Corneal Biomechanical Properties and Glaucoma-Related Quantitative Traits in the EPIC-Norfolk Eye Study

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PURPOSE. We examined the association of corneal hysteresis (CH) with Heidelberg retina tomograph (HRT)– and Glaucoma Detection with Variable Corneal Compensation scanning laser polarimeter (GDxVCC)–derived measures in a British population.

METHODS. The EPIC-Norfolk Eye Study is nested within a multicenter cohort study—the European Prospective Investigation of Cancer. Ocular response analyzer (ORA), HRT3, and GDxVCC measurements were taken at the research clinic. Three ORA measurements were taken per eye, and the single best value used. Participants meeting predefined criteria were referred for a second examination, including Goldmann applanation tonometry (GAT) and central corneal thickness (CCT) measurement. Generalized estimating equation models were used to examine the associations of CH with HRT and GDxVCC parameters, adjusted for disc area. The GDxVCC analyses were adjusted further for typical scan score to handle atypical retardation.

RESULTS. There were complete research clinic data from 5134 participants. Corneal hysteresis was associated positively with HRT rim area ($P < 0.001$), and GDxVCC retinal nerve fiber layer (RNFL) average thickness ($P = 0.006$) and modulation ($P = 0.003$), and associated negatively with HRT linear cup-to-disc ratio (LCDR, $P < 0.001$), after adjustment for Goldmann-correlated IOP and other possible confounders. In the 602 participants undergoing the second examination, CH was associated negatively with LCDR ($P = 0.008$) after adjustment for GAT, CCT, and other possible confounders.

CONCLUSIONS. Lower CH was associated with HRT and GDxVCC parameters in a direction that is seen in glaucoma and with ageing. Further research is required to establish if this is a causal relationship, or due to residual confounding by age, IOP, or CCT.

Keywords: biomechanics, cornea, epidemiology, glaucoma, optic disk, scanning laser polarimetry

Thinner central corneal thickness (CCT) has been found to be associated with the incidence of primary open angle glaucoma (POAG) in patients with ocular hypertension¹ and in healthy population-based samples.^{2,3} Thinner CCT also has been associated with an increased risk of POAG progression⁴ and more advanced POAG at presentation.⁵ While these findings may well be explained by the known influence CCT has on tonometry readings,⁶ another contributing factor might be that CCT is reflecting ocular biomechanics in general. A thinner CCT may be associated with lamina cribrosa and posterior sclera properties that increase susceptibility to POAG.

Corneal hysteresis (CH) and corneal resistance factor (CRF) are measures derived by the Reichert ocular response analyzer (ORA), and are thought to provide a more complete characterization of biomechanical properties than CCT.⁷ Lower CH has been associated with the presence⁸ and severity of POAG,^{9–11} as well as structural¹² and functional^{13–15} progression of POAG. However, CH is related to the IOP level as well as

CCT and participant age, with CH being lower with higher IOP, thinner CCT, and greater age.¹⁶ Thus, the association between CH, and the presence, severity, and progression of POAG is at least partially confounded by IOP, IOP measurement, and participant age.

Primary open angle glaucoma is an optic neuropathy characterized by accelerated loss of retinal ganglion cells (RGCs).¹⁷ Manifestations of RGC loss include optic disc cupping and thinning of the retinal nerve fiber layer (RNFL).¹⁷ If factors that determine RGC loss in POAG also affect RGC loss in normal individuals, examination of the determinants of optic disc and RNFL measures at a population level may provide insight into the etiology of POAG. The approach of examining a continuous quantitative trait related to POAG in largely healthy participants, rather than comparing cases of POAG with controls, has been fruitful in the search for genetic associations with POAG.¹⁸ To date, to our knowledge no study has

examined whether CH and CRF are associated with glaucoma-related quantitative traits in a population-based sample.

The aim of our study was to examine the association of ORA-derived corneal biomechanical measures with Heidelberg Retina Tomograph (HRT)- and Glaucoma Detection with Variable Corneal Compensation scanning laser polarimeter (GDxVCC; Carl Zeiss Meditec, Inc., Dublin, CA)-derived optic disc and RNFL measures in a British population.

