Determining possible shared genetic architecture between myopia and primary open-angle glaucoma

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Definition of advanced POAG in ANZRAG

ANZRAG recruits cases of advanced glaucoma Australia-wide through ophthalmologist referral. The cohort also included participants enrolled in the Glaucoma Inheritance Study in Tasmania (GIST) who met the criteria for ANZRAG. This cohort has been described previously [1]. Advanced POAG was defined as best-corrected visual acuity worse than 6/60 due to POAG, or a reliable 24-2 Visual Field with a mean deviation of worse than -22db or at least 2 out of 4 central fixation squares affected with a Pattern Standard Deviation of < 0.5%. The less severely affected eye was also required to have signs of glaucomatous disc damage. Clinical exclusion criteria for this advanced POAG study were: i) pseudoexfoliation or pigmentary glaucoma, ii) angle closure or mixed mechanism glaucoma; iii) secondary glaucoma due to aphakia, rubella, rubeosis or inflammation; iv) infantile glaucoma, v) glaucoma in the presence of a known associated syndrome. Controls were drawn from the Australian Cancer Study (225 oesophageal cancer cases, 317 Barrett's oesophagus cases and 552 controls) or from a study of inflammatory bowel diseases (303 cases and 595 controls).

Phenotyping in the Rotterdam Study

Intraocular pressure (IOP)

In all three Rotterdam Study cohorts, IOP was measured for both eyes with Goldmann applanation tonometry (Haag-Streit, Bern, Switzerland) see Supplementary Table 1. The measurement was done twice. If the second measurement was different from the first measurement, a third measurement was performed and the median of all three values was taken.

Optic nerve head parameters

The optic nerve head was assessed with ImageNet (RS-I and RS-II) or Heidelberg Retina Tomograph 2 (RS-III) (see Supplementary Table 1). Details of the optic nerve head assessment have been described elsewhere 1,2. In brief, for RS-I and RS-II, images were analyzed by two trained

technicians using the ImageNet retinal nerve fiber layer height module. A total of four points on the disc margin were marked by the technicians. These points were used by ImageNet to define a retinal zero-reference plane. All points within the ellipse and at least 150μm below the zero-reference plane were considered as cup. We used the VCDR as our ImageNet outcome measure. Images with 25% or more bad points were excluded. For RS-III HRT 2 was used. The HRT obtains during one scan, three series of 16 to 64 confocal frontal slices. From each of these series, a 3-dimensional image of the ONH is reconstructed, from which the software calculates several optic disc parameters. All HRT 2 data was converted to HRT 3. The inter-observer variability and agreement for both systems have been described elsewhere 3. Imaging was performed after entering the participant's keratometry data into the software and after adjusting the settings in accordance with the refractive error.

Axial length and spherical equivalent

Axial length was measured using the Lenstar LS900 (Laméris Ootech) for participants in the Rotterdam Study I and II or the A-scan function of the PacScan 300 AP (Sonomed Escalon) for participants in the Erasmus Rucphen Family Study and the Rotterdam Study III. Measurements of axial length were introduced in a later phase of the Rotterdam Study I, II, and III; therefore, measurements of axial length were available in 5686 study participants of these studies. For each eye the spherical equivalent was calculated using the standard formula: spherical equivalent=spherical component +(cylindrical value/2). The mean spherical equivalent of both eyes was included.

Retinal nerve fiber layer measured by Optical Coherence Tomography (OCT)

From March 2009 to June 2014, participants underwent a standard ophthalmic examination after pharmacological mydriasis, which included fundus photography of the macula and optic nerve, and OCT scanning. Initially, participants' eyes were scanned with the Topcon 3D OCT-1000 (n = 2242; Topcon optical Co, Tokyo, Japan). Due to an update during the study, from August 2011 onward, this device was replaced with the Topcon 3D OCT-2000 (n = 3019). The macula and optic nerve head was scanned in the horizontal direction in an area of 6 × 6 × 1.68 mm with 512 × 512 × 480 voxels for OCT-

1000 and in an area of 6 × 6 × 2.30 mm with 512 × 512 × 885 voxels for OCT-2000, allowing to detect structures with a resolution of 5 μm. Thickness of the peripapillary retinal nerve fiber layer (RNFL) was measured automatically by Topcon's built-in segmentation algorithm. This was done in 12 peripapillary segments of 30° each, and average RNFL thickness was derived from the calculation circle.

