *N* (%)	14,921	8819 (59.1)
History of smoking, *N* (%)	14,655	10,293 (70.2)
Cigarettes per day	9485	15.1 ± 11.1
OAG, *N* (%)	12,921	329 (2.5)
IOP, mm Hg*	13,204	14.4 ± 3.5
Vertical cup-disc ratio	10,462	0.3 ± 0.2
Diabetes mellitus, *N* (%)	10,965	1459 (13.3)
BMI, kg/m^2	13,476	26.9 ± 4.1
Hypertension, *N* (%)	14,188	8703 (61.3)
Alcohol intake, drinks/week	13,265	1.0 ± 1.4
Coffee intake, cups/day	12,316	3.3 ± 2.3
Total caloric intake, kcal/day	9701	2089.0 ± 594.1
Anti-asthmatic inhaler usage, *N* (%)	14,899	936 (6.3)
Systemic steroid usage, *N* (%)	14,899	227 (1.5)
COPD, *N* (%)	14,899	623 (4.2)
IOP-lowering medication *N* (%)	13,396	354 (2.6)
Glaucoma intervention (surgery or SLT) (*N*, %)	13,396	209 (1.6)

*Measured IOP is adjusted by 1.3 for participants on glaucoma medicines and excludes those who received glaucoma surgery or SLT.

BMI, body mass index; COPD, chronic obstructive pulmonary disease; IOP, intraocular pressure; OAG, open-angle glaucoma, SLT, selective laser trabeculoplasty.

$P < 0.001$; Supplementary Table S8), and a moderate correlation with directly measured CPD (0.45 CPD per SD, 95% CI = 0.19 to 0.70, $P < 0.001$). There was also a strong correlation between the smoking intensity GRS and the measured number of CPD (0.68 CPD per SD, 95% CI = 0.43 to 0.93, $P < 0.001$) although the

Table 5. Multivariable Linear and Logistic Regression Analyses for Glaucoma Outcome Measures and the Genetic Risk Score (GRS) of Smoking Initiation and Intensity Versus Directly Measured Smoking Behaviors*,†

	Outcome Trait											
	OAG			OAG (IOP Added to Model)			IOP (mm Hg)‡			vCDR		
Exposure Trait	OR (95% CI)	*P* Value	*N*	OR (95% CI)	*P* Value	*N*	Estimate (95% CI)	*P* Value	*N*	Estimate (95% CI)	*P* Value	*N*
GRS for smoking initiation (Z-score)	0.84 (0.73, 0.98)	0.02	6512	0.88 (0.75, 1.03)	0.10	6,420	−0.09 (−0.17 to −0.01)	0.04	6496	0.00 (−0.004 to 0.004)	0.88	6408
Directly measured history of smoking	1.11 (0.80, 1.54)	0.54	7437	1.09 (0.75, 1.57)	0.66	7328	−0.08 (−0.25 to 0.10)	0.38	7417	−0.006 (−0.02 to 0.003)	0.21	7316
GRS for smoking intensity (Z-score)	1.04 (0.91, 1.18)	0.60	7652	0.93 (0.79, 1.08)	0.34	6420	−0.06 (−0.14 to 0.02)	0.13	6496	−0.003 (−0.007 to 0.001)	0.13	6408
Directly measured CPD	1.00 (0.98, 1.02)	0.99	4966	1.00 (0.99, 1.02)	0.73	4904	−0.002 (−0.01 to 0.007)	0.65	4965	1.0E-4 (−0.0005 to 0.0004)	0.77	4898

*All effect sizes are per standard deviation increase in the exposure trait.

†All models were adjusted for age, sex, body mass index, type 2 diabetes mellitus, chronic obstructive pulmonary disease, hypertension, alcohol consumption, coffee consumption, total caloric intake, anti-asthmatic inhaler usage, and systemic corticosteroid usage. Age, body mass index, alcohol consumption, coffee consumption and total caloric intake are defined as continuous variables. The remaining covariates are defined as binary variables.

‡Measured IOP is adjusted by 1.3 for participants on glaucoma medicines and excludes those who received glaucoma surgery or selective laser trabeculoplasty.

CI, confidence interval; CPD, cigarettes per day; IOP, intraocular pressure; OAG, open-angle glaucoma; OR, odds ratio; vCDR, vertical cup-disc ratio.

GRS for smoking intensity was not associated with the exposure of any history of smoking ($P = 0.39$).

In the Rotterdam Study, the GRS for smoking initiation was inversely associated with OAG (OR = 0.84 per SD, 95% CI = 0.73 to 0.98, $P = 0.02$; Table 5). Like the MR experiments, the GRS for smoking initiation was also correlated with lower IOP (−0.09 mm Hg per SD, 95% CI = −0.17 to −0.01, $P = 0.04$), but not significantly correlated with vCDR ($P = 0.88$). When adding IOP to the multivariable model for OAG, the inverse relationship persisted but was no longer significant (OR = 0.88 per SD, 95% CI = 0.75 to 1.03, $P = 0.10$). There was no significant relationship between the directly assessed history of smoking and glaucoma ($P = 0.54$), IOP ($P = 0.38$), or vCDR ($P = 0.21$). In contrast to the MR experiments, the GRS for smoking intensity was not associated with either glaucoma ($P = 0.60$), IOP ($P = 0.13$), or vCDR ($P = 0.13$). There was no association between directly assessed CPD and any of our glaucoma outcome measures ($P \geq 0.65$).

