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Genetic Risk Score Is Associated with Vertical Cup-to-Disc Ratio and Improves Prediction of Primary Open-Angle Glaucoma in Latinos

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Purpose: Genome-wide association studies have identified multiple genetic variants associated with vertical cup-to-disc ratio (VCDR). Genetic risk scores (GRS) examine the aggregate genetic effect of individual variants on a trait by combining these separate genetic variants into a single measure. The purpose of this study was to construct GRS for VCDR and to determine whether the GRS are associated with VCDR and whether the GRS increase the discriminatory ability for primary open-angle glaucoma (POAG) in a Latino population.

Design: Population-based genetic association study.

Participants: A total of 4018 Latino participants recruited from Los Angeles.

Methods: Weighted and unweighted GRS were constructed using 68 previously reported VCDR single nucleotide polymorphisms (SNPs), as well as SNPs from our own genome-wide association data. Linear and logistic regression analyses examined the associations of GRS with VCDR and POAG, respectively. To evaluate the discriminatory ability of the GRS for POAG, we conducted receiver operating characteristic (ROC) analyses.

Main Outcome Measures: The relationship between GRS and VCDR in Latinos.

Results: The GRS were associated significantly with VCDR ($P < 0.0001$), after adjusting for age, gender, central corneal thickness, intraocular pressure, and education. The weighted GRS explained an additional 2.74% of the variation in VCDR. Adding the weighted GRS derived from previously reported SNPs resulted in a moderate improvement in the discriminatory ability for POAG during ROC analyses, yielding an area under the ROC curve (AUC) of 0.735 (95% CI, 0.701−0.768). When our own SNPs were used, the AUC increased significantly to 0.809 (95% CI, 0.781−0.837; $P < 0.0001$). We obtained similar results for the unweighted GRS.

Conclusions: To our knowledge, we identified a novel association between GRS and VCDR and its improvement in the discriminatory ability of POAG in a Latino population. *Ophthalmology 2017;■:1−7 Published by Elsevier on behalf of the American Academy of Ophthalmology*

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The morphologic features of the optic disc commonly are assessed during routine ophthalmic examinations to monitor and diagnose multiple ocular diseases, including glaucoma. In particular, the vertical cup-to-disc ratio (VCDR) is an important clinical measurement to identify glaucomatous damage to the optic nerve. Accordingly, identifying factors that affect VCDR not only will aid in uncovering the biological mechanisms regulating this ocular trait, but also may assist in predicting ocular disease.

Population-based epidemiologic studies have identified multiple factors associated with VCDR, including higher intraocular pressure (IOP) and lower body mass index.[1−3] Other identified factors, such as male gender[1,2] or older age,[1−4] have shown an association with VCDR in several studies and no association in other studies.[5,6] Diastolic blood pressure also has been associated positively[1] and negatively[2] with VCDR. Despite the identification of conventional risk factors, these systematic and ocular traits account for less than 4% of the variation in VCDR, suggesting that other factors may contribute to this ocular trait.[2]

Vertical cup-to-disc ratio also has a demonstrated genetic component, with heritability estimates of 48% to 66% for this trait.[7,8] Genome-wide association studies (GWASs) have identified multiple loci associated with VCDR, including *ATOH7*, *SIX1*, *CHEK2*, and *SCYL1*.[9−12] Despite the identification of genetic variants associated with VCDR, each variant confers only a modest effect and individually has limited predictive power. Genetic risk scores (GRSs) examine the aggregate genetic effect by combining these separate genetic variants into a single measure. A previous study identified a polygenetic model for VCDR, and subsequently primary open-angle glaucoma (POAG), using various variant-significance thresholds.[13] However, this study was conducted in individuals of European descent and may not be generalizable to other ethnic groups. Latinos, a

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traditionally underrepresented racial group in ocular genetic research, exhibit a high prevalence of POAG.[14] As such, examining the association between an aggregate measure of genetic risk and VCDR will further our understanding of the determinants of this trait. Additionally, the generation of GRS for an endophenotype for POAG will enable an opportunity to evaluate whether the addition of this genetic information improves the discriminatory ability for POAG compared with traditional risk factors. In this study, we examine the association between genetic risk scores and VCDR and the discriminatory ability for POAG in a Latino population.

Methods

Ethics Statement

The institutional review board at the University of Illinois at Chicago approved the following research. All clinical investigation was performed according to the principles stated in the Declaration of Helsinki.

