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A common glaucoma-risk variant of *SIX6* alters retinal nerve fiber layer and optic disc measures in a European population: The EPIC-Norfolk Eye Study

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ABSTRACT

Purpose: A common missense variant in the *SIX6* gene (rs33912345) is strongly associated with primary open-angle glaucoma (POAG). We aimed to examine the association of rs33912345 with optic disc and retinal nerve fiber layer (RNFL) measures in a European population.

Methods: We examined participants of the population-based EPIC-Norfolk Eye Study. Participants underwent confocal laser scanning tomography (Heidelberg Retina Tomograph II, HRT) to estimate optic disc rim area and vertical cup-disc ratio (VCDR). Scanning laser polarimetry (GDxVCC) was used to estimate average RNFL thickness. The mean of right and left eye values was considered for each participant. Genotyping was performed using the Affymetrix UK Biobank Axiom Array. Multivariable linear regression with the optic nerve head parameter as outcome variable and dosage of rs33912345 genotype as primary explanatory variable was used, adjusted for age, sex, disc area, axial length and intraocular pressure. We further repeated analyses stratified into age tertiles.

Results: In total, 5,433 participants with HRT data and 3,699 participants with GDxVCC data were included. Each "C" allele of rs33912345 was associated with a smaller rim area ($-0.030mm^2$ [95% CI -0.040, -0.020], $P=5.4x10^{-9}$), a larger VCDR (0.025 [95% CI 0.017, 0.033], $P=3.3x10^{-10}$) and a thinner RNFL (-0.39μm [95% CI -0.62, -0.15], $P=0.001$). The RNFL association was strongest in the oldest age tertile, whereas rim area and VCDR associations were strongest in the youngest and oldest age tertiles.

Conclusions: The protein coding *SIX6* variant rs33912345, previously associated with POAG, has a functional effect on glaucoma-associated optic nerve head traits in Europeans.

Keywords: POAG, glaucoma, SIX6, rs33912345, genetic association, retinal nerve fiber layer (RNFL) thickness, optic nerve rim area, vertical cup-disc ratio (VCDR), scanning laser polarimetry (SLP), confocal laser scanning tomography (CLST)

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INTRODUCTION

Glaucoma is a group of diseases that are characterized by a progressive loss of retinal ganglion cells, optic nerve cupping, and visual field loss. Glaucoma is a leading cause of blindness worldwide and is predicted to affect over 60 million people by the year 2040.[1] Primary open angle glaucoma (POAG), the most common form, is a complex inherited disease. Complex inherited disorders may cluster in families but do not have a Mendelian inheritance pattern. Rather, complex inherited traits, far more common than single gene disorders, are inherited through the interaction of multiple genetic influences in combination with environmental factors. Genome-wide association studies (GWAS) for POAG have radically altered the genetic landscape for this disorder through the identification of robust associations between multiple genetic loci and POAG.[2]

Genetic association of variants in the *SIX1*/*SIX6* locus were first made with glaucoma-related optic nerve traits including optic disc area and vertical cup-disc ratio (VCDR).[3–5] Shortly thereafter, an association was identified between this locus and POAG.[6–8] These associations have been observed in European and Asian subjects.

A common missense variant in *SIX6* (rs33912345), a bi-allelic single nucleotide polymorphism (SNP), is strongly associated with POAG.[7,9] This *SIX6* variant codes for an amino acid substitution (Asn141His) in the *SIX6* protein. Association studies in European and Asian populations have found the His141 variant is associated with POAG-risk (conversely, the Asn141 variant is protective).[7,9,10] Two independent research groups using a zebrafish model have demonstrated functional consequences of the Asn141His variant that include effects on eye size and optic nerve volume.[7,9] In addition, one group reported that among POAG patients, retinal nerve fiber layer (RNFL) thickness was significantly thinner in patients who were homozygous for the POAG-risk variant (His141) compared to POAG patients homozygous for the protective (Asn141) variant.[7] The effect on RNFL thickness was corroborated in a dataset containing POAG cases, glaucoma suspects, and controls in subjects of European descent.[11] This study used a POAG-associated non-coding variant, rs10483727, which is in high linkage disequilibrium with rs33912345.[7] In participants of the population-based Singapore Chinese Eye Study (SCES), the His141 POAG-risk variant was associated with thinner peripapillary RNFL in individuals without glaucoma.[10] The SCES study provided the first evidence that the Asn141His

variant influences RNFL thickness in an ostensibly normal population rather than having a more limited glaucoma-specific effect. These studies have observed an effect on RNFL thickness that is limited to the superior and inferior sectors of the peripapillary region.[7,9,11]

The purpose of this study was to determine if the common *SIX6* coding variant Asn141His is associated with differences in optic nerve head and RNFL parameters in the EPIC-Norfolk Eye Study, a large European population-based dataset.