LD score regression

To perform LD score regression analyses. GWAS data were harmonized using the "munge_sumstats.py" function. For analyses, we used the pre-computed LD scores for Europeans "eur_w_ld_ch" available at https://github.com/bulik/ldsc/wiki. The ldsc.py function (with all default settings) was used to estimate the genetic correlation between traits.

Supplementary Table 1. Phenotyping methods in the Rotterdam Study

Cohort	IOP measurement	Optic nerve head assessment
RS-I	Goldmann applanation tonometry (Haag-Streit, Bern, Switzerland)	ImageNet and stereoscopic fundus camera (Topcon TRC-SS2; Tokyo Optical Co., Tokyo, Japan)
RS-II	Goldmann applanation tonometry (Haag-Streit, Bern, Switzerland)	ImageNet and stereoscopic fundus camera (Topcon TRC-SS2; Tokyo Optical Co., Tokyo, Japan)
RS-III	Goldmann applanation tonometry (Haag-Streit, Bern, Switzerland)	Heidelberg Retina Tomograph 2, Heidelberg Engineering, Heidelberg, Germany

RS; Rotterdam Study

Supplementary Table 2. Genotyping and Imputation methods in the RS and ANZRAG studies

			1000 Genomes Project reference panel Imputations	
Study	**genotyping arrays**	**Quality Control**	**version**	**Tool used for imputations**
RS-I	Illumina 550K	MAF < 0.05, SNP callrate < 0.95 and/or HWE *p*-value < 1 x 10^{-7}	Phase 1 integrated release v3, march 2012, all populations	MaCH and Minimac
RS-II	Illumina 550K	MAF < 0.05, SNP callrate < 0.95 and/or HWE *p*-value < 1 x 10^{-7}	Phase 1 integrated release v3, march 2012, all populations	MaCH and Minimac
RS-III	Illumina 610K and 660K	MAF < 0.05, SNP callrate < 0.95 and/or HWE *p*-value < 1 x 10^{-7}	Phase 1 integrated release v3, march 2012, all populations	MaCH and Minimac
ANZRAG	Illumina Omni1M/ OmniExpress	MAF < 0.01, SNP callrate < 0.97 and/or HWE *p*-value<0.0001 in controls and P<5e-10 in cases	Phase 1 Europeans March 2012 release	IMPUTE2

RS; Rotterdam Study, MAF; Minor allele frequency, HWE; Hardy-Weinberg equilibrium, ANZRAG; Australian & New Zealand Registry of Advanced Glaucoma

Supplementary Table 3. Number of SNPs per *P*-value category

Score	**P-value threshold**	***n* of SNPs***
S1	5.0×10^{-8}	152
S2	5.0×10^{-7}	214
S3	5.0×10^{-6}	334
S4	5.0×10^{-5}	661
S5	5.0×10^{-4}	1815
S6	0.005	7303
S7	0.01	11763
S8	0.05	37999
S9	0.1	63106
S10	0.5	183871
S11	0.8	228198
S12	1	243938

*Number of SNPs in each *P*-value category according to the summary statistics results from the meta-analysis of myopia [4] -excluding the Rotterdam Study-.

Supplementary Table 4. Number of individuals in the RS-I-II-III with IOP-lowering medication/laser or surgery

Cohort	n of participants with IOP-lowering medication	n of participants with IOP-lowering laser or surgery *
RS-I	112	59
RS-II	40	36
RS-III	35	12
Total	187	107

* patients with IOP-lowering laser or surgery were removed from the analyses

Supplementary Table 5. Association of the polygenic risk scores for myopia with axial length

Score	*MEGA GWAS P-value threshold**	*n of SNPs**	*% variance explained*	*P-value*
REF			8.12	NA
S1	5.0×10^{-8}	152	5.23	9.76×10^{-63}
S2	5.0×10^{-7}	214	5.35	2.75×10^{-64}
S3	5.0×10^{-6}	334	5.45	1.88×10^{-65}
S4	5.0×10^{-5}	661	5.07	6.71×10^{-61}
S5	5.0×10^{-4}	1815	**6.12**	1.63×10^{-73}
S6	0.005	7303	5.74	6.64×10^{-69}
S7	0.01	11763	5.55	1.11×10^{-66}
S8	0.05	37999	4.93	2.92×10^{-59}
S9	0.1	63106	4.66	4.77×10^{-56}
S10	0.5	183871	4.26	3.22×10^{-51}
S11	0.8	228198	4.23	6.05×10^{-51}
S12	1	243938	4.23	6.29×10^{-51}

Predictive power of the calculated PRSs for myopia in the RS (n = 10,792). REF; reference model refers to axial length ~ age + sex + 5PCs + cohort (without the PRS). In bold the maximum variance explained by the PRSs.

