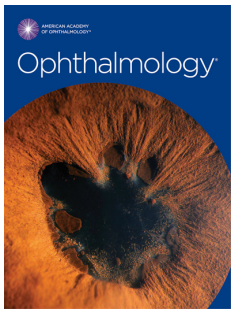

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GWAS identifies two common loci associated with pigment dispersion syndrome/pigmentary glaucoma and implicate myopia in its development

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Abstract

Purpose – To identify genetic variants associated with pigment dispersion syndrome and pigmentary glaucoma in unrelated patients, and to further understand the genetic and potentially causal relationships between pigment dispersion syndrome and associated risk factors.

Design – A two-stage genome-wide association meta-analysis with replication and subsequent in-silico analyses including Mendelian randomisation.

Subjects – A total of 574 cases with pigmentary glaucoma and/or pigment dispersion syndrome and 52,627 controls of European descent.

Methods – Genome-wide association analyses were performed in four cohorts and meta-analysed in three stages: first a discovery meta-analysis of three cohorts, secondly replication was performed in the fourth cohort, thirdly all four cohorts were meta-analysed to increase statistical power. Two-sample Mendelian randomisation was utilised to determine whether refractive error and intraocular pressure exert causal effects over pigment dispersion syndrome.

Results – Significant association was present at two novel loci for pigment dispersion syndrome/pigmentary glaucoma. These loci and follow up analyses implicate the genes *GSAP* (lead SNP: rs9641220, $p=6.0x10^{-10}$) and *GRM5/TYR* (lead SNP: rs661177, $p=3.9x10^{-9}$) as important factors in disease risk. Mendelian randomisation showed significant evidence that negative refractive error (myopia) exerts a direct causal effect over pigment dispersion syndrome ($p=8.9x10^{-7}$).

Main Outcome Measures – A) The association of genetic variants with pigment dispersion syndrome and, B) whether myopia exerts causal effects over pigment dispersion syndrome.

Conclusions – Common SNPs relating to the *GSAP* and *GRM5/TYR* genes are associated risk factors for the development of pigment dispersion syndrome and pigmentary glaucoma. Although myopia is a known risk factor, this study is the first to use genetic data to demonstrate that myopia is, in part, a cause of pigment dispersion syndrome and pigmentary glaucoma.

Introduction

Glaucoma is the leading cause of irreversible blindness worldwide[1] and pigmentary glaucoma (PG) is one of the most common secondary glaucomas[2]. Though not as prevalent as primary open-angle glaucoma (POAG), PG has a disproportionate disease burden as it has a much younger average age of onset, in mid-thirties for men and late forties for women[3]. Therefore, it is important that patients are identified early and provided with medical intervention, to prevent many years of blindness in severely affected individuals.

Pigment dispersion syndrome (PDS) is a precursor of PG and to glaucomatous damage. PDS is characterised by the abnormal dispersion of pigment from the iris pigment epithelium. The most well-known cause for this is friction between zonules and the posterior iris due a peripheral iris concavity[4]. Once released, the pigment may accumulate in the trabecular meshwork, resulting in obstruction of aqueous humour outflow and cell atrophy[3, 5]. The obstruction of aqueous humour outflow leads to elevated intraocular pressure (IOP) and glaucomatous optic neuropathy. The incidence of progression to PG in PDS patients is estimated to be 15% within 15 years after initial diagnosis[6], and up to 50% during their lifetime[7].

It has long been hypothesised that PDS and PG have a genetic origin[2, 8-10]; familial studies have identified associated risk between PG and mutations in the *PMEL* gene[11] and a locus on chromosome 7[12]. Additionally, mutations in the *Gpnmb* and *Tyrp1* genes result in PG-like pathology in murine models, though no association has thus far been reported in human orthologues for these genes[13].

The prevalence of PDS in European populations may be as common as 2.45%[14], so it is probable that common genetic risk factors contribute to sporadic PDS and PG

cases. We have previously demonstrated that 45% of PG risk is explained by common genetic variants[10]. However, to our knowledge, no sufficiently well powered genome-wide studies of genetic factors influencing PDS or PG have been conducted. Here we report for the first time the results of an international meta-analysis of genome-wide association studies (GWAS) of 574 cases and 52,627 controls of European descent from four centres, and report the first common genetic loci associated with PDS/PG at genome-wide level.

Methods

Participants and phenotyping

Total numbers of cases and controls are summarised in Table 1.

King's College London (KCL) cohort – Full details of this cohort have been previously described[15]. Briefly, all participants were of confirmed European ancestry, with PG cases recruited in Germany and controls recruited in South London. 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. Controls were selected based on absence of any clinical signs of glaucoma or of PDS. All participants gave full written informed consent.

All participants were genotyped using the Human Omni Express Exome 8v1-2 BeadChip (Illumina). Following QC, genotypes were then imputed to the Haplotype Reference Consortium panel[16] using the Michigan Imputation Server[17]. For the final analyses there were 227 PG cases and 291 controls.

Principal component analysis confirmed that despite different nationalities cases and controls were suitably matched[15], as Northern European populations share recent common ancestry and genetic background[18].