METHODS

The EPIC-Norfolk Eye Study is a cross-sectional population-based study of eye disease nested within a pan-European prospective cohort study—The European Prospective Investigation into Cancer (EPIC).¹⁹ Details of the EPIC-Norfolk Eye Study design, participant recruitment, methods, and baseline characteristics have been reported previously.²⁰ In brief, EPIC-Norfolk is one of the UK arms of EPIC, and recruited and examined 25,639 participants aged 40 to 79 years between 1993 and 1997 for the baseline examination.²¹ Detailed ophthalmic examination formed part of the third health examination of EPIC-Norfolk, and this has been termed the EPIC-Norfolk Eye Study.²⁰ In total, 8623 participants attended the Eye Study between 2004 and 2011. A subset of these participants (those meeting predefined criteria described below) had a further examination by a glaucoma specialist at the major local ophthalmic department. 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 and Waveney NHS Research Governance Committee (2005EC07L). All participants gave written, informed consent.

Measurements (EPIC-Norfolk Eye Study Research Clinic)

All participants were examined in the research clinic and measurements were done without pupil dilation by trained nursing staff following standard operating procedures, as detailed previously.²⁰ The ORA (software version 3.01; Reichert, New York, NY) was used to measure CH, CRF, Goldmann-correlated IOP (IOPg), and corneal-compensated IOP (IOPcc). Three ORA readings were taken per eye following a demo puff. The ORA measurements with a poor quality pressure waveform were repeated. The ORA software derived a waveform score that indicated the quality of each reading and the single best value for each eye was considered. While it has been suggested that applying a quality cutoff for the waveform score may be unnecessary,²² we excluded eyes with a very low best waveform score (<3.5) based on manufacturer advice and other normative data.²³

Scanning laser ophthalmoscopy of each eye was done using the HRT II device (Heidelberg Retina Tomogram II; Heidelberg Engineering, Heidelberg, Germany) after entering the participant's keratometry and refraction (Auto-Refractor 500; Humphrey Instruments, San Leandro, CA). If the HRT image quality was poor (topography standard deviation > 40 μ m) a repeat scan was done. Contours around the disc margins were drawn manually and subsequently checked by an ophthalmologist (and redrawn if necessary). The HRT software subsequently was updated to Glaucoma Module Premium Edition (software version 3.1) and data exported following this. This derived data that are equivalent to HRT3-derived parameters. Three HRT parameters were considered: rim area, linear cup-to-disc ratio (LCDR), and mean RNFL thickness, based on a principal

components analysis of all HRT variables for the Eye Study cohort.²⁴ Only scans with a topography standard deviation $\leq$ 40 μ m were included in analyses.

The RNFL measurements were taken using the GDxVCC scanning laser polarimeter, (Carl Zeiss Meditec, Inc.). Spherical equivalent values derived from the autorefractor were inputted. Initially a corneal scan was taken, 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 2.4 and 3.2 mm, centered on the optic disc. Only scans with a quality score of at least 7 were included in the analyses, as per manufacturer recommendation. Parameters considered were the average RNFL thickness and RNFL modulation (standard deviation) within the annulus, and the nerve fiber indicator (NFI), a neural network-derived parameter designed to discriminate maximally between glaucomatous eyes and healthy controls.²⁵

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 value calculated. Height and weight were measured at the third health examination, with participants wearing light clothing and no shoes. Height was measured to 0.1 cm using a stadiometer, and weight was measured to the nearest 0.1 kg using digital scales (Tanita UK, Ltd., Middlesex, UK). Body mass index (BMI) was calculated as weight/height². Educational level was ascertained at the first health examination and was classified into four groups according to the highest qualification achieved.

Measurements (Referral Hospital Clinic)

Participants meeting predefined criteria at the research clinic visit were referred for examination by a glaucoma specialist (DCB) at the major local ophthalmic department (Norfolk and Norwich University Hospitals NHS Foundation Trust).²⁰ The referral criteria were any one of the following: best-corrected visual acuity > 0.34 LogMAR in either eye, IOP > 24 mm Hg in either eye, IOP > 21 mm Hg in either eye with $\geq$ 3 borderline HRT sectors on Moorfields Regression Analysis, GDx RNFL average thickness/standard deviation/superior thickness/inferior thickness measures outside normal limits in either eye (one reading at $P < 0.5\%$, two readings at $P < 1\%$, or three readings at $P < 5\%$), any HRT sector Moorfields Regression Analysis outside normal limits, manifest abnormalities on fundus photography in either eye.²⁰ Examination included measurement of IOP by Goldmann applanation tonometry (GAT, one measurement per eye) and ultrasound pachymetry (Pachmate DGH 55, mean of 10 readings per eye; DGH Technology, Exton, PA).

