Association of a Primary Open-Angle Glaucoma Genetic Risk Score With Earlier Age at Diagnosis

Bao Jian Fan, MD, PhD; Jessica Cooke Bailey, PhD, MA; Rob P. Igo Jr, PhD; Jae H. Kang, ScD; Tahani Boumenna, BA; Murray H. Brilliant, PhD; Donald L. Budenz, MPH; John H. Fingert, MD, PhD; Terry Gaasterland, PhD; Douglas Gaasterland, MD; Michael A. Hauser, PhD; Peter Kraft, PhD; Richard K. Lee, MD, PhD; Paul R. Lichter, MD; Yutao Liu, MD, PhD; Syoko E. Moroi, MD; Jonathan S. Myers, MD; Margaret A. Pericak-Vance, PhD; Anthony Realini, MD; Douglas J. Rhee, MD; Julia E. Richards, PhD; Robert Ritch, MD; Joel S. Schuman, MD; William K. Scott, PhD; Kuldev Singh, MD; Arthur J. Sit, SM, MD; Douglas Vollrath, MD, PhD; Robert N. Weinreb, MD; Gadi Wollstein, MD; Donald J. Rhee, MD; Julia E. Richards, PhD; Robert Ritch, MD; Joel S. Schuman, MD; Louis R. Pasquale, MD; Janey L. Wiggs, MD, PhD

IMPORTANCE Genetic variants associated with primary open-angle glaucoma (POAG) are known to influence disease risk. However, the clinical effect of associated variants individually or in aggregate is not known. Genetic risk scores (GRS) examine the cumulative genetic load by combining individual genetic variants into a single measure, which is assumed to have a larger effect and increased power to detect relevant disease-related associations.

OBJECTIVE To investigate if a GRS that comprised 12 POAG genetic risk variants is associated with age at disease diagnosis.

DESIGN, SETTING, AND PARTICIPANTS A cross-sectional study included individuals with POAG and controls from the Glaucoma Genes and Environment (GLAGEN) study and the National Eye Institute Glaucoma Human Genetics Collaboration (NEIGHBOR) study. A GRS was formulated using 12 variants known to be associated with POAG, and the alleles associated with increasing risk of POAG were aligned in the case-control sets. In case-only analyses, the association of the GRS with age at diagnosis was analyzed as an estimate of disease onset. Results from cohort-specific analyses were combined with meta-analysis. Data collection started in August 2012 for the NEIGHBOR cohort and in July 2008 for the GLAGEN cohort and were analyzed starting in March 2018.

MAIN OUTCOMES AND MEASURES Association of a 12 single-nucleotide polymorphism POAG GRS with age at diagnosis in individuals with POAG using linear regression.

RESULTS The GLAGEN study included 976 individuals with POAG and 1140 controls. The NEIGHBOR study included 2132 individuals with POAG and 2290 controls. For individuals with POAG, the mean (SD) age at diagnosis was 63.6 (9.8) years in the GLAGEN cohort and 66.0 (13.7) years in the NEIGHBOR cohort. For controls, the mean (SD) age at enrollment was 65.5 (9.2) years in the GLAGEN cohort and 68.9 (11.4) years in the NEIGHBOR cohort. All study participants were European white. The GRS was strongly associated with POAG risk in case-control analysis (odds ratio per 1-point increase in score = 1.24; 95% CI, 1.21-1.27; \( P = 3.4 \times 10^{-66} \)). In case-only analyses, each higher GRS unit was associated with a 0.36-year earlier age at diagnosis (\( \beta = -0.36; 95\% \) CI, \(-0.56\) to \(-0.16\); \( P = 4.0 \times 10^{-4}\)). Individuals in the top 5% of the GRS had a mean (SD) age at diagnosis of 5.2 (12.8) years earlier than those in the bottom 5% GRS (61.4 [12.7] vs 66.6 [12.9] years; \( P = 5.0 \times 10^{-4}\)).

CONCLUSIONS AND RELEVANCE A higher dose of POAG risk alleles was associated with an earlier age at glaucoma diagnosis. On average, individuals with POAG with the highest GRS had 5.2-year earlier age at diagnosis of disease. These results suggest that a GRS that comprised genetic variants associated with POAG could help identify patients with risk of earlier disease onset impacting screening and therapeutic strategies.
Primary open-angle glaucoma (POAG), a leading cause of blindness worldwide, is a genetically complex disease associated with multiple genetic and environmental risk factors. Genome-wide association studies have now identified over 20 loci associated with POAG in European white, Asian, and multiethnic populations. Although genetic variants located within these associated regions are known to influence disease risk, the clinical effect of associated variants individually or in aggregate is currently not known. A genetic risk score (GRS) examines the cumulative genetic load by combining individual genetic variants into a single measure, which is assumed to have a larger effect and increased power to detect relevant disease-related associations. While the heritability of POAG is substantial overall, the genetic effect may be largest in patients with earlier onset of disease. Identifying risk variants associated with earlier disease onset could influence monitoring and therapeutic strategies. In this study, we performed a retrospective cross-sectional study to evaluate the association of a cumulative dose of 12 established POAG genetic risk variants with age at diagnosis.