GERA – The Genetic Epidemiology Research in Adult Health and Aging (GERA) cohort[19, 20] consists of 110,266 adult (18 years and older) members of the Kaiser Permanente Northern California (KPNC) Medical Care Plan who consented to participate in the Research Program on Genes, Environment, and Health. This study included 50,885 adults who self-reported as non-Hispanic white. Pigmentary glaucoma cases were identified in the KPNC electronic health record system based on the following International Classification of Diseases, Ninth Revision (ICD-9) diagnosis code: 365.13, and corresponding ICD-10 code: H40.13x. All selected pigmentary glaucoma cases (N=64) had at least two diagnoses of pigmentary glaucoma, and at least one of the diagnoses was made by a Kaiser Permanente ophthalmologist. Our control group (N=50,821) included all the non-cases who have no diagnosis of any type of glaucoma (ICD-9: 365.xx and ICD-10: H40.xxx). All study procedures were approved by the Institutional Review Board of the Kaiser Foundation Research Institute and all participants gave full written informed consent.

DNA samples from GERA individuals were extracted from Oragene kits (DNA Genotek Inc., Ottawa, ON, Canada) at KPNC and genotyped at the Genomics Core Facility of the University of California, San Francisco (UCSF). DNA samples were genotyped at over 665,000 single nucleotide polymorphisms (SNPs) on Affymetrix Axiom arrays (Affymetrix, Santa Clara, CA, USA)[21, 22]. Genotype quality control (QC) procedures were conducted as previously described[20], after an updated genotyping

algorithm with an advanced normalization step specifically for SNPs in batches not recommended or flagged by the outlier plate detector. Subsequently, on the EUR array, variants were excluded if: >3 clusters were identified; the number of batches was <38/42; and the ratio of expected allele frequency variance across packages was <100. Further, variants were excluded if heterozygosity >.52 or <.02, and if an association test between Reagent kit v1.0 and v2.0 had P<10-4. Before imputation, we additionally removed variants with call rates <90%. Genotypes were then pre-phased with Eagle[23] v2.3.2, and then imputed with Minimac3[17] v2.0.1, using two reference panels. Variants were preferred if present in the EGA release of the Haplotype Reference Consortium (HRC; n=27,165) reference panel[16], and from the 1000 Genomes Project Phase III release if not (n=2,504; e.g., indels)[24].

Harvard cohort – PDS and PG cases were recruited at the Massachusetts Eye and Ear or the New York Eye and Ear Infirmary of Mount Sinai and controls were recruited from the Massachusetts Eye and Ear. All cases and controls underwent a complete ocular examination. PDS cases were diagnosed on the presence of pigment dispersion at slit-lamp examination, Krukenberg spindles, presence of a hyperpigmented trabecular meshwork, and an absence of glaucomatous features. PG was diagnosed on the basis of the presence of glaucomatous optic neuropathy accompanied by visual field loss, elevated IOP (>21 mm Hg), in addition to PDS. Controls had no evidence of PDS features as well as normal IOP (<21 mmHg) and no evidence of optic nerve damage from glaucoma. Ethical approval was provided by the Massachusetts Eye and Ear Human Studies Committee and the New York Eye and Ear Infirmary institutional review board and all participants gave full written informed consent.

All cases and controls were genotyped on the Illumina BeadChip Global Screening Array. Principal component analysis was used to confirm that all participants were of European ancestry and any outliers were excluded. Any closely related participants with a relatedness of pihat>0.05 were also excluded. Genotypes were then imputed to the Haplotype Reference Consortium panel[16] using the Michigan Imputation Server[17].

For the final analyses, there were 146 PDS cases (58 with PG) and 145 controls post QC.

Moorfields cohort – PDS and PG cases were recruited at Moorfields Eye Hospital following a full ophthalmic assessment. PDS cases were diagnosed on the presence of pigment dispersion at slit-lamp examination, Krukenberg spindles, presence of a hyperpigmented trabecular meshwork, and an absence of glaucomatous features. PG was diagnosed on the basis of the presence of glaucomatous optic neuropathy accompanied by visual field loss, elevated IOP (>21 mm Hg), in addition to PDS. Controls were extracted from a pool of 80,000 randomly selected participants in the UK Biobank cohort. Exclusions included any individual with any ICD9 or ICD10 code for any ophthalmic disease. 1370 controls were then selected in a 1:10 case:control ratio and were matched with cases for age, sex, and the first 20 genetic principal components.

All cases and controls were genotyped on the Affymetrix UK Biobank Axiom Array. Genotypes were then called together to prevent artefacts from batch effects and were then imputed to the Haplotype Reference Consortium panel[16] using the Michigan Imputation Server[17] as previously described[25].

For the final analyses there were 137 PDS cases (46 with PG) and 1370 matched controls of confirmed European ancestry through principal component analysis.

For cases, ethical approval was provided by the Moorfields and Whittington Research Ethics Committee. For controls, ethical approval granted and overseen by the UK Biobank Ethics and Governance Council. All participants provided full written informed consent.

Association analyses

GWAS methods – Genome-wide association analysis was performed separately in each cohort. In the KCL, Harvard, and Moorfields cohorts, PDS and PG case/control status was used as the outcome variable in a Firth logistic regression performed using the PLINK2[26] software under the assumption of additive allelic effects. Adjustments were made for sex and the first three principal components in all three cohorts, with additional adjustments for age in the KCL and Moorfields cohorts. SNPs were excluded from these analyses based on the following criteria: minor allele frequency<0.01, imputation score<0.7, Hardy-Weinberg Equilibrium $p<1x10^{-4}$, or call rate<0.05.

