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Genetic architecture of open angle glaucoma and related determinants

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ABSTRACT

Background Although the vertical cup-disc ratio (VCDR) and intraocular pressure (IOP) are important determinants of open angle glaucoma (OAG), it is unclear to what extent the genetic origin of these traits overlap with those of OAG. We evaluated whether the same genes that determine VCDR and IOP also predict OAG.

Methods Genetic risk scores were constructed from single nucleotide polymorphisms (SNPs) using genome wide association data of 9326 participants from the Rotterdam Study cohorts (mean±SD age: 64.6±9.1 years). These risk scores were used to calculate the explained variance of VCDR and IOP in an independent cohort (Erasmus Rucphen Family study) consisting of 1646 participants (mean±SD age: 46.8±14.1 years) and the OAG risk in a subset of the Rotterdam Study cohorts. To evaluate false positive findings, we generated two new variables containing randomly sampled values to serve as a negative control.

Results The explained variance of VCDR increased when increasing the number of SNPs included in the risk score, suggesting a polygenic model. We found no clear evidence for a similar model for IOP, suggesting that a small number of SNPs determine the susceptibility to IOP. The SNPs related to IOP in terms of p values contributed little to VCDR. The risk scores associated with VCDR were also associated significantly with OAG. This suggests a common polygenic background for VCDR and OAG.

Conclusions We found evidence for a polygenic model underlying one of the major traits of OAG, VCDR, and OAG itself. The IOP did not show any evidence for such a model.

INTRODUCTION

Open angle glaucoma (OAG) is a neurodegenerative disease that leads to progressive damage to the retinal ganglion cells and nerve fibres, resulting in glaucomatous visual field loss. It is one of the leading causes of irreversible blindness in the world, affecting over 60 million persons worldwide.¹ Although the aetiology of OAG is still obscure, it has long been recognised that genetic factors play a role in its pathogenesis. Relatives of patients with OAG have an increased risk of developing OAG.² Several rare genetic variants associated with a high risk of disease have been implicated in familial forms of OAG, but these variants only explain a small percentage of OAG patients.³ This raises the

question whether the genetic aetiology of OAG is determined by a large number of rare variants with major effects on the disease risk (rare variant, common disease hypothesis) or whether there are multiple common variants underlying the disease (common variant—common disease hypothesis).

Genome wide association studies (GWAS) have identified a large number of common variants (single nucleotide polymorphisms (SNPs)) with a low risk for a large number of common diseases (eg, type 2 diabetes, Alzheimer's disease, and various forms of cancer), but also for diseases that are less common such as multiple sclerosis and Crohn's disease.⁴ GWAS have also been powerful in studies on quantitative risk factors of diseases such as blood pressure, glucose values, and height.⁴ More recently, GWAS were used to identify polygenic forms of inheritance for schizophrenia, implicating tens of thousands of genetic variants covering the whole genome rather than a distinct number of variants.⁵ The essence of the latter approach is that a gene discovery sample is used to define risk scores, and the risk is subsequently predicted in an independent sample. In this approach, not only the variants that are genome wide significant are used, but also those who do not reach this threshold. This method can also be applied to test for overlapping of genes between diseases and their underlying liability.

If there is a polygenic model that explains part of the susceptibility to a disease, the classical theory of Fisher predicts that the number of risk alleles is distributed according to the Gaussian distribution in the population.⁶ It is easy to show that if there are >30 genetic variants, no person in the population carries zero risk alleles but everyone carries a gradient number of risk alleles.⁷ Thus, a polygenic model implies that individuals in a population always carry risk alleles for a disease and that the liability to disease increases with the number of risk alleles one carries. It also follows from this model that underlying a disease that is defined as present or absent, there is probably a quantitative trait. The persons in the tail of the distribution of this trait are considered to be 'diseased'. If the quantitative trait (or risk factor) is related to the genotypic distribution and increases with the number of risk alleles, the risk of disease will behave accordingly.

If we translate this to OAG, there are two clinical measures consistently associated with this disease

and may thus determine its (polygenic) liability to OAG. First, OAG is characterised by damage to the optic nerve head, which is visible as an (increased) excavation (cupping) upon ophthalmological examination. The extent of the excavation is commonly quantified as the vertical cup-to-disc ratio (VCDR), ranging from 0 to 1.⁸ Both an increase in VCDR and an unusually large VCDR at a single observation may indicate glaucomatous changes of the optic nerve head.⁹ The second clinical measure that may determine the (polygenic) liability to OAG is the intraocular pressure (IOP). Current treatment for OAG is based on lowering the IOP. VCDR as well as IOP are highly heritable, making these two variables potentially important endophenotypes for OAG.¹⁰ Until now it is unknown to what extent the genes involved in VCDR overlap with those involved in IOP, and to what extent they actually predict OAG.