Discussion

This study investigated the genetic relationship between smoking and glaucoma using large publicly available GWAS summary statistic datasets. From MR experiments, we detected a consistent pattern of inverse associations with modest effect sizes between genetically predicted smoking and glaucoma. Individual-level data from the Rotterdam Study provided additional evidence for an inverse relationship, with the smoking initiation GRS associated with both lower measured IOP and lower odds of clinically determined OAG. Notably, there were no associations between the reported smoking behaviors and measured IOP and glaucoma in the Rotterdam Study. Smoking cessation remains an important ocular and systemic health goal; yet, the inverse genetic underpinnings between smoking predisposition and glaucoma risk warrant further research. Interestingly, smoking cessation shares heritability with smoking initiation and smoking intensity[16] and one US Food and Drug Administration (FDA)-approved smoking cessation agent, bupropion, was associated with a reduced risk of OAG.[40,41]

This study utilized genome-wide genetic correlations to estimate the overall shared architecture between two complex traits. We provide evidence that our global genetic correlations had adequate sample sizes to detect genetic correlations between smoking and glaucoma traits (see Supplementary Table S1). Although our findings were not significant following Bonferroni correction, the data are consistent in magnitude and direction with previously published reports using smoking summary-level datasets that contained smaller sample sizes. Khawaja et al. demonstrated a weak but significant inverse relationship between smoking initiation and IOP ($r_g = -0.13$, SE = 0.04, $P = 0.002$).[23] Furthermore, Gharahkhani et al. found inverse trends between OAG and smoking initiation ($r_g = -0.04$, SE = 0.06, $P = 0.48$) and smoking intensity ($r_g = -0.07$, SE = 0.06, $P = 0.24$) using a different smoking meta-analysis cohort.[22] Therefore, we hypothesize that the consistent trend of weak inverse global genetic correlations may reflect an underlying biology driven by specific genetic regions or weakened by opposite effect sizes at different loci.[42,43]

Whereas our bivariate global genetic analyses examined all common variants across the genome, our MR experiments used only genome-wide significant SNPs of the smoking traits to identify associations with glaucoma phenotypes. Of note, there were no overlapping genome-wide significant SNPs between the smoking initiation and smoking intensity traits (see Supplementary Tables S2, S3) despite their modest r_g (0.28; see Supplementary Table S6). This suggests the biology related to these two traits is somewhat different and subsequent associations with glaucoma endophenotypes would have a different genetic basis. The MR experiments revealed a significant inverse association between genetically predicted smoking initiation and lower IOP. However, the result was only reproducible under the MR-PRESSO and multivariable MR methods. Furthermore, whereas the MR test statistics for the smoking initiation IV, which contained hundreds of SNPs, suggested significant pleiotropy, it was balanced as indicated by the MR-Egger intercept test (i.e. some pleiotropic SNPs likely had a positive effect on the outcome, whereas others had a negative effect, and together these canceled each other; see Supplementary Table S4). We still observed an inverse significant association between the smoking initiation GRS and lower IOP in the Rotterdam Study. Overall, we believe the association between a genetic predisposition to smoking initiation and lower IOP has scientific rigor but may not be exclusively causal in nature.

The MR experiments also revealed an inverse relationship between smoking intensity and OAG. On the other hand, in the Rotterdam Study, the smoking initiation GRS, but not the smoking intensity GRS, was associated with a reduced risk of clinically determined OAG. Although we performed multiple MR sensitivity analyses, which demonstrated a consistent association, and we did not find any evidence of a violation of the MR assumptions (see Supplementary Table S5), it is still possible that these assumptions

were not completely met. Given the null association between the smoking intensity GRS and OAG in the Rotterdam Study, this may suggest that the smoking intensity SNPs could have affected the outcome outside the exposure-outcome biological pathway (violation of assumption 3 of MR experiments).[44,45] Even so, it does not discount the inverse relation between the smoking initiation GRS and OAG in the Rotterdam Study.

The inverse association detected between the smoking initiation GRS and OAG was surprising, but not unexpected. The randomized placebo-controlled United Kingdom Glaucoma Treatment Study (UKGTS) similarly found an inverse association between smoking initiation and decreased rates of glaucoma progression based on visual field testing.[46] In contrast, a recent retrospective study detected an association between increased visual field loss and smoking intensity (defined by pack years and not CPD) but no association with smoking initiation.[47] Although this study accounted for BMI and alcohol consumption, the findings may be vulnerable to residual confounding from other unmeasured lifestyle-related factors associated with smoking.[48,49] Interestingly, when we adjusted for IOP, we found that the association between the smoking initiation GRS and reduced risk of OAG persisted but lost significance. As glaucoma and IOP are strongly correlated ($r_g = 0.68$; see Supplementary Table S7), genetically predicted smoking initiation may decrease the odds of glaucoma via an IOP-dependent pathway. To our knowledge, there has been no overlap in significantly associated SNPs identified for smoking initiation and glaucoma, likely due to the higher significance threshold required in GWAS studies. An exploratory review of our smoking initiation IV and genetically determined IOP revealed SNPs of weak effect sizes (see Supplementary Figure S1b); however, the rs28441558 SNP appeared to have the strongest inverse effect size with IOP. This SNP is located in *CHD3* and encodes an ATPase that is part of the nucleosome remodeling and deacetylase (NuRD) complex.[50] Although further research is needed, the genetic connection between smoking and lower IOP may be related to epigenetic modifications.[51,52]