Statistical Analysis

We excluded participants who reported (on nurse interview or postal questionnaire) a history of glaucoma therapy, corneal refractive surgery, or other corneal pathology, as these may affect IOP and corneal biomechanical measurements. Analyses were done on data from participants with complete data for all variables of interest. Pearson product-moment correlation coefficients were calculated to examine how ORA variables, GAT, and CCT were related. Linear regression models were used to examine the associations of CH and CRF with HRT and GDx measures. All models were adjusted for disc area given the recognized association between disc area and optic nerve head structural measures.^{26–36} All models with GDx measures were adjusted further linearly for typical scan score (TSS) to handle scans exhibiting atypical retardation, as we have described previously.³⁷ Initially, crude associations were examined by

TABLE 1. Correlation Matrix for IOPg, IOPcc, CH, and CRF in 8213 Eyes of 5134 Participants

	IOPg	IOPcc	CH	CRF
IOPg	1.00			
IOPcc	0.88, $P < 0.001$	1.00		
CH	−0.03, $P = 0.002$	−0.50, $P < 0.001$	1.00	
CRF	0.62, $P < 0.001$	0.17, $P < 0.001$	0.77, $P < 0.001$	1.00

including only the ORA measure, disc area, and TSS for GDx models, or the ORA measure and disc area for HRT models. Multivariable models then were used to examine whether the associations persisted with further adjustment for potential confounding factors, including IOPg. The factors adjusted for were IOPg, age, sex, height, BMI, education level, axial length, and lens status, based on previous descriptive analysis of GDx³⁷ and HRT²⁴ measures in the cohort. To examine whether the associations were modified by IOP level, we repeated analyses stratified by IOPg tertile. We tested further for evidence of effect modification by including an interaction term between IOPg and CH or CRF as continuous measures in the multivariable regression models.

Since the ORA derives biomechanical measures and IOP measures from the same waveform, it may not be possible to adjust optimally for “true” IOP using ORA-derived IOP. It is possible that ORA-derived CH adjusted for IOPg still is describing some attribute of the underlying “true” IOP. Therefore, we examined further the associations in a subset of participants who had GAT and CCT measured at a follow-up visit. For the subset analysis, similar adjusted linear regression models were used to examine the biomechanical associations with HRT and GDx measures, as described above, adjusting for GAT instead of IOPg, and further adjusting for CCT.

Data from both eyes of participants were considered, and generalized estimating equation models used to account for the correlation between eyes. Statistical significance should be interpreted against the background of the multiple tests conducted and we have highlighted P values of <0.01 as potentially significant in analyses. Stata version 12.1 (StataCorp LP, College Station, TX) was used for all analyses.

RESULTS

Participants

There were good quality ORA measurements, and good quality HRT and GDxVCC scans, together with complete data for covariables, from 8458 eyes of 5302 participants who attended the research clinic. Following exclusion of participants reporting a history of glaucoma medication ($n = 120$), history of a glaucoma procedure ($n = 37$), or history of corneal refractive surgery ($n = 26$), data were available from 8213 eyes of 5134 participants with a mean age of 67 years (range, 48–90); 57% were women. Compared to participants attending the EPIC-Norfolk Eye Study ($n = 8623$), but excluded from analyses ($n = 3489$), included participants were younger (mean age 67.2 vs. 70.8 years, $P < 0.001$), had better visual acuity in the better seeing eye (median LogMAR acuity -0.04 vs. 0.02 , $P < 0.001$), and more were women (57% vs. 53%, $P < 0.001$).

Of the 5134 participants included in the primary analyses, a proportion underwent a further examination at the referral hospital clinic, including GAT and CCT measurements (see above). Complete data from 918 eyes of 602 participants who attended the referral hospital clinic were available; the mean

age of these participants was 68 years (range, 49–88) and 51% were women.

