Association of CAV1/CAV2 Genomic Variants with Primary Open-Angle Glaucoma Overall and by Gender and Pattern of Visual Field Loss

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Purpose: The CAV1/CAV2 (caveolin 1 and caveolin 2) genomic region previously was associated with primary open-angle glaucoma (POAG), although replication among independent studies has been variable. The aim of this study was to assess the association between CAV1/CAV2 single nucleotide polymorphisms (SNPs) and POAG in a large case-control dataset and to explore associations by gender and pattern of visual field (VF) loss further.

Design: Case-control study.

Participants: We analyzed 2 large POAG data sets: the Glaucoma Genes and Environment (GLAUGEN) study (976 cases, 1140 controls) and the National Eye Institute Glaucoma Human Genetics Collaboration (NEIGHBOR) consortium (2132 cases, 2290 controls).

Methods: We studied the association between 70 SNPs located within the CAV1/CAV2 genomic region in the GLAUGEN and NEIGHBOR studies, both genotyped on the Illumina Human 660WQuadv1C BeadChip array and imputed with the Markov Chain Haplotyping algorithm using the HapMap 3 reference panel. We used logistic regression models of POAG in the overall population and separated by gender, as well as by POAG subtypes defined by type of VF defect (peripheral or paracentral). Results from GLAUGEN and NEIGHBOR were meta-analyzed, and a Bonferroni-corrected significance level of $7.7 \times 10^{-4}$ was used to account for multiple comparisons.

Main Outcome Measures: Overall POAG, overall POAG by gender, and POAG subtypes defined by pattern of early VF loss.

Results: We found significant associations between 10 CAV1/CAV2 SNPs and POAG (top SNP, rs4236601; pooled $P = 2.61 \times 10^{-4}$). Of these, 9 were significant only in women (top SNP, rs4236601; pooled $P = 1.59 \times 10^{-4}$). Five of the 10 CAV1/CAV2 SNPs were associated with POAG with early paracentral VF (top SNP, rs17588172; pooled $P = 1.07 \times 10^{-4}$), and none of the 10 were associated with POAG with peripheral VF loss only or POAG among men.

Conclusions: CAV1/CAV2 SNPs were associated significantly with POAG overall, particularly among women. Furthermore, we found an association between CAV1/CAV2 SNPs and POAG with paracentral VF defects. These data support a role for caveolin 1, caveolin 2, or both in POAG and suggest that the caveolins particularly may affect POAG pathogenesis in women and in patients with early paracentral VF defects. Ophthalmology 2014;121:508-516 © 2014 by the American Academy of Ophthalmology.

Primary open-angle glaucoma (POAG) is a leading cause of blindness worldwide, affecting more than 35 million people and is characterized by retinal ganglion cell death and defects in the visual field (VF) that ultimately cause functional visual loss. Primary open-angle glaucoma has a genetic component, with contributions from both rare, highly penetrant alleles (MYOC, OPTN) and common risk alleles with smaller effects (CAV1/CAV2, TMCO1, SIX1/SIX6, CDKN2BAS, DKK3, CRIM1, OPA1, PRPS1, ATP1A2, ABCA4, C tip4p).
and 8q22).\textsuperscript{5–8} The genomic region that includes CAV1 and CAV2 initially was identified in a genome-wide association study using cases and controls from Iceland.\textsuperscript{9} Significant associations in this region also were observed in the Glaucoma Genes and Environment (GLAUGEN) study using a sample consisting of 976 cases and 1140 controls.\textsuperscript{10} However, 3 other smaller studies including 545 cases and 297 controls from Iowa,\textsuperscript{11} 220 cases and 405 controls from Saudi Arabia,\textsuperscript{12} and 272 cases and 165 controls from Barbados\textsuperscript{13} have not replicated the overall association between CAV1/CAV2 single nucleotide polymorphisms (SNPs) and POAG. This is likely because of modest associations that necessitate large sample sizes for detection.

