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Genetic Heritability of Pigmentary Glaucoma and Associations With Other Eye Phenotypes

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IMPORTANCE Mechanisms behind pigmentary glaucoma, a form of early-onset glaucoma that may potentially lead to severe visual impairment or blindness, are poorly understood.

OBJECTIVE To calculate the single-nucleotide polymorphism (SNP) heritability of pigmentary glaucoma and identify genetic associations with the disease.

DESIGN, SETTING AND PARTICIPANTS This genome-wide association study included affected individuals from Germany and control participants from the United Kingdom. Genome-wide information was obtained for patients with pigmentary glaucoma and control participants free of glaucoma by using the Illumina Human Omni Express Exome 8v1-2 chip and genomic imputation. The SNP heritability of pigmentary glaucoma was estimated through a restricted maximum likelihood analysis. Associations between the genetic variants and pigmentary glaucoma obtained from age, sex, and principal component–adjusted logistic regression models were compared with those of SNPs previously associated with other eye phenotypes using Pearson product-moment correlations. Data were collected from November 2008 to January 2018, and analysis was completed between April 2018 and August 2019.

MAIN OUTCOMES AND MEASURES An estimate of SNP-explained heritability for pigmentary glaucoma; correlations of effect sizes between pigmentary glaucoma and iris pigmentation and myopia; and correlations of effect sizes between pigmentary glaucoma and other eye phenotypes.

RESULTS A total of 227 affected individuals (mean [SD] age, 58.7 [13.3] years) and 291 control participants (mean [SD] age, 80.2 [4.9] years) were included; all were of European ancestry. The SNP heritability of pigmentary glaucoma was 0.45 (SE, 0.22; $P = 6.15 \times 10^{-10}$). Twelve SNPs previously reported with genome-wide significant association with eye pigmentation were associated with pigmentary glaucoma's SNP heritability (4.9% SNP heritability; 0.022; $P = 6.0 \times 10^{-4}$). Pigmentary glaucoma SNP effect sizes were correlated moderately for myopia (r, 0.42 [95% CI, 0.14-0.63]; $P = 4.3 \times 10^{-3}$) and more strongly with those for iris pigmentation ($r = -0.69$ [95% CI, −0.91 to −0.20]; $P = .01$), although this was nonsignificant per a strict adjusted significance threshold ($P < .01$).

CONCLUSIONS AND RELEVANCE These findings support the conclusion that pigmentary glaucoma may have a genetic basis and be highly heritable. Variants associated with lighter eye color and myopia appear to be associated with increased risk of pigmentary glaucoma, but no shared genetic basis with primary open-angle glaucoma (or its quantitative endophenotype of cup-disc ratio) was observed.

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Supplemental content

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Glaucoma is currently the leading cause of irreversible blindness worldwide.[1] Although various subtypes of glaucoma are pathogenetically different, they all result in loss of retinal ganglion cells, with visible damage to the optic nerve head, thinning of retinal nerve fiber layer, and progressive visual field loss, as well as blindness if the condition is left untreated.[2] Pigmentary glaucoma (PG) is not as common as primary open-angle glaucoma (POAG), but it affects patients at a much earlier age. The mean age at diagnosis for PG is during the fifth decade of life[3]; the initial age at onset is likely to be younger, given the lack of symptoms and screening performed at this age, which result in a late diagnosis.

Pigment dispersion syndrome (PDS) is a precursor to PG. This condition is characterized by the atypical accumulation of iris pigment, predominantly deposited in the trabecular meshwork but also throughout the anterior segment of the eye,[4] where the pigment is phagocytosed.[5] Pigment dispersion syndrome alone is a relatively benign condition, with an estimated population prevalence of 2.45% in European individuals.[6] Although the trabecular meshwork can accommodate substantial accumulation of pigment without intraocular pressure (IOP) increases,[7] pigment buildup may lead to phagocytic capacity overload, trabecular epithelium cell death (which may cause irreversible changes of the denuded trabecular beams), aqueous outflow obstruction, elevated IOP, and glaucomatous optic disc changes.[8] About 15% of patients with PDS will develop PG within 15 years from initial diagnosis,[9] and up to 50% will develop it at some point during the patient's lifetime.[10] Factors that determine whether a patient has PDS or PDS evolves into PG are currently unknown, although male sex, myopia, European ancestry, and a family history of PG have been associated with a higher risk of PG.[11]

