Published in final edited form as:
J Glaucoma. 2013 September ; 22(7): 517–525. doi:10.1097/IJG.0b013e31824d4fd8.

The NEIGHBOR Consortium Primary Open Angle Glaucoma Genome-wide Association Study: Rationale, Study design and Clinical variables

Janey L Wiggs[*], **Michael A Hauser**, **Wael Abdrabou**, **R Rand Allingham**, **Donald L Budenz**, **Elizabeth DelBono**, **David S Friedman**, **Jae H Kang**, **Douglas Gaasterland**, **Terry Gaasterland**, **Richard K Lee**, **Paul R Lichter**, **Stephanie Loomis**, **Yutao Liu**, **Cathy McCarty**, **Felipe A Medeiros**, **Sayoko E Moroi**, **Lana M Olson**, **Anthony Realini**, **Julia E Richards**, **Frank W Rozsa**, **Joel S Schuman**, **Kuldev Singh**, **Joshua D Stein**, **Douglas Vollrath**, **Robert N Weinreb**, **Gadi Wollstein**, **Brian L Yaspan**, **Sachiko Yoneyama**, **Don Zack**, **Kang Zhang**, **Margaret Pericak-Vance**, **Louis R Pasquale**, and **Jonathan L Haines**

Abstract

Primary open-angle glaucoma (POAG) is a common disease with complex inheritance. The identification of genes predisposing to POAG is an important step toward the development of novel gene-based methods of diagnosis and treatment. Genome-wide association studies (GWAS) have successfully identified genes contributing to complex traits such as POAG however, such studies frequently require very large sample sizes, and thus, collaborations and consortia have been of critical importance for the GWAS approach. In this report we describe the formation of the NEIGHBOR consortium, the harmonized case control definitions used for a POAG GWAS, the clinical features of the cases and controls and the rationale for the GWAS study design.

INTRODUCTION

Primary open-angle glaucoma (POAG) is the most common glaucoma subtype in the Western world. Among people older than 70 years, the prevalence of POAG is 6% in white populations, 16% in black populations and 3% in Asians (1). By the year 2020, 5.9 million people are estimated to be bilaterally blind from open-angle glaucoma (2,3) Current therapies directed at reducing intraocular pressure can slow disease progression, but do not prevent retinal ganglion cell apoptosis. The development of primary and secondary preventative strategies and treatments for POAG will require more information about the underlying mechanisms responsible for the disease, particularly information about the molecular events contributing to disease pathogenesis. The identification and characterization of genes predisposing to POAG can define the proteins and molecular pathways that underlie disease development, information that could lead to the development of biomarkers for early molecular diagnosis and treatment.

A family history of glaucoma is a major risk factor for POAG, and the prevalence of POAG in first-degree relatives of affected patients is between 4 and 10 times that of the general

[*]Corresponding author, Janey L Wiggs MD, PhD, Associate Professor of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114, 617 573 6440 (office), janey_wiggs@meei.harvard.edu.

population (4–7). The higher concordance of glaucoma among monozygotic twins compared to dizygotic twins is also consistent with a significant genetic predisposition (8, 9). While POAG has a significant heritability, the adult-onset POAG genes that have emerged from family based linkage studies account for only a small fraction of the overall POAG population (10). Recent genome-wide association studies (GWAS) have identified a small number of POAG candidate genes: a single genomic region containing the genes for caveolins 1 and 2 (CAV1/CAV2) in an Icelandic sample (OR 1.3) (11) and a POAG GWAS using cases selected for advanced disease successfully identified two genes of more signficant effect TMCO1 (OR 1.5) and CDKN2BAS (OR 1.3) (12). Together these three genes account for less than 10% of the population attributable risk (13). These results suggest that there are multiple genes awaiting discovery and that datasets with large sample sizes and well-defined phenotypes are needed to delineate the complex genetic architecture of POAG. In addition to the recent POAG GWAS success, genome-wide studies have successfully identified genetic factors contributing to other complex ocular disorders, including AMD (14–16) myopia (17, 18) and exfoliation syndrome (19). The formation of multiple consortia and collaborations has been crucial for the success of the GWAS approach by increasing the sample size to enhance the statistical power and to enable the replication of findings from individual studies and establishing common methods of analysis (20,21)

The NEIGHBOR (**NEI G**laucoma **H**uman genetics colla**BOR**ation) consortium is a unique collaborative effort involving investigators at 12 institutions located throughout the United States. The goal of the consortium is to identify genetic variants associated with POAG using an initial approach of genome-wide association studies. The eventual outcome of this work is to elucidate the molecular pathogenesis of POAG making it possible to implement effective screening and prevention strategies and to develop novel therapies. The consortium has harmonized clinical definitions and genotyping platforms with the GLAUGEN POAG GWAS that is part of the GENEVA consortium (22), allowing for inter-study validation and a combined meta-analysis of at least 3500 cases and 3500 controls. This combined dataset is one of the largest POAG case control study populations collected to date and will provide sufficient power to investigate the complex genetic architecture of POAG. Described in this report is the organization of the NEIGHBOR consortium, the harmonized case control definitions, the clinical features of the cases and controls and the rationale for the GWAS study design.

METHODS

The NEIGHBOR consortium includes samples from the NEIGHBOR study as well as the GLAUGEN study. Cases and controls for the NEIGHBOR study were collected from 12 sites in parallel with the collection of cases and controls from 3 sites for the GLAUGEN study. For these genome-wide association studies, approval was obtained by the institutional review boards of the: Massachusetts Eye and Ear Infirmary, Brigham and Women's Hospital, Duke University, Johns Hopkins University, Marshfield Clinic, Stanford University, University of Pittsburgh, University of West Virginia, University of Miami, University of Michigan, University of California, San Diego and Vanderbilt University.

Case and control definitions

The NEIGHBOR and GLAUGEN studies used the same definitions for all cases and controls. All clinical data was reviewed and copies of the original visual field tests were re-evaluated for criteria meeting the case definition described below by the coordinating center at the Massachusetts Eye and Ear Infirmary. Cases and controls were at least 35 years old and were European-derived or Hispanic Caucasians. People of Asian or African descent

were excluded. Individuals with greater than 8 diopters of myopia or 8 diopters of hyperopia were excluded as either cases or controls. Both cases and controls had slit lamp examinations that did not reveal secondary causes for elevated intraocular pressure, including narrow angles, exfoliation syndrome, pigment dispersion syndrome, anterior segment dysgenesis or chronic inflammatory changes. We identified narrow or occudable angles as those where the filtering portion of the trabecular meshwork was not visible for at least 180 degrees. Alternatively if the patient was status post prophylactic laser iridotomy prior to the development of reproducible visual field loss, the angles were regarded as narrow or occludable. In addition to the above, cases had evidence of optic nerve disease defined as: 1) visual field loss, consistent with nerve fiber layer defects, reproduced on two reliable visual fields defined as: fixation loss ≤ 33%; false positive rate ≤ 20% and false negative rate ≤ 20% consistent with nerve fiber layer loss regardless of optic nerve appearance, or 2) One visual field showing a defect consistent with nerve fiber layer loss associated with a corresponding vertical cup-to-disc ratio (vCDR) of at least 0.7, or 3) vertical cup-to-disc ratios of at least 0.8 in both eyes. Elevated intraocular pressure (IOP) was not a feature of our case definition. Additionally controls were excluded if there was documentation of IOP > 22 mm Hg in either eye and if the vCDR was greater than 0.6 in either eye or if there was CDR asymmetry of more than 0.2. Unaffected individuals with a known primary family history of glaucoma (first degree relatives) were excluded as controls.