CAV1 and CAV2 code for caveolin 1 and caveolin 2, which are members of the caveolin protein family. These proteins inhibit endothelial nitric oxide synthase (eNOS; coded by the gene NOS3, or nitric oxide synthase) activity within the caveolae, which are specialized invaginations of the plasma membrane that are especially prevalent in endothelial plasma membranes.\textsuperscript{13} This interaction alters nitric oxide generation and hence may lead to changes in vascular tone\textsuperscript{14,15} and trabecular meshwork function,\textsuperscript{16} both of which have been implicated in POAG pathogenesis.\textsuperscript{17}

Estrogen receptors are expressed in retinal ganglion cells,\textsuperscript{18} and estrogen is neuroprotective in animal models of POAG.\textsuperscript{8,19} Higher estrogen levels affect the expression of NOS3,\textsuperscript{20} leading to increased nitric oxide production, which may be protective against POAG.\textsuperscript{13,21} Our group reported gene—environment interactions between NOS3 SNPs and postmenopausal hormone use with high-tension POAG\textsuperscript{22} and between age at menarche and NOS3 SNPs with overall POAG.\textsuperscript{23} Because eNOS (NOS3) directly interacts with caveolin 1 (CAV1),\textsuperscript{24} there is a strong rationale to assess the impact of gender on the association of CAV1/CAV2 genomic variations with POAG.

The interaction between caveolin 1 and eNOS in the caveolae of the plasma membranes suggests that the CAV1/CAV2 genomic region SNPs may be associated with the POAG clinical subgroups that exhibit systemic vascular dysregulation. Several clinical parameters have been observed, with higher frequency in POAG cases exhibiting systemic vascular dysregulation, including paracentral VF loss and disc hemorrhages.\textsuperscript{25} Furthermore, emerging evidence suggests that enzymes that influence vascular physiology, such as soluble guanylyl cyclase, are associated with early paracentral loss in POAG patients. Buys et al\textsuperscript{26} demonstrated that soluble guanylyl cyclase knockout mice, which have defective nitric oxide signaling, develop open-angle glaucoma and that variants in the genomic region containing genes for the \( \alpha1 \) and \( \beta1 \) subunits of soluble guanylate cyclase are associated with paracentral VF loss in women. Because the caveolins are an integral part of the nitric oxide signaling pathway, there is interest in whether the CAV1/CAV2 genomic region SNPs associated with POAG are also associated with the POAG subgroup that is defined by early paracentral visual loss. In this study, we investigated the association between SNPs located in the CAV1/CAV2 genomic region and overall POAG, as well as overall POAG separately by gender and by POAG subgroups defined by pattern of early VF loss.

Methods

Study Populations

We used 2 POAG case-control groups in this study: the GLAUGEN study and the National Eye Institute Glaucoma Human Genetics Collaboration (NEIGHBOR) study. The GLAUGEN study (976 cases, 1140 controls) consists of 2 longitudinal cohort studies, the Nurses’ Health Study and the Health Professionals Follow-up Study, and 1 clinic-based study from the Massachusetts Eye and Ear Infirmary, the Genetic Etiologies of Primary Open-Angle Glaucoma study. The NEIGHBOR study (2132 cases, 2290 controls) consists of clinic-based case-control studies from 12 sites across the United States. Details of these studies, along with inclusion criteria, have been published previously.\textsuperscript{9,27} The institutional review boards of the Massachusetts Eye and Ear Infirmary, Harvard School of Public Health, the Brigham and Women’s Hospital, the University of Pittsburgh, Johns Hopkins University, Duke University, the University of West Virginia, the University of Miami, the University of Michigan, Stanford University, the Marshfield Clinic, and the University of California, San Diego, approved this study. Informed consent was obtained from all participants.

Case and Control Definition

Definitions of POAG cases and controls have been described previously.\textsuperscript{27} Briefly, cases had VF defects consistent with nerve fiber layer pathologic features occurring in the setting of a slit-lamp biomicroscopic examination that did not reveal any significant findings (aside from possible media opacities) and open angles, regardless of intraocular pressure (IOP). Visual field loss was either reproduced on a subsequent test, with the same region of the VF exhibiting VF loss on both VF reports, or if it was not, there were signs suggestive of glaucomatous cupping as indicated by a cup-to-disc ratio of more than 0.7. Controls were under ophthalmic surveillance, with an eye examination within the previous 2 years indicating cup-to-disc ratio of less than 0.6 and IOP of less than 22 mmHg.