Supplementary Table 6. Association between PRSs for myopia and POAG endophenotypes in individuals with high myopia (≤ -6D) from the Rotterdam study.

High Myopia group (≤ -6D, n = 232)

		IOP			**Disc area**			**Cup area**			**VCDR**		
Score	MEGA GWAS *P*-value threshold	*P*	adj.R^2	% of variance	*P*	adj.R^2	% of variance	*P*	adj.R^2	% of variance	*P*	adj.R^2	% of variance
REF		NA	0.031	3.11	NA	0.234	23.35	NA	0.131	13.07	NA	0.201	20.09
S1	5.0×10^{-8}	0.08	0.040	0.89	0.58	0.231	*	0.44	0.129	*	0.46	0.199	*
S2	5.0×10^{-7}	0.26	0.032	0.13	0.24	0.235	0.16	0.31	0.131	0.02	0.35	0.200	*
S3	5.0×10^{-6}	0.42	0.029	*	0.24	0.235	0.15	0.69	0.127	*	0.73	0.197	*
S4	5.0×10^{-5}	0.66	0.027	*	0.16	0.237	0.40	0.56	0.128	*	0.75	0.197	*
S5	5.0×10^{-4}	0.54	0.028	*	0.55	0.231	*	0.71	0.127	*	0.47	0.199	*
S6	0.005	0.05	0.044	1.25	0.91	0.230	*	0.90	0.126	*	0.80	0.197	*
S7	0.01	0.06	0.042	1.14	1.00	0.230	*	0.96	0.126	*	0.86	0.197	*
S8	0.05	**0.03**	**0.047**	**1.62**	0.74	0.230	*	0.77	0.127	*	0.77	0.197	*
S9	0.1	**0.01**	**0.054**	**2.31**	0.62	0.231	*	0.69	0.127	*	0.83	0.197	*
S10	0.5	0.13	0.037	0.60	0.91	0.230	*	0.63	0.127	*	0.91	0.197	*
S11	0.8	0.10	0.039	0.74	0.83	0.230	*	0.56	0.128	*	0.83	0.197	*
S12	1	0.11	0.038	0.73	0.81	0.230	*	0.57	0.128	*	0.84	0.197	*

IOP; intraocular pressure, VCDR; vertical cup-disc ratio; *P; P*-value of the association of the PRS for myopia and the studied POAG endophenotype, adj.R2; adjusted R^2, % of variance; percentage of the variance of the studied POAG endophenotype explained by the PRS for myopia, REF; reference model refers to POAG endophenotype ~ age + sex + 5PCs + cohort (without the PRS). NA; Not applicable. **In bold** PRS showing a suggestive association ($P < 0.05$) but no significant after correction for multiple tests. *PRS in which the tested model does not improve the variance explained compared to the reference model.

Supplementary Table7. Association between PRSs for myopia and POAG endophenotypes in individuals with moderate myopia (≤ -3D) from the Rotterdam study.

Moderate Myopia group (-5.99 to -3 D, n = 771)

		IOP			**Disc area**			**Cup area**			**VCDR**		
Score	META GWAS *P*-value threshold	*P*	adj.R^2	% of variance	*P*	adj.R^2	% of variance	*P*	adj.R^2	% of variance	*P*	adj.R^2	% of variance
REF		NA	0.052	5.20	NA	0.265	26.46	NA	0.078	7.77	NA	0.199	19.85
S1	5.0×10^{-8}	0.37	0.052	*	0.51	0.264	*	0.50	0.077	*	0.73	0.198	*
S2	5.0×10^{-7}	0.78	0.051	*	0.68	0.264	*	0.66	0.077	*	0.82	0.197	*
S3	5.0×10^{-6}	0.61	0.051	*	1.00	0.264	*	0.79	0.076	*	0.79	0.197	*
S4	5.0×10^{-5}	0.77	0.051	*	0.74	0.264	*	0.69	0.077	*	0.78	0.198	*
S5	5.0×10^{-4}	0.28	0.052	0.02	0.66	0.264	*	0.85	0.076	*	0.74	0.198	*
S6	0.005	0.10	0.054	0.21	0.47	0.264	*	0.60	0.077	*	0.76	0.198	*
S7	0.01	0.13	0.054	0.17	0.39	0.264	*	0.41	0.077	*	0.75	0.198	*
S8	0.05	0.38	0.052	*	0.36	0.264	*	0.97	0.076	*	0.82	0.197	*
S9	0.1	0.13	0.054	0.16	0.35	0.265	*	0.89	0.076	*	0.89	0.197	*
S10	0.5	0.12	0.054	0.18	0.42	0.264	*	0.87	0.076	*	0.71	0.198	*
S11	0.8	0.11	0.054	0.20	0.37	0.264	*	0.76	0.076	*	0.54	0.198	*
S12	1	0.11	0.054	0.20	0.38	0.264	*	0.77	0.076	*	0.55	0.198	*