METHODS

Participants

The European Prospective Investigation into Cancer (EPIC) study is a pan-European prospective cohort study designed to investigate the aetiology of major chronic diseases.[12] EPIC-Norfolk, one of the UK arms of EPIC, recruited and examined 25,639 participants aged 40-79 years between 1993 and 1997 for the baseline examination.[13] Recruitment was via general practices in the city of Norwich and the surrounding small towns and rural areas, and methods have been described in detail previously.[13] Since virtually all residents in the United Kingdom are registered with a general practitioner through the National Health Service, general practice lists serve as population registers. Therefore, the EPIC-Norfolk study is essentially a population-based study of persons receiving medical care in the Norfolk region of the United Kingdom. Ophthalmic assessment formed part of the third health examination and this is termed the EPIC-Norfolk Eye Study.[14] In total, 8,623 participants were seen for the ophthalmic examination, between 2004 and 2011. The EPIC-Norfolk Eye Study was carried out following the principles of the Declaration of Helsinki and the Research Governance Framework for Health and Social Care. The study was approved by the Norfolk Local Research Ethics Committee (05/Q0101/191) and East Norfolk & Waveney NHS Research Governance Committee (2005EC07L). All participants gave written, informed consent.

Ocular measurements

Confocal laser scanning tomography (CLST) of each eye was carried out using the Heidelberg Retina Tomograph II (HRT II, Heidelberg Engineering, Heidelberg, Germany) following entering the participant's keratometry. If image quality was poor (topography standard deviation >40μm) a repeat scan was undertaken. Only scans above this quality threshold were included in

the analyses. Contours around the disc margins were manually drawn and subsequently checked by an ophthalmologist (and re-drawn if necessary). The HRT software automatically places a reference plane parallel to the peripapillary retinal surface located 50 μm below the retinal surface as measured along the contour line in the papillomacular bundle (350° to 356°). The HRT software was subsequently updated to Glaucoma Module Premium Edition (software version 3.1) and data exported following this. This derived data that is equivalent to HRT-3 derived parameters. We analysed the following parameters: rim area (the area of the neuroretinal rim; equal to the area enclosed by the contour line and located above the reference plane), vertical cup-disc ratio and disc area (the area bounded by the contour line). A descriptive analysis of HRT measures in the EPIC-Norfolk Eye Study has been previously reported.[15]

Scanning laser polarimetry (SLP) measurements of the RNFL were taken using the GDxVCC (Carl Zeiss Meditec, Inc., Dublin, California), without pupil dilation, according to a standardised operating procedure. Spherical equivalent values derived from an auto-refractor (Auto-Refractor 500, Humphrey Instruments, San Leandro, California, USA) were inputted. Initially a 'corneal' scan was taken (to infer the anterior segment contribution to radial asymmetry of the total phase retardance which is then taken into account for the estimate of RNFL retardance), followed by the RNFL scan. Scans were repeated to aim for a quality score of at least 7. The software automatically delineated an annulus, with an inner and outer diameter of 2.4mm and 3.2mm, centred on the optic disc. We analysed the average RNFL thickness parameter, representing the average thickness within the annulus. The GDxVCC assumes that RNFL thickness is linearly related to retardance and reports an estimate of RNFL thickness calculated using a linear conversion factor of 0.67 nm/μm.[16] Only scans with a quality score of at least 7 and a typical scan score of at least 70 were included in the analyses, based on manufacturer recommendation. A descriptive analysis of GDx measures in the EPIC-Norfolk Eye Study has been previously reported.[17]

Axial length was measured using a Zeiss IOLMaster Optical Biometer (Carl Zeiss Meditec Ltd, Welwyn Garden City, UK). Five measurements were taken per eye and a mean was calculated. Refractive error was measured using a Humphrey Auto-Refractor 500 (Humphrey Instruments, San Leandro, California, USA) and spherical equivalent calculated as [sphere + 0.5 * cylinder]. Intraocular pressure (IOP) was measured using a non-contact appliance, the Ocular Response Analyser (ORA; Reichert, Corp., Buffalo, NY). Three readings were taken per eye and the best

signal value of the Goldmann-correlated measure (IOPg) and the corneal compensated measure (IOPcc) were used based on the best quality pressure waveform as assessed by the ORA software. All ocular examinations were carried out without pupil dilation.