Familial aggregation and recurrences in relatives[4,11] suggest a genetic component for PG. A previous genetic linkage study suggested that a locus in the 7q35-7q36 region is linked to PDS and PG,[12] and more recently variants in the premelanosome protein gene (*PMEL*) have been identified in a family study.[13] The relative importance of genetic factors remains unknown; DBA/2J murine models that are homozygous for both a premature stop codon in the transmembrane glycoprotein NMB gene (*Gpnmb*) and a recessive tyrosinase-related protein 1 (*Tyrp1*) mutation show iris pigment dispersion and elevated IOP, causing glaucomatous damage,[14,15] but no association has yet been detected between any variants within their human orthologues and PG,[16,17] nor have any other genetic variants been reported for association with either PDS or PG outside of familial studies.

In this study, we further elucidate the genetics of PG through a genome-wide association study (GWAS) of affected individuals and control participants to estimate the proportion of PG risk that can be attributed to common genetic variants (the single-nucleotide polymorphism [SNP] heritability) in a population of unrelated individuals. We also evaluate the similarities in genetic architecture between PG and common ocular risk factors, some of which were previously hypothesized as risk factors to PG, such as myopia and eye pigmentation, but also IOP, POAG, and vertical cup-disc ratio.

Key Points

Question How heritable is pigmentary glaucoma, and are there shared genetic risks with other eye phenotypes?

Findings This genome-wide association study of 227 affected individuals and 291 control participants estimated that 45% of disease variance is associated with common single-nucleotide polymorphisms. Pigmentary glaucoma appeared genetically distinct from primary open-angle glaucoma and its endophenotypes, but some single-nucleotide polymorphisms associated with eye color and myopia were correlated with those for pigmentary glaucoma.

Meaning This study's findings support that pigmentary glaucoma may be heritable and found evidence for shared genetic architecture between myopia and iris pigmentation with pigmentary glaucoma.

Methods

Participants

All participants gave full written informed consent to participate in the study. Recruitment of all affected individuals and control participants followed the Ethical Principles for Medical Research Involving Human Subjects of the Helsinki Declaration. For our study, we used data from individuals affected by PG cases from Germany. All cases were examined by the same ophthalmologist, and PG was diagnosed on the basis of the presence of glaucomatous optic neuropathy accompanied by visual field loss, elevated IOP (>21 mm Hg), presence of Krukenberg spindles, and presence of a hyperpigmented trabecular meshwork. Recruitment of these patients was approved by the ethics committees of the University of Tübingen and the University of Würzburg.

The control participants were aging individuals recruited from eye clinics in South London, United Kingdom. (Ethical approval was obtained from National Research Ethics Committee–Brent.) These individuals were selected based on absence of any clinical sign of glaucoma. Specifically, these participants showed no sign of pathological disc changes, elevated IOP, or pigmented trabecular meshwork. The age difference between the 2 groups was intentional and designed to include supercontrol participants who were unlikely to develop glaucoma, to minimize classification errors.

DNA Extraction and Genotyping

Patient DNA was extracted from peripheral blood lymphocytes using the Magnetic Separation Module I from Chemagen using DNA chemistry (Chemagic DNA Blood Kit Special [Chemagen AG]). Control DNA was extracted from whole, frozen EDTA blood using the Nucleon BACC 3 kit (Gen-Probe Life Sciences Ltd). Both affected individuals and control participants were genotyped using the Human Omni Express Exome 8v1-2 BeadChip (Illumina). Genotypes were submitted to stringent quality-control pipelines, following established best practices previously described,[18] which assured that only high-quality individual genotypes were included, such as individuals

with sufficiently high genotype success rates (>0.95), who showed no excessive relatedness to other participants in the sample (<0.05) and were of nonadmixed European ancestry after principal component analyses (PCA), among other criteria. Polymorphic sites were excluded from subsequent analyses whenever they had a low genotype success rate (<0.95) or were in Hardy-Weinberg disequilibrium ($P < 1 \times 10^{-4}$). Most quality-control analyses were carried out using the PLINK software version 1.9 (GRAIL Inc).[19] Principal component analyses ascertained the full European ancestry of the participants by overlaying them to control participants from the HapMap project phase 3. Genotypes were imputed up to the Haplotype Reference Consortium panel,[20] using the Michigan Imputation Server.[20,21]