IOP; intraocular pressure, VCDR; vertical cup-disc ratio; *P;* p-value of the association of the PRS for myopia and the studied POAG endophenotype, adj.R2; adjusted R^2, % of variance; percentage of the variance of the studied POAG endophenotype explained by the PRS for myopia, REF; reference model refers to POAG endophenotype ~ age + sex + 5PCs + cohort (without the PRS). NA; Not applicable, *PRS in which the model does not improve the variance explained compared to the reference model.

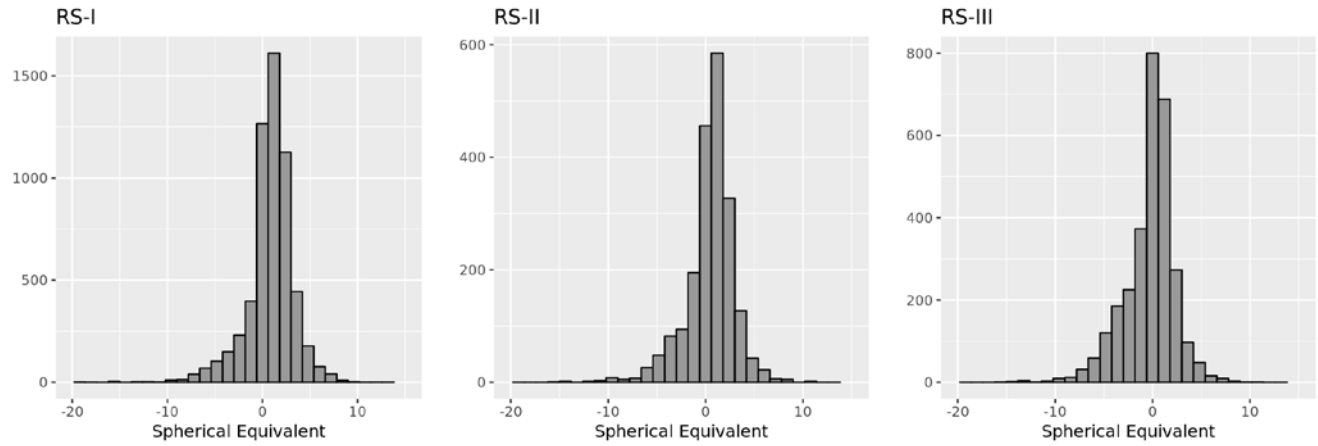
Supplementary Table 8. Association between PRSs for myopia and RNFL when adjusting for axial length in the Rotterdam study

Participants with RNFL and axial length data available (n=2038)			
	META GWAS-P-value threshold	*P-value*	*adj.R2*
REF		NA	0.413514
S1	5x10-8	0.738664	*
S2	5x10-7	0.566423	*
S3	5x10-6	0.865468	*
S4	5x10-5	0.983378	*
S5	5x10-4	0.91292	*
S6	5x10-3	0.593786	*
S7	0.01	0.75268	*
S8	0.05	0.470877	*
S9	0.1	0.423305	*
S10	0.5	0.440658	*
S11	0.8	0.419209	*
S12	1	0.418954	*

