Endothelial Nitric Oxide Synthase Gene Variants and Primary Open-Angle Glaucoma

Interactions With Hypertension, Alcohol Intake, and Cigarette Smoking

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Objective: To evaluate whether an association between risk of any of the factors of hypertension, alcohol intake, and cigarette smoking and the risk of primary open-angle glaucoma (POAG) depended on nitric oxide synthase 3 (NOS3) gene variants.

Methods: Two functional single-nucleotide polymorphisms (SNPs) (T-786C [rs2070744] and Glu298Asp [rs1799983]) and 2 tagging SNPs (rs7830 and rs3918188) were evaluated in nested case-control studies from the Nurses’ Health Study (1980-2002) and the Health Professionals’ Follow-up Study (1986-2002). Participants were 40 years of age or older and white, and were followed up biennially. We included 527 incident case patients with POAG and 1539 control participants, matched by cohort, age, and eye examination at the matched case patients’ diagnosis dates. Cohort-specific relative risks were estimated using multivariable conditional logistic regression and were pooled using meta-analytic methods.

Results: The association between hypertension and POAG depended on T-786C SNP variants. Compared with TT homozygotes without hypertension, the TT homozygotes with hypertension were at significantly higher risk of POAG (relative risk, 1.45 [95% confidence interval, 1.01-2.08]); however, among carriers of the variant (C) allele, hypertension was not associated with POAG ($P_{interaction}=.007$). Similarly, compared with CC homozygotes with the rs7830 tagging SNP who never smoked, CC homozygotes who were past or current smokers were at significantly higher risk of POAG (relative risk, 1.63 [95% confidence interval, 1.15-2.31]); however, among carriers of the variant allele (A), smoking was not associated with POAG ($P_{interaction}=.004$). Interactions were not observed with alcohol intake.

Conclusions: The associations between hypertension and cigarette smoking in relation to POAG depended on NOS3 SNPs.

Arch Ophthalmol. 2011;129(6):773-780

The nitric oxide synthase 3 (NOS3) enzyme catalyzes the production of nitric oxide, which influences the tone of luminal structures with smooth muscle. NOS3 is of interest in glaucoma because this isoform is in the human outflow pathway and in the ocular endothelial cells in the vasculature for retinal ganglion cells. Dysfunction in the ocular vascular endothelium and in the trabecular meshwork cell relaxation could lead to primary open-angle glaucoma (POAG). Su et al reported that patients with POAG, including both those with normal intraocular pressure (IOP) and those with elevated IOP, failed to exhibit flow-mediated vasodilation. Among cases of normal-tension glaucoma, Henry et al demonstrated that the brachial artery failed to dilate in response to acetylcholine, which triggers endothelial cell–mediated relaxation. Feke and Pasquale documented unstable retinal blood flow in response to physiologic alterations in ocular perfusion pressure, which implicates dysfunction in nitric oxide–mediated responses. Nitric oxide also influences trabecular meshwork cell volume and outflow facility, which, in turn, influences IOP, an established risk factor for POAG.

Although single-nucleotide polymorphisms (SNPs) in the NOS3 gene influence nitric oxide levels, exogenous factors such as systemic hypertension, cigarette smoking, and alcohol consumption may also alter endothelial cell–derived luminal tone, partially via nitric oxide–dependent mechanisms.

Therefore, we evaluated whether an association between any of the factors of hypertension, alcohol intake, and cigarette smoking and the risk of POAG depended on nitric oxide synthase 3 (NOS3) gene variants. We used data from 2 large case-
control studies nested within the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS), in which exposure information was collected prospectively prior to any diagnoses of POAG.

**METHODS**

**STUDY POPULATION**

The NHS began in 1976, when 121,700 US-registered nurses (aged 30-55 years) returned a questionnaire on health-related exposures.29 The HPFS started in 1986 with 51,529 US male health professionals (aged 40-75 years) who responded to a similar health questionnaire that was mailed to them. Participants have been followed with the use of biennial questionnaires to update information on lifestyle factors and newly diagnosed illnesses, such as glaucoma.30 Follow-up rates were high (>95%) of the total possible person-time through 2002. The human research committees of the Brigham and Women's Hospital, the Massachusetts Eye and Ear Infirmary, and the Harvard School of Public Health approved our study.

**BLOOD AND CHEEK SAMPLE COLLECTION**

From 1989 to 1990, blood samples were obtained from 32,826 of 121,700 women (27%), and from 1993 to 1995, blood samples were obtained from 18,225 of 51,529 men (33%). From 2001 to 2004, buccal cell samples were obtained from 29,684 women who did not provide a blood sample. The follow-up rate has been greater than 95% of the total possible person-time for both of these subcohorts.

Blood samples were obtained with heparin sodium as the anticoagulant. They were sent by mail within 24 hours of being obtained; aliquoted into plasma, red blood cells, and buffy coat components; and stored in liquid nitrogen freezers. All buccal cell samples were obtained using a single “swish-and-spit” procedure. Subjects were provided a small bottle of mouthwash and a small cup with a cap seal and were asked to swish the mouthwash and then spit into the cup, which was returned by mail.24 Within a week of receipt, samples were processed and DNA was extracted.

**CASE AND CONTROL ASCERTAINMENT**

First, we ascertained POAG cases with the biennial questionnaire, in which we asked whether participants received an eye examination and a diagnosis of “glaucoma.” Second, we sought permission from participants with self-reported glaucoma to retrieve their medical records. We contacted the eye care providers who made the diagnosis for all visual field (VF) tests to date and for the completion of a glaucoma questionnaire; this questionnaire included items for maximal IOP, the status of the filtration apparatus, optic nerve structural information, and information on prior ophthalmic surgery and/or any VF loss. Relevant medical records were also accepted in lieu of questionnaires. To determine case status, all the ophthalmic information from questionnaires or medical records and from VF test results were evaluated in a standardized manner by a glaucoma specialist (L.R.P.).