**Methods**

This study has been approved by the institutional review boards of Massachusetts Eye and Ear, Harvard School of Public Health, Brigham and Women's Hospital, University of Pittsburgh, Johns Hopkins University, Duke University, University of West Virginia, University of Miami, University of Michigan, Stanford University, Marshfield Clinic, and the University of California, San Diego. Written informed consent was obtained from all participants. Data collection started in August 2012 for the NEIGHBOR cohort and in July 2008 for the GLAUGEN cohort and were analyzed starting in March 2018.

The Glaucoma Genes and Environment Study (GLAUGEN) genome-wide association study is part of the Genes Environment Association (GENEVA) studies, and the National Eye Institute Glaucoma Human Genetics Collaboration (NEIGHBOR) genome-wide association study is a collaboration of 12 groups in the United States. Detailed information on these data sets has been described previously. Both the GLAUGEN and NEIGHBOR data sets include individuals with POAG and controls.

A harmonized definition of POAG used the following criteria: (1) open anterior segment angles; (2) reproducible glaucomatous visual field loss on reliable tests; or (3) an eye with a cup-disc ratio of at least 0.7 with 1 visual field showing glaucomatous loss; and (4) no identifiable secondary cause for optic nerve disease. Elevated intraocular pressure (IOP) was not a criterion for POAG definition, but if present, there had to be no secondary causes on anterior segment examination.

The GRS was constructed based on the lead single-nucleotide polymorphisms (SNPs) for loci previously associated with POAG in European white populations (Table 1). For this study, the SNPs we selected for the GRS were the lead SNPs from the loci with statistical evidence for association with POAG in European white populations. The GRS corresponds with the unweighted sum of all POAG risk alleles (ie, dosage) under the assumption that all risk alleles have the same effect on the measure selected for analysis (age at diagnosis). To create the GRS, the risk alleles at each SNP were aligned in each data set.

To test for association, regression analyses were performed separately for the GLAUGEN and NEIGHBOR data sets using SAS, version 9.4 (SAS Institute). Logistic regression was performed for each SNP and for the cumulative GRS with POAG. Linear regression was performed for each SNP and for the cumulative GRS with age at diagnosis. Potential confounding factors such as sex, DNA source, and population structure (eigenvector 1, 2 and 6 for GLAUGEN and eigenvector 1 and 2 for NEIGHBOR) were included as covariates in the regression models. Age at diagnosis between individuals with POAG in the bottom 5% of the GRS and those in the top 5% of the GRS was compared using the t test. Histograms with kernel density curves and boxplots for age at diagnosis in the bottom 5% GRS and in the top 5% GRS were generated using the R package ggplot2 (R Foundation for Statistical Computing).

Meta-analysis was conducted to combine the results of the GLAUGEN and NEIGHBOR data sets. The inverse-variance weighting method was applied based on the regression coefficients and standard errors estimated from each study as implemented in the METAL software.

**Results**

The GLAUGEN data set included 2116 individuals (976 individuals with POAG [46.1%] and 1140 controls [53.9%]), and the NEIGHBOR data set included 4422 individuals (2132 individuals with POAG [48.2%] and 2290 controls [51.8%]). The mean (SD) age at diagnosis was 63.6 (9.8) years for individuals with POAG in the GLAUGEN cohort and 66.0 (13.7) years for individuals with POAG in the NEIGHBOR cohort. The mean (SD) age at enrollment was 65.5 (9.2) years for GLAUGEN controls and 68.9 (11.4) years for NEIGHBOR controls. In the GLAUGEN group, of 976 individuals with POAG, 570 (58.4%) were women, and of 1140 controls, 682 (59.8%) were women. In the NEIGHBOR group, of 2132 individuals with POAG, 1153 (54.1%) were women, and of 2290 controls, 1297 (56.5%) were women.

All 12 lead SNPs were associated with POAG in case-control analyses in the meta-analysis of NEIGHBOR and GLAUGEN data sets (Table 1), and the GRS created using the 12
SNPs selected for this study was significantly associated with POAG risk (odds ratio, 1.24; 95% CI, 1.21-1.27; \( P = 3 \times 10^{-66} \)). For individuals with POAG, the mean (SD) number of risk alleles was 14.0 (2.2), ranging from 6 to 21.