In GERA, we ran a Firth logistic regression of pigmentary glaucoma and each SNP using PLINK[26] v1.9 (www.cog-genomics.org/plink/1.9/) with the following covariates: age, sex, and genetic ancestry principal components (PCs). We modelled data from each genetic marker using additive dosages to account for the uncertainty of imputation[27]. Eigenstrat[28] v4.2 was used to calculate the PCs[19]. The top 10 ancestry PCs were included as covariates, as well as the percentage of Ashkenazi ancestry to adjust for genetic ancestry, as described previously[19].

Meta-analysis

Meta-analyses were performed using the summary statistics from each cohort in a fixed-effects, inverse-variance method implemented using the METAL[29] software. SNPs were excluded from these results if they were not available for testing in all cohorts or if they displayed considerable heterogeneity across cohorts (I^2>0.7). The first stage meta-analysis included the three cohorts with the greatest enrichment of PG: The KCL, GERA, and Harvard cohorts. The final meta-analysis included all four cohorts.

Conditional analysis

Following the same procedure outlined by Yang et al., conditional analysis was conducted in GCTA[30] using results from the final meta-analysis as input, to identify independently associated variants with PDS.

SNP Heritability calculations

The variance of PG explained by the SNPs of interest was calculated using restricted maximum-likelihood (REML) analysis implemented by GCTA[30] in the KCL cohort of PG only cases. Results were transformed from the observed scale to the liability scale under the assumption of a PG population prevalence of 0.37%, in accordance with the literature[10].

LD score regression

The intercept from LD score regression was calculated to identify the presence of any possible inflation[31] which is more informative than the use of the Devlin genomic inflation factor[32] alone. LD score regression was then utilised to test for genetic correlation between PDS/PG, using results from the final meta-analysis as input, and a selection of ocular and pigmentation traits of interest, taken from a combination of

previously published studies, these included refractive error and myopia[33], IOP[34], VCDR[35], POAG[36], eye color[37], and hair color[38].

Functional annotation and gene-based association analysis

Follow-up analyses with Functional Mapping and Annotation (FUMA)[39] of genome-wide association studies and Multi-marker Analysis of GenoMic Annotation (MAGMA)[40] were used to perform functional annotation and conduct gene-set analysis of results from the final PDS/PG meta-analysis following the same procedures described elsewhere[39, 40]. A Bonferroni adjusted significance threshold for the MAGMA gene-based association analysis was set at $p<2.84x10^{-6}$ to correct for 17,601 tested genes.

Regulatory and functional enrichment analysis

Analyses to identify any enrichment in regulatory and functional annotations in PDS/PG was performed using GARFIELD[41]. Corrections for linkage disequilibrium were applied for these tests and the summary statistics from the final meta-analysis were used as input.

Gene pathway enrichment analysis

Summary statistics from the final meta-analysis were analysed by MAGENTA[42] to identify any enriched association in gene pathways for canonical gene sets, Gene Ontology gene sets, and transcription factor target gene sets[43]. The original databases used were acquired from the Molecular Signatures Database (version MSigDB v6.1)[44] and were then modified for compatibility. An enrichment cut-off for the 95th percentile was applied as recommended[42]. A 5% false discovery rate was applied to results to correct for multiple testing.

Gene-based association

Summary statistics from the second stage meta-analysis were used as input for analysis by S-PrediXcan[45] to test for association between PDS/PG and whole gene expression. As a reference eQTL database was not available for relevant ocular tissues, this analysis was performed across all tissue types available in the GTEx[46] database. This approach is appropriate as many eQTL effects are shared across tissue types[47]. A significance threshold of $p<2.5x10^{-7}$ was set for this analysis to correct for multiple testing arising from all gene-tissue pairs.

Determining if association is driven by gene expression

The SMR[48] methodology was utilised to determine if association between PDS and the *GSAP* gene is mediated through variation in gene expression levels. eQTL data for brain cerebellum tissue was used for this analysis as this was the tissue displaying the strongest results in the S-Predixcan analysis. Additionally, among the tissues available, neural tissues are among the best models for ocular tissues as they derive from the same dermal layer during development. The same methodology was also applied using methylation (mQTL) data[49] as DNA methylation can exert effects over gene expression. As each gene uses a single SNP to correct for LD, the Bonferroni adjusted significance thresholds were set at $p<7.4x10^{-6}$ and $p<5.9x10^{-7}$ for eQTL and mQTL data respectively.

Mendelian randomisation

Two-sample Mendelian randomisation was used to test for causal inference between myopia and IOP over PDS. Summary data for myopia was taken from the largest myopia and refractive error GWAS published to date[33], as was summary data for IOP[34]. Uncorrelated lead SNPs (N=286) from each associated genomic region

($p<5x10^{-8}$) were selected as the genetic instrument variables to prevent confounding errors from correlated SNPs. A combination of the inverse-variance weighted (IVW) and Egger methods implemented in the R package MendelianRandomization[50] were selected as the most appropriate for this data, with the IVW method providing the best effect size estimates and the Egger method being more robust to allow the detection of violations of Mendelian randomisation assumptions, particularly in the context of pleiotropy. Heterogeneity was tested and is reported using the I^2 statistic.

Results

We conducted our genome-wide analyses in three stages. In the first discovery stage, we meta-analysed GWAS summary results obtained from three cohorts, all of European ancestry, which were predominantly PG cases (349 cases of PG and 88 cases of PDS). The Devlin genomic inflation factor for this analysis[32] (λ_{GC}) was 1.042 indicating that this analysis was not subject to inflated results.