NEIGHBOR consortium participating sites

Duke—Cases and controls were examined by Dr. Allingham or one of the other faculty members in the glaucoma service in the Duke Eye Center. For this study, Caucasian patients from the Southeastern US have been collected. An additional set of controls was selected from 7500 subjects who had undergone cardiac catheterization at Duke Medical Center and who are part of the CATHGEN study (23) with blood samples collected at the time of catheterization. Included in the CATHGEN study are 1496 individuals who have been examined at the Duke Eye Center and controls were selected from this group. Additionally, 182 individuals of Mexican descent from the Nogales region of Northern Mexico were enrolled as part of the Duke dataset.

Johns Hopkins—Cases were identified by ophthalmologists from the Johns Hopkins glaucoma service. Controls were individuals that were also identified as controls for genetic studies of age-related macular degeneration, and included a group characterized in nursing homes located in the Baltimore area.

Marshfield—Cases and controls were drawn from the Marshfield eMERGE dataset, who were part of the eMERGE consortium. The eMERGE (the Electronic MEdical Records and Genomics) study was established by the National Human Genome Research Institute (NHGRI) to develop, disseminate, and apply approaches to research that combine DNA biorepositories with electronic medical record (EMR) systems for large-scale, high-throughput genetic research (24). To be eligible for the eMERGE study, subjects in the population-based Marshfield Clinic Personalized Medicine Research Project (PMRP) had to be aged 50 and older and have had an ophthalmic examination in the previous five years. All participants in the PMRP are patients at Marshfield Clinic. ICD-9 codes were used to search the Marshfield Clinic electronic health record for diagnoses of glaucoma. These records were then manually reviewed to confirm glaucoma diagnosis and to abstract IOP, CDR and visual field data. The records of controls were similarly reviewed to confirm control status.

Miami—Cases and controls were identified from the glaucoma clinic and general ophthalmology clinics at Bascom Palmer Eye Institute by Drs. Lee and Budenz.

Michigan—Cases and controls were identified from the Kellogg Eye Center clinics by Dr. Moroi, Dr. Lichter, or another board certified glaucoma specialist. These subjects were Caucasians who come primarily from the Great Lakes States and most reported western European ancestry.

CIGTS—The Collaborative Initial Glaucoma Treatment Study (CIGTS) is a randomized, controlled, multi-center clinical trial designed to determine whether patients with newly diagnosed open-angle glaucoma (primary, pigmentary, or exfoliative) are managed better by initial treatment with medications or by immediate filtration surgery (25). In this geographically dispersed sample, 607 patients were recruited from 14 different clinical centers around the United States. Blood samples were obtained from a total of 224 cases of European descent diagnosed with primary open-angle glaucoma who otherwise met the NEIGHBOR study criteria.

AGIS (Advanced Glaucoma Intervention study)—This study of advanced open-angle glaucoma patients who have failed medical therapy was designed to compare two treatment sequences: Argon laser trabeculoplasty (ALT) then Trabeculectomy then Trabeculectomy (the ATT sequence) or Trabeculectomy-ALT-Trabeculectomy (the TAT sequence) (26). In this geographically dispersed sample, 591 subjects were recruited from 11 clinical centers around the United States, of which 64 individuals of European descent had supplied blood samples.

Pittsburgh—Cases and controls were selected from the UPMC Eye Center Glaucoma and Comprehensive Ophthalmology Services by Dr. Wollstein. The glaucoma subjects were examined by Dr. Schuman and other board certified glaucoma specialists, and controls were recruited from the Comprehensive Ophthalmic Service. The majority of patients originated from the Northeastern US or Midwestern US geographic area, and were of European descent.

Stanford—Cases and controls were enrolled from the glaucoma and comprehensive ophthalmology services. All study subjects were Caucasian, with the majority residing in Northern California. All glaucoma patients were examined by Dr. Kuldev Singh and controls were examined by other board-certified Stanford eye care providers.

UC San Diego—Cases included in the NEIGHBOR study were recruited from the Hamilton Glaucoma Center (Drs. Weinreb and Medeiros). The controls were recruited from the Comprehensive Ophthalmic Services at the UC San Diego Shiley Eye Center, and were examined by Dr. Zhang or another board-certified ophthalmologist. The majority of patients originated from the Southwestern US geographic area, and were of European descent.

West Virginia—Cases were identified by glaucoma specialists Drs. Charlton and Realini. Control individuals were enrolled from the WVU Eye Institute comprehensive ophthalmology clinics after examination by a board-certified ophthalmologist. The majority of patients were of European decent, resided in West Virginia and had the unique feature of representing the Appalachian population.

GLAUGEN Samples—Our planned genomic studies include a meta-analysis with 1000 cases and 1183 controls collected with harmonized case and controls definitions for the GLAUGEN (**Glau**coma **gen**etics) study that is part of the GENEVA (GENEVA Genes Environment Association Studies) project (22). The GLAUGEN study includes glaucoma patients selected from two cohort studies and a clinic based case control sample: the Nurses' Health Study (NHS), the Health Professionals' Follow-up Study (HPFS); and the

Massachusetts Eye and Ear Infirmary (MEEI) case control group. All participants are Caucasian of European or Latino descent. In addition to clinical phenotype data, the cases and controls from the NHS and HPFS have extensive environmental exposure data. The cases from MEEI were examined by Drs. Wiggs, Pasquale or another board-certified glaucoma specialist. Controls from MEEI were identified by a board-certified ophthalmologist primarily from the Comprehensive Ophthalmology service. Cases from NHS and HPFS were identified via 3-step process that has been previously described and validated (6). Briefly we followed up on self-reported cases to determine if they met our case definition predicated on the presence of reproducible visual loss consistent with nerve fiber layer dropout on reliable visual fields. We required evidence that absence of secondary causes of elevated IOP and secondary causes of visual field loss such as optic nerve drusen were absent. Controls were matched to cases on gender, type of DNA sample (blood or cheek cell), age, and whether they reported that they received an eye exam within the same 2-year period when cases were diagnosed. Additional information about the GLAUGEN study can be found on the dbGaP website: (http://www.ncbi.nlm.nih.gov/gap?term=GLAUGEN).

Collection of DNA

For the majority of individuals in this study DNA was purified from peripheral blood (Table 1), while in 9% percent, DNA was purified from buccal cells collected using the Swish and Spit procedure (27), or from saliva (28). We previously performed a pilot study showing that buccal cell samples represent a feasible source of DNA for the performance of high-throughput genotyping (29). From blood DNA was isolated using Puregene (QIAGEN, Valencia, CA), DNAzol (Invitrogen, Carlsbad, CA), and from buccal cells, the Purgene Buccal Cell kit (QIAGEN, Valencia, CA, USA).