The aim of this study was to investigate whether there is a polygenic model in which many variants apply to VCDR and IOP and whether these genes also predict the risk of OAG. For this study, we used the Rotterdam Study I, Rotterdam Study II, and Rotterdam Study III (RS-I, RS-II, and RS-III, respectively) as discovery cohorts to derive genetic risk scores that capture the genes involved in VCDR and IOP. Next, we assessed whether these risk scores predicted VCDR and IOP in an independent target cohort (the Erasmus Rucphen Family (ERF) study). For the prediction of OAG, RS-I served as the target cohort, because no OAG cases were included in ERF.

METHODS

Study populations

The RS-I is a prospective population based cohort study of 7983 residents aged 55 years and older living in Ommoord, a suburb of Rotterdam, the Netherlands. Baseline examination for the ophthalmic part took place between 1991 and 1993; follow-up examinations were performed from 1997 to 1999 and from 2002 to 2006.^{11 12} RS-I included 188 OAG cases that either already had OAG at baseline or developed OAG during follow-up.

The RS-II and RS-III are two other prospective, population based, cohort studies of, respectively, 3011 residents aged 55 years and older, and 3392 residents aged 45 years and older. The rationale and study design are similar to that of the RS-I.¹¹ The baseline examination of RS-II took place between 2000 and 2002; the follow-up examination was performed from 2004 to 2005. Baseline examination of RS-III took place between 2006 and 2009.¹³

The ERF Study is a family based cohort in a genetically isolated population in the southwest of the Netherlands with over 3000 participants aged between 18 and 86 years. Cross-sectional examinations took place between 2002 and 2005. The rationale and study design of this study have been described elsewhere.^{14 15} All measurements in these studies were conducted after the Medical Ethics Committee of the Erasmus University had approved the study protocols and all participants had given a written informed consent in accordance with the Declaration of Helsinki.

Ophthalmic examination

The ophthalmic assessment in RS-I and RS-II, both for baseline and follow-up, included a medical history, autorefractometry, keratometry, IOP measurement, visual field testing, and optic nerve head imaging with simultaneous stereoscopic photography (ImageNet; Topcon Corporation, Tokyo, Japan) of both eyes after mydriasis with tropicamide 0.5% and phenylephrine 2.5% eye drops.

RS-III was similar to RS-I except for optic nerve head imaging with Heidelberg Retina Tomograph 2 (HRT; Heidelberg Engineering, Dossenheim, Germany). The ophthalmic assessment in ERF included a medical history, autorefractometry, keratometry, IOP measurement, and optic nerve head imaging with HRT of both eyes after pharmacologic mydriasis.

In all cohorts we measured IOP at baseline and at every follow-up round with Goldmann applanation tonometry (Haag Streit, Bern, Switzerland) after applying oxybuprocaine 0.4% eye drops. The median value of three successive measurements was recorded.¹⁶

The OAG definition was based on the presence of glaucomatous visual field loss. Details have been described elsewhere. Neither VCDR nor IOP were part of the diagnostic criteria of OAG.^{17 18}

ImageNet, which was used for optic nerve head imaging in RS-I and RS-II, takes simultaneous stereoscopic images of the optic disc at a fixed angle of 20°, using a simultaneous stereoscopic fundus camera (Topcon TRC-SS2; Tokyo Optical Co, Tokyo, Japan). Images were analysed using the ImageNet retinal nerve fibre layer height module. On each stereoscopic pair of optic disc images four points were marked on the disc margin, defined as the inner border of the peripapillary ring or the outer border of the neural rim, if a scleral ring was visible. Next, the software drew an ellipse using these points to outline the disc margin and to determine the cup. The amount of correspondence between the marked points on the two images of the stereoscopic pair is expressed as a 'bad points' percentage, which indicates the percentage of points lacking correspondence. This percentage can be used as an indicator of image quality. Images with 25% or more bad points were excluded.¹⁹

HRT 2, used for optic nerve head imaging in RS-III and ERF, uses a focused 670 nm diode laser light beam to acquire scans of the optic nerve head region, using the confocal principle. The HRT obtains, during one scan, three series of 16 to 64 confocal frontal slices. From each of these series, a three dimensional image of the optic nerve head is reconstructed, from which the software calculates several optic disc parameters. To define the cup, the HRT places a reference plane 50 µm below the peripapillary retinal surface in the region of the papillomacular bundle. Imaging was performed after entering the participant's keratometry data into the software and after adjusting the settings in accordance with the refractive error.