Research Clinic Analyses (8213 Eyes of 5134 Participants)

The mean (SD) of ORA variables were: IOPg 16.0 (3.8) mm Hg, IOPcc 16.7 (3.7) mm Hg, CH 10.2 (1.6) mm Hg, and CRF 10.4 (1.8) mm Hg. The correlation matrix for the ORA variables is presented in Table 1. The IOPg and IOPcc were correlated strongly ($r = 0.88$, $P < 0.001$), as were CH and CRF ($r = 0.77$, $P < 0.001$). Corneal hysteresis was correlated very poorly with IOPg ($r = -0.03$, $P = 0.002$), and correlated moderately with IOPcc ($r = -0.50$, $P < 0.001$). The CRF was correlated moderately with IOPg ($r = 0.62$, $P < 0.001$), but only very weakly correlated with IOPcc ($r = 0.17$, $P < 0.001$). The mean (SD) for the HRT and GDx variables were: rim area 1.42 (0.34) mm², LCDR 0.44 (0.18), HRT RNFL thickness 0.22 (0.07) mm, GDx RNFL thickness 56.7 (6.2) μ m, RNFL modulation 22.4 (4.7) μ m, and NFI 18.5 (9.5).

Table 2 presents the crude associations of ORA variables with HRT variables (adjusted for disc area only) and GDxVCC variables (adjusted for disc area and TSS). Higher CH was associated significantly with a larger rim area, smaller LCDR, thicker HRT RNFL, thicker GDxVCC RNFL, more RNFL modulation, and a lower NFI. Similar associations were observed for CRF, though less statistically significant. While higher IOPg was associated with a smaller rim area and larger LCDR, it was not associated with any GDxVCC measure. Higher IOPcc was associated with a smaller rim area, larger LCDR, thinner HRT RNFL thickness, and a higher NFI. Table 3 presents the associations of CH and CRF with HRT and GDxVCC variables, adjusted for IOPg and other potential confounders. Both CH and CRF remained positively associated with rim area, GDxVCC RNFL thickness, and RNFL modulation, and negatively associated with LCDR. Neither CH nor CRF was associated with HRT RNFL thickness or NFI in the adjusted analyses. We also repeated the analyses presented in Table 3 using the mean of all CH or CRF measurements with a waveform score ≥ 3.5 for each eye rather than the single best value; results were very similar (data not shown). To examine whether the level of IOPg modified the effect of CH or CRF on the outcome measures, we examined the same associations presented in Table 3 stratified by IOPg tertile (Supplementary Table S1). The associations of CH and CRF with HRT and GDxVCC variables did not appear to differ significantly between IOPg tertiles, and there was no evidence of statistical interaction ($P > 0.01$ for all interaction terms). The associations between CH or CRF and GDxVCC variables were no longer significant at the $P < 0.01$ level in each tertile, possibly due to reduced statistical power.

Referral Hospital Clinic Analyses (918 Eyes of 602 Participants)

The correlation matrix for the ORA variables (measured at the research clinic visit), and GAT and CCT (measured at the referral hospital clinic) for participants with complete data is presented in Supplementary Table S2. Goldmann applanation tonometry IOP was correlated strongly with IOPg ($r = 0.72$, $P < 0.001$) and IOPcc ($r = 0.67$, $P < 0.001$), correlated moderately with CRF ($r = 0.52$, $P < 0.001$), correlated weakly with CCT ($r = 0.25$, $P < 0.001$), and there was very poor correlation between GAT and CH ($r = -0.03$, $P = 0.38$). The CCT was correlated moderately with CH ($r = 0.41$, $P < 0.001$), CRF ($r = 0.60$, $P < 0.001$), and IOPg ($r = 0.41$, $P < 0.001$).

Crude associations of CH, CRF, GAT, and CCT with HRT parameters (adjusted for disc area only) and GDxVCC