POAG ascertainment

Following pre-defined criteria, participants with study measurements suggestive of glaucoma were referred for further examination by a glaucoma specialist at the regional University Hospital.[14] Those criteria included any one of: best-corrected visual acuity >0.34 LogMAR in either eye, IOP >24mmHg in either eye, IOP >21mmHg in either eye with ≥3 borderline HRT II sectors on Moorfields Regression Analysis, GDx RNFL average thickness/standard deviation/superior thickness/inferior thickness measures outside normal limits in either eye (1 reading at P<0.5% or 2 readings P<1% or 3 readings P<5%), any HRT II sector Moorfields Regression Analysis outside normal limits, manifest abnormalities on fundus photography in either eye. POAG status was ascertained at this specialist examination and additionally, a diagnosis refinement process was undertaken by a second glaucoma specialist who independently reviewed the test results of all participants classified as glaucoma and a proportion of participants who were not classified as having glaucoma, ensuring that International Society of Geographical & Epidemiological Ophthalmology (ISGEO) principles were observed.[18] POAG was defined as the presence of a glaucomatous optic disc together with either a corresponding visual field defect or otherwise unexplained non-specific visual field loss, open angles on gonioscopy, and absence of secondary causes of glaucoma.[19] A glaucomatous disc was defined as one with focal or diffuse neuro-retinal rim narrowing, and may possess, though not necessary for the definition, additional characteristic features such as bared circumlinear vessels, disc haemorrhages or nerve fibre layer defects.[19] Pseudoexfoliative and pigmentary glaucoma were defined as secondary glaucoma in this study and therefore did not contribute to POAG cases. We defined controls as participants not meeting referral criteria for glaucoma on initial ophthalmic assessment and participants who attended the University Hospital for further examination and were not classified as having or being suspect for any type of glaucoma or ocular hypertension.

Genotyping

Genotyping was performed using the Affymetrix UK Biobank Axiom Array. SNP exclusion criteria included: call rate < 95%, abnormal cluster pattern on visual inspection, plate batch effect evident by significant variation in minor allele frequency, and/or Hardy-Weinberg equilibrium $P < 10^{-7}$. Sample exclusion criteria included: DishQC < 0.82 (poor fluorescence signal contrast), sex discordance, sample call rate < 97%, heterozygosity outliers (calculated separately for SNPs with minor allele frequency >1% and <1%), rare allele count outlier, and impossible identity-by-descent values. Following these exclusions, there were no ethnic outliers (i.e. all included participants were of European descent).

Statistical analysis

Descriptive statistics were calculated as means (SD) for continuous variables and count (%) for categorical variables. Crude associations between rs33912345 genotype and ocular parameters were examined using the chi-squared test. To further test these associations adjusted for co-variables, we used multivariable linear regression with the optic nerve head parameter as the outcome variable and dosage of rs33912345 genotype as the primary explanatory variable. Specifically, genotype was coded as 0/1/2 dependent on the number of "C" (His141, POAG-risk) alleles at rs33912345 in each individual. This genotype dosage parameter was then considered as a continuous explanatory variable deriving regression effects per "C" allele. This is equivalent to assuming an additive genetic model. We constructed models adjusted for age, sex and disc area, and additional models adjusted for age, sex, disc area, axial length and IOPg. Additionally, we carried out sensitivity analyses adjusting for IOPcc instead of IOPg, and adjusting for spherical equivalent instead of axial length. To test whether the association of interest was affected by age we performed regression analyses stratified by age tertile. Logistic regression was used to examine the association of rs33912345 genotype with POAG status adjusted for age and sex, and separately further adjusted for disc area.

RESULTS

Of the 8,623 participants of the EPIC-Norfolk Eye Study, complete genetic and optic nerve head data that passed quality control measures were available for 5,859 participants for HRT-derived parameters and 4,537 participants for GDx-derived parameters. For the multivariable analyses, complete data for all covariables were available for 5,433 participants for HRT-derived parameters and 3,699 participants for GDx-derived parameters. Participants with incomplete

imaging data were excluded from further analysis. Compared to participants who were excluded, included participants were significantly younger ($P < 0.001$ for both HRT and GDx) but of a similar sex distribution ($P = 0.84$ for HRT; $P = 0.40$ for GDx). Descriptive characteristics of included participants are presented in **Table 1** and ocular characteristics by genotype are presented in **Table 2**. There were significant crude associations between rs33912345 genotype and rim area, VCDR and RNFL thickness. The association between rs33912345 genotype and disc area was not significant at the 1% level. There were no significant associations between rs33912345 genotype and axial length, spherical equivalent, IOPg or IOPcc.

Genotype at rs33912345 was highly significantly associated with both HRT and GDx optic nerve-head parameters following adjustment for age, sex and disc area (**Table 3**). The "C" (risk) allele was associated with smaller rim area, larger VCDR, and thinner RNFL. The magnitudes of the difference per risk allele as a percentage of the population standard deviation of each parameter were 9.0%, 7.7% and 7.7% for rim area, VCDR and RNFL thickness, respectively. Results were similar and remained highly significant after further adjustment for axial length and IOPg. Results were similar when adjusted for IOPcc instead of IOPg (**Supplementary Table A, Supplemental Digital Content 1, http://links.lww.com/IJG/A201**) and slightly weaker, but remained highly significant, following exclusion of all participants with glaucoma (**Supplementary Table A, Supplemental Digital Content 1, http://links.lww.com/IJG/A201**). Associations between rs33912345 genotype and sectoral parameters of GDx RNFL thickness and HRT rim area are presented in **Supplementary Table B, Supplemental Digital Content 1, http://links.lww.com/IJG/A201**. There were no strong patterns of the association by anatomical region that were consistent across both imaging modalities, although the association between rs33912345 genotype and GDx RNFL thickness was more robust superiorly and inferiorly than temporally or nasally.