In RS-III all HRT 2 data were converted to HRT 3. As an indicator of image quality we used the topographic SD of the scan, which is a measure of the variability among the three series of a single HRT scan. Scans with a topographic SD exceeding 50 µm were excluded. The inter-observer variability and agreement for both systems have been described elsewhere²⁰ (Ramdas *et al*, unpublished data, 2010).

Genotyping

In the RS-I, RS-II, and RS-III cohorts, DNA was genotyped using the Illumina Infinium II HumanHap arrays according to the manufacturer's protocols. After exclusion of participants for reasons of low quality DNA, a total of 5974 participants were available with genotyping data from RS-I (HH550 v3.0), 2157 participants were from RS-II, and 2082 from RS-III (HH550Duo and HH610Quad). Details are described elsewhere.²¹

In ERF, DNA was genotyped on four different platforms (Illumina 6k, Illumina 318K, Illumina 370K, and Affymetrix 250K), which were then merged and imputed to 2.5 million SNPs hapmap using build 36 HapMap (release 22) CEU population as a reference cohort. After exclusion of participants for

whom genotyping data were unavailable, 2385 had genotyping data.

Extensive quality control analyses have been performed in each cohort.

Statistical analysis

Statistical analysis within the discovery cohorts

If data of both eyes were available, we randomly chose one. In case of missing or unreliable baseline data of both eyes, we used follow-up data whenever possible. For each of the discovery cohorts (comprising RS-I, RS-II, and RS-III) linear regression models were used to examine the associations between the risk scores and VCDR and IOP, adjusted for age and gender. For VCDR we also adjusted for optic disc area. For IOP the mean value of both eyes was included and adjusted for IOP lowering treatment. This adjustment was done by adding 30% upon the measured IOP for medical treatment and by fixing the IOP at 30 mm Hg for surgical/laser treatment in the past.

To summarise the results through the three discovery cohorts, we performed a meta-analysis with Metal for Linux (<http://www.sph.umich.edu/csg/abecasis/metal>) using the inverse variance method of each effect size estimate.²²

All statistical analyses were performed using SPSS version 15.0.0 for Windows (SPSS Inc, 2006), PLINK²³ and R statistical package version 2.10.1 for Mac (<http://www.r-project.org/>).

Risk scores within the independent cohort

The risk score profiling method tests the association of a genetic score variable that reflects a combined effect of a number of selected SNPs with a trait. Details of this method have been described elsewhere.⁵ The results of the SNPs of the meta-analysed discovery cohorts were categorised according to p value and examined in an independent cohort (ERF). The p value thresholds were as follow: $p < 10^{-10}$, $p < 10^{-9}$, $p < 10^{-8}$, $p < 10^{-7}$, $p < 10^{-6}$, $p < 10^{-5}$, $p < 10^{-4}$, $p < 10^{-3}$, $p < 10^{-2}$, $p < 0.1$, $p < 0.2$, $p < 0.3$, $p < 0.4$, $p < 0.5$, $p < 0.6$, $p < 0.7$, $p < 0.8$, $p < 0.9$, $p < 1.0$. For each category the effect (β) of each SNP was multiplied by the corresponding number of alleles of each participant to calculate a score for that particular SNP. The mean of all these scores for the respective participant was the risk score. In case of missing SNP data from a participant of the replication cohort, the allele frequencies were used and multiplied by the betas. Next, we ran linear regression analyses with the VCDR as the outcome measure and the risk scores adjusted for age and gender as the determinant in order to calculate the proportion of explained variance (PEV) of each group of SNPs (ie, p value threshold) as determined in the discovery cohort. A value of $p \leq 0.05$ was considered as statistically significantly associated with the trait.

The same analysis was also done with IOP as the outcome. To examine whether a 'non-ophthalmic' variable reveals the same

results we performed the same analysis for a negative control. For this variable we used a sampling method without replacement. We sampled the VCDR respectively IOP in the target cohort, and thus the distribution remained the same. In addition, we created a second normal distributed variable by assigning a random number (with mean 10; SD 1) to all participants.

For the analysis of the family based data (ERF) we used SOLAR version 4.1.5 for Linux to adjust for the pedigree structure.²⁴

Finally, we applied the same approach for the OAG cases of RS-I by calculating the Nagelkerke R^2 of OAG for the VCDR and IOP risk scores. This was done using logistic regression analyses adjusted for age and gender. Since, as a matter of course, individuals with OAG may have an increased VCDR and/or IOP, we performed a secondary analysis in which we excluded RS-I from the meta-analysis of the discovery cohorts to avoid possible biased results. Differences in general characteristics between cases and controls were analysed with independent t tests and χ^2 statistics.