The discovery stage meta-analysis identified association at genome-wide significance for one genomic locus, located on chromosome 7 (q11.23) spanning across the gamma secretase activator protein (*GSAP*) gene (lead SNP rs9641220, $p=3.6x10^{-9}$). The lead SNP had an odds ratio of 1.83 (SE=0.10, effect allele=T), this is a relatively large effect size especially for the most common allele (allele frequency in meta-analysis: cases=0.71, controls=0.62), which corresponds to a relative risk of approximately 1.21 per allele.

Association at a second locus on chromosome 11q14.2-14.3, within the glutamate metabotropic receptor 5 gene (*GRM5*), was strongly suggestive (lead SNP

rs661177, OR=1.73, SE=0.10, $p=6.7x10^{-8}$), but just above the conventional genome-wide significance threshold ($p<5x10^{-8}$).

At a second stage we sought to replicate these results in one further independent cohort of white British ancestry, recruited at London's Moorfields Hospital. Despite the smaller size of the replication cohort, association with diagnosis of PDS/PG was significant (replication significance threshold set at p=0.025) and in the same direction for both the *GSAP* (OR=1.40, SE=0.14, p=0.014 for rs9641220) and the *GRM5* loci (OR=1.42, SE=0.13, p=0.0084 for rs661177).

For the third stage, we performed a meta-analysis of all four cohorts. The strength of association at the *GSAP* and *GRM5* loci increased in this meta-analysis and both loci were associated with genome-wide significance ($p=6.0x10^{-10}$ for rs9641220 and $p=3.9x10^{-9}$ for rs661177). No additional loci were associated at genome-wide significant levels (Figure 1). The effect estimates of the lead SNPs rs9641220 and rs661177 were consistent across the 4 studies and were not heterogeneous (heterogeneity I-scores = 0 and 34.4, p= 0.44 and 0.21 respectively). There was no evidence of inflation in this final meta-analysis, $\lambda_{GC}=1.047$. LD score regression[31] further confirmed results from our meta-analysis were not inflated with an intercept of 1.02 (SE=0.007).

Conditional analysis identified no other independent sources of association other than the lead SNPs at each locus.

The association of *GRM5* with pigmentation traits[38] is a consequence of strong LD between *GRM5* and the adjacent gene *TYR*. Interestingly in our analysis, though the lead SNP is situated within *GRM5*, upstream of *TYR*, there were also strongly associated SNPs on the opposing side downstream of *TYR* (rs11018567, $p=8.7x10^{-}$

[8], 410kb distance from rs661177). As there is no recombination in this genomic region between *GRM5* and *TYR* in Europeans (see regional plot Figure 2) and these SNPs are in strong LD, *TYR* is a good candidate as the functional gene for this associated locus, given its role in ocular pigmentation[51].

We performed a partitioned heritability analysis in the King's College London (KCL) sample (all PG cases) to determine the proportion of PG risk explained by the two lead SNPs identified in our meta-analysis. Collectively, the two SNPs had a SNP heritability (h^2_{SNP}) of 0.031, SE=0.029, $p=1.9x10^{-7}$. This means the two SNPs explain an estimated 6.9% of heritable PG risk among sporadic cases (total PG h^2_{SNP}=0.45 as previously reported[10]).

LD score regression was then used to test the genetic correlation between PDS and multiple ocular and pigmentation traits. Significant genetic correlation (after adjustment for multiple testing) was found between PDS/PG and three ocular traits: POAG, IOP, and spherical equivalent, and nominally with VCDR (Table 2). However, no genetic correlation was identified with eye color despite the phenotypic correlation.

The MAGMA[40] gene-based test identified significant association for two genes with PDS/PG: *GRM5* ($p=4.4x10^{-7}$) and *FAM83D* ($p=2.4x10^{-6}$) (Manhattan plot shown in Figure 3). *FAM83D* plays a regulatory role in mitosis and spindle activity[52]; further study is needed to determine how this gene is connected with PDS/PG, pending replication.

Functional enrichment analysis identified no significant regulatory or functional enrichment for PDS in this study (Supplementary Figures S1-S3).

Gene-set enrichment analysis conducted using MAGENTA[42] identified 17 significantly enriched gene sets at a 5% false discovery rate. The enriched gene sets, summarised in Table 3, are predominately transcription factor targets for genes important for development such as *PAX8* and *FOXO4*.

We performed a gene-based analysis using S-Predixcan[45] which incorporates eQTL data to test for association between PDS/PG with overall gene expression levels. Significant association was present with *GSAP* in 10 tissues (significant results provided in Table 4) with a consistent direction across the tissue types. These results provide strong support that increased expression of *GSAP* is associated with PDS/PG risk. Unfortunately, expression data for *GRM5, TYR,* and *FAM83D* were not available for testing in this expression dataset.

As the gene-based association tests performed by S-Predixcan showed association with the *GSAP* gene, and associated SNPs at the *GSAP* locus are significant expression quantitative trait loci (eQTLs) in multiple GTEx tissues[46] (rs9641220, $p=7.0x10^{-24}$ in brain cerebellum), we sought to determine if association at this locus is mediated through variation in gene expression using SMR[48]. Following adjustment for multiple correction, the SNP at the *GSAP* locus (rs7778041) showed a significant causative effect over PDS/PG that is mediated through *GSAP* expression levels ($p=1.1x10^{-7}$). The corresponding test for heterogeneity (HEIDI[48]) analysis was non-significant (p=0.64), indicating that the SMR result is a product of causality and not pleiotropy. Further SMR analysis using methylation (mQTL) data supported these results, as methylation changes can lead to changes in gene expression, with two significant methylation probes (cg02407048 and cg14288326) situated in *GSAP* ($p=1.8x10^{-8}$ and $p=2.6x10^{-8}$ respectively) and not a product of pleiotropy (HEIDI p=0.51 and p=0.54 respectively). SMR did not identify any significant causality for

eQTLs or mQTLs at the *GRM5* locus, suggesting that association at this region is not mediated through variation in gene expression at this locus.