Visual Field Scoring

For each participant, we obtained the earliest available reliable VF that demonstrated defects consistent with nerve fiber layer pathologic characteristics. Most VFs (>70%) were performed with Humphrey Visual Field Analyzers (Carl Zeiss, Dublin, CA), although other types of visual function data derived from perimeters such as the Dicon Perimeter (Vision Systems, Inc., Taron Springs FL) or Octopus Perimeter (Haag-Streit, Bern, Switzerland) were used if no Humphrey VFs were available. Reliable VFs were defined based on having fixation loss of 33% or less, false-positive rates of 20% or less, and false-negative rates of 20% or less. Regardless of VF type, each VF underwent systematic review whereby the pattern deviation plot was subdivided into paracentral, Bjerrum, nasal step, and temporal wedge zones above and below the horizontal meridian (Fig 1). We examined these regions for clusters of 3 or more contiguous points with retinal sensitivity depression of 0.5 log unit (−5 dB) relative to age-matched controls. Fields with isolated loss in the paracentral zone only without loss in other zones were labeled as paracentral loss cases. If, in the judgment of the scorer, the VF represented initial functional loss, the case was designated as an early paracentral loss case. If the other zones were involved without loss in the paracentral zone, then the case was categorized as having only peripheral loss. Patients with both paracentral and peripheral VF loss that were considered to have advanced functional deficits and were excluded from secondary analysis based on type of VF loss. Two reviewers (L.R.P. and S.J.L.) assessed the VFs masked to
Figure 1. Paracentral and peripheral visual field loss definitions: representative grey scale and pattern deviation plot for (A) peripheral visual field loss and (B) paracentral visual field loss in 2 right eyes. A. The red boxes indicate each possible peripheral visual field loss region, including inferior and superior nasal steps, temporal wedge, and Bjerrum regions. A cluster of 3 or more points with sensitivity of −5 decibels (dB) or more in any of these regions represents peripheral visual field loss. In this case, there is visual field loss in the inferior nasal step zone. B. The blue boxes indicate the superior and inferior paracentral visual field loss regions. A cluster of 3 or more points with sensitivity of −5 dB or more in either of these regions represents paracentral visual field loss. In this case, there is visual field loss in the inferior paracentral region. There is overlap between the paracentral zone and Bjerrum areas, consisting of the second row of points. For the Bjerrum area to be considered to be involved, there must be at least 1 point in the third row from the top or bottom that has a retinal sensitivity of −5 dB or more. This figure illustrates the approach used for Humphrey visual fields. Subjects with other types of perimetric data (such as the Dicon or Octopus visual fields) were included and a similar strategy was used to grade the equivalent of the pattern deviation plot. Less than 1% of visual fields were kinetic tests, and they were excluded from analyses related to pattern of field loss.

Figure 2. The CAV1/CAV2 genomic region, including all single nucleotide polymorphisms (SNPs) examined in this study and the 9 significantly associated SNPs, which are shown using the UCSC genome browser CAV1/CAV2 region (http://genome.ucsc.edu; accessed April 2, 2013). Single nucleotide polymorphisms that are significant overall are highlighted in black. H3K27Ac histone marks in human umbilical vascular endothelial cells (typically found in genomic regions with regulatory activity) are indicated by the blue peaks. DNaseI hypersensitivity sites are represented by black rectangles.
genotype status, and any differences were adjudicated to arrive at a consensus designation.

**Genotyping and Imputation**

We used the Illumina Human 660WQuadv1C BeadChip array (Illumina, San Diego, CA) to genotype all samples. Genotyping for GLAUGEN study participants occurred at the Broad Institute (Cambridge, MA), whereas genotyping for NEIGHBOR consortium participants was performed at the Center for Inherited Disease Research (Baltimore, MD). Details regarding quality control and data cleaning steps have been described previously. All data were imputed with the Markov Chain Haplotyping algorithm (MACH) (University of Michigan Center for Statistical Genetics; available at: http://www.sph.umich.edu/csg/abecasis/MACH; accessed September 9, 2012) to the HapMap 3 reference panel.