Power calculations

Power was calculated using the online program CaTS (30).

RESULTS

Collection of cases and controls

Reproducible visual field defects corresponding to glaucomatous nerve damage is the centerpiece of our case definition. We selected the automated visual field for our case definition because it is widely used among glaucoma practices in the United States and the standardized protocols produce data that is readily harmonizable. While there was no requirement for the type of perimetry performed, 93% of cases had full static threshold visual field testing, and the majority of these were Humphrey visual field tests. We developed a standardized methodology using a visual field review form for evaluating and extracting visual field information across all of these visual field platforms. For individuals with advanced glaucoma or other medical issues that prevented effective Humphrey testing, Goldmann testing was used (81 cases). Additionally 98 cases (3.8%) did not have visual fields available for collection, and these individuals were enrolled based on vCDR of >0.8 in both eyes.

The NEIGHBOR consortium collected 2,517 POAG cases and 2,428 controls, and an additional 1,000 cases and 1,183 controls were collected for the GLAUGEN study. The cases and controls collected for both studies are Caucasians, mainly of European descent. For the NEIGHBOR study, 53% of the cases and 54% of the controls are female with a mean age of 66.4 years for the cases and 68 years for the controls (Table 1). In the GLAUGEN study, 59% of the cases and 60% of the controls are female with a mean age of 63.6 years for the cases and 65.5 years for the controls (Table 1). For NEIGHBOR, 24% of

the cases had a first degree family member affected by POAG, while 43% of the GLAUGEN cases had a family history of disease involving a primary relative (Table 2). Examination of the summary data reveals differences between the cases and controls that may be both clinically and statistically significant. Any difference between cases and controls that might confound the association between genetic polymorphisms and POAG will be accounted for in multivariable models (described further below).

Clinical variables

In addition to the phenotype data defining the case and control status additional clinical variables relevant to glaucoma has been collected for both the NEIGHBOR and GLAUGEN studies (Tables 1 and 2). Information on case and control clinical features and additional data collection by site is listed in Supplementary Tables 1 and 2.

IOP—Elevation of intraocular pressure (IOP) was not a criterion for case definition; however we did record IOP information at enrollment for 94% of cases and 94% of controls for NEIGHBOR and 85% of cases and 35% of controls for GLAUGEN. Among NEIGHBOR cases, 67% had documentation of highest IOP prior to initiation of therapy, and for 45% of cases these initial IOPs were greater than 21mmHg. All of the GLAUGEN cases had information about highest IOP prior to initiation of therapy and of these 28% were above 21 mmHg. Among the various sites, methods of IOP measurements varied, however the majority of measurements were made with a Goldmann tonometer.

CCT—Central corneal thickness (CTT), an important factor influencing intraocular pressure measurements and risk for POAG (31) was recorded for 36% of cases and 12% of controls for NEIGHBOR and 32% of the cases and none of the controls for GLAUGEN. The mean CCT for NEIGHBOR cases was 543.0 nm (standard deviation 40.9; range, 374–853) and for GLAUGEN cases was 549.2 (standard deviation 37.1; range, 469–688). The mean CCT for NEIGHBOR controls was 544.3 nm (standard deviation 44.9; range, 402–875).

Refractive error—Myopia has been suggested as a risk factor for POAG and myopic individuals may have more severe disease (32). Refractive error, recorded as spherical equivalence was available for 46% of NEIGHBOR cases and 53% of NEIGHBOR controls as well as 47% of GLAUGEN cases and 31% of GLAUGEN controls. While we excluded those with known high myopia (worse than 8D), we did not exclude participants when this data was not available. NEIGHBOR cases had a mean spherical equivalence of −0.79 (standard deviation 2.41; range (−8.00 to +7.5) and GLAUGEN cases had a mean spherical equivalence of −0.46 (standard deviation +2.32; range (−7.75 to +1.13). NEIGHBOR and GLAUGEN controls had similar values for refractive error (Table 1).

Height, weight and BMI—Recent studies suggest that BMI (body mass index) may be associated with POAG risk (33, 34). BMI, height and weight data were collected on 9% of NEIGHBOR cases and 25% of NEIGHBOR controls. The mean BMI was 27.2 kg/mm^2 for the NEIGHBOR cases (standard deviation 5.3; range 17–51) and 27.4 kg/mm^2 for the NEIGHBOR controls (standard deviation 5.5; range 16–60). BMI, height and weight was also collected for 51% of GLAUGEN cases and 82% of GLAUGEN controls. For GLAUGEN the mean BMI for cases was 24.9 (3.72 standard deviation; range 17.4–48.1) and for controls was 25.1 (3.60 standard deviation; range 16.6–50.3).

History of LTP and filtering surgery—Glaucoma therapy requiring laser trabeculoplasty (LTP) or filtering surgery could be indicative of progressive severe disease, although this may not always be the case since surgery may sometimes be elected by choice rather than because of progression. This information is available for a limited number of

NEIGHBOR and GLAUGEN cases (NEIGHBOR, 5% LTP; 7% filtering surgery; GLAUGEN 28% LTP, 14.7% filtering surgery).

History of diabetes and hypertension—Systemic diagnosis of diabetes and hypertension may influence POAG disease risk (35, 36). Of the NEIGHBOR subjects, 8% of cases and 2% of controls had documentation of a diagnosis of diabetes, and 23% of cases and 31% of controls had documentation of systemic hypertension. For GLAUGEN 11% of cases and 42% of controls had information on diabetes and 43.8% of cases and 49.7% of controls had information for hypertension.

History of smoking—Smoking exposure was available as 'ever' or 'never' for 37% of NEIGHBOR cases and 68% of NEIGHBOR controls and 98.2% of GLAUGEN cases and 99.6% of GLAUGEN controls.

Systemic medication use—Information on the use of systemic beta-blockers, calcium channel blockers and ACE inhibitors was available for a limited number of cases and controls in both the NEIGHBOR and GLAUGEN studies.

Power and effect size

The power of a single-stage case control study using the NEIGHBOR data-set (n=2500) or the combined NEIGHBOR-GLAUGEN data-set (n=3500) to detect an association between a marker SNP (single nucleotide polymorphism) and POAG for a range of genetic relative risks (RR), minor allele frequencies (MAF), and genetic models (fully dominant or additive) is shown in Table 3. We calculated power for a marker using a specific type I error rate of 3.3×10^{-6}. This does not control the experiment-wide type I error rate--the probability of finding *any* false positive--at the .05 level, which is typically 5×10^{-8} (depending on the number of SNPs analyzed) for genome-wide studies. However, as the goal of the genetic association study is to identify promising candidates for further investigation, we will accept an increased chance of false positives in return for more power to detect true positives (limit false negatives) (37). For a type I error rate of 3.3×10^{-6} we expect approximately 1.65 false positive results.