Only autosomal SNPs that were available in all four cohorts (~520 000 SNPs) were included. To have less overlap of linkage disequilibrium blocks we did not use imputed data. SNPs that deviated significantly from the Hardy–Weinberg equilibrium ($p < 0.0001$) or that had a minor allele frequency < 0.05 were excluded from the present study.

RESULTS

Study samples

Of the 5974 participants that were genotyped in the RS-I cohort, 5312 had valid VCDR data. The remaining 662 were excluded because of missing or unreliable data. From the RS-II cohort 2048 from 2157 genotyped participants were included in this study. For 109 persons we did not have (reliable) data. From the RS-III cohort a total of 1966 participants were included. The target cohort for the quantitative analysis (ERF) included 1646 participants. Table 1 summarises the general characteristics for the discovery cohorts and the replication cohort. The participants of the target set were significantly younger ($p < 0.001$), and had lower VCDR values ($p < 0.001$). The latter difference can be explained by the use of different measurement techniques for optic disc imaging (since we analysed the cohorts separately and subsequently meta-analysed the findings, this difference will not influence our findings). The distributions of VCDR and IOP for the four cohorts are shown in supplementary figure S1. Genetic outliers of non-European ancestry were excluded. No institutional heterogeneity between the cohorts or residual population sub-stratification was noticed after inspecting the genotype data.²⁵ Inflation factors for the included cohorts for VCDR and IOP ranged from 1.024 to 1.061 and from 1.006 to 1.037, respectively.

Table 1 Characteristics of the four study populations, presented as mean \pm SD (range) unless stated otherwise

	Discovery cohorts			Target cohort ERF
	RS-I	RS-II	RS-III	
Total sample size (N)	5312	2048	1966	1646
Age (years)	68.0 \pm 8.4 (55–99)	64.3 \pm 7.8 (55–98)	55.6 \pm 5.5 (45–89)	46.8 \pm 14.1 (18–84)
Gender, N (%) women	3099 (58.3)	1109 (54.2)	1102 (56.1)	942 (57.2)
Vertical cup-disc ratio*	0.50 \pm 0.14 (0.00–0.89)	0.50 \pm 0.14 (0.05–0.87)	0.42 \pm 0.17 (0.00–1.00)	0.46 \pm 0.15 (0.00–0.84)
Intraocular pressure (mm Hg)†	14.8 \pm 3.4 (5.0–58.5)	14.4 \pm 3.4 (7.0–31.5)	13.6 \pm 3.0 (5.0–30.0)	14.8 \pm 3.0 (6.0–30.0)

*In RS-I and RS-II measured with ImageNet and in RS-III and ERF with Heidelberg Retina Tomograph.

†Sample sizes for intraocular pressure analyses were 5794 for RS-I, 2102 for RS-II, 2041 for RS-III, and 2035 for ERF. RS, Rotterdam Study; ERF, Erasmus Rucphen Family.

Risk score analysis

For VCDR, age and gender explained 0.3% of the total variance. Figure 1A presents the PEV attributable to the risk scores when risk scores from the VCDR analysis in the discovery sample were used to predict VCDR in the target sample ERF. Figure 1B shows the PEV when the same risk scores were used to predict a random variable (negative control). For VCDR, the risk score based on the p value threshold of $p < 10^{-10}$ (first bar) consisted of nine SNPs (table 2) and explained only 0.1% (in addition to age and gender; region above the dotted line) of the variance in VCDR (figure 1A) in the target population. Increasing the number of SNPs included in the risk scores (ie, increasing the p value thresholds for the SNPs to be included) resulted in a gradual increase in the PEV until the $p < 10^{-2}$ threshold was used (consisting of 7260 SNPs; $p = 8.68 \times 10^{-4}$). The PEV increased up to 1.0% ($p = 1.91 \times 10^{-4}$) at the threshold of $p < 0.2$ for the SNPs to be included in the risk score and then stabilised. This suggests that there is a polygenic model underlying the genetics of VCDR. No similar pattern was observed for the negative controls—that is, the random variable used to evaluate false positive associations other than due to confounding by

admixture (lowest observed $p = 0.204$). For the second negative control, based on sampling without replacement, increasing the p value thresholds for the SNPs did not have any effect on the PEV, neither for the negative control based on random generated numbers (figure 1B; supplementary figure S2).

Figures 1C,D present the corresponding graphs for IOP. For IOP, age and gender explained 2.5% of the total variance, while the first risk score based on a threshold of $p < 10^{-8}$ explained an additional 0.2% (region above the dotted line) of the variation in IOP in the target sample ERF. This score included only one SNP and did not reach nominal significance ($p = 0.093$). However, the addition of more SNPs did not yield any increase in PEV. None of the more extended risk scores were even marginally significant.