Finally, we performed Mendelian randomisation (MR) to elucidate whether refractive error and IOP exert causative effects over PDS and PG. Our results infer that refractive error does exert a causative effect, with each one diopter decrease in spherical equivalent translating to an odds ratio of 1.4 in PDS/PG risk (Table 5), with no evidence of pleiotropic effects (evidenced by a non-significant Egger intercept). The heterogeneity score (I^2) of 9.6%, p=0.11 indicates that the variants used in this analysis are suitable.

The inverse variance weighted methods indicate that IOP does exert causative effects over PDS/PG, however there is some variation in significance for the Egger methods (Table 4) and evidence of heterogeneity (I^2=26.1%, p=0.019). Therefore, there is uncertainty regarding IOP's effects on PDS.

Discussion

The is the first GWAS to identify genetic factors significantly associated with PDS among sporadic cases. SNPs at these two loci account for 6.9% of PG SNP heritability and allow greater insight into the genetic aetiology of PDS and PG.

The strongest association identified was for the gene *GSAP*. Our combination of analyses indicates that increased expression of this gene, relative to the general population, increases the risk of PDS and PG. Gamma secretase is primarily known and studied for its role in Alzheimer's disease, as it interacts with the amyloid precursor protein C-terminal fragment[53], but it also plays a functional role in

pigmentation. Gamma secretase is required by tyrosinase (TYR) and its related proteins (TYRP1 and TYRP2) to correctly target melanocytes in melanin production[54]. Impaired gamma secretase function in murine models results in defective ocular pigmentation and tyrosinase mislocalisation, meanwhile pigmentation is blocked in mice receiving treatment with gamma secretase inhibitors to their primary melanocytes[55]. The gamma secretase complex also plays a functional role in eliminating the premelanosome (PMEL) and glycoprotein nmb (GPNMB) C-terminal fragments[56]; treatment with DAPT (a gamma secretase inhibitor) stabilises the C-terminal fragments for both these proteins.

There is strong functional evidence to support this association, as gamma secretase interacts with TYR, TYRP1, and TYRP2 in ocular pigmentation[54]. Mutations in the *TYRP1* mouse orthologue (*Tyrp1*) in the DBA/2J line, result in a murine model for PG[13] (in combination with a mutation in *Gpnmb*). In addition, gamma secretase also has a role in cleaving *GPNMB* (the human orthologue of the second mutated gene in PG murine models) and *PMEL* C-terminal fragments. *PMEL* mutations have previously been identified as causing PG in a family study[11], therefore *GSAP* is functionally connected to all previously identified genes associated with PG.

Alternatively, the association between increased *GSAP* expression and PG may result from increased *GSAP* expression increasing the production of amyloid-β[53]. The neurotoxic effects of amyloid-β could then result in optic nerve neuropathy and the progression of glaucomatous damage. There is some suggestive evidence linking amyloid-β and POAG from the association between *APBB2* and POAG in Afro-Caribbean cohorts[57], and other Alzheimer's risk loci associated with POAG in a multi-ethnic meta-analysis[58]. However, this hypothesised model only accounts for association between *GSAP* and PG, but not with PDS. Further *in vivo* and *in vitro*

411 analyses are required to develop and support a model for how increased *GSAP*
412 expression affects PDS and PG aetiology.

413 *GRM5* has previously been reported to be associated with retinal detachment[59] and
414 pigmentation in hair[60] and skin[61-64]. Retinal detachment occurs in 6.6% of PDS and
415 7.6% of PG patients[65], which is a greater prevalence that can be accounted for by
416 the associated myopia. However, there is strong LD across the genomic region over
417 both the *GRM5* and *TYR* genes. As previously discussed, TYR interacts with gamma
418 secretase in ocular pigmentation[66], is also a prominent pigmentation gene
419 associated with eye, hair, and skin pigmentation[38, 51, 67] and mutations within the *TYR*
420 gene also cause oculocutaneous albinism[66]. Lighter eye color correlates with PDS
421 and PG risk at an observational[68, 69] and genetic[10] level. Therefore, we hypothesise
422 *TYR* as the most probable functional candidate at this locus, which may explain in
423 part the association between lighter eye color and PG risk. Association between
424 *GRM5* and retinal detachment[59] adds additional complexity to this locus, as there is
425 a high incidence of retinal detachment among PDS and PG patients[65]. A possible
426 explanation for this comorbidity could be that, given the strong LD in this region,
427 there may be a risk haplotype containing both genetic risk for PDS influenced by
428 *TYR,* and genetic risk for retinal detachment risk influenced by *GRM5*. Further study
429 is required to determine if this occurs.

430 A third gene, *FAM83D*, is associated with PDS in one of our gene-based analyses
431 and introduces an interesting, novel candidate for further research. However, further
432 replication is required before we can conclusively determine if this is a true
433 association.