In case-only analyses, each higher GRS unit was associated with a 0.36-year earlier age at diagnosis (\( \beta = -0.36; 95\% \text{ CI}, -0.56 \text{ to } -0.16; P = 4.0 \times 10^{-4} \); Table 2). Four SNPs were individually nominally associated with earlier age at diagnosis: AFAP1 (\( \beta = -0.71; 95\% \text{ CI}, -1.31 \text{ to } -0.11; P = .02 \)), FOXC1 (\( \beta = -1.23; 95\% \text{ CI}, -1.93 \text{ to } -0.53; P = 6.0 \times 10^{-4} \)), CDKN2B-AS1 (\( \beta = -0.73; 95\% \text{ CI}, -1.39 \text{ to } -0.08; P = .03 \)), and GAS7 (\( \beta = -0.88; 95\% \text{ CI}, -1.50 \text{ to } -0.27; P = .005 \)). Individuals in the top 5% of the GRS (equivalent to a mean of 18.3 risk allele dosage; range, 17.3-21.0) had a mean (SD) age at diagnosis of 5.2 (12.8) years earlier than those in the bottom 5% of the GRS (equivalent to a mean of 9.4 risk allele dosage; range, 6.0-10.1); mean [SD] ages in groups, 61.4 [12.7] vs 66.6 [12.9] years; \( P = 5.0 \times 10^{-4} \); Figure).

**Discussion**

In this study, we created a cumulative GRS for 12 SNPs previously shown to be significantly associated with POAG in European white individuals. We investigated the effect of this GRS on age at diagnosis, a disease feature with clinical relevance. Our results show that individuals with the largest number of risk variants (top fifth percentile) for the GRS have, on average, a 5.2-year earlier age at diagnosis compared with patients in the bottom fifth percentile (Figure). Patients with earlier disease onset will require more years of treatment and may be more likely to become blind from...
Association of a Primary Open Angle Glaucoma Genetic Risk Score With Earlier Age at Diagnosis

Figure. Distribution of Age at Diagnosis Among Cases in the Bottom and Top 5% of the Genetic Risk Score (GRS)

Conclusions

In summary, using a 12-SNP GRS, we have shown that an increased load of genetic risk variants was associated with an earlier age at disease diagnosis. These findings could lead to the development of screening tests that identify patients at risk for early disease, allowing for timely initiation of preventive treatment and surveillance strategies.

ARTICLE INFORMATION

Accepted for Publication: May 19, 2019.
Published Online: August 22, 2019.

Author Affiliations:
Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear, Boston (Fan, Boumslama, Wiggs); Institute for Computational Biology, Case Western Reserve University School of Medicine, Cleveland, Ohio (Bailey, Haines); Department of Population and Quantitative Health Sciences, Case Western Reserve University School of Medicine, Cleveland, Ohio (Bailey, Igo, Haines); Channing Division of Network Medicine, Brigham and Women’s Hospital/Harvard Medical School, Boston, Massachusetts (Kraft); Department of Biostatistics, Harvard T. H. Chan School of Public Health, Harvard Medical School, Boston, Massachusetts (Kraft); Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, Florida (Lee); Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor (Lichter, Moroi, Richards); Department of Cellular Biology and Anatomy, Augusta University, Augusta, Georgia (Liu); Wills Eye Hospital, Philadelphia, Pennsylvania (Myers); Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, Florida (Pericak-Vance, Scott); Department of Ophthalmology, WVU Eye Institute, Morgantown, West Virginia (Realini); Department of Ophthalmology, Case Western Reserve University School of Medicine, Cleveland, Ohio (Rhee); Einhorn Clinical Research Center, New York Eye and Ear Infirmary of Mount Sinai, New York (Ritch); Department of Ophthalmology, NYU Langone Medical Center, NYU School of Medicine, New York, New York (Schuman, Wollstein); Department of Ophthalmology, Stanford University, Palo Alto, California (Singh); Department of Ophthalmology, Mayo Clinic, Rochester, Minnesota (Sit); Department of Genetics, Stanford University, Palo Alto, California (Vollrath); Department of Ophthalmology, Hamilton Glaucome Center and Shiley Eye Institute, University of California at San Diego, La Jolla (Weinreb); Wilmer Eye Institute, Johns Hopkins University Hospital, Baltimore, Maryland (Zack); Department of Ophthalmology, Icahn School of Medicine, Mount Sinai Hospital, New York (Pasquale).

Author Contributions: Drs Fan and Wiggs had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Pericak-Vance, Rhee, Ritch, Schuman, Singh, Pasquale, Wiggs.