Study Sample, Vertical Cup-to-Disc Ratio Measurement, and Glaucoma Criteria

This research was conducted using previously published data on VCDR[11] and POAG.[14,15] The data were collected from the Los Angeles Latino Eye Study, the largest population-based study of visual impairment and ophthalmic diseases in Latinos. All study participants underwent detailed ophthalmic examinations described elsewhere.[16] Briefly, stereoscopic optic disc photographs were obtained and evaluated using a stereoscopic viewer (Asahi viewer; Pentax, Englewood, CO) to examine the optic nerve. The Humphrey Automated Field Analyzer II (Carl Zeiss Meditech, Dublin, CA) was used to test peripheral vision and a Swedish interactive threshold algorithm standard C24 was used to evaluate the visual field. Using the stereoscopic photographs, a certified ophthalmologist measured the VCDR for the left and right eyes. The average VCDR between the eyes was used for downstream analysis. If one of the measurements was missing, the value from the other eye was used as the final measurement. The presence of POAG was determined by agreement of 3 glaucoma specialists using all clinical data with the following criteria: the presence of an open angle; congruent, characteristic, or compatible glaucomatous visual field abnormality; evidence of characteristic or compatible glaucomatous optic disc damage in at least 1 eye; or a combination thereof. All participants included in this study were 40 years of age or older.

Genotyping and Quality Control

A total of 4996 Latinos were genotyped through the Los Angeles Latino Eye Study and the Mexican American Glaucoma Genetic Study using either the Illumina OmniExpress BeadChip Kit (730 522 markers; Illumina, Inc., San Diego, CA) or the Illumina Hispanic/SOL BeadChip (approximately 2.5 million markers; Illumina, Inc.). The software Illumina GenomeStudio (version 2011.1; Illumina, Inc.) was used to call single nucleotide polymorphisms (SNPs). Genotype quality control and imputation procedures have been described elsewhere.[11,17] Briefly, PLINK (version 1.90) was used to perform quality control on the genotype data.[18] Additionally, study participants with a genotyping call rate of less than 97%, inconsistencies between reported and genetically inferred gender, missing VCDR measurements, and duplicates were excluded. Haplotype phasing was conducted using SHAPEIT2,[19] and imputation was performed using Minimac3[20] and the 1000 Genomes Project reference panels.[17] Imputed SNPs of low quality (i.e., Rsq $<$ 0.80) and those with a minor allele frequency of less than 1% were excluded. After applying these quality control parameters, 4018 unrelated participants and more than 6.8 million SNPs remained for downstream analysis.

Single Nucleotide Polymorphism Selection and Construction of Genetic Risk Scores

Unweighted and weighted GRSs were constructed based on SNPs previously associated with VCDR.[9–12] Risk alleles were defined as alleles associated with an increase in VCDR. If a SNP was reported in multiple studies, the weight from the largest study sample was used. To ensure the weights for SNPs were on the same scale, SNPs from studies using untransformed VCDR values were retained. Additionally, all SNPs, except for rs2159128 (imputation Rsq, 0.67), were well imputed. This resulted in 68 SNPs to be used for the construction of the GRS. Using a previous candidate gene approach,[21] we also constructed unweighted and weighted GRS based on the lead SNP (most significant SNP) from our GWAS results within ±100 kb of the 68 previously reported SNPs. Moreover, unweighted and weighted GRS were generated from our previous genome-wide association data using all independent SNPs (PLINK pruned at $r^2 = 0.2$) with $P < 1 \times 10^{-3}$.[11] The unweighted GRS were calculated as the summation of the number of risk alleles across the genetic variants under the assumption that all risk alleles have the same effect. The weighted GRS were constructed by multiplying the VCDR-increasing allele by the effect size as reported in the corresponding study and summing these values together.

Statistical Analysis

Univariate analyses were performed to describe the characteristics of the study sample. Clinical variables (i.e., age, gender, body mass index, systolic blood pressure [SBP], central corneal thickness [CCT], IOP, and type 2 diabetes), potential environmental and socioeconomic confounders (i.e., smoking status, education, and income), and GRS were included in this analysis. Simple and multiple linear regression analyses were conducted to evaluate the association between VCDR and these variables. Because the raw VCDR values were not normally distributed in our dataset, inverse normally transformed VCDR was used for all analyses. Stepwise selection was applied to retain significant covariates at a significance cutoff of $P \leq 0.05$. Additional variance of VCDR explained by the GRS also was examined.

To investigate the relationship between GRS and POAG, logistic regression analyses were performed. We created quintiles of the unweighted and weighted GRS to compare individuals with low GRS with individuals with higher GRS based on the odds of POAG. Stepwise selection was conducted to retain significant covariates at a significance cutoff of $P \leq 0.05$. Receiver operating characteristic (ROC) curve analyses were conducted and the areas under the ROC curve (AUCs) were calculated to examine the improvement in the discriminatory ability of POAG when the GRSs are added into a model with traditional risk factors. All statistical analyses were performed using SAS software version 9.4 (SAS, Inc., Cary, NC) and R software version 3.3.[22]

Results

Table 1 presents a summary of the study sample characteristics and simple linear regression results between VCDR and the variables