We further examined the genotype-optic nerve head measure associations stratified into age tertiles (**Table 4**). For GDx RNFL thickness, the association with rs33912345 was not apparent in the youngest tertile, borderline in the middle tertile and strongly significant in the oldest tertile. For HRT measures, the pattern with age was less clear. The association of HRT

measures with rs33912345 was mostly evident in the youngest and oldest tertiles and weaker for the middle tertile.

To test for rs33912345 genotype association with POAG in the study population, we identified 174 POAG cases and 5,176 controls (see criteria in Methods). Genotype at rs33912345 was significantly associated with POAG (odds ratio [95% CI] per "C" allele: 1.31 [1.06 – 1.63], P = 0.013), adjusted for age and sex. The association was slightly stronger after further adjusting for disc area (odds ratio [95% CI] per "C" allele: 1.35 [1.09 – 1.69], P = 0.007). This represents a 1.8 fold (1.35^2) increased risk of POAG in participants homozygous for the "C" allele compared to participants with no "C" allele. We additionally examined the associations between rs33912345 and optic nerve head parameters among only participants with POAG (**Supplementary Table C, Supplemental Digital Content 1, http://links.lww.com/IJG/A201**). Despite the large reduction in statistical power (only 164 participants with data for the fully adjusted analyses), the "C" allele was still associated with a smaller rim area and larger VCDR with nominal significance ($P < 0.05$). There were only 81 participants with POAG and complete data for the fully adjusted GDx analysis; the association between rs33912345 and RNFL thickness in these participants was not significant (P = 0.13) but retained the expected direction of effect (a thinner RNFL for each "C" allele).

DISCUSSION

We report the common *SIX6* missense variant rs33912345 (Asn141His) significantly influences RNFL and optic disc glaucoma-associated traits in participants of European ancestry enrolled in the EPIC-Norfolk Eye Study. This is the largest reported population-based study to date examining this common, POAG-risk associated *SIX6* variant. The magnitude of this effect remained robust in ostensibly normal subjects with no known history of glaucoma. These results are consistent with previous reports in other populations.[7,10,11] Taken together with animal model studies[7,9], these data provide strong support that the Asn141His *SIX6* protein-coding variant is the first common genetic variant that is associated with POAG risk and has a consistent functional effect on glaucoma-associated traits in multiple populations.

SIX6 is a highly conserved protein that plays a central role in ocular development. The *SIX6* His141 protein-coding variant is conserved in vertebrates ranging from zebrafish to primates. The protective Asn141 variant is found exclusively in modern humans and appears to have arisen

in populations located in East Africa (communication, Sarah Tishkoff, 06-23-2017). The allele frequency of the His141 variant versus the Asn141 varies widely between global populations. For example, the allele frequency of the His141 variant was 40% (95% CI 39.1 – 40.7) in the European population that participated in the EPIC-Norfolk Eye Study. This is in close agreement with the His141 allele frequency observed in other populations of European ancestry that contributed to the 1000 Genomes Project, which ranges from 31% in Finns to 41% of residents in Utah.[20] Therefore, in this European population the protective Asn141 variant (60%) is more common than the POAG-risk His141 variant (40%). However, the more prevalent allele in the Asian population is His141. In Asians, the His141 POAG-risk allele frequency is approximately 80% and the protective allele frequency is approximately 20%.[20] The effect of these variants in people of African ancestry is not known. It is difficult or impossible to test in African populations from West and South Africa where the allele frequency of the POAG-risk variant (His141) approaches 100%, or stated another way, these populations are almost uniformly homozygous for His141. Therefore, the protective Asn141 is either absent or too rare to allow accurate statistical testing.[20,21] This has led to speculation that the very high allele frequency of the His141 POAG-risk variant has contributed to the higher prevalence of POAG in many African populations and their diaspora, for example African American and Afro-Caribbean populations.[22–25] A different variant in the *SIX1*/*SIX6* region (rs11849906) has been associated with normal-tension glaucoma in African Americans;[26] this suggests there may be different functional variants within the *SIX1*/*SIX6* region and these may be differentially apparent in different ethnicities. Studies that have examined differences in measured optic nerve parameters and RNFL thickness attributable to race have drawn mixed conclusions. Some investigators have reported significantly larger optic nerve parameters, including VCDR, optic disc area, and optic cup volume in African Americans or those of African ancestry.[27,28] However, other investigators found that the differences in VCDR were lost after adjustment for disc area.[29,30] In this study, it is interesting to note that allele-related associations with optic nerve parameters were strengthened after correction for disc area.