To evaluate whether there is a shared genetic component between VCDR and OAG and IOP and OAG, we tested the association of risk scores based on VCDR and IOP in the discovery sample with OAG cases and controls in RS-I. Table 3 shows the descriptive data of the OAG cases and controls in RS-I. A total of 5304 participants of RS-I had complete data for optic nerve head measurements, IOP, and reliable visual fields. Of these, 171 were classified as having OAG. This classification

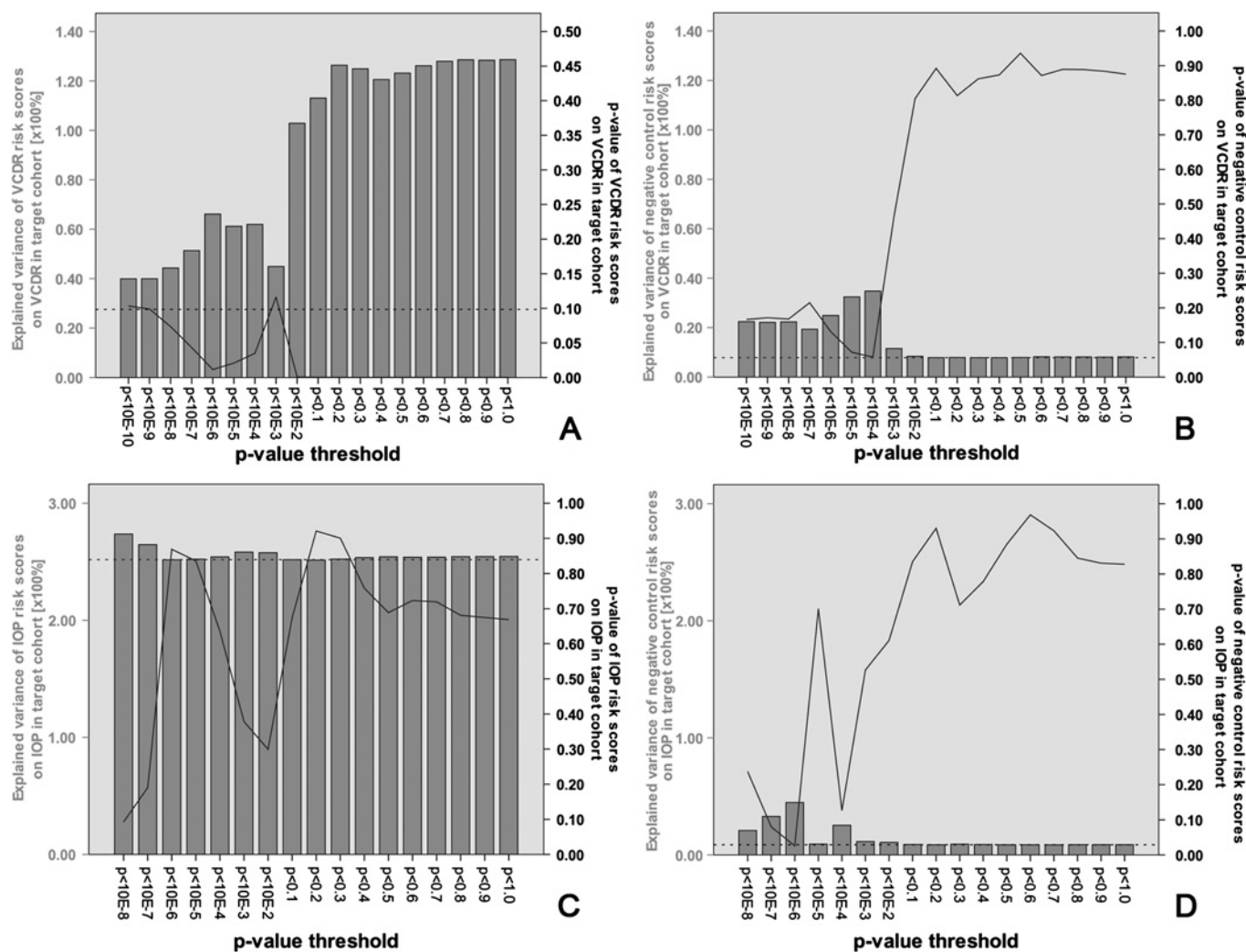


Figure 1 The explained variance of risk scores of the vertical cup-disc ratio (VCDR) and intraocular pressure (IOP) for each p value threshold for their respective trait in the target cohort Erasmus Rucphen Family (ERF) (A and C, respectively); and the explained variance of the risk scores of VCDR and IOP for each p value threshold for the negative control (B and D, respectively). The y1-axis (left; histogram) depicts the explained variance (in %) against different p value thresholds on the x-axis that were used to construct the risk scores. The y2-axis (right; line) depicts the p values from the association analysis of the risk scores with the trait. Dotted line represents the attribution of age and gender to the explained variance.