For the power calculation we assumed that each SNP is causative and that the disease prevalence in a Caucasian population is 2% (38, 39). A strict interpretation of Hardy-Weinberg distribution for a disease with prevalence of 2%, would indicate that the minor allele frequencies (MAFs) for SNPs associated with POAG will be 0.15 or higher. For a SNP with an MAF of 0.15 and the conservative dominant genetic model, we have a 75% chance to detect an allele with a relative risk (RR) of 1.4 in the NEIGHBOR data-set alone and in the combined data-set 59% power to detect an allele with 1.3 RR. For the additive genetic model, which may more accurately reflect inheritance of a complex trait such as POAG (considering multiple effects of multiple risk factors) there is more than a 50% chance that a risk allele with a MAF of 0.15 and RR of 1.3 will be detected, and more than an 85% chance of detecting a risk allele of 1.3 RR over a range of MAFs in the combined data-set. Risk alleles for complex traits are typically common and the alleles we are seeking could have MAFs of 0.30 – 0.40. For example, the complement factor H allele associated with macular degeneration has a MAF of 0.40 in the Caucasian population (40). In the NEIGHBOR data-set alone we have considerable power to detect POAG risk alleles with MAF greater than 0.30, and in the combined data-set have power to detect risk alleles with MAF of 0.3–0.4 over a broad range of effect sizes.

GWAS study design

Considering the complex genetic architecture of POAG and the likelihood that we will need to detect alleles of moderate relative risk we have designed our study to have maximal power using the largest possible sample size. Harmonization with the GLAUGEN study will make it possible to combine data-sets in a meta-analysis of over 3500 cases and 3500 controls.

Our design is to initially use the NEIGHBOR and GLAUGEN samples as independent datasets allowing for inter-study confirmation of results. We will examine each set independently, using a single-stage screening design and look for consistency across datasets. However, the power to detect alleles of modest effect size is optimal when the data is analyzed in the full combined dataset (Table 3). We expect that the combined analysis will, in addition to replicated effects across both datasets, identify more modest effects that will be worthy of additional study.

Prior to testing for association genotype data from both studies will be tested for quality using the following quality control filters: exclusion of SNPs with missing rate ≥ 2%, > 1% discordance in known duplicated samples, Hardy-Weinberg equilibrium $P < 1\times10^{-3}$ and minor allele frequency < 0.01. We will also exclude samples based on gender misidentification, unexpected duplicates and cryptic relatedness (IBD k>0.025). Confounding differences in population substructure between cases and controls will be evaluated by principle components analysis and corrected for by including selected eigenvectors as co-variates in the following logistic regression model. Ethnicity outliers will not be excluded from the analysis. To test for association, for each SNP, we will construct a score test of the null hypothesis $b_g=0$ from the logistic model (A): logit Pr[D] = a + b_x X + b_g G. Here X is a vector of potential confounders (fixed for all markers), age, sex, population stratification (eigen vectors and principle component analyses) DNA source, study site and family history of glaucoma. G is a vector of dummy indicators for heterozygote and homozygote minor allele genotypes, yielding a 2 degree of freedom (2df) test that makes no assumptions about the disease model. This test provides a powerful test for association across a range of (unknown) true dominance patterns (dominant, recessive, etc.). The score test is asymptotically equivalent to the likelihood ratio test comparing the full alternative model (A) to the constrained null model (N): logit Pr[D] = a + b_x X, but has the advantages of computational speed (iterative estimation of the nuisance parameters a, b_x and needs to be only performed once, under the null; after that the test statistic for each marker is constructed using simple matrix algebra) and robustness to sparse data (e.g. small numbers of homozygotes).

Data sharing

Individual genotype data will be available from the dbGaP website (http://www.ncbi.nlm.nih.gov/gap). Aggregate and individual phenotype data for the clinical variables in Tables 1 and 2 will also be included in the material contributed to dbGaP. Our consortium plans to continue to extract phenotype data for the genotyped cases and controls from existing medical records and other clinical data. The newly acquired clinical information will be added to the NEIGHBOR heritable overall operational database (The NEIGHBORHOOD). Data deposited in the NEIGHBORHOOD will be accessible through a data usage plan maintained by the consortium.

DISCUSSION

Our collaborative consortium has collected clinical information and DNA on 2,517 POAG cases and 2,428 controls. Initially we will perform a genome-wide association study to

identify genes that contribute to POAG pathogenesis. The substantial collection of clinical data relevant to glaucoma will facilitate future studies investigating specific gene-phenotype correlations for affected individuals, although these studies will be limited to those subgroup with relevant information. The NEIGHBOR consortium includes samples from two landmark NEI-funded clinical trials (CIGTS, AGIS) investigating treatment outcomes. The samples from these well-characterized cases with important longitudinal clinical data could lead to the identification of genetic risk factors associated with progressive disease and therapeutic response. Our case control definitions and genotyping platforms have been harmonized with the collection of 1,000 cases and 1,183 controls that are part of the GENEVA GLAUGEN study. Together we have created one of the largest POAG case control data set currently available for genetic studies.

Over 800 GWAS completed for diseases with complex inheritance have provided important insights that may also be applicable to the underlying genetic architecture of complex traits such as POAG (41). The initial rationale for GWAS of common diseases was the ‘common disease, common variant’ hypothesis, yet recent studies have shown that most common variants actually confer modest changes in risk (10–50%), including the recently reported association between POAG and the *CAV1/CAV2* SNPs, and the *TMCO1* and *CDKN2BAS* SNPs (11, 12, 42). These findings suggest that the POAG genetic risk factors awaiting discovery will have low to moderate relative risks and gene discovery will likely require secondary analyses that can address the phenotypic heterogeneity as well as the underlying molecular complexity.

Patients affected by POAG have a range of phenotypes that could result from varied disease mechanisms. As a result, true gene associations accounting for specific aspects of the POAG phenotype may be obscured when the data is analyzed in aggregate. One way to approach this problem is to focus on ‘subphenotypes’, or ‘endophenotypes’ which define POAG subgroups that are more phenotypically homogeneous. Quantitative endophenotypes can also increase the power of the study and reduce misclassification. Genetic variants contributing to several POAG-related quantitative endophenotpes have recently been identified using quantitative trait analytical methods including optic nerve cup-to-disc ratio (CDR), optic nerve size (43, 44) and CCT (45–47). Importantly, some variants associated with structural optic nerve parameters have also been shown to be risk factors for POAG (48, 49), providing support for this overall approach to POAG gene discovery. Clinical information for several quantitative traits related to glaucoma are included in our dataset including IOP, CCT and CDR.

Secondary analyses can define complex molecular interactions that predispose to disease pathogenesis. Analysis for gene-gene and gene-environment interactions can identify disease associations that are biologically more significant than single gene or environmental risk factors. In previous studies we showed that an interaction between variants in the *NOS3* gene and lifestyle choices such as postmenopausal hormone use (50) and cigarette smoking (51) was significantly associated with POAG in women than either *NOS3* variants or hormone replacement alone. Similarly, a molecular pathway may have a greater contribution to disease heritability than any individual gene in the pathway. For example, genetic variants in an axon guidance pathway collectively predispose to Parkinson’s disease, even though none of the individual variants had highly significant disease association (52). Another successful example of the pathway approach comes from work in macular degeneration showing that multiple complement factor genes, in addition to complement factor H, are associated with the disease (53). Although the environmental exposure data is limited in the current GLAUGEN-NEIGHBOR dataset, the additional data contributed to the NEIGHBORHOOD (as described above) will support future secondary analysis of gene-environment and gene-gene interactions including pathway studies.