TABLE 2. Linear Regression Results From 8213 Eyes of 5134 Participants

	HRT Measures						GDx Measures											
	Rim Area			Linear Cup-to-Disc Ratio			Mean RNFL Thickness			Average RNFL Thickness			RNFL Modulation			Nerve Fiber Indicator		
	β	95% CI	P	β	95% CI	P	β	95% CI	P	β	95% CI	P	β	95% CI	P	β	95% CI	P
IOPg, mm Hg	-0.005	(-0.006, -0.003)	<0.001*	0.003	(0.002, 0.004)	<0.001*	0.000	(-0.001, 0.000)	0.38	0.011	(-0.022, 0.044)	0.52	0.018	(-0.010, 0.046)	0.22	0.042	(-0.016, 0.100)	0.16
IOPcc, mm Hg	-0.006	(-0.008, -0.005)	<0.001*	0.004	(0.003, 0.005)	<0.001*	-0.001	(-0.001, -0.000)	0.003*	-0.025	(-0.057, 0.007)	0.13	-0.019	(-0.047, 0.009)	0.18	0.082	(0.026, 0.138)	0.004*
CH, mm Hg	0.013	(0.009, 0.016)	<0.001*	-0.007	(-0.009, -0.005)	<0.001*	0.002	(0.001, 0.003)	<0.001*	0.160	(0.087, 0.232)	<0.001*	0.166	(0.102, 0.230)	<0.001*	-0.233	(-0.360, -0.106)	<0.001*
CRF, mm Hg	0.004	(0.001, 0.007)	0.022	-0.002	(-0.004, 0.000)	0.07	0.002	(0.001, 0.002)	0.001*	0.150	(0.079, 0.222)	<0.001*	0.152	(0.091, 0.213)	<0.001*	-0.133	(-0.258, -0.008)	0.037

Models were constructed with HRT or GDx measures as the dependent variable and IOPg, IOPcc, CH, or CRF as an explanatory variable. Both HRT and GDx models were adjusted for optic disc area, and GDx models were adjusted further for TSS.

* $P < 0.01$.

parameters (adjusted for disc area and TSS) are presented in Supplementary Table S3. Table 4 presents the associations of CH and CRF with HRT and GDxVCC outcome measures, adjusted for GAT, CCT, and other potential confounders (as listed above). The coefficients for GAT and CCT in these models also are presented in Table 4. The CH, CRF, and CCT were associated positively with rim area and associated negatively with LCDR ($P < 0.01$ or borderline $P < 0.02$). The only significant association with GDxVCC measures was a positive association between CCT and RNFL modulation. The GAT was not associated with any HRT or GDxVCC measure.

DISCUSSION

We found CH and CRF to be associated significantly with optic nerve head anatomical quantitative traits as measured by two different techniques—scanning laser ophthalmoscopy and scanning laser polarimetry. Lower CH or CRF was associated with a smaller rim and thinner RNFL; this direction of association is the same as that seen in glaucoma and with ageing. The associations were apparent in crude analyses, and remained following adjustment for IOPg and other possible confounders (namely age, sex, height, BMI, education level, axial length, and lens status). In the smaller subset of participants with GAT and CCT measures, CH and CRF remained associated with rim area and LCDR, but lost significance in the GDxVCC models following further adjustment for GAT and CCT.

Our findings supported existing evidence that an association exists between CH and glaucoma. If this relationship is causal, it might suggest that a lower CH reflects an eye that is biomechanically more susceptible to glaucomatous change, and that this effect is detectable even among the spectrum of values in healthy participants. It is important to consider whether residual confounding contributed to the detected association. Possible confounders include age (older participants have lower CH,³⁸ as well as smaller optic disc rims,²⁴ and thinner RNFL³⁷) and IOP (eyes with higher IOP have lower CH¹⁶ and smaller optic disc rims²⁴). However, the association remained following statistical adjustment for age and IOP. It is unlikely that tonometry error contributed to the association in our population-based sample, as participants were not selected on the basis of IOP, and the association was apparent in analyses not adjusted for IOP. Possible tonometry error complicates interpretation of hospital-based studies, as it may not be clear whether participants have been selected on the basis of IOP, at least at some stage of their care.

Mangouritsas et al.⁸ found CH to be significantly lower in 108 glaucoma patients compared to 74 controls, in an unadjusted analysis. It is possible this finding is explained entirely by confounding; the glaucoma patients were older, had higher IOP, and had thinner CCT, all of which are associated with lower CH. Lower CH also has been associated with severity of disease in an analysis of quantitative traits in 191 patients with confirmed or suspected glaucoma.¹⁰ The CH was found to be associated positively with MD and GDx RNFL average thickness in univariable models. However, the associations were no longer statistically significant following adjustment for potential confounders, including age and CCT.¹⁰ In three retrospective studies^{12–14} and a recent prospective study,¹⁵ lower CH was associated with an increased chance of glaucoma progression, suggesting predictive value at baseline assessment. Interpretation of these studies may be complicated by the possibility that a lower CH (and CCT) results in an underestimate of IOP and, therefore, undertreatment in a clinical setting. In a study of 117 POAG patients with asymmetric visual fields, lower CH was associated with the