Drafting of the manuscript: Fan, Cooke Bailey, Pericak-Vance, Ritch, Pasquale, Wiggs.

Critical revision of the manuscript for important intellectual content: Igo, Kang, Boumnenna, Brilliant, Budenz, Fingert, G.A. Gaasterland, D.G. Gaasterland, Hauser, Kraft, Lee, Lichter, Liu, Moroi, Myers, Pericak-Vance, Realini, Rhee, Richards, Ritch, Schuman, Scott, Singh, Sit, Vollrath, Weinreb, Wollstein, Zack, Haines, Pasquale, Wiggs.

Statistical analysis: Fan, Cooke Bailey, Igo, Boumnenna, Kraft, Ritch, Pasquale, Wiggs.

Obtained funding: Brilliant, Liu, Pericak-Vance, Ritch, Weinreb, Pasquale, Wiggs.

Administrative, technical, or material support: Kang, Budenz, Fingert, Hauser, Lee, Lichter, Liu, Moroi,
Realini, Schuman, Scott, Singh, Vollrath, Weinreb, Haines, Pasquale, Wiggs.

**Supervision:** Hauser, Myers, Rhie, Richards, Ritch, Pasquale, Wiggs.

**Conflict of Interest Disclosures:** Dr Hauser reports grants from National Institutes of Health (NIH)/National Eye Institute (NEI) during the conduct of the study. Dr Kraft reports grants from NIH during the conduct of the study. Dr Moroi reports grants from University of Michigan during the conduct of the study. Dr Myers reports grants from NEI during the conduct of the study. Dr Reinalini reports being a consultant for New World Medical, Aerie Pharmaceuticals, iViSci, and iStarMed outside the submitted work. Dr Rhee reports grants from Allergan and Glaukos; grants and personal fees from Ivinxis; and personal fees from Bausch + Lomb, Aerie Pharmaceuticals, and Ocular Therapeutix outside the submitted work. Dr Richards reports grants from NIH and BrightFocus Foundation outside the submitted work; and book royalties from Elsevier. Dr Schuman reports grants from the NIH during the conduct of the study. Dr Scott reports grants from the NIH and Foundation Fighting Blindness during the conduct of the study; grants from the NIH and the BrightFocus Foundation outside the submitted work; and book royalties from Elsevier. Dr Schuman reports grants from the NIH during the conduct of the study; personal fees from Brain Canada outside the submitted work; and grants from the NIH and BrightFocus Foundation outside the submitted work. Dr Sit reports grants from Aerie Pharmaceuticals, Glaucovit, and Novartis outside the submitted work. Dr Vitale reports grants from the NIH and Foundation Fighting Blindness during the conduct of the study; personal fees from Alcon, Allergan, Aerie Pharmaceuticals, Glaucovit, and Novartis outside the submitted work. Dr Vitale reports grants from the NIH and Foundation Fighting Blindness during the conduct of the study; personal fees from Alcon, Allergan, Aerie Pharmaceuticals, and Glaucovit outside the submitted work. Dr Vollrath reports grants from the NIH during the conduct of the study. Dr Weinreb reports personal fees from Aerie Pharmaceutical, Allergan, Eyenova, Galimedix Therapeutics, and Unity Biotechnology; nonfinancial support from Heidelberg Engineering, Carl Zeiss Meditec, Genentech, Konan, Optovue, Topcon, Optos, CenterVue, and Bausch + Lomb outside the submitted work; a patent to Toromedes pending; and a patent to Carl Zeiss Meditec with royalties paid. Dr Haines reports grants from the NIH during the conduct of the study. Dr Pasquale reports grants from the NEI during the conduct of the study. Dr Wiggs reports grants from the NIH during the conduct of the study; grants from Aerie Therapeutics outside the submitted work, and other support from Maze Therapeutics outside the submitted work. No other disclosures are reported.

**Funding/Support:** This work was supported by the National Institutes of Health/National Eye Institute (grants R01 EY022305, Dr Wiggs; R01 EY015473, Dr Pasquale; and P30 EY14104, Dr Wiggs). The National Institutes of Health grants supported the maintenance of the Nurses Health Study and Health Professionals Follow-up, allowing these health care professionals to contribute to this analysis (grants CA186107, CA87449, CA167552, CA53075, HL135464, and National Eye Institute grant R01 EY015473, Dr Pasquale). The National Human Genome Research Institute supported the Glaucoma Genes and Environment study (grants HG004728, Dr Pasquale; HG004424 [Broad Institute to support genotyping]; HG004446).

**Role of the Funder/Sponsor:** The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**REFERENCES**