**Single Nucleotide Polymorphism Selection**

All SNPs within 50 kb upstream of CAV2, in between CAV2 and CAV1, and within 50 kb downstream of CAV1 were selected using the UCSC Genome Browser Table Browser tool (February 2009 CRCCh37/hg19 assembly, Common SNPs(137) track; UCSC Genome Browser, available at: www.genome.ucsc.edu; accessed September 9, 2012). Subsequently, we used the SNAP proxy search application (CEU population panel, distance limit of 500 bp, using a combination of 1000 Genomes Pilot 1, HapMap 22, and HapMap 3 to maximize number of included SNPs; Broad Institute, available at: http://www.broadinstitute.org/mpg/snap/lsearch.php; accessed September 9, 2012) to obtain a list of SNPs in strong linkage disequilibrium (LD; \( R^2 \geq 0.8 \)) with the selected SNPs. Of these, 70 SNPs were in both the GLAUGEN and NEIGHBOR data sets and were evaluated in these analyses. The genomic locations of the SNPs included in this study are shown in Figure 2.

**Statistical Analysis**

Logistic regression was performed separately in the GLAUGEN and NEIGHBOR studies (\( \lambda \) inflation factor, 1.009 and 1.034, respectively) using ProbABEL (Erasmus Medical Center, available at: http://www.genabel.org/packages/ProbABEL; accessed September 9, 2012). Subsequently, the results were pooled using the inverse weighted variance method based on regression coefficients and standard errors using the program METAL (University of Michigan Center for Statistical Genetics, available at: http://www.sph.umich.edu/csg/abecasis/metal; accessed September 9, 2012), with the GENOMICCONTROL option on to correct for any residual population stratification or relatedness. In the GLAUGEN sample, we controlled for age, DNA source (blood or cheek), gender, site, method of extraction, and 3 principal components that adjust for population stratification. In the NEIGHBOR sample, we controlled for age, gender, site, and 2 principal components. We performed an assessment for heterogeneity before combining data from the 2 studies. The analyses were run first using all participants, and then analyses were performed using men only and women only, as well as cases with early paracentral VF loss (minimal involvement of the other portions of the VF was possible) and cases with only peripheral VF loss (only involvement of the temporal wedge region, Bjerrum areas, nasal step zones, or a combination thereof in either eye) versus controls. We implemented Bonferroni correction to account for multiple comparisons based on the number of LD blocks and the number of analyses. Sixty-five of the 70 SNPs analyzed fell into 1 of 8 LD blocks. Five SNPs were not in LD with any other SNPs (Fig 3, available at http://aaojournal.org). We corrected for the 8 LD blocks, 5 independent SNPs, and the 5 analysis outcomes (by POAG overall, among women only, among men only, by paracentral VF loss, and by peripheral VF loss) to obtain a significance level of \( 7.7 \times 10^{-4} \) (13 LD blocks \( \times 5 \) analyses = 65; 0.05/65 = \( 7.7 \times 10^{-4} \)).

### Results

The mean age of participants in GLAUGEN and NEIGHBOR was similar, although participants in NEIGHBOR were slightly older (Table 1). Cases in the NEIGHBOR consortium had lower IOP at study entry than cases in the GLAUGEN study (16.1 vs. 18.0 mmHg), higher cup-to-disc ratio (0.76 vs. 0.67), higher pattern standard deviation on the earliest VF

### Table 1. Demographic and Ocular Features of Glaucoma Genes and Environment (GLAUGEN) and National Eye Institute Glaucoma Human Genetics Collaboration (NEIGHBOR) Cases and Controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>GLAUGEN Cases</th>
<th>Controls</th>
<th>NEIGHBOR Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>976</td>
<td>1140</td>
<td>2132</td>
<td>2290</td>
</tr>
<tr>
<td>Mean age (SD), yrs</td>
<td>63.6 (9.8)</td>
<td>65.5 (9.2)</td>
<td>66.6 (13.7)</td>
<td>68.9 (11.4)</td>
</tr>
<tr>
<td>Mean IOP (SD), mmHg*</td>
<td>18.0 (5.6)</td>
<td>N/A</td>
<td>16.1 (6.0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Mean CDR (SD)*</td>
<td>0.67 (0.19)</td>
<td>N/A</td>
<td>0.76 (0.15)</td>
<td>N/A</td>
</tr>
<tr>
<td>Mean MD (SD)*</td>
<td>5.83 (4.94)</td>
<td>N/A</td>
<td>-8.38 (6.72)</td>
<td>N/A</td>
</tr>
<tr>
<td>Mean PSD (SD)*</td>
<td>5.62 (3.03)</td>
<td>N/A</td>
<td>6.67 (3.50)</td>
<td>N/A</td>
</tr>
<tr>
<td>Female (%)</td>
<td>58</td>
<td>60</td>
<td>52</td>
<td>55</td>
</tr>
<tr>
<td>Cases with early paracentral VF loss (%)</td>
<td>18</td>
<td>N/A</td>
<td>2</td>
<td>N/A</td>
</tr>
<tr>
<td>Cases with only peripheral VF loss (%)</td>
<td>52</td>
<td>N/A</td>
<td>23</td>
<td>N/A</td>
</tr>
</tbody>
</table>

CDR = vertical cup-to-disc ratio; IOP = intraocular pressure; MD = mean deviation; N/A = not available; PSD = pattern standard deviation; SD = standard deviation; VF = visual field.