Statistical Analyses

The variance explained by genotyped SNPs, a proxy for genetic heritability, was estimated using restricted maximum-likelihood (REML) analysis as implemented in the Genome-wide Complex Trait Analysis (GCTA) software version 1.26.0 (Yang).[22,23] This method correlates genetic similarities with phenotypic variability within a population sample. This is conceptually similar to family-based heritability models in which genetic similarity is modeled on the basis of family relationships, while GCTA takes advantage of the smaller degrees of similarity between any 2 individuals in a population sample. The GCTA software calculates the proportion of trait variance explained by the collective additive effect sizes of all available SNPs. This method shows equivalence to traditional family-based heritability calculations; however, because of limitations discussed elsewhere,[24] the estimates of variability produced only include the genomic variation measured using a given platform and so are lower than family-based heritability. Significance tests for the SNP heritability estimate are performed by testing against the null hypothesis (SNP heritability = 0), with significant results indicating confidence that the estimate is greater than 0. To distinguish between these methods, results obtained using the REML methodology are hereinafter described as *SNP heritability*, as opposed to *narrow-sense heritability* obtained from family-based studies. To apply this method to case-control data, results are transformed from the linear scale to the liability scale, so the calculated SNP heritability is a quantification of the proportion of disease risk that is explained by genotyped SNPs. For our analysis, directly genotyped, pruned SNPs (ie, those not in linkage disequilibrium with each other) were used for the SNP heritability calculations. This model requires the value of disease prevalence to be known to make calculations of the genetic heritability of discrete phenotypic traits possible. Because no population prevalence of PG was published at the time of writing, we made estimates based on the population prevalence of PDS and the incidence of PG in patients with PDS. From the reported PDS prevalence 2.45%[6] and the assumption of a PDS-to-PG conversion rate of 15%,[9] we inferred and subsequently used 0.37% as the value of the most likely prevalence of PG in a European population.

The association between PG status and each available polymorphism (after imputation and quality control) was tested using logistic regression, as implemented in the PLINK 1.9 software,[25] adjusting for age, sex, and the first 3 principal components. Because a GWAS of this size has insufficient power to identify associated variants with genome-wide significance, it is inherently underpowered for methods, such as linkage disequilibrium score regression,[26] to estimate SNP heritability and test for genetic correlation between other traits of interest. Instead, we used the Pearson correlation test to compare effect sizes (regression coefficients) of SNPs associated with other phenotypes of interest and their association with PG.

For comparison of SNP effect sizes, we used summary statistics from the most well-powered GWAS for the traits of interest that have been published to our knowledge to date. For PG and eye pigmentation, we used regression coefficients for 12 SNPs with genome-wide significant associations for eye color.[27] For myopia, we used coefficients from 45 reported polymorphic loci[28] that passed the quality-control criteria in our data set. For IOP, we used 133 significantly associated SNPs identified by Khawaja et al[29] using conditional analysis. For POAG, we used the 18 SNPs reported by Choquet et al[30] as having genome-wide significance. For vertical cup-disc ratio, we used the 18 polymorphisms associated at a GWAS level.[31] In addition to the correlation of effect sizes, the amount of PG risk variance on the liability scale (partitioned heritability) explained by the subsets of polymorphisms associated with each phenotype was calculated using REML analysis, as described. Bonferroni correction for multiple testing was applied for the partitioned heritability and correlation analyses, with $P < .01$ determined to be significant.

These SNPs were also used as instruments in mendelian randomization (MR) analysis to test if either iris pigmentation or IOP have detectable potential causality in PG. These analyses were conducted in the R package Mendelian Randomization version 0.3.02018 (R Foundation for Statistical Computing). Data were collected from November 2008 to January 2018, and analysis was completed between April 2018 and August 2019.

Results

A total of 227 individuals with PG were included. All were of self-reported European origin. Their mean (SD) age was 58.7 (13.3) years. In addition, 291 control participants (mean [SD] age, 80.2 [4.9] years), all of self-reported European ancestry, were included. The European origin of all participants was confirmed. Affected individuals and control participants were suitably matched for ancestry when the first 3 principal components were compared (eFigures 1, 2, and 3 in the Supplement).