Table 2 Number of single nucleotide polymorphisms (SNPs) included in each p value category presented as N (%)

p Value threshold	Vertical cup-disc ratio	Intraocular pressure
<10 ⁻¹⁰	9 (0.0)	—
<10 ⁻⁹	10 (0.0)	—
<10 ⁻⁸	12 (0.0)	1 (0.0)
<10 ⁻⁷	18 (0.0)	2 (0.0)
<10 ⁻⁶	34 (0.0)	4 (0.0)
<10 ⁻⁵	61 (0.0)	13 (0.0)
<10 ⁻⁴	179 (0.0)	88 (0.0)
<10 ⁻³	966 (0.2)	755 (0.1)
<10 ⁻²	7260 (1.4)	6429 (1.2)
<0.1	60029 (11.5)	56798 (10.9)
<0.2	114122 (21.8)	110338 (21.1)
<0.3	166995 (31.9)	163261 (31.2)
<0.4	219096 (41.9)	215470 (41.2)
<0.5	270510 (51.7)	267418 (51.2)
<0.6	321288 (61.5)	318625 (60.9)
<0.7	371823 (71.1)	369764 (70.7)
<0.8	422175 (80.8)	420547 (80.4)
<0.9	472496 (90.4)	471532 (90.2)
<1.0	522762 (100.0)	522782 (100.0)

was based on the presence or absence of glaucomatous visual field loss. OAG cases were significantly older and more often men compared to controls. Not surprisingly, OAG cases had a larger VCDR and an increased IOP compared to controls, although these measures were not part of the classification process.

Figure 2A displays the PEV (Nagelkerke R²) of OAG in RS-I attributable to VCDR genetic risk scores. Age and gender explained 4.0% of the variance of OAG and the first risk score based on the p value threshold of p<10⁻¹⁰ (first bar) explained only an additional 0.3% of the OAG variance. However, when more SNPs with higher p values were added to the score, the PEV increased sharply from p<10⁻² and kept on increasing up to the inclusion of SNPs in the risk score with p<0.3 in the discovery set. The latter score explained up to 4.7% of the variance of OAG above the attribution of age and gender, which is more than what is explained by age and gender together. The pattern of figure 2A suggests a polygenic model for OAG as well and also a large genetic component shared by both VCDR and OAG. In a secondary analysis we excluded RS-I from the meta-analysis of the discovery cohorts and found a similar trend (figure 3A). Nevertheless, as a consequence the PEV was much lower due to a major reduction in power. Results for IOP were similar to VCDR if we included RS-I, in that the risk scores were significantly associated with OAG (figure 2B). However, in contrast to VCDR, the evidence for a polygenic model underlying IOP disappeared in the secondary analyses (ie, after excluding RS-I from the meta-analysis of the discovery cohorts; figure 3B).

DISCUSSION

In the present study of GWAS data from Caucasian participants, we found evidence for a polygenic model underlying VCDR, one of the major known measures for OAG. In contrast, IOP did not show significant evidence for a polygenic model in the current study. Of these two traits, VCDR is the most relevant trait related to the diagnosis of OAG, while IOP is the most relevant trait for OAG treatment in patients with elevated IOP. Of interest is the finding that the risk scores based on the VCDR also predicted OAG. Although both VCDR and elevated IOP are putative endophenotypes for OAG, only VCDR showed evidence for a joint polygenic origin with OAG.

In the approach of the present study a large discovery cohort is crucial, while the size of the target cohort is less important. Therefore, we combined data of three independent cohorts from the Rotterdam Study, and used a single target cohort (ERF). We also repeated our analysis in which we used RS-III as a second replication cohort, rather than as one of the discovery cohorts. The results were similar, further supporting the evidence for a polygenic model of VCDR (data not shown).

At first sight, it seems unexpected that we did not find a similar model for IOP as for VCDR, because an elevated IOP has been implicated as the most important determinants of both glaucomatous optic neuropathy (often referred to as an increased VCDR) and glaucomatous visual field loss. In some patients with OAG the IOP is not elevated. This form of disease is referred to as normal tension glaucoma, which represents about 15–40% of all patients with OAG.²⁶ In these patients, however, IOP lowering also causes a decrease in the rate of disease progression, suggesting that IOP matters in normal tension glaucoma as well. Due to the relatively low number of cases in our study, we did not separately analyse patients with normal tension glaucoma. For both traits, IOP and VCDR, strong evidence of genetic correlation with OAG susceptibility has been found. These two traits also correlate with each other in our population (Pearson's p<0.001) and in other populations.²⁷ Nonetheless, when we tested whether the risk scores based on IOP predicted VCDR in the target cohort we found 0.54% as the highest significant PEV for VCDR of IOP related SNPs (at p<10⁻⁸; data not shown). This finding suggests there is little overlap in the genes determining IOP and VCDR. Combined with the current findings this suggests that there are other, possibly rare, variants with strong effects or yet unidentified environmental risk factors that determine IOP. However, a drawback of our analysis of IOP is that we did not adjust for the central corneal thickness, while the measurement of IOP may also be affected by central corneal thickness.²⁸