SNP rs33912345 was strongly associated with the amount of optic nerve head rim tissue; however, we did not find evidence of association between rs33912345 with axial length, refractive error, or IOP. This suggests that the rs33912345 variant affects the optic nerve head by an IOP-independent mechanism, and that any developmental effect is independent of eye size.

Investigators in another study had similar conclusions when a lack of association between IOP and two major POAG-risk loci, *SIX6* and *CDKN2B-AS*, was found in a large multi-ancestry genome-wide association meta-analysis.[31] Identification of measurable IOP-independent risk factors for POAG carries important clinical implications. IOP is widely used to stratify patients' risk for glaucoma. However, we are currently not able to predict which patients are more or less susceptible to glaucomatous nerve damage at a given level of IOP, and we rely on observation over a period to determine the rate of disease progression. Identifying measurable IOP-independent risk factors may eventually help predict which patients are more susceptible to IOP damage at presentation. In turn, this may help improve risk stratification and guide personalized treatment. This could potentially reduce under- and over- treatment of glaucoma patients and suspects.

A physiological loss of optic nerve axons with normal aging has been suggested for decades, based on age-related differences in cross-sectional studies.[32–35] The use of imaging technologies such as OCT has enabled study of the RNFL with high resolution *in vivo*. Longitudinal OCT studies have demonstrated within individual loss of RNFL with age, and variation in the rate of loss between individuals.[36,37] It is tempting to speculate that the rs33912345 variant, broadly represented in all global populations, might contribute to individual variation in age-related loss of the RNFL.

GDxVCC and HRT II were the imaging technologies used for the EPIC-Norfolk Eye Study. All previous studies examining the association between RNFL and *SIX6* POAG-associated variants have used OCT. Based on age stratified by tertile we observed a genotype-associated difference in HRT measurement of both VCDR and optic disc rim area, in all age stratified tertiles (**Table 4**). The association was strongest in the younger and older age tertiles. The genotype-associated difference for RNFL thickness (GDx) was significant only in the oldest age tertile (> age 69.6) although there was a trend for the middle age tertile ($P = 0.06$). Although these data are cross-sectional, they suggest that changes in the optic nerve topography precede retinal nerve fiber changes, which is consistent with in vivo imaging studies in animal models of experimental glaucoma.[38–40] Different times of onset have been reported for the appearance of glaucomatous damage measured by SLP (GDxVCC) compared with CSLT (HRTII) in studies of non-human primate glaucoma models. SLP is a measure of RNFL retardance produced by the parallel

arrangement of neurotubules within RGC axons of the RNFL versus OCT, which measures total RNFL tissue thickness, including non-axonal elements.[40]

We found evidence for the rs33912345 association with structural parameters to be regionally distinct for GDx RNFL thickness but not for HRT rim area or VCDR. The effect of the *SIX6* common variant was strongest in the SLP-derived inferior and superior RNFL sectors, and less apparent nasally and temporally. This is in agreement with OCT studies in Singapore Chinese people[10] and a small cohort of European descent.[11] In contrast, the genetic association with CSLT-derived rim area did not appear to have a regional predilection in our study. To the best of our knowledge, our study is the first to examine the association between *SIX6* variation and regional optic disc measures. It is unclear why there are different regional patterns for GDx and HRT derived measures. It is possible that rs33912345 variation has differential effects on RNFL versus the optic disc, or that

SLP is more susceptible to regional variation than CSLT. A regional effect of rs33912345 on RNFL thinning is of broad clinical interest. Relative sparing of optic nerve axons in the nasal and temporal peripapillary region would favor preservation of the central and temporal visual islands, considered hallmarks of advanced glaucomatous optic neuropathy. In addition, we observed the same effect on RNFL in study participants without glaucoma, which was also observed in the Singapore Chinese Asian population.[10] These data support the notion that the genetic effect of POAG-risk variant His141 underlies a biological process that broadly contributes to loss of RNFL with age. Specifically, the His141 variant might contribute to manifest glaucoma in some persons, and age-related loss of RNFL in others who will never be diagnosed as having glaucoma. Additionally, these data, observed in persons with ostensibly healthy eyes and normal intraocular pressure, suggests that changes in optic nerve anatomy associated with RGC loss, which is consistent with the pathology observed in glaucoma, is a *genetically influenced component of normal aging*.