This study has several strengths. We estimated global genetic correlations and performed MR analyses using well-powered GWAS data and then validated our results using individual-level data. These genetic approaches, which can serve as proxies for smoking behaviors, mitigate bias from reverse causation, residual confounding, and survival bias.[10] Furthermore, our analyses were limited to participants of European descent, thereby minimizing bias due to population stratification and LD score mismatch. Although global genetic correlation estimates can be difficult to interpret and all assumptions for MR experiments cannot be formally tested,[53,54] we used several approaches including MR sensitivity analyses and external validation. For the latter, we took several potential confounders into account. Sample overlap is an increasingly common issue in two-sample MR analyses with many studies contributing genetic data to multiple GWAS and genetic consortia. However, given the strength of our IV (F statistics all >45) and the small degree of overlap (<10%), it is unlikely that this biased our MR results significantly.[55] To confirm this, we performed sensitivity analyses using summary statistics from GSCAN which excluded UK Biobank and 23andMe participants, and our results remained essentially unchanged (see Supplementary Tables S9, S10). This study also has limitations. By restricting the study to European participants, our findings may not be generalizable to people of other ancestries. For the MR experiments, the estimates are best viewed as a test of causal association rather than true effect size and our IVs may have limitations that affect their validity. Concerning the Rotterdam Study cohort, we were unable to examine associations between the smoking traits and all six glaucoma-related traits due to reduced sample sizes.

In conclusion, this study reveals that the genetic architectures that contribute to starting and maintaining cigarette smoking are not shared with the genetic propensity to higher IOP or increased risk of glaucoma. There may be a weak inverse relation between genetic loci that contribute to starting smoking and lower IOP. Similarly, there may be a weak inverse relationship between genetic predisposition to higher smoking intensity and reduced OAG risk. The biology of the smoking traits we studied are complex and polygenic. Our validation efforts suggest that it is an individual's genetic liability to smoking behaviors and not the reported smoking behavior that is responsible for the inverse relationships we report. Discovering and understanding the functional significance of shared loci among smoking traits, IOP, and OAG will be important next steps for better understanding glaucoma pathogenesis and environmental influences.

Data Availability

The summary statistics used in this study are available through the GWAS Catalog under the study accession identifiers, OAG: GCST90011766, GCST90011767, GCST90011770); mRNFL:

GCST90014266; mGCIPL: GCST90014267; VCDR: GCST004075; smoking initiation: GCST007474; smoking intensity: GCST007459; and alcohol consumption: GCST007472. The summary statistics for coffee consumption can be found through the Northwestern Digital Hub (https://digitalhub.northwestern.edu/catalog). The summary statistics for AI-VCDR are available at https://xikunhan.github.io/site/publication/. The IOP data using UK Biobank and Epic-Norfolk can be requested through the UK Biobank Access Management System (https://www.ukbiobank.ac.uk/) and the Epic Norfolk website (https://www.epic-norfolk.org.uk/). Data from the Rotterdam Study can be obtained upon request. Requests should be directed toward the management team of the Rotterdam Study (datamanagement.ergo@erasmusmc.nl), which has a protocol for approving data requests. Due to restrictions based on privacy regulations and informed consent of the participants, data cannot be made freely available in a public repository.

URLS

GSCAN website: https://conservancy.umn.edu/handle/11299/201564

GWAS Catalog: https://www.ebi.ac.uk/gwas/home

IBM SPSS Statistics for statistical computing: https://www.ibm.com/analytics/spss-statistics-software

LD Score Regression: https://github.com/bulik/ldsc

Mendelian Randomization R package: https://cran.r-project.org/web/packages/MendelianRandomization/index.html

MRPRESSO R package: https://github.com/rondolab/MR-PRESSO

Northwestern Digital Hub: https://digitalhub.northwestern.edu/catalog

R programming language and software environment for statistical computing: https://cran.r-project.org/

TwoSampleMR R package: https://mrcieu.github.io/TwoSampleMR/

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Members of the International Glaucoma Genetics Consortium

Tin Aung, Kathryn Burdon, Li Chen, Ching-Yu Cheng, Jamie Craig, Angela Cree, Victor de Vries, Sjoerd Driessen, John Fingert, Paul Foster, Puya Gharahkhani, Christopher Hammond, Caroline Hayward, Alex Hewitt, Pirro Hysi, Nomdo Jansonius, Fridbert Jonansson, Jost Jonas, Michael Kass,

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