We performed a GWAS using SNPs available after imputation. This analysis did not produce any associations of genome-wide significance. This analysis confirmed lack of genomic inflation (genomic inflation factor $\lambda = 1$). Under the assumption of a 0.37% population prevalence and despite the low sample size, REML analysis found that a portion of PG variance was associated collectively with all the polymorphisms from the genotyping array (0.45 [SE, 0.22]; $P = 6.15 \times 10^{-10}$).

A proportion of SNP heritability was associated with SNPs that have previously been associated with other ocular traits (**Table 1**), such as iris color (SNP heritability, 0.022 [4.9%];

$P = 6.0 \times 10^{-4}$) and myopia (SNP heritability, 0.02 [4.4%]; $P = .04$). By contrast, we were unable to detect any contribution to PG heritability of loci associated with IOP, POAG, and vertical cup-disc ratio (Table 1).

In addition, genetic correlations between myopia and eye pigmentation with PG were found (**Table 2**). Genetic susceptibility loci associated with myopia were significantly correlated, with a higher risk for PG after correction for multiple testing ($r = 0.42$ [95% CI, 0.14-0.63]; $P = 4.3 \times 10^{-3}$), and genetic factors predisposing to darker iris colors were negatively correlated with PG ($r = -0.69$ [95% CI, −0.91 to −0.20]; $P = .01$). There was a correlation between PG and POAG-associated loci ($r = 0.67$ [95% CI, 0.29-0.86]; $P = 2.6 \times 10^{-3}$) and IOP-associated loci ($r = 0.22$ [95% CI, 0.05-0.37]; $P = .01$).

We further tested the lysyl oxidase like 1 (*LOXL1*) locus in this sample, because exfoliation glaucoma is a phenotype that is susceptible to being misdiagnosed as PG, given increased pigment found in the iridocorneal angle in this condition. No SNPs in the *LOXL1* locus approached statistical significance, including the most strongly associated SNP, rs3825942 (for exfoliation glaucoma[32]). The lack of an association in this region confirms the phenotyping quality in our cohort.

The MR analyses did not identify any direct potential causality of IOP or eye color in PG. (More information is in eTables 1 and 2 in the Supplement.)

Table 1. Single-Nucleotide Polymorphism (SNP) Heritability of Pigmentary Glaucoma, Accounted for by the SNPs Associated With Other Eye Phenotypes

Phenotype	No. of SNPs	SNP Heritability (SE)	*P* Value
Pigmentation	12	0.022 (0.013)	6.0×10^{-4}
Myopia	45	0.02 (0.012)	.04
Intraocular pressure	133	0.011 (0.015)	.31
Primary open-angle glaucoma	18	7.9×10^{-4} (5.5×10^{-3})	.50
Vertical cup-disc ratio	18	1.8×10^{-3} (5.9×10^{-3})	.50

Table 2. Correlation Between the Effect Sizes for Single-Nucleotide Polymorphisms (SNPs) Associated With Other Phenotypes and Their Effect Sizes for Pigmentary Glaucoma

Phenotype	No. of SNPs	Pearson *r* (95% CI)	*P* Value
Pigmentation	12	−0.69 (−0.91 to −0.20)	.01
Myopia	45	0.42 (0.14-0.63)	4.3×10^{-3}
Intraocular pressure	133	0.22 (0.05-0.37)	.01
Primary open-angle glaucoma	18	0.67 (0.29-0.86)	2.6×10^{-3}
Vertical cup-disc ratio	18	0.43 (−0.04 to 0.75)	.07

Discussion

To our knowledge, this is the first attempt to evaluate the SNP heritability of PG to date. Despite observations of familial aggregation, and epidemiologic association with factors that are significantly heritable, the apparent sporadic nature of PG and its low population prevalence have made classic family-based studies of heritability nearly impossible. Here, we have overcome these difficulties by exploring the phenotypic variance attributable to measured genomic polymorphisms as a proxy for heritability.