Strengths of our study include the large sample size and the availability of genetic data together with glaucoma-related traits measured by two distinct imaging technologies, as well as data on potentially important covariables such as IOP and axial length. A potential weakness of this study is that it is cross-sectional, not longitudinal. Therefore, we can only compare findings between defined subjects grouped by age, rather than trends determined by grouping individual

subjects rate of change. We can only state at which aged-tertile measured differences were observed. Similarly, lacking younger age groups, we are unable to determine the *age of onset* for a specific observation. *SIX6* plays a critical role in vertebrate ocular development.[41] It is possible that these traits present early in life. A cross-sectional or longitudinal study that incorporates younger age groups would address these and other important questions. Future studies may examine other anatomical attributes of RNFL, such as changes in the retinal ganglion cell layer of the macular region, measurements that may be at least as robust as those of the peripapillary RNFL.[42] Further, this study did not examine functional attributes of optic nerve integrity, for example, visual field parameters. A limitation of the ascertainment of rs33912345 status in our study was that we did not validate the genotyping results, by sequencing for example. Nevertheless, the allele frequency at rs33912345 observed in our study is similar to other European populations (see above) and we observed a significant association with POAG, replicating the findings from other larger studies.[7,9] Finally, we conducted multiple statistical tests in this analysis. While our primary results were significant after Bonferroni correction, the possibility of chance findings should be considered, especially for the smaller sub-group analyses.

In conclusion, these findings in the European population, combined with similar data in the Asian population, support the hypothesis that Asn141His, a common variant of *SIX6*, plays a functional role in POAG risk. This information provides a powerful tool to dissect the molecular mechanisms that contribute to both pathogenesis and protection of POAG, the most common form of glaucoma and a leading cause of irreparable vision loss and blindness in man.

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Table 1: Descriptive characteristics for participants included in HRT and GDx analyses.
Mean values (SD) are presented for age and count (%) for sex and rs33912345 genotype.

	HRT (n = 5,859)	**GDx** (n = 4,537)
Age (years)	67.8 (7.7)	67.2 (7.5)
Sex		
Men	2619 (45%)	2051 (45%)
Women	3240 (55%)	2486 (55%)
rs33912345		
AA	2130 (36%)	1649 (36%)
AC	2795 (48%)	2174 (48%)
CC	934 (16%)	714 (16%)

Table 2: Ocular phenotypes by rs33912345 genotype. Mean values (SD) are presented. *P*-values are for chi-squared tests. *P*-values < 0.01 are in bold.

	rs33912345 genotype			*P*-value
	AA	**AC**	**CC**	
HRT measures				
Disc area (mm^2)	1.882 (0.413)	1.855 (0.414)	1.856 (0.418)	0.039
Disc rim area (mm^2)	1.446 (0.335)	1.398 (0.328)	1.377 (0.332)	**<0.001**
Vertical cup-disc ratio	0.325 (0.227)	0.347 (0.227)	0.367 (0.230)	**<0.001**
GDx measures				
Average RNFL thickness (μm)	55.60 (5.21)	55.26 (5.32)	54.92 (5.33)	**0.003**
Axial length (mm)	23.55 (1.18)	23.55 (1.19)	23.60 (1.18)	0.37
Spherical equivalent (D)	0.19 (2.27)	0.16 (2.22)	0.10 (2.29)	0.26
IOPg (mmHg)	16.11 (3.62)	16.07 (3.68)	16.03 (3.59)	0.57
IOPcc (mmHg)	16.86 (3.65)	16.84 (3.65)	16.85 (3.40)	0.93