All OAG cases were only derived from RS-I. A potential problem in the interpretation of the findings on OAG might be that the OAG cases derived from RS-I were also included in the discovery cohorts for the VCDR genetic risk scores. Although this may create an autocorrelation between the VCDR risk scores defined in the discovery set and OAG prediction

Table 3 Descriptive data of open angle glaucoma cases and controls in RS-I, presented as mean±SD (range) unless stated otherwise

	Cases (N=171)	Controls (N=5133)	p Value	RS-I (total N=5304)
Age (years)	77.4±7.2 (56–94)	74.0±7.5 (55–99)	<0.001*	74.1±7.5 (55–99)
Gender, N (%) women	81 (47.4)	3013 (58.7)	<0.001*	3094 (58.3)
Vertical cup-disc ratio	0.59±0.15 (0.15–0.89)	0.50±0.14 (0.00–0.83)	<0.001*	0.50±0.14 (0.00–0.89)
Intraocular pressure (mm Hg)	16.8±4.7 (5.5–39.7)	14.7±3.2 (5.0–35.8)	<0.001*	14.7±3.3 (5.0–39.7)

*Significant at p=0.05.
RS, Rotterdam Study.

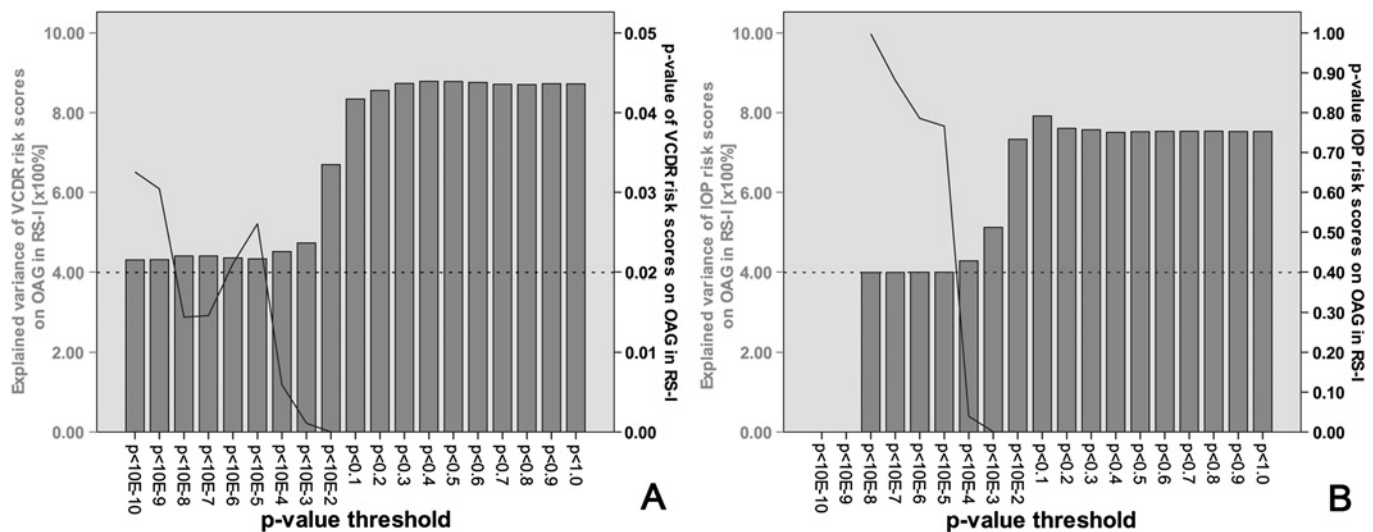


Figure 2 The Nagelkerke R^2 of risk scores of the vertical cup-disc ratio (VCDR) and intraocular pressure (IOP), adjusted for age and gender, for each p value threshold on open angle glaucoma (OAG) in Rotterdam study I (RS-I) (A and B, respectively). The y1-axis (left; histogram) depicts the explained variance (in %) against different p value thresholds on the x-axis, which were used to construct risk scores. The y2-axis (right; line) depicts the p values from the association analysis of the risk scores with the trait.

conducted in the same set, this problem also occurred for the IOP scores. As the IOP score did not associate with the risk of OAG, it appears unlikely that any autocorrelation bias explained the overlap in genes involved in VCDR and OAG. Furthermore, it is important to realise that the diagnosis for OAG in RS-I was primarily based on glaucomatous visual field loss, thus not on VCDR or IOP. Moreover, the results did not alter when we excluded RS-I from the discovery cohorts.