*Means are mean of both eyes.

1 These statistics are based on Humphrey Visual Field Analyzer data available for 859 GLAUGEN study participants and 1369 NEIGHBOR consortium participants. Missing data reflect the fact that some participants underwent visual field tests other than Humphrey tests.

2 These statistics are based on Humphrey Visual Field Analyzer data available for 865 GLAUGEN study participants and 1371 NEIGHBOR consortium participants. Because PSD spuriously declines as MD worsens, subjects with MD worse than ~13 dB were excluded.
(6.67 vs. 5.62 dB), and more depressed mean defect (MD) on the earliest VF (−8.38 vs. −5.83 dB). Females comprised 58% of GLAUGEN cases and 52% of NEIGHBOR cases. For NEIGHBOR, 2% of cases had early paracentral VF loss, whereas in GLAUGEN, 18% of cases had early paracentral VF loss.

Overall, 10 SNPs were significant at a Bonferroni-corrected P value of 7.7×10−6 (top SNP, rs4236601: pooled P = 2.61×10−2; odds ratio [OR], 1.26; 95% confidence interval [CI], 1.16−1.38; Table 2). Four of the 10 significant SNPs are located in a regulatory region between the CAV1 and CAV2 genes, 2 are located in the 3′ untranslated region of CAV2, and 4 are in the second intron of CAV1 (Fig 2; Fig 4, available at http://aaojournal.org). The top SNP, rs4236601, is located within the binding site for the transcription factor c-FOS, and SNPs rs10256914, rs10270569, rs3779512, and rs4736740 are in DNaseI hypersensitivity sites (regions of DNA that are gene promoters or other regulatory sites) active in human vascular endothelial cells (Fig 4, available at http://aaojournal.org; regulatory regions determined from the ENCyclopedia Of DNA Elements (ENCODE) data in the UCSC Genome Browser, available at: www.genome.ucsc.edu; accessed September 9, 2012).

When stratified by gender, 9 of the 10 SNPs showed significant associations among women (top SNP, rs4236601; pooled P = 1.59×10−2; OR, 1.30; 95% CI, 1.15−1.46; Table 3), but none were significant in men (top SNP, rs17588172; pooled P = 0.002). Tests of the SNP by gender interactions yielded no significant associations between CAV1/CAV2 SNPs and POAG (P > 0.18), but the slightly stronger ORs in women are suggestive of a differential effect.

Of the total 3108 cases, 224 had early paracentral VF loss only, 993 had peripheral VF loss only, and the remaining 1891 cases

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### Table 2. Top 10 Significant CAV1/CAV2 Single Nucleotide Polymorphisms Associated with Primary Open-Angle Glaucoma in a Meta-analysis of the Combined Glaucoma Genes and Environment (GLAUGEN) and National Eye Institute Glaucoma Human Genetics Collaboration (NEIGHBOR) Dataset