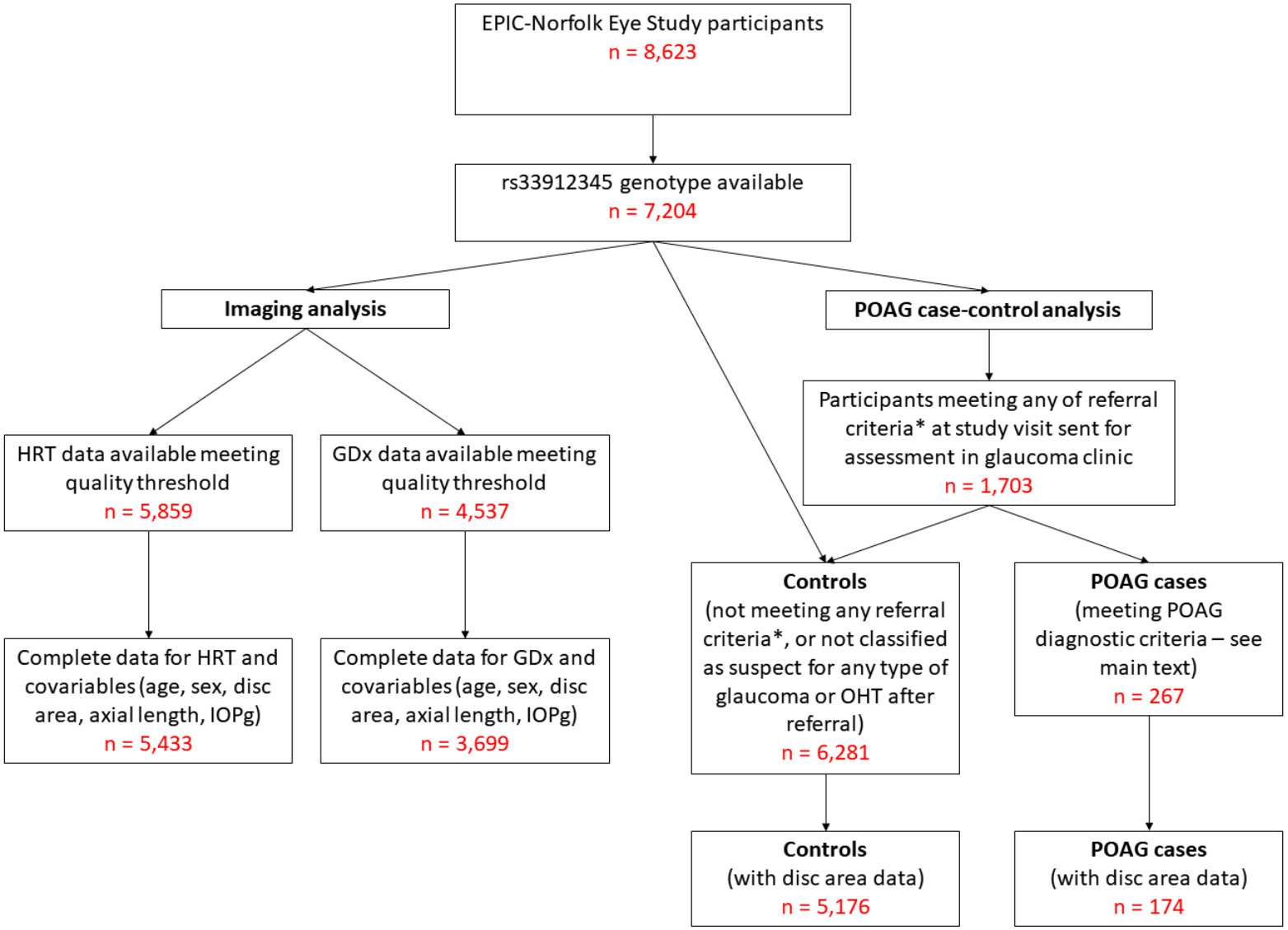
Table 3: Association between rs33912345 genotype and optic nerve head parameters.
Results are from multivariable linear regression models with the optic nerve head parameter as the dependent variable and genotype dosage as an explanatory variable. Parameters additionally adjusted for are indicated in the table. *P*-values < 0.008 (Bonferroni-corrected significance threshold for 6 tests) are in bold.

	Model 1 (adjusted for age, sex and disc area)			**Model 2 (adjusted for age, sex, disc area, axial length and IOPg)**		
	Change per risk allele (C)	**95% CI**	***P*-value**	**Change per risk allele (C)**	**95% CI**	***P*-value**
HRT rim area (mm^2)	-0.030	(-0.039, -0.020)	**2.56E-09**	-0.030	(-0.040, -0.020)	**5.36E-09**
HRT VCDR	0.025	(0.017, 0.032)	**4.33E-10**	0.025	(0.017, 0.033)	**3.27E-10**
GDx RNFL thickness (μm)	-0.406	(-0.637, -0.176)	**0.001**	-0.386	(-0.624, -0.149)	**0.001**

Table 4: Association between rs33912345 genotype and optic nerve head parameters stratified by age tertile. Results are from multivariable linear regression models with the optic nerve head parameter as the dependent variable and genotype dosage as an explanatory variable, adjusted for sex, disc area, axial length and IOPg. *P*-values < 0.006 (Bonferroni-corrected significance threshold for 9 tests) are in bold.

	Lowest age tertile			**Middle age tertile**			**Oldest age tertile**		
	Difference per risk allele	95% CI	*P*-value	Difference per risk allele	95% CI	*P*-value	Difference per risk allele	95% CI	*P*-value
HRT measures	n = 1811			n = 1811			n = 1811		
Age range	48.49 - 63.79			63.80 - 70.96			70.97 - 89.65		
HRT rim area (mm^2)	-0.045	(-0.062, -0.029)	**<0.001**	-0.019	(-0.036, -0.003)	0.023	-0.024	(-0.043, -0.005)	0.013
HRT VCDR	0.032	(0.020, 0.045)	**<0.001**	0.017	(0.003, 0.030)	0.014	0.027	(0.012, 0.041)	**<0.001**
GDx measures	n = 1233			n = 1232			n = 1234		
Age range (years)	48.49 - 63.19			63.20 - 69.54			69.55 - 88.26		
GDx average thickness (μm)	-0.077	(-0.499, 0.345)	0.72	-0.379	(-0.778, 0.020)	0.06	-0.688	(-1.102, -0.274)	**0.001**