There is significant genetic correlation between PDS and POAG in this analysis, as seen in our previous analysis of a subset of individuals in this study[10]. However, the lead SNPs associated with POAG[70] did not account for any of the PG SNP heritability in the previous study, nor do the lead SNPs associated with PDS in this study show significant association with POAG. This indicates that the strongest genetic risk factors for both diseases are specific to their respective condition, but there is some shared genetic architecture through genes of lower effect size. An alternative possibility is that the POAG cases in previous GWAS may include PG patients, as some PDS cases undergo a “burn out” phase during middle age[71]. It is postulated that pigment stops being released from the iris due to pupillary miosis raising the peripheral iris forward so it is no longer in contact with the zonules and age-related lens enlargement[71]. Alternatively, the onset of presbyopia and loss of iris concavity, seen during ultrasound biomicroscopy examination, decreases contact between the iris and zonules[8]. In both scenarios, pigment accumulated in the trabecular meshwork is gradually removed; however any glaucomatous damage that occurred during the active PDS phase will persist[72]. Therefore, if diagnosed later in life, glaucoma signs will be present in the absence of PDS. These cross-sectional data cannot determine if this occurs, though it is certainly an area ripe for future study.

Factors that determine whether or not PDS will progress to PG is of great interest in PG research. Factors influencing IOP variation, genetic or otherwise, are strong candidates, with the obvious hypothesis that a combination of higher IOP and PDS results in PG. Our analyses identified a genetic correlation between PDS (enriched for PG) and IOP, though the MR analysis was not conclusive in determining the nature of their relationship. The inverse variance weighted method did indicate IOPs’

459 causative effects over PDS, but there was discrepancy in results from the Egger
460 method. The Egger tests are more resistant to violations of MR assumptions than the
461 inverse variance weighted methods. It is possible this discrepancy between methods
462 is a product of elevated IOP being a diagnostic criterion for PG, which is highly
463 enriched in our sample, leading to ascertainment bias. A second possibility could be
464 the outcome sample containing a combination of PDS and PG cases, and that
465 elevated IOP risk only exerts effects on PG but not PDS. However, the non-
466 significant intercept results indicate that pleiotropy is not the source of this
467 discrepancy. It may not be possible to conclusively determine whether normal IOP
468 variation elicits a causative role due to the limitation from potential ascertainment
469 bias.

470 Further analysis of potential factors responsible for progression from PDS to PG was
471 not possible, due to limitations in sample size preventing cases of PDS only and PG
472 from being stratified.

473 Genetic correlation and MR analyses confirm the genetic link between PDS/PG and
474 myopia[10], and support case series data showing that PG patients are more myopic
475 than PDS subjects, which is itself associated with myopia [7]. As MR analyses are
476 analogous to a randomised controlled trial[73], our MR analysis was able to determine
477 if the association between myopia and PDS/PG is causal in nature. Indeed, the MR
478 analysis suggests myopia is causative of PDS/PG, possibly the larger size of myopic
479 eyes leads to posterior bowing of the iris[71] and friction between the iris and zonules,
480 resulting in more pigment release.

481 This study is the largest genetic analysis of PDS and PG to date, albeit it is still small
482 by GWAS-standards. Despite this, we provide strong evidence for two genomic loci,

and suggestive evidence for a third, associated with PDS and PG and explored their relationship with known PG genetic factors. Our findings also demonstrate that myopia exerts direct causal effects in the development of PDS and PG.

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Figure 1 – A Manhattan plot from the second stage meta-analysis of all four cohorts. The red line shows the genome-wide significance threshold at $p=5x10^{-8}$. The blue line shows the suggestive significance line at $p=1x10^{-6}$.

Figure 2 – A locus plot of *GRM5*, *TYR* and neighbouring genes. Recombination rate (shown in blue on the y-axis) indicate that there is no recombination between *GRM5* and *TYR*.

Figure 3 – A Manhattan plot from the MAGMA gene-based association analysis. The red line shows the genome-wide significance threshold at $p=2.84x10^{-6}$.

Supplementary Figure S1 – Enrichment of PDS/PG variants in for translational features. Radial plot shows the enrichment (odds ratio) for each feature (dots on outside of circle). Small dots on the outer side of plot show if the enrichment is significant (if dot is present) or not (if there is no dot) for the threshold $p<10^{-5}$.

Supplementary Figure S2 – Enrichment of PDS/PG variants in histone modifications. Radial plot shows the enrichment (odds ratio) for each modification (dots on outside of circle). Small dots on the outer side of plot show if the enrichment is significant (if dot is present) or not (if there is no dot) for the threshold $p<10^{-5}$.

Supplementary Figure S3 – Enrichment of PDS/PG variants in DNaseI Hypersensitive sites. Radial plot shows the enrichment (odds ratio) for each cell type (dots on outside of circle). Small dots on the outer side of plot show if the enrichment is significant (if dot is present) or not (if there is no dot) for the threshold $p<10^{-5}$.

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711

Cohort	Total N	Controls	All cases	PG cases	PDS only
KCL	518	291	227	227	0
GERA	50885	50821	64	64	0
Harvard	291	145	146	58	88
Moorfields	1507	1370	137	46	91
Total	53201	52627	574	395	179

Table 1 – A summary of the total numbers of participants in each cohort and the number of cases that were PG or PDS only in each cohort.

Phenotype	rg	SE	P
POAG	0.533	0.108	7.49E-07
IOP	0.505	0.095	1.10E-07
Spherical equivalent	-0.342	0.081	2.41E-05
VCDR	0.257	0.118	0.029
Hair color	0.042	0.064	0.51
Eye color	0.00	0.049	9.90E-01

Table 2 – Genetic correlations between PDS and other ocular traits. rg is the Spearman's correlation r value, SE is the standard error, and P is the respective p-value.