Our POAG genotype-phenotype dataset will also be used to validate genes that are implicated in POAG by *in vitro* studies or in animal models. An attractive feature of this approach is the potential for a more targeted analysis using a minimum number of tests, which can increase the power of our dataset to detect alleles of smaller effect sizes.

The potential outcome of this study is the identification of genes that contribute to POAG pathogenesis with the eventual goals of developing gene-based screening and therapeutics. Even though the POAG genetic architecture is expected to consist of multiple risk alleles of small to moderate effect size the realization of these goals remains possible. The discovery of POAG-predisposing genetic variants could inform the development of novel therapies targeting the underlying molecular events responsible for disease. Genetic risk factors with small effect size can be successfully translated as therapeutic targets that can favorably modify a disease process. An example is the *PPARG* gene associated with Type 2 diabetes where a modest association of OR 1.2 has led to the development of novel therapies for diabetes using *PPARG* agonists, an approach that has benefited millions of patients (54). Testing for a panel of risk alleles of small to moderate effect can effectively identify individuals with increased disease risk. Emerging studies suggest that the aggregate mutation load created by contributions from multiple risk factors may be a useful metric for establishing an overall risk assessment in disorders with complex inheritance such as POAG (55, 56). Although the development of clinically useful gene-based screening tests and therapies will require additional research, the discovery of POAG genetic risk factors is the critical first step toward the overall goal of preventing vision loss from this common blinding disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Collecting and processing samples for the NEIGHBOR dataset was supported by the National Eye Institute through ARRA grants 3R01EY015872-05S1 (Wiggs) and 3R01EY019126-02S1 (Hauser, M). Funding for the collection of cases and controls was provided by NIH grants: EY015543 (Allingham), EY006827 (Gaasterland), HL73042 (Goldschmidt), HL073389 (Hauser, E); EY13315 (Hauser, M); CA87969 (Kang), CA49449 (Kang), CA55075 (Kang), EY009149 (Lichter), HG004608 (McCarty), EY008208 (Medeiros), EY015473 (Pasquale), EY012118 (Pericak-Vance), EY015682 (Realini), EY011671 (Richards), EY09580 (Richards), EY013178 (Schuman), RR015574 (Spirou), EY015872 (Wiggs), EY010886 (Wiggs), EY009847 (Wiggs), EY011008 (Zangwill), EY144428 (Zhang), EY144448 (Zhang), EY18660 (Zhang). Drs. Wiggs, and Pasquale are also supported by the Harvard Glaucoma Center for Excellence, and the Margolis Fund. Dr. Yaspan is supported in part by NIH grant T32EY021453. Dr. Yutao Liu is supported in part by the Glaucoma Research Foundation, National Glaucoma Research of American Health Assistance Foundation, and The Glaucoma Foundation. Drs. Wiggs, Pasquale and Richards are supported by Research to Prevent Blindness. A full listing of collaborators for GLAUGEN can be found in dbGaP at The Primary Open-Angle Glaucoma Genes and Environment (GLAUGEN) Study. Study Accession: phs000308.v1.p1. Additionally, Drs. Cynthia Grosskreutz, Teresa Chen, Doug Rhee, A. Tim Johnson, Colm O'Brien, Ms. Katy Downs, and the CIGTS investigators and AGIS investigators helped identify patients and controls for these studies.

REFERENCES

1. Rudnicka AR, Mt-Isa S, Owen CG, Cook DG, Ashby D. Variations in primary open-angle glaucoma prevalence by age, gender, and race: a Bayesian meta-analysis. Invest Ophthalmol Vis Sci. 2006; 47(10):4254–4261. [PubMed: 17003413]
2. Congdon NG, Friedman DS, Lietman T. Important causes of visual impairment in the world today. JAMA. 2003; 290:2057–2060. [PubMed: 14559961]
3. Pizzarello L, Abiose A, Ffytche T, et Pizzarello L, Abiose A, Ffytche T, et al. Vision 2020: The right to sight. A global initiative to eliminate avoidable blindness. Arch Ophthalmol. 2004; 122:615–620. [PubMed: 15078680]

4. Sung VC, Koppens JM, Vernon SA, Pawson P, Rubinstein M, King AJ, Tattersall CL. Longitudinal glaucoma screening for siblings of patients with primary open angle glaucoma: the Nottingham Family Glaucoma Screening Study. Br J Ophthalmol. 2006 Jan; 90(1):59–63. [PubMed: 16361669]

5. Tielsch JM, Sommer A, Katz J, Royall RM, Quigley HA, Javitt J. Racial variations in the prevalence of primary open-angle glaucoma. The Baltimore Eye Survey. JAMA. 1991 Jul 17; 266(3):369–374. [PubMed: 2056646]

6. Kang JH, Willett WC, Rosner BA, Hankinson SE, Pasquale LR. Prospective study of alcohol consumption and the risk of primary open-angle glaucoma. Ophthalmic Epidemiol. 2007 May-Jun; 14(3):141–147. [PubMed: 17613849]

7. Wang X, Harmon J, Zabrieskie N, Chen Y, Grob S, Williams B, Lee C, Kasuga D, Shaw PX, Buehler J, Wang N, Zhang K. Using the Utah Population Database to assess familial risk of primary open angle glaucoma. Vision Res. 2010 Nov 23; 50(23):2391–2395. [PubMed: 20858511]

8. Teikari JM. Genetic factors in open-angle (simple and capsular) glaucoma: a population-based twin study. Acta Ophthalmol Scand. 1987; 65(6):715–720.

9. Gottfredsdottir MS, Sverrisson T, Musch DC, Stefansson E. Chronic open-angle glaucoma and associated ophthalmic findings in monozygotic twins and their spouses in Iceland. J Glaucoma. 1999 Apr; 8(2):134–139. [PubMed: 10209731]

10. Fan BJ, Wiggs JL. Glaucoma: genes, phenotypes, and new directions for therapy. J Clin Invest. 2010 Sep 1; 120(9):3064–3072. [PubMed: 20811162]

11. Thorleifsson G, Walters GB, Hewitt AW, et al. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma. Nat Genet. 2010 Oct; 42(10):906–909. [PubMed: 20835238]

12. Burdon KP, Macgregor S, Hewitt AW, et al. Genome-wide association study identifies susceptibility loci for open angle glaucoma at TMCO1 and CDKN2B-AS1. Nat Genet. 2011 Jun; 43(6):574–578. [PubMed: 21532571]

13. Ramdas WD, Amin N, van Koolwijk LM, et al. Genetic architecture of open angle glaucoma and related determinants. J Med Genet. 2011 Mar; 48(3):190–196. [PubMed: 21059592]

14. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. Science. 2005; 308(5720):385–389. [PubMed: 15761122]

15. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. Science. 2005; 15;308(5720):419–421.

16. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science. 2005; 308(5720):421–424. [PubMed: 15761121]

17. Solouki AM, Verhoeven VJ, van Duijn CM, Verkerk AJ, Ikram MK, Hysi PG, Despriet DD, van Koolwijk LM, Ho L, Ramdas WD, Czudowska M, Kuijpers RW, Amin N, Struchalin M, Aulchenko YS, van Rij G, Riemslag FC, Young TL, Mackey DA, Spector TD, Gorgels TG, Willemse-Assink JJ, Isaacs A, Kramer R, Swagemakers SM, Bergen AA, van Oosterhout AA, Oostra BA, Rivadeneira F, Uitterlinden AG, Hofman A, de Jong PT, Hammond CJ, Vingerling JR, Klaver CC. A genome-wide association study identifies a susceptibility locus for refractive errors and myopia at 15q14. Nat Genet. 2010 Oct; 42(10):897–901. [PubMed: 20835239]

18. Hysi PG, Young TL, Mackey DA, Andrew T, Fernández-Medarde A, Solouki AM, Hewitt AW, Macgregor S, Vingerling JR, Li YJ, Ikram MK, Fai LY, Sham PC, Manyes L, Porteros A, Lopes MC, Carbonaro F, Fahy SJ, Martin NG, van Duijn CM, Spector TD, Rahi JS, Santos E, Klaver CC, Hammond CJ. A genome-wide association study for myopia and refractive error identifies a susceptibility locus at 15q25. Nat Genet. 2010 Oct; 42(10):902–905. [PubMed: 20835236]

19. Thorleifsson G, Magnusson KP, Sulem P, et al. Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma. Science. 2007; 317:1397–1400. [PubMed: 17690259]

20. Manolio TA, Rodriguez LL, Brooks L, Abecasis G, Collaborative Association Study of Psoriasis; Ballinger D, Daly M, Donnelly P, Faraone SV, International Multi-Center ADHD Genetics Project; Frazer K, Gabriel S, Gejman P, Molecular Genetics of Schizophrenia Collaboration; Guttmacher A, Harris EL, Insel T, Kelsoe JR, Bipolar Genome Study; Lander E, McCowin N, Mailman MD, Nabel E, Ostell J, Pugh E, Sherry S, Sullivan PF, Major Depression Stage 1

Genomewide Association in Population-Based Samples Study; Thompson JF, Warram J, Genetics of Kidneys in Diabetes (GoKinD) Study. Wholley D, Milos PM, Collins FS. GAIN Collaborative Research Group. New models of collaboration in genome-wide association studies: the Genetic Association Information Network. Nat Genet. 2007 Sep; 39(9):1045–1051. [PubMed: 17728769]

21. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007 Jun 7; 447(7145):661–678. [PubMed: 17554300]
22. Cornelis MC, Agrawal A, Cole JW, Hansel NN, Barnes KC, Beaty TH, Bennett SN, Bierut LJ, Boerwinkle E, Doheny KF, Feenstra B, Feingold E, Fornage M, Haiman CA, Harris EL, Hayes MG, Heit JA, Hu FB, Kang JH, Laurie CC, Ling H, Manolio TA, Marazita ML, Mathias RA, Mirel DB, Paschall J, Pasquale LR, Pugh EW, Rice JP, Udren J, van Dam RM, Wang X, Wiggs JL, Williams K, Yu K. for the GENEVA Consortium 2010. The gene, environment association studies consortium (GENEVA): maximizing the knowledge obtained from GWAS by collaboration across studies of multiple conditions. Genet Epidemiol. 2010 May; 34(4):364–372. [PubMed: 20091798]
23. Wang L, Hauser ER, Shah SH, Pericak-Vance MA, Haynes C, Crosslin D, Harris M, Nelson S, Hale AB, Granger CB, Haines JL, Jones CJ, Crossman D, Seo D, Gregory SG, Kraus WE, Goldschmidt-Clermont PJ, Vance JM. Peakwide mapping on chromosome 3q13 identifies the kalirin gene as a novel candidate gene for coronary artery disease. Am J Hum Genet. 2007 Apr; 80(4):650–663. [PubMed: 17357071]
24. McCarty CA, Chisholm RL, Chute CG, Kullo IJ, Jarvik GP, Larson EB, Li R, Masys DR, Ritchie MD, Roden DM, Struewing JP, Wolf WA. eMERGE Team. The eMERGE Network: a consortium of biorepositories linked to electronic medical records data for conducting genomic studies. BMC Med Genomics. 2011 Jan 26.4:13. [PubMed: 21269473]
25. Musch DC, Lichter PR, Guire KE, Standardi CL. The Collaborative Initial Glaucoma Treatment Study: study design, methods, and baseline characteristics of enrolled patients. Ophthalmology. 1999 Apr; 106(4):653–662. [PubMed: 10201583]
26. The Advanced Glaucoma Intervention Study (AGIS): 1. Study design and methods and baseline characteristics of study patients. Control Clin Trials. 1994 Aug; 15(4):299–325. [No authors listed]. [PubMed: 7956270]
27. Hayney MS, Poland GA, Lipsky JJ. A noninvasive 'swish and spit' method for collecting nucleated cells for HLA typing by PCR in population studies. Hum Hered. 1996 Mar-Apr;46(2):108–111. [PubMed: 8666410]
28. Rylander-Rudqvist T, Håkansson N, Tybring G, Wolk A. Quality and quantity of saliva DNA obtained from the self-administrated oragene method--a pilot study on the cohort of Swedish men. Cancer Epidemiol Biomarkers Prev. 2006 Sep; 15(9):1742–1745. [PubMed: 16985039]
29. Loomis SJ, Olson LM, Pasquale LR, Wiggs J, Mirel D, Crenshaw A, Parkin M, Rahhal B, Tetreault S, Kraft P, Tworoger SS, Haines JL, Kang JH. Feasibility of High-Throughput Genome-Wide Genotyping using DNA from Stored Buccal Cell Samples. Biomark Insights. 2010 May 20.5:49–55. [PubMed: 20520743]
30. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Optimal designs for two-stage genome-wide association studies. Genet Epidemiol. 2007 Nov; 31(7):776–788. (2007). [PubMed: 17549752]
31. Dueker DK, Singh K, Lin SC, Fechtner RD, Minckler DS, Samples JR, Schuman JS. Corneal thickness measurement in the management of primary open-angle glaucoma: a report by the American Academy of Ophthalmology. Ophthalmology. 2007 Sep; 114(9):1779–1787. [PubMed: 17822980]
32. Perera SA, Wong TY, Tay WT, Foster PJ, Saw SM, Aung T. Refractive error, axial dimensions, and primary open-angle glaucoma: the Singapore Malay Eye Study. Arch Ophthalmol. 2010 Jul; 128(7):900–905. [PubMed: 20625053]
33. Pasquale LR, Willett WC, Rosner BA, Kang JH. Anthropometric measures and their relation to incident primary open-angle glaucoma. Ophthalmology. 2010 Aug; 117(8):1521–1529. [PubMed: 20382429]
34. Ramdas WD, Wolfs RC, Hofman A, de Jong PT, Vingerling JR, Jansonius NM. Lifestyle and risk of developing open-angle glaucoma: the Rotterdam study. Arch Ophthalmol. 2011 Jun; 129(6): 767–772. [PubMed: 21320952]