Supplementary Figure A: Flow chart describing the derivation of participants included in the primary analyses. *referral criteria were any one of: best-corrected visual acuity >0.34 LogMAR in either eye, IOP >24mmHg in either eye, IOP >21mmHg in either eye with ≥3 borderline HRT II sectors on Moorfields Regression Analysis, GDx RNFL average thickness/standard deviation/superior thickness/inferior thickness measures outside normal limits in either eye (1 reading at P<0.5% or 2 readings P<1% or 3 readings P<5%), any HRT II sector Moorfields Regression Analysis outside normal limits, manifest abnormalities on fundus photography in either eye.



Supplementary Table A: Sensitivity analyses for association between rs33912345 genotype and optic nerve head parameters. Results are from multivariable linear regression models with the optic nerve head parameter as the dependent variable and genotype dosage as an explanatory variable. The first model is for all included participants without excluding glaucoma (n = 5,433 for HRT, n = 3,699 for GDx) and adjustment for IOPcc rather than IOPg. The second model is for all participants following exclusion of participants with glaucoma or history of glaucoma medication or procedure (following exclusion: n = 5,196 for HRT, n = 3,572 for GDx). *P*-values < 0.01 are in bold.

	Sensitivity analysis 1: All participants with alternative IOP adjustment (adjusted for age, sex, disc area, axial length and IOPcc)			**Sensitivity analysis 2: Glaucoma excluded (adjusted for age, sex, disc area, axial length and IOPg)**		
	Difference per risk allele (C)	**95% CI**	***P*-value**	**Difference per risk allele (C)**	**95% CI**	***P*-value**
HRT rim area (mm^2)	-0.030	(-0.040, -0.020)	**5.66E-09**	-0.025	(-0.034, -0.015)	**3.35E-07**
HRT VCDR	0.025	(0.017, 0.033)	**3.62E-10**	0.022	(0.014, 0.029)	**4.81E-08**
GDx RNFL thickness (μm)	-0.389	(-0.627, -0.151)	**0.001**	-0.319	(-0.549, -0.089)	**0.007**

Supplementary Table B: Association between rs33912345 genotype with sectoral optic nerve head (HRT) and RNFL thickness (GDxVCC) parameters. Results are from multivariable linear regression models with the sectoral optic nerve head parameter as the dependent variable and genotype dosage as an explanatory variable, adjusted for age, sex, disc area, axial length and IOPg. *P*-values < 0.01 are in bold.

	Difference per risk allele	95% CI	*P*-value
HRT rim area			
Superotemporal rim area (mm^2)	-0.006	(-0.007, -0.004)	**<0.001**
Temporal rim area (mm^2)	-0.007	(-0.011, -0.004)	**<0.001**
Inferotemporal rim area (mm^2)	-0.004	(-0.006, -0.002)	**<0.001**
Superonasal rim area (mm^2)	-0.005	(-0.006, -0.003)	**<0.001**
Inferonasal rim area (mm^2)	-0.004	(-0.005, -0.002)	**<0.001**
Nasal rim area (mm^2)	-0.005	(-0.007, -0.002)	**<0.001**
GDx RNFL thickness			
Superior thickness (µm)	-0.683	(-1.023, -0.344)	**<0.001**
Inferior thickness (µm)	-0.641	(-0.998, -0.284)	**<0.001**
Temporal thickness (µm)	-0.327	(-0.617, -0.038)	0.027
Nasal thickness (µm)	0.409	(0.051, 0.768)	0.025

Supplementary Table C: Association between rs33912345 genotype and optic nerve head parameters in participants with primary open-angle glaucoma (POAG). Results are shown for the associations among POAG patients with complete data available for genotype, optic nerve parameter and covariables (numbers included in each analysis are shown). We present results for two models: the first model is adjusted for age, sex and disc area; the second model is adjusted for age, sex, disc area, axial length and IOPg.

	Adjusted for age, sex and disc area				Adjusted for age, sex, disc area, axial length and IOPg			
	Number of participants	**Change per risk allele (C)**	**95% CI**	***P*-value**	**Number of participants**	**Change per risk allele (C)**	**95% CI**	***P*-value**
HRT rim area (mm^2)	174	-0.073	(-0.128, -0.018)	**0.009**	164	-0.067	(-0.123, -0.010)	**0.020**
HRT VCDR	174	0.044	(0.011, 0.077)	**0.010**	164	0.042	(0.008, 0.076)	**0.015**
GDx RNFL thickness (μm)	86	-1.608	(-3.230, 0.014)	**0.052**	81	-1.242	(-2.847, 0.362)	**0.13**