Gene set category	Gene Set	Nominal p-value	FDR q-value
TFT	TTGTTT_FOXO4_01	1.30E-05	6.25E-03
TFT	CTTTGT_LEF1_Q2	1.00E-04	8.00E-03
TFT	IK2_01	1.58E-04	1.23E-02
TFT	CAGGTG_E12_Q6	7.00E-05	1.52E-02
TFT	PAX8_01	3.00E-04	1.53E-02
TFT	ZF5_B	1.00E-04	1.58E-02
TFT	HLF_01	2.00E-04	1.61E-02
TFT	CTTTGA_LEF1_Q2	2.00E-04	1.80E-02
TFT	GGGAGGRR_MAZ_Q6	6.20E-05	1.85E-02
TFT	AACTTT_UNKNOWN	2.00E-04	1.93E-02
TFT	TGCTGAY_UNKNOWN	1.00E-03	1.97E-02
TFT	CAGCTG_AP4_Q5	2.00E-04	2.00E-02
TFT	CCAWNWWNNNGGC_UNKNOWN	9.00E-04	2.70E-02
Canonical	T_CELL_SIGNAL_TRANSDUCTION	3.21E-02	3.21E-02
TFT	NF1_Q6_01	9.00E-04	4.47E-02
TFT	TATA_C	1.60E-03	4.68E-02
TFT	HNF4_Q6	1.50E-03	4.80E-02

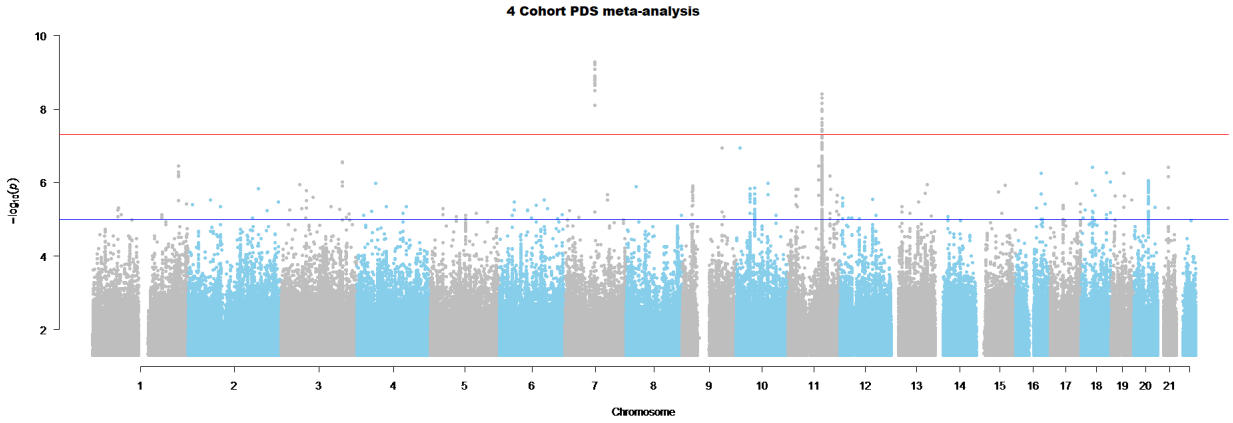
Table 3 – Enriched gene-sets. Gene-set category refers to whether the gene-set is canonical or a transcription factor target (TFT). Gene set is the given name for each gene set. Nominal p-value is the unadjusted p-value and FDR q-value is the q-value for association at a 5% false discovery rate.

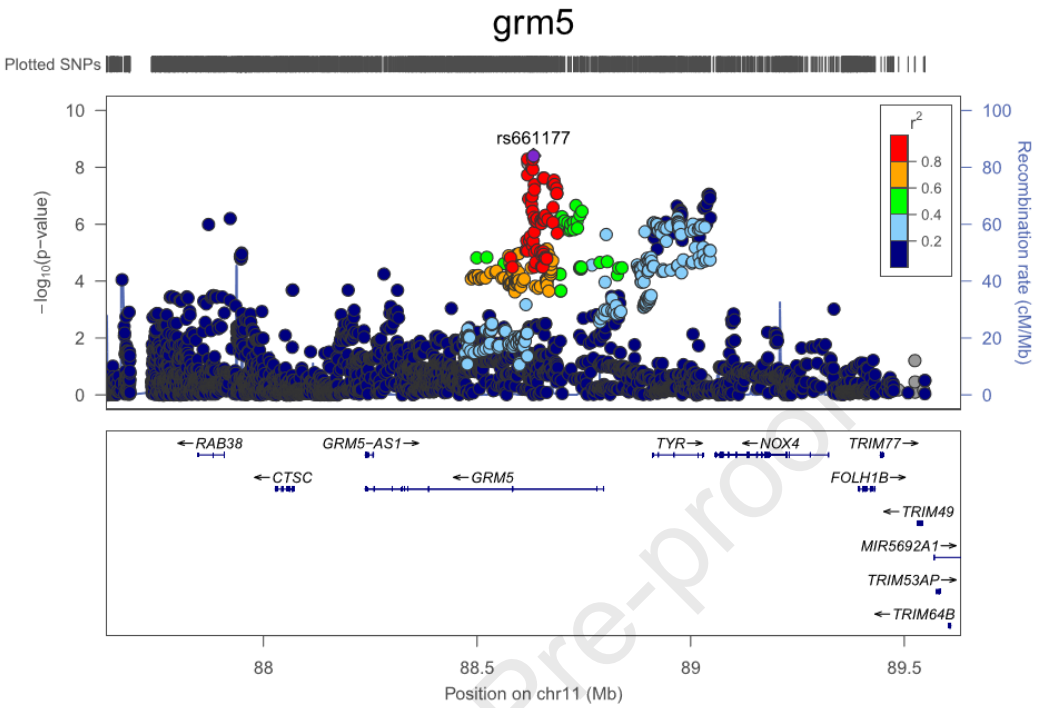
Gene	Tissue	Effect size	P
GSAP	Brain Frontal Cortex BA9	1.45	4.13E-10
GSAP	Brain Cerebellum	1.08	4.31E-10
GSAP	Brain Cortex	1.14	4.92E-10
GSAP	Artery Aorta	1.37	5.78E-10
GSAP	Muscle Skeletal	1.06	1.81E-09
GSAP	Brain Hypothalamus	3.20	3.85E-09
GSAP	Brain Anterior cingulate cortex BA24	0.73	8.12E-09
GSAP	Esophagus Mucosa	2.77	2.35E-08
GSAP	Artery Tibial	1.06	7.97E-08
GSAP	Stomach	1.83	8.01E-08