| SNP       | Position | Reference Allele | Pooled Odds Ratio (95% Confidence Interval) | GLAUGEN | NEIGHBOR | Pooled *
|-----------|----------|------------------|---------------------------------------------|--------|----------|--------
| rs4236601 | 49021016 | A                | 1.26 (1.16−1.38)                            | 0.003  | 1.89×10−5 | 2.61×10−7 |
| rs6969706 | 49020340 | T                | 1.26 (1.15−1.38)                            | 0.003  | 2.54×10−4 | 3.58×10−7 |
| rs10256914| 49031338 | C                | 1.24 (1.13−1.35)                            | 9.13×10−4 | 6.77×10−4 | 3.69×10−6 |
| rs17588172| 49019406 | G                | 1.22 (1.12−1.32)                            | 7.80×10−4 | 0.001  | 5.78×10−6 |
| rs10270569| 49033664 | T                | 1.23 (1.12−1.35)                            | 0.002  | 6.70×10−4 | 6.43×10−6 |
| rs1052990 | 49012056 | G                | 1.21 (1.11−1.31)                            | 0.001  | 0.011   | 1.09×10−5 |
| rs10227696| 49024030 | A                | 1.24 (1.12−1.37)                            | 0.007  | 0.001   | 2.98×10−5 |
| rs4370748 | 49046276 | G                | 1.13 (1.11−1.36)                            | 0.007  | 0.002   | 6.68×10−5 |
| rs10278782| 49011162 | G                | 1.22 (1.10−1.35)                            | 0.02   | 0.002   | 1.49×10−4 |
| rs3779512 | 49027534 | T                | 1.15 (1.26−1.25)                            | 0.026  | 0.03    | 7.60×10−4 |

CAV1 = caveolin 1; CAV2 = caveolin 2; SNP = single nucleotide polymorphism.

*Single nucleotide polymorphisms significant at Bonferroni-corrected P < 7.7×10−6 (13 linkage disequilibrium blocks×5 analyses = 65; 0.05/65 = 7.7×10−6) appear in boldface. P > 0.20 for heterogeneity between GLAUGEN and NEIGHBOR for all SNPs.

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### Table 3. Top 10 Significant CAV1/CAV2 Single Nucleotide Polymorphisms Overall Associated with Primary Open-Angle Glaucoma in Women Only and Men Only in a Meta-analysis of the Combined Glaucoma Genes and Environment (GLAUGEN) and National Eye Institute Glaucoma Human Genetics Collaboration (NEIGHBOR) Dataset

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Reference Allele</th>
<th>Women Only (1682 Cases and 1937 Controls)</th>
<th>Men Only (1426 Cases and 1493 Controls)</th>
<th>SNP×Gender Interaction P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4236601</td>
<td>49021016</td>
<td>A</td>
<td>1.30 (1.15−1.46)</td>
<td>1.23 (1.07−1.40)</td>
<td>0.003</td>
</tr>
<tr>
<td>rs6969706</td>
<td>49020340</td>
<td>T</td>
<td>1.29 (1.15−1.46)</td>
<td>1.22 (1.07−1.40)</td>
<td>0.004</td>
</tr>
<tr>
<td>rs10256914</td>
<td>49031338</td>
<td>C</td>
<td>1.29 (1.14−1.45)</td>
<td>1.18 (1.03−1.35)</td>
<td>0.02</td>
</tr>
<tr>
<td>rs17588172</td>
<td>49019406</td>
<td>G</td>
<td>1.22 (1.09−1.36)</td>
<td>1.23 (1.08−1.40)</td>
<td>0.002</td>
</tr>
<tr>
<td>rs10270569</td>
<td>49033664</td>
<td>T</td>
<td>1.28 (1.14−1.45)</td>
<td>1.17 (1.02−1.34)</td>
<td>0.02</td>
</tr>
<tr>
<td>rs1052990</td>
<td>49012056</td>
<td>G</td>
<td>1.21 (1.08−1.35)</td>
<td>1.22 (1.07−1.38)</td>
<td>0.003</td>
</tr>
<tr>
<td>rs10227696</td>
<td>49020430</td>
<td>A</td>
<td>1.35 (1.18−1.54)</td>
<td>1.12 (0.96−1.31)</td>
<td>0.14</td>
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<tr>
<td>rs4370748</td>
<td>49046276</td>
<td>G</td>
<td>1.33 (1.17−1.53)</td>
<td>1.11 (0.95−1.29)</td>
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<tr>
<td>rs10278782</td>
<td>49011162</td>
<td>G</td>
<td>1.32 (1.15−1.51)</td>
<td>1.10 (0.94−1.28)</td>
<td>0.22</td>
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<tr>
<td>rs3779512</td>
<td>49027534</td>
<td>T</td>
<td>1.22 (1.09−1.36)</td>
<td>1.07 (0.95−1.22)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

CAV1 = caveolin 1; CAV2 = caveolin 2; SNP = single nucleotide polymorphism.