As far as we know, the current study is the first to describe the phenomenon of a polygenic model for optic disc cupping (expressed in the VCDR). We further showed that the same risk scores predicted OAG. This finding sheds new light on the aetiology of OAG. The amount of variance explained by the VCDR risk scores exceeded that of age and gender. Nevertheless,

the PEV of both was still too low to allow the prediction of OAG in persons at risk.

Rare variants with large effects have also been implicated in the aetiology of OAG. However, the current data suggest that many SNPs (probably with very small effects) may collectively account for a substantial proportion of variation in VCDR. This implies that many common variants have not been identified yet, because of low effect estimates of risks associated with single SNPs. Larger GWAS are needed to detect those variants and to further replicate our findings.

In conclusion, the present study has three major implications. First, in our large epidemiological study there is little overlap between the genes involved in VCDR and IOP. Second, the current study provides evidence for a polygenic model in VCDR

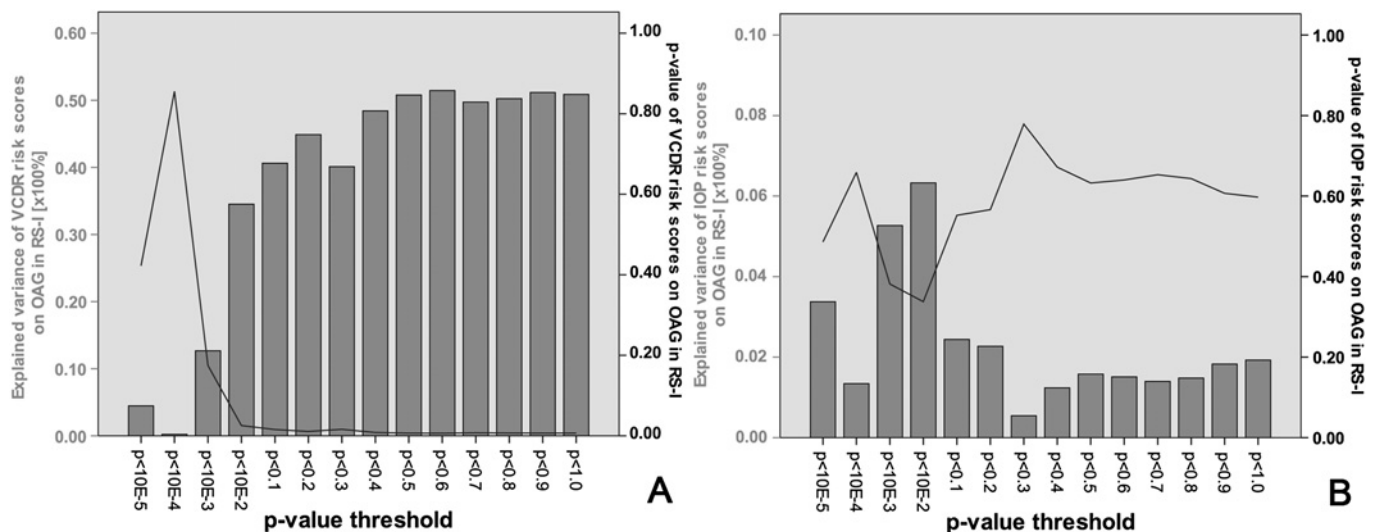


Figure 3 The Nagelkerke R^2 of risk scores of the vertical cup-disc ratio (VCDR) and intraocular pressure (IOP), adjusted for age and gender, for each p value threshold on open angle glaucoma (OAG) in Rotterdam Study I (RS-I) (A and B, respectively) after excluding RS-I from the discovery cohort. To display the pattern more clearly the attribution of age and gender has been subtracted. The y1-axis (left; histogram) depicts the explained variance (in %) against different p value thresholds on the x-axis, which were used to construct risk scores. The y2-axis (right; line) depicts the p values from the association analysis of the risk scores with the trait.

involving many common SNPs that are not shared by a random variable, but specific to VCDR itself. This polygenic basis is not shared by the IOP, which may suggest that either the trait IOP has underlying rare variants, or the variants involved in IOP have such small effects that they were not well detected in the discovery set because of lack of power. Third, the genetics of VCDR overlap convincingly with that of OAG.

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