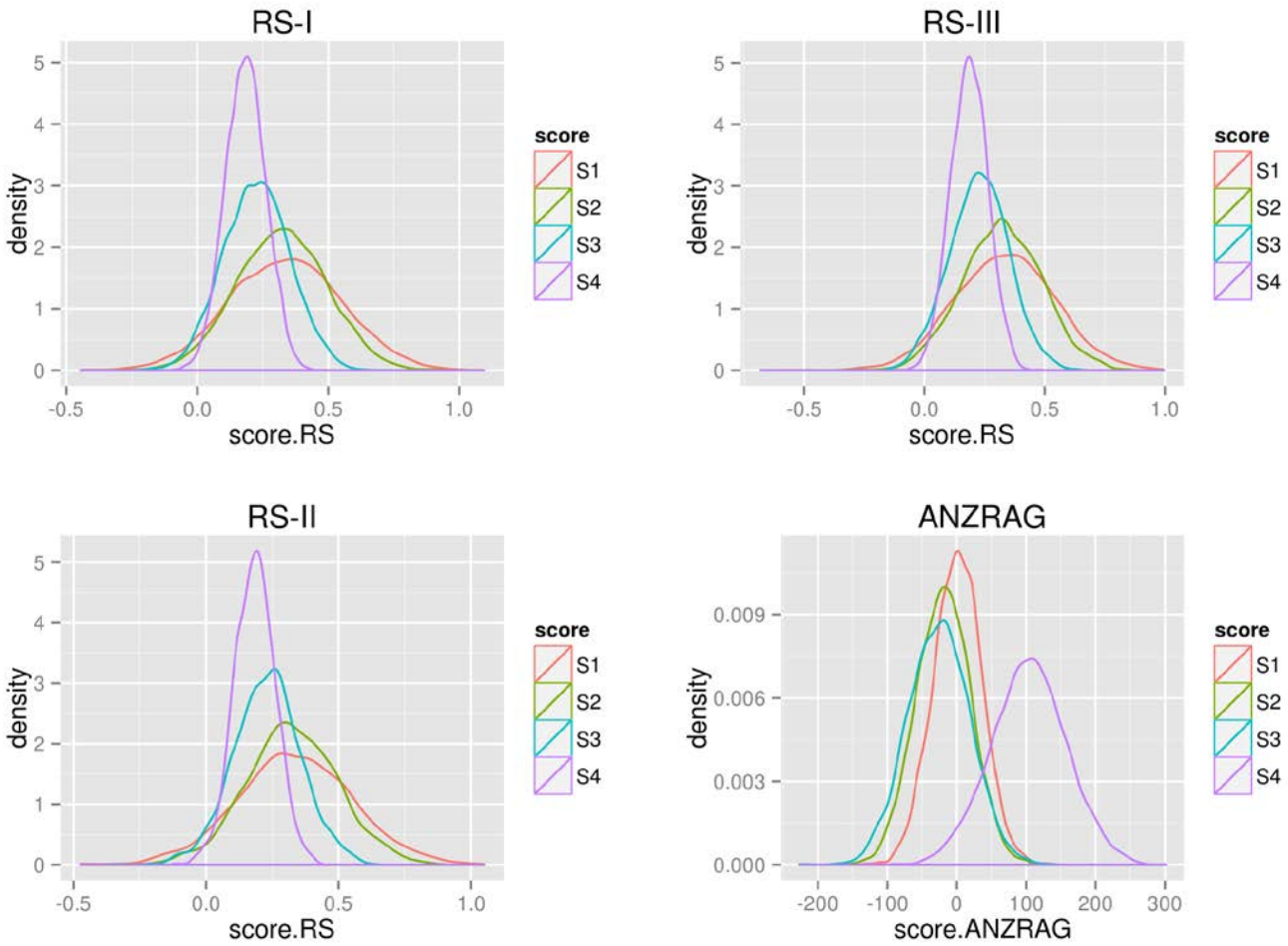
P; p-value of the association of the PRS for myopia and the studied POAG endophenotype, adj.R2; adjusted R2, REF; reference model refers to RNFL ~ age + sex + 5PCs + cohort + OCT device + axial length (without the PRS). NA; Not applicable, *PRS in which the model does not improve the variance explained compared to the reference model.

Supplementary Figures

Supplementary Figure 1. Distribution of Spherical Equivalent in RS-I-II-III



Supplementary Figure 2. Distribution of polygenic risk score in the RS-I-II-III and ANZRAG



Distribution of the first four scores (S1, S2, S3, S4). RS; Rotterdam Study (population-based study), ANZRAG; the Australian & New Zealand Registry of Advanced Glaucoma (case-control study).

Reference

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