*Single nucleotide polymorphisms significant at Bonferroni-corrected P < 7.7×10−6 (13 linkage disequilibrium blocks×5 analyses = 65; 0.05/65 = 7.7×10−6) appear in boldface. P > 0.20 for heterogeneity between GLAUGEN and NEIGHBOR for all SNPs.
Table 4. Top 10 Significant CAV1/CAV2 Single Nucleotide Polymorphisms Overall Associated with 2 Subtypes of Primary Open-Angle Glaucoma Defined by Location of Visual Field Defects (Paracentral versus Peripheral) in Meta-analysis of the Combined Glaucoma Genes and Environment (GLAUGEN) and National Eye Institute Glaucoma Human Genetics Collaboration (NEIGHBOR) Dataset

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Reference Allele</th>
<th>Pooled Odds Ratio (95% Confidence Interval)</th>
<th>Pooled P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4236601</td>
<td>49021016</td>
<td>A</td>
<td>1.53 (1.23–1.91)</td>
<td>1.45×10⁻⁴</td>
</tr>
<tr>
<td>rs6997066</td>
<td>49020340</td>
<td>T</td>
<td>1.53 (1.23–1.91)</td>
<td>1.58×10⁻⁴</td>
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<tr>
<td>rs10256914</td>
<td>49031338</td>
<td>C</td>
<td>1.47 (1.18–1.84)</td>
<td>5.49×10⁻⁴</td>
</tr>
<tr>
<td>rs17588172</td>
<td>49019406</td>
<td>G</td>
<td>1.52 (1.23–1.87)</td>
<td>1.07×10⁻⁴</td>
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<tr>
<td>rs10275069</td>
<td>49033664</td>
<td>T</td>
<td>1.42 (1.14–1.77)</td>
<td>0.002</td>
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<tr>
<td>rs1025990</td>
<td>49012056</td>
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<td>1.48 (1.20–1.83)</td>
<td>2.89×10⁻⁴</td>
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<tr>
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<td>A</td>
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<tr>
<td>rs4730748</td>
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<td>49011162</td>
<td>G</td>
<td>1.29 (1.01–1.66)</td>
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<tr>
<td>rs3779512</td>
<td>49027534</td>
<td>T</td>
<td>1.17 (0.95–1.44)</td>
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Discussion

In this study, we confirmed the association between genetic variants in the CAV1/CAV2 genomic region and POAG overall and have shown that these associations may differ by gender and for subtypes of POAG defined by pattern of VF loss. Our group initially replicated the CAV1/CAV2 findings in the GLAUGEN study alone, and here we have shown that meta-analyzing the results from the GLAUGEN study and with the NEIGHBOR study confirmed the association between CAV1/CAV2 genomic region SNPs and POAG overall. For example, for the CAV1/CAV2 SNP rs4236601, the strength of statistical association was enhanced in the combined dataset (pooled P = 2.61×10⁻⁷) when compared with either the GLAUGEN (P = 0.003) or NEIGHBOR (P = 1.89×10⁻⁵) dataset alone. The differences in the statistical strength of the association in GLAUGEN and NEIGHBOR probably reflects the different case numbers in each cohort (Table 5, available at http://aaojournal.org).29 Our combined GLAUGEN and NEIGHBOR analysis is the largest POAG case-control sample currently available. It is possible that studies failing to replicate the POAG association with CAV1/CAV2 SNPs were underpowered because of smaller sample size. It is also interesting that the robust association initially observed in the Icelandic population included fewer cases and hence had lower power (n = 1263 cases; power, 61%; P = 5.0×10⁻¹⁰) than the study combining GLAUGEN and NEIGHBOR (n = 3108 cases; power, 86%; P = 2.61×10⁻⁷), yet the observed associations were more significant than in the GLAUGEN and NEIGHBOR combined dataset (Table 5, available at http://aaojournal.org).8,29 This could be the result of a founder effect or a stronger allele effect in the Icelandic population.

We found greater significance and stronger associations between the CAV1/CAV2 region SNPs and POAG in women than in men, supporting the impact of estrogen on the nitric oxide pathway. Previously, our group found that NOS3 interacts with reproductive factors such as age at menarche and postmenopausal hormone use.3 Similarly, Magalhães da Silva et al10 found an association between NOS3 SNPs and POAG in women but not in men. Because the gene product of NOS3 (eNOS) directly interacts with caveolin 1, these genetic associations may reflect the altered protein interactions that influence the risk of POAG, especially in women.