35. Pasquale LR, Kang JH, Manson JE, Willett WC, Rosner BA, Hankinson SE. Prospective study of type 2 diabetes mellitus and risk of primary open-angle glaucoma in women. Ophthalmology. 2006 Jul; 113(7):1081–1086. [PubMed: 16757028]

36. Duggal P, Klein AP, Lee KE, Klein R, Klein BE, Bailey-Wilson JE. Identification of novel genetic loci for intraocular pressure: a genomewide scan of the Beaver Dam Eye Study. Arch Ophthalmol. 2007 Jan; 125(1):74–79. [PubMed: 17210855]

37. Kraft P. Efficient two-stage genome-wide association designs based on false positive report probabilities. Pac Symp Biocomput. 2006:523–534. [PubMed: 17094266]

38. Klein BE, Klein R, Sponsel WE, Franke T, Cantor LB, Martone J, Menage MJ. Prevalence of glaucoma. The Beaver Dam Eye Study. Ophthalmology. 1992 Oct; 99(10):1499–1504. [PubMed: 1454314]

39. Tielsch JM, Katz J, Singh K, Quigley HA, Gottsch JD, Javitt J, Sommer A. A population-based evaluation of glaucoma screening: the Baltimore Eye Survey. Am J Epidemiol. 1991 Nov 15; 134(10):1102–1110. [PubMed: 1746520]

40. Chen Y, Zeng J, Zhao C, Wang K, Trood E, Buehler J, Weed M, Kasuga D, Bernstein PS, Hughes G, Fu V, Chin J, Lee C, Crocker M, Bedell M, Salasar F, Yang Z, Goldbaum M, Ferreyra H, Freeman WR, Kozak I, Zhang K. Assessing susceptibility to age-related macular degeneration with genetic markers and environmental factors. Arch Ophthalmol. 2011 Mar; 129(3):344–351. [PubMed: 21402993]

41. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TF, McCarroll SA, Visscher PM. Finding the missing heritability of complex diseases. Nature. 2009 Oct 8; 461(7265):747–753. (2009). [PubMed: 19812666]

42. Wiggs JL, Kang JH, Yaspan BL, Mirel D, Laurie C, Crenshaw A, Brodeur W, Gogarten S, Olson LM, Abdrabou A, DelBono E, Loomis S, Haines JL. Pasquale LR for the GENEVA consortium. Common Variants Near CAV1 and CAV2 are Associated with Primary Open-Angle Glaucoma in Caucasians from the Continental United States. Hum Mol Genet. 2011 Dec 1; 20(23):4707–4713. [PubMed: 21873608]

43. Ramdas WD, van Koolwijk LM, Ikram MK, Jansonius NM, de Jong PT, Bergen AA, Isaacs A, Amin N, Aulchenko YS, Wolfs RC, Hofman A, Rivadeneira F, Oostra BA, Uitterlinden AG, Hysi P, Hammond CJ, Lemij HG, Vingerling JR, Klaver CC, van Duijn CM. A genome-wide association study of optic disc parameters. PLoS Genet. 2010 Jun 10.6(6):e1000978. [PubMed: 20548946]

44. Macgregor S, Hewitt AW, Hysi PG, Ruddle JB, Medland SE, Henders AK, Gordon SD, Andrew T, McEvoy B, Sanfilippo PG, Carbonaro F, Tah V, Li YJ, Bennett SL, Craig JE, Montgomery GW, Tran-Viet KN, Brown NL, Spector TD, Martin NG, Young TL, Hammond CJ, Mackey DA. Genome-wide association identifies ATOH7 as a major gene determining human optic disc size. Hum Mol Genet. 2010 Jul 1; 19(13):2716–2724. Epub 2010 Apr 15. [PubMed: 20395239]

45. Desronvil T, Logan-Wyatt D, Abdrabou W, Triana M, Jones R, Taheri S, Del Bono E, Pasquale LR, Olivier M, Haines JL, Fan BJ, Wiggs JL. Distribution of COL8A2 and COL8A1 gene variants in Caucasian primary open angle glaucoma patients with thin central corneal thickness. Mol Vis. 2010 Oct 29.16:2185–2191. [PubMed: 21139683]

46. Vithana EN, Aung T, Khor CC, Cornes BK, Tay WT, Sim X, Lavanya R, Wu R, Zheng Y, Hibberd ML, Chia KS, Seielstad M, Goh LK, Saw SM, Tai ES, Wong TY. Collagen-related genes influence the glaucoma risk factor, central corneal thickness. Hum Mol Genet. 2011 Feb 15; 20(4): 649–658. [PubMed: 21098505]

47. Vitart V, Bencić G, Hayward C, Skunca Herman J, Huffman J, Campbell S, Bućan K, Navarro P, Gunjaca G, Marin J, Zgaga L, Kolcić I, Polasek O, Kirin M, Hastie ND, Wilson JF, Rudan I, Campbell H, Vatavuk Z, Fleck B, Wright A. New loci associated with central cornea thickness include COL5A1, AKAP13 and AVGR8. Hum Mol Genet. 2010 Nov 1; 19(21):4304–4311. [PubMed: 20719862]

48. Fan BJ, Wang DY, Pasquale LR, Haines JL, Wiggs JL. Genetic Variants Associated with Optic Nerve Vertical Cup-to-Disc Ratio Are Risk Factors for Primary Open Angle Glaucoma in a US

Caucasian Population. Invest Ophthalmol Vis Sci. 2011 Mar 28; 52(3):1788–1792. [PubMed: 21398277]

49. Ramdas WD, van Koolwijk LM, Lemij HG, et al. Common genetic variants associated with open-angle glaucoma. Hum Mol Genet. 2011 Jun 15; 20(12):2464–2471. [PubMed: 21427129]
50. Kang JH, Wiggs JL, Rosner BA, Hankinson SE, Abdrabou W, Fan BJ, Haines J, Pasquale LR. The relation between endothelial nitric oxide synthase gene variants and primary open-angle glaucoma: Interactions with gender and postmenopausal hormone use. Invest Ophthalmol Vis Sci. 2010; 51:971–979. (2010). [PubMed: 19815736]
51. Kang JH, Wiggs JL, Rosner BA, Haines J, Abdrabou W, Pasquale LR. Endothelial nitric oxide synthase gene variants and primary open-angle glaucoma: Interactions with hypertension, alcohol and cigarette smoking. Arch Ophthalmol. 2011; 129:773–780. [PubMed: 21670344]
52. Lesnick TG, Papapetropoulos S, Mash DC, Ffrench-Mullen J, Shehadeh L, de Andrade M, Henley JR, Rocca WA, Ahlskog JE, Maraganore DM. A genomic pathway approach to a complex disease: axon guidance and Parkinson disease. PLoS Genet. 2007 Jun.3(6):e98. (2007). [PubMed: 17571925]
53. Dinu V, Miller PL, Zhao H. Evidence for association between multiple complement pathway genes and AMD. Genet Epidemiol. 2007 Apr; 31(3):224–237. (2007). [PubMed: 17266113]
54. Bénardeau A, Benz J, Binggeli A, Blum D, Boehringer M, Grether U, Hilpert H, Kuhn B, Märki HP, Meyer M, Püntener K, Raab S, Ruf A, Schlatter D, Mohr P. Aleglitazar, a new, potent, and balanced dual PPARalpha/gamma agonist for the treatment of type II diabetes. Bioorg Med Chem Lett. 2009 May 1; 19(9):2468–2473. [PubMed: 19349176]
55. Gourraud PA, McElroy JP, Caillier SJ, Johnson BA, Santaniello A, Hauser SL, Oksenberg JR. Aggregation of multiple sclerosis genetic risk variants in multiple and single case families. Ann Neurol. 2011 Jan; 69(1):65–74. [PubMed: 21280076]
56. Zaghloul NA, Katsanis N. Functional modules, mutational load and human genetic disease. Trends Genet. 2010 Apr; 26(4):168–176. [PubMed: 20226561]