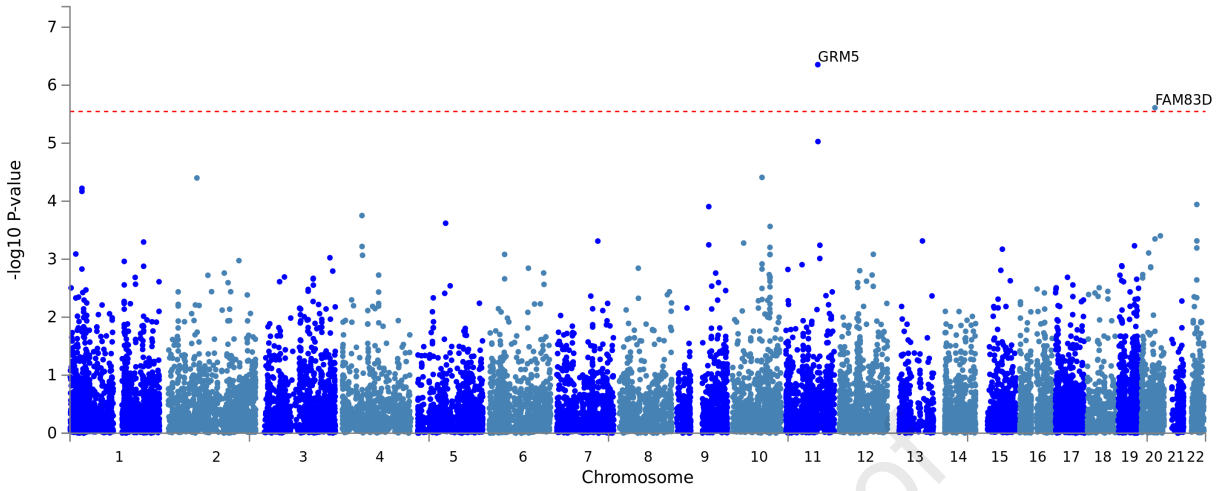
Table 4 – Significantly ($p<2.5x10^{-7}$) associated genes in the S-Predixcan analysis. Gene is the HGNC gene symbol. Tissue is the tissue type the eQTL reference data was taken from. Effect size is the S-Predixcan computed relative effect size. P is the association p-value.

Exposure phenotype	Model	Beta	SE	P-value
Refractive error	IVW	-0.349	0.071	$8.86x10^{-7}$
	Egger	-0.387	0.148	0.009
	Egger intercept	0.003	0.011	0.769
IOP	IVW	0.424	0.092	$4.05x10^{-6}$
	Egger	0.402	0.238	0.091
	Egger intercept	0.003	0.03	0.923

Table 5 – Mendelian randomisation results. Model is the Mendelian randomisation model applied where IVW is the inverse-variance weighted, Egger is the Egger model with its corresponding Egger intercept. Beta is the effect size of the exposure phenotype over PDS for the IVW and Egger rows, and the intercept value for the Egger intercept rows. SE is the corresponding standard error for values in the Beta column, and P-value is the corresponding p-values for each row. Significant values in the IVW and Egger rows indicate a causal effect over PDS, whilst a significant, non-zero Egger intercept indicates failure of the InSIDE assumption.







From: Kozareva, Diana <diana.kozareva@kcl.ac.uk>
Sent: Monday, July 12, 2021 09:16
To: Hammond, Chris
Subject: Re: Acknowledgement

Morning Chris,

I confirm, I am happy my name to be added to the acknowledgment section.
Is there original email that I need to reply to?

Many thanks

Diana Kozareva
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Eugen Gramer <EugenGramer@t-online.de>
Wed 21/07/2021 15:15
To: Simcoe, Mark
Cc: Nicole Weisschuh <nicole.weisschuh@uni-tuebingen.de>

You don't often get email from eugengramer@t-online.de. Learn why this is important

Dear Mark Simcoe,

Thank you for mentioning me in the acknowledgements in your significant publication "Genetic analyses identify two common loci associated with pigment dispersion syndrome/pigmentary glaucoma and implicate myopia in its development". I am very honoured and happy to agree.

All the best and best regards

Eugen Gramer

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We report the first genetic loci associated with sporadic pigment dispersion syndrome and pigmentary glaucoma in unrelated cases. Further analysis indicate that myopia exerts causal effects in the development of these conditions.