We also found, despite a small number of cases, significant and stronger associations between the CAV1/CAV2 region and POAG cases with early paracentral VF loss. Manifest VF loss in glaucoma commonly begins peripherally and proceeds toward the center of vision; however, visual loss can commence in the paracentral region. This type of VF deficit can decrease quality of life substantially, making reading and driving more difficult.31,32 Some...
studies, although not all,33–35 have suggested that paracentral VF loss is more likely to develop in patients with IOP levels in the normal range (<22 mmHg), indicating that risk factors other than increased IOP may contribute to this POAG subtype.36,37 One such factor is p53—a functional polymorphism in p53 was found to be associated with POAG and paracentral VF loss.38 A p53 SNP, which is thought to be functionally proapoptotic, may render the metabolically active maculopapillary nerve fiber bundles vulnerable to cell death, resulting in paracentral VF loss, seen in some POAG cases. Another major factor is systemic vascular dysregulation, which has been associated with early paracentral VF loss.25 The CAV1/CAV2–NOS3 pathway could contribute to abnormalities in systemic vascular tone and vasospastic phenomena. Recently, SNPs located in a genomic region near the GUCY1A3/GUCY1B3 genes coding for soluble guanylyl cyclase also have been associated with paracentral VF loss in POAG.26 Interestingly, the GUCY1A3/GUCY1B3 genomic region was associated with paracentral VF primarily in women, and soluble guanylyl cyclase serves as the intracellular receptor for nitric oxide, downstream of the interaction between eNOS and caveolin 1. CAV1 knockout mice have been studied in vascular-related diseases such as atherosclerosis and pulmonary hypertension, indicating a role for caveolin 1 in endothelial cell dysfunction, but the ocular phenotype of this mouse model has not been explored.39,40 Thus, more research into the genetic factors that determine vascular dysregulation in relation to POAG with paracentral VF loss is warranted.

The intergenic region between CAV1/CAV2 contains several regulatory elements including H3K27Ac histone marks (indicating an active regulatory region), DNaseI hypersensitivity sites, and transcription factor binding sites (Fig 2; Fig 4, available at http://aaojournal.org). rs4236601 is the top SNP in our analysis and also in the Icelandic study.8 This SNP falls in the regulatory region 5′ of CAV1. Several transcription factors are predicted to bind in this region, including c-FOS, a transcription factor known to be active in vascular endothelial cells, especially in response to shear stress.41,42 Additionally, the DNaseI sites in this region are active in human vascular endothelial cell lines, and several of the significantly associated SNPs are located in these active regulatory sites. Although preliminary, these results suggest that the associated SNPs may contribute to regulation of CAV1 and CAV2 gene expression in human vascular endothelial cells.

There are several limitations to our study. The NEIGHBOR study contained very few cases with early paracentral VF defects. Most NEIGHBOR participants with POAG were prevalent clinic cases, which made it difficult to obtain VFs at initial disease onset to determine the type of early VF loss. Many of the GLAUGEN cases were incident cases identified during prospective follow-up of a population for several disease end points, including glaucoma. Thus, there was greater opportunity in GLAUGEN to access the initial VF that showed glaucomatous loss. Test results of the SNP and gender interactions were negative because the tests may have been underpowered or because the true interaction involves some other gender-specific trait that remains unknown. It could also be argued that our subgroup analyses may be underpowered and hence the differential significance between women only and men only and between paracentral VF loss and peripheral VF loss could represent false-negative results. Despite the smaller case numbers, however, the women only and men only analyses both were powered adequately to detect a significant association (power, 93% and 84%, respectively; Table 5, available at http://aaojournal.org).29 The paracentral VF loss analysis was underpowered, with a power of 31%, but still found significant associations. The peripheral VF loss analysis did have adequate power (99%). Thus, we can conclude that the differences found in the subgroup analyses most likely are not spurious.

In this study, we confirmed the association of CAV1/CAV2 SNPs with POAG overall and found additional evidence that the relationship between CAV1/CAV2 and POAG may be stronger in women and for POAG with early paracentral VF defects only. Additionally, this study contributes to the emerging evidence that the nitric oxide signaling pathway plays an important role in POAG pathogenesis. Further study of the impact of CAV1/CAV2 genetic variation on nitric oxide signaling could lead to new therapeutic targets for the treatment of POAG.

References


Footnotes and Financial Disclosures

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