TABLE 3. Multivariable Linear Regression Results From 8213 Eyes of 5134 Participants

	HRT Measures						GDx Measures					
	Rim Area			Linear Cup-to-Disc Ratio			Mean RNFL Thickness			Average RNFL Thickness		
	Coef	95% CI	P	Coef	95% CI	P	Coef	95% CI	P	Coef	95% CI	P
CH, mm Hg	0.011	(0.007, 0.014)	<0.001*	-0.006	(-0.008, -0.004)	<0.001*	0.001	(0.000, 0.002)	0.042	0.102	(0.029, 0.176)	0.006*
CRF, mm Hg	0.013	(0.009, 0.017)	<0.001*	-0.007	(-0.010, -0.005)	<0.001*	0.001	(0.000, 0.002)	0.041	0.122	(0.036, 0.208)	0.006*
										0.115	(0.039, 0.191)	0.003*
										0.098	(0.034, 0.163)	0.003*
										-0.012	(-0.140, 0.116)	0.85
										-0.016	(-0.167, 0.134)	0.83

Models were constructed with HRT or GDx measures as the dependent variable and CH or CRF as an explanatory variable. All models were adjusted for IOPg, optic disc area, age, sex, height, BMI, education level, axial length, and lens status. Additionally, GDx models were adjusted for TSS.

* $P < 0.01$.

worse eye in multivariable models, while CCT and GAT were not associated significantly with the worse eye.¹¹ The CH had the best discriminative ability for the eye with the worse visual field.¹¹ While this CH asymmetry may be indicating asymmetric biomechanical properties of the eyes, it also may be reflecting asymmetric “true” IOP. So, while there is a growing amount of evidence that CH and glaucoma are linked statistically, the complexity of the relationships between CH, age, IOP, and CCT makes it difficult to determine whether CH is involved independently in the pathogenesis of glaucoma.

To our knowledge, our study is the first to demonstrate an association of CH and CRF with glaucoma-related traits across the spectrum of values in a population-based sample. It might be that causative mechanisms in glaucoma result in a lesser degree of RGC loss even in eyes without glaucoma, and may be what determines healthy variation in optic nerve head parameters, at least in part. The approach of examining quantitative traits related to a disease rather than comparing cases of a disease to controls has some advantages in a population-based setting. Firstly, there is a reduced chance of outcome misclassification bias, a particular problem for glaucoma (the prevalence of which can vary greatly depending on the classification used).³⁹ Objective measuring devices, such as the HRT or GDx, are unlikely to misclassify a participant significantly for parameters, such as rim area or LCDR. Secondly, there is greater statistical power from examining a continuous outcome variable compared to a binary outcome. Thirdly, information is used from all participants with measurements in the population, the majority of whom usually are healthy. For uncommon diseases, the limited number of cases in a population-based study can limit power to detect small associations in case-control analyses. There also are limitations when using a quantitative trait approach. Firstly, if an inference is to be made regarding glaucoma pathogenesis, there would be a lack of specificity of discovered associations with a particular form of glaucoma. For example, it may be open-angle glaucoma or angle-closure glaucoma, or both, contributing to an association with rim area. This lack of specificity means an association with one particular type of glaucoma may not be detected (if the effect signal driven by the subset is not detectable in the complete sample), and it cannot be concluded which type of glaucoma any discovered association is important for. Secondly, what determines variation across the whole range of the trait may not be what determines risk of extreme values at one end of the spectrum and, hence, disease risk. Thirdly, diseases other than the one of interest may affect the quantitative trait. For example, multiple sclerosis, as well as glaucoma, can affect RNFL measurements.⁴⁰ Despite these limitations, the quantitative trait approach has been successful in the search for genetic associations of POAG. Genomic regions found to be associated with vertical cup-to-disc ratio in largely healthy participants⁴¹ were later found to be important in POAG after sufficient cases were collected to power the analyses.^{42,43}

In the subset of participants in our study who underwent a second examination, we also found that CCT was associated with glaucoma-related traits; this was the case in crude analyses and adjusted analyses. It is important to be cautious in interpreting these findings given the selected nature of this subset of participants. For example, one of the criteria for a second examination was IOP > 24 mm Hg. Participants with measured IOP > 24 may have either a “true” IOP > 24 mm Hg, or a “true” IOP ≤ 24 mm Hg and a cornea that is harder to applanate (which may be associated with a thicker CCT). Therefore, within this selected subset, CCT may be reflecting variation in “true” IOP, even in crude analyses. We found a modest correlation between CH and CCT. When CH and CCT were included together in the same model, both were