There are a number of conclusions we can draw from our analyses. First, our findings are consistent with a possible genetic component in the cause of PG. The SNP heritability estimate of 0.45 is in line with those of many different human complex phenotypes and diseases (for example, body height, which also has an estimated SNP heritability of 0.45).[24] The SNP-based estimates of heritability are usually lower than true narrow-sense heritability estimates from family-based studies. For example, the classic twin-based narrow-sense heritability of body height is estimated to be around 0.8.[33]

Second, our analyses suggest that PG has a distinctive risk profile from POAG. This is compatible with prior reports of no significant difference in the family history of POAG in patients with PG compared with nonglaucomatous PDS.[34] Genetic variants associated with IOP explain sizeable proportions of POAG risk.[29] Although IOP-associated and POAG-associated variants were correlated with PG risk, their actual contribution to its SNP heritability was insignificant. It is possible that an existing genetic predisposition toward higher IOP may be associated with patients showing symptoms of glaucoma, but it is equally possible that the genetic correlation observed may simply reflect the fact that elevated IOP was a formal criterion for PG diagnosis in the cases of this study's participants. The findings of the MR analysis did not support the potential causality of IOP in PG. This may be due to the large standard errors for outcome (PG) estimates, or because the MR-Egger test was able to detect and correct for horizontal pleiotropy.[35] Much like the GWAS analysis, statistical power is the limiting factor preventing any certain conclusions being drawn from MR analysis in this data set. There was no evidence for sharing of genetic risk factors with other endophenotypes correlated with POAG, such as vertical cup-disc ratio.

Third, our analyses point to shared genetic risks between iris pigmentation, myopia, and PG. It has previously been noted that PDS and PG primarily affect European individuals, especially those with lighter eye color.[36] It is not clear whether the contribution of genetic factors involved in eye pigmentation is based on pathophysiology or if it is a consequence that PG signs (eg, transillumination, pigment in angle) may be more noticeable in patients with lighter eye colors.[4]

Although the contribution of genetic variants associated with myopia was nominal, it is consistent with previous observations published in the literature that led to suggestions about a molecular basis for which PG is more frequent in patients with myopia. One possible explanation, as proposed by Campbell, is that myopic eyes of male patients are elongated with iris bowing, which causes mechanical rubbing between the iris and so-called packets of zonular fibers, displacing iris pigment.[37] Kaiser-Kupfer et al[38] presented cases inconsistent with this theory, suggesting additional, other (unknown) factors in PG pathogenesis. Our results complement these studies, suggesting that myopia accounts for a proportion of risk but other genetic factors should be considered. Finally, in cataract surgery, so-called iris bounce

(lens-iris diaphragm retropulsion syndrome) is more common in individuals with high myopia.[39]

One of the strengths of our study is the systematic nature of our analyses. We used genome-wide information to evaluate SNP heritability and the contribution of different sets of genetic variants to it. Pigmentary glaucoma is a relatively rare condition, and it is practically difficult to collect the sample sizes required to estimate heritability through family-based studies or use the power of genetic association studies to identify genes and highlight pathophysiological mechanisms. Our study took advantage of prior knowledge and identified mechanisms that contribute toward PG in the general population.

Limitations

This study also has limitations, including limited statistical power. This prevented the use of established, systematic methods such as linkage disequilibrium score regression, which has been successful in identifying genetic correlations for traits analyzed in larger cohorts. Although some aspects of the limited statistical power were mitigated by hypothesis-driven analyses, measuring genetic-risk sharing with phenotypes already suspected to be involved in PG, our results have large confidence intervals of estimates. Typically, larger sample sizes are required for REML analyses to calculate SNP heritability with a reasonable margin of error. The relatively limited power of our modest cohort is reflected in the standard error (0.22) for the SNP heritability estimate of 0.45. There was also an unsurprising absence of associations, given the sample size. While we attempted to maximize our cohort's informativeness using strict phenotyping and high-quality control data, it is also very likely that other genetic variants had potentially high levels of correlation with PG, which our study was not sufficiently well powered to identify.

Conclusions

We provide evidence that PG may be a heritable condition and that there is shared genetic risk with iris pigmentation and myopia. These results point to some possible mechanisms that may contribute to this disease, which can be better explored by future work in the field.

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Concept and design: Hysi, Hammond.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Simcoe, Hammond.
Critical revision of the manuscript for important intellectual content: All authors.
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