Table 1

Features of Cases and Controls for the NEIGHBOR and GLAUGEN Studies

	NEIGHBOR		GLAUGEN	
	Cases	**Controls**	**Cases**	**Controls**
Number	2517	2428	1000	1003
Gender (% Female)	53	55	59	60
DNA source	92% blood 8% buccal	96% blood 4% buccal	50% blood 50% buccal	36% blood 64% buccal
Age	66.4 ± 13.5 (35–107)	68.1 ± 11.6 (36–97)	63.6 + 9.8 (40–87)	65.5 + 9.2 (40–91)
IOP (mmHg)	16.2 ± 6.4 (1–52) N= 2365	14.6 ± 2.8 (4–21) N= 2282	19.1 + 6.0 (5–45) N= 844	15.1 + 2.5 (9–21) N= 350
vCDR	0.76 ± 0.18 (0.1–1.0) N= 1961	0.31 ± 0.12 (0.1–0.6) N= 2167	0.71 + 0.19 (0.1–1.00) N= 965	0.30 + 0.11 (0.1–0.7) N= 348
CCT	543.0 ± 40.9 (374–853) N= 909	544.3 ± 44.9 (402–875) N= 305	549.2 ± 37.1 (469–688) N= 321	NA
Refractive error (D) (spherical equivalent)	−0.79 ± 2.41 (−8.00, +7.50) N= 1156	−0.21 ± 2.22 (−7.75, +8.00) N= 1311	−0.46 + 2.32 (−7.75, +1.13) N= 470	−0.47 ± 2.15 (−6.75,6.87) N= 308
Height (cm)	167.1 ± 9.9 (149–198) N= 221	167.6 ± 9.8 (145–198) N= 572	169.2 + 9.7 (147–198) N= 509	166.6 + 9.4 (150–211) N= 832
Weight (kg)	76.0 ± 18.5 (45–170) N= 220	78.2 ± 18.8 (41–167) N= 572	71.5 + 14.1 (44–156) N= 508	72.3 + 13.2 (44–133) N= 826
BMI	27.2 ± 5.3 (17.0–51.0) N= 220	27.4 ± 5.5 (16.0–60.0) N= 567	24.9 + 3.72 (17.4–48.1) N= 508	25.1 + 3.60 (16.6–50.3) N= 826

All data is the value obtained at study enrollment. Gender, DNA source and age are available for 100% of cases and controls for both the NEIGHBOR and GLAUGEN studies. For features that are not available on all cases and controls, the number of cases of controls with data for that feature (N) are listed below the mean, standard deviation and range. Abbreviations: IOP, intraocular pressure; vCDR, vertical cup-to-disc ratio; D, Diopters; BMI, body mass index.

Table 2

Percent of cases and controls with data for selected clinical variables for the NEIGHBOR and GLAUGEN studies.

		NEIGHBOR		GLAUGEN	
Variable		**Cases (%)**	**Controls (%)**	**Cases (%)**	**Controls (%)**
Family history of disease in primary relatives	Yes	24	0	43.0	9.6
	No	24	57	56.5	90.5
	NA	56	41	0.1	0
Family history of disease in secondary relatives	Yes	10	2	NA	NA
	No	32	57	NA	NA
	NA	58	41	NA	NA
History of IOP >21	Yes	46	0	26.4	0
	No	21	48	58.0	0
	NA	33	52	15.6	100
History of LTP	Yes	5	0	28.0	0
	No	8	0	72.0	0
	NA	87	100	0	100
History of glaucoma filtering surgery	Yes	7	0	14.7	6.1
	No	6	0	85.3	93.8
	NA	87	100	0	0.1
History of diabetes	Yes	8	12	11.0	42.1
	No	36	47	89.0	57.8
	NA	56	41	0	0.1
History of hypertension	Yes	23	31	43.8	49.7
	No	22	31	56.2	44.0
	NA	55	38	0	6.3
Smoking history	Ever	12	23	50.8	15.1
	Never	25	45	47.4	84.5
	NA	63	32	1.8	0.3
Systemic Beta-blocker use (at enrollment)	Yes	5	4	14.6	8.9
	No	21	8	85.2	90.8

Variable		NEIGHBOR		GLAUGEN	
		Cases (%)	Controls (%)	Cases (%)	Controls (%)
Systemic Calcium channel blocker use (at enrollment)	NA	74	88	0.2	0.3
	Yes	4	2	8.9	12.1
	No	22	10	90.9	87.6
	NA	74	88	0.2	0.3
Systemic ACE inhibitor use (at enrollment)	Yes	5	2	12.2	9.6
	No	21	10	87.6	90.5
	NA	74	88	0.2	0

Abbreviations: IOP, intraocular pressure; LTP, laser trabeculoplasty; ACE, Angiotensin-Converting Enzyme.

Table 3

Power (%) to detect a given POAG marker with specified RR for single-stage design

		n=2500	n=3500	n=2500	n=3500	n=2500	n=3500	n=2500	n=3500
MAF	**Model**	**RR=1.20**	**RR=1.20**	**RR=1.30**	**RR=1.30**	**RR=1.40**	**RR=1.40**	**RR=1.5**	**RR=1.5**
.05	Dominant	1	1	4	11	19	43	48	79
	Additive	1	1	5	15	24	51	57	86
10%	Dominant	2	5	18	41	57	86	89	99
	Additive	3	10	30	59	75	95	96	>99
15%	Dominant	4	10	30	59	75	95	96	>99
	Additive	9	24	56	85	93	>99	>99	>99
20%	Dominant	5	13	36	67	80	97	98	>99
	Additive	17	39	73	94	98	>99	>99	>99
30%	Dominant	5	13	32	62	85	98	98	>99
	Additive	29	58	88	99	>99	>99	>99	>99
40%	Dominant	5	15	35	65	85	98	98	>99
	Additive	35	82	91	>99	>99	>99	>99	>99

Abbreviations: MAF, mean allele frequency; RR, relative risk; D, dominant; A, additive.