TABLE 4. Results From 12 Multivariable Linear Regression Models, Each Representing Data From 918 Eyes of 602 Participants

	HRT Measures					GDx Measures				
	Rim area			Linear Cup-to-Disc Ratio		Mean RNFL Thickness		Average RNFL Thickness		Nerve Fiber Indicator
	β	95% CI	P	β	95% CI	P	95% CI	β	95% CI	
CH, mm Hg	0.017	(0.003, 0.030)	0.015	-0.009	(-0.016, -0.002)	0.008*	0.001	0.064	(-0.223, 0.352)	0.66
GAT, mm Hg	0.003	(-0.003, 0.008)	0.30	-0.001	(-0.004, 0.002)	0.42	0.000	0.110	(-0.005, 0.225)	0.06
CCT, mm Hg	0.001	(0.000, 0.002)	0.001*	-0.001	(-0.001, -0.000)	0.002*	0.000	0.013	(-0.000, 0.026)	0.06
CRF, mm Hg	0.021	(0.007, 0.035)	0.003*	-0.011	(-0.018, -0.004)	0.002*	0.003	0.244	(-0.058, 0.545)	0.11
GAT, mm Hg	-0.002	(-0.008, 0.004)	0.56	0.001	(-0.002, 0.004)	0.40	-0.001	0.064	(-0.061, 0.188)	0.32
CCT, mm Hg	0.001	(0.000, 0.002)	0.010	0.000	(-0.001, -0.000)	0.018	0.000	0.008	(-0.006, 0.023)	0.24

Models were constructed with a HRT or GDx measure as the dependent variable, and either CH or CRF as an explanatory variable. All models also contained both GAT and CCT, and age, sex, height, BMI, systolic blood pressure, education level, and lens status. The HRT models were adjusted further for disc area, and GDx models adjusted further for TSS.
* $P < 0.01$.

associated significantly with rim area and LCDR. Therefore, it appears that CH does provide some relevant information that CCT does not, at least for this small subset of participants examined. Interestingly, we did not find GAT to be associated with any glaucoma-related traits.

It is important to note that, while many of the associations we described were highly statistically significant, the regression coefficients were small and, therefore, do not explain a great deal of the variability of the glaucoma-related traits. The associations between corneal biomechanical parameters and optic disc parameters are interesting from an etiological point of view and may provide insights into pathophysiological processes. However, at present, the regression models do not appear to be useful for prediction. The main strengths of our study are the large sample size and the data on glaucoma-related traits from two different instruments. There are several limitations of our study. While the study is population-based, it is likely that eligible residents of Norfolk with greater degrees of visual impairment were unable to participate in the study due to difficulty with completion of questionnaires and travel to the research clinic. However, it seems unlikely that the associations we were examining would be systematically different in those not taking part. Similarly, while approximately 40% of participants who did attend the Eye Study were excluded, it is unlikely that the associations we were examining would be in the opposite direction to those we observed in the included participants. Therefore, the likely effect of any selection bias or exclusion would be to truncate distributions and reduce power to detect associations. The EPIC-Norfolk Eye Study currently is cross-sectional, which limits understanding of the temporal sequence of the associations. For example, we were unable to determine if a lower CH results in anatomic changes in the direction of glaucoma, or whether an eye that already is predisposed to glaucoma later has a lower CH. A proportion of GDxVCC scans are complicated by atypical retardation pattern, and while we have demonstrated an effective method for handling this,³⁷ residual artefact cannot be excluded. However, it does not seem likely that the association between CH and glaucoma-related traits would be affected significantly by atypical retardation. Another limitation relates to the secondary analyses on the subset of participants undergoing a second examination at the referral hospital clinic; this subset no longer is population-based, and has been selected on several criteria already described. This selection bias limits the generalizability of the findings for the subset part of the study and complicates interpretation of the findings, as already discussed.

In summary, we found CH and CRF to be associated with glaucoma-related quantitative traits in a Caucasian population-based sample independent of age, IOP, and CCT; this may provide insights into the pathophysiological processes leading to glaucoma.

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