Only participants with “definite” or “probable” POAG were included as case patients. For definite POAG cases, we required documentation of gonioscopy showing that angles were not occludable in either eye; slitlamp biomicroscopy showing no indication in either eye of pigment dispersion syndrome, uveitis, exfoliation syndrome, trauma, or rubesis; and 2 or more reliable VF test results showing reproduced defects that were consistent with glaucoma. For probable POAG cases, the slitlamp examination and VF criteria were also required, but documentation of pupil dilation without subsequent adverse events was accepted instead of gonioscopy. Among the case patients, more than 70% met the criteria for “definite POAG.” For VF tests, there was no requirement for the type of perimetry performed; however, in 95% of cases, full static threshold testing was completed, and in less than 1% of cases, kinetic VF tests were used. For static threshold or suprathreshold testing, we considered the VF test reliable if the fixation loss rate was 33% or less, if the false-positive rate was 20% or less, and if the false-negative rate was 20% or less. For kinetic VF tests, we considered the field reliable unless there was a notation by the examiner to the contrary.

We included 527 case patients with glaucoma and 1539 control participants (373 NHS case patients and 1078 control participants; 154 HPFS case patients and 461 control participants) who were at least 40 years of age and white (<20 case patients were of Latino ethnicity). The control participants were matched by sex, type of DNA sample (blood or cheek cell), year of birth, and ethnicity (Latino or not), and they were required to have had an eye examination during the same period as the diagnosis of the matched case patient. Approximately 3 control participants were matched to each case patient, using incidence density sampling.

**GENOTYPING**

Two functional SNPs (T-786C [rs2070744] and Glu298Asp [rs1799983]) and 2 tagging SNPs (rs3918188 and rs7830) were genotyped. The tagging SNPs corresponded to the NOS3 linkage disequilibrium blocks and were selected using Haploview version 4.1 (Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, Massachusetts) according to the HapMap release 22 data from the CEU population,25 with the minimum minor allele frequency set to 0.01. Along with the 2 functional SNPs, the 2 tagging SNPs (rs3918188 and rs7830) captured the majority (88%) of alleles at $r^2 > 0.8$ across the whole gene, including the 5’ and 3’ untranslated regions.

For DNA extraction, 50 µL ofuffy coat or 20 µL of cheek cells were diluted with 150 µL of phosphate-buffered saline and processed via the QIAmp (Qiagen Inc, Chatsworth, California). Genotyping was performed by quantitative polymerase chain reaction (TaqMan Assay; Applied Biosystems Inc, Foster City, California). The reverse transcription–polymerase chain reaction amplification was performed with the ABI Prism 7000 Sequence Detection System (Applied Biosystems Inc). The thermal cycler parameters (model 2720; Applied Biosystems Inc) were set per manufacturer’s instructions. The genotyping success rate was more than 90% for all 4 SNPs included in our study. Plates that passed quality-control measures (including Hardy-Weinberg equilibrium tests) were included, and in 5% of samples that underwent repeat genotyping, there was more than 95% concordance on genotyping calls.

**ASSESSMENT OF HYPERTENSION, CIGARETTE SMOKING, AND ALCOHOL INTAKE**

**Hypertension**

In the baseline questionnaires, participants were asked whether they received a physician diagnosis of hypertension during the preceding 2 years. Self-reported hypertension was previously validated in the NHS26 and the HPFS.27
Cigarette Smoking

In the NHS, we first ascertained the participants’ smoking status (current, past, or never smoker) in 1976. In the HPFS, we obtained information on smoking in 1986. In subsequent 2-year follow-up questionnaires, we updated participants’ smoking status.

Alcohol Intake

Semi-quantitative food frequency questionnaires were administered every 4 years during the study periods in the NHS and the HPFS. For alcohol assessment, the food frequency questionnaire included separate items for beer (bottle or can, containing 13.2 g of alcohol), wine (4-oz glass with 10.8 g of alcohol), and liquor (1 drink or shot with 15.1 g of alcohol). From 1984 in the NHS and from 1986 in the HPFS, consumption of red wine and consumption of white wine were asked separately. The total alcohol intake for each participant was computed by summing the contributions from beer, wine, and liquor, taking into account the frequency of consumption. The mean intake of alcohol based on the food frequency questionnaire and the diet records were very similar (9.0 g/d), and the correlation between methods was 0.90 in the NHS and 0.86 in the HPFS.30

STATISTICAL METHODS

We analyzed the cohort-specific data separately with conditional logistic regression, adjusting for potential confounders. Then, we pooled the results using meta-analytic methods, incorporating random effects.29 We used SAS version 9.1.3 (SAS Institute Inc, Cary, North Carolina) for analyses, and a value of P < .05 was considered statistically significant. In addition, to address the multiple testing issue, we also calculated adjusted P values using an optimized false discovery rate approach.30

Information on exposures and potential confounders was obtained from the biennial questionnaires and was updated through the questionnaire immediately before the diagnosis date of the index case patient. For alcohol intake, we averaged the intake data from all the available food frequency questionnaires up to the date of the diagnosis of the index case patient. Potential confounders were family history of glaucoma, body mass index (calculated as weight in kilograms divided by height in meters squared); NA, not applicable.

Sex, No. 373 1078
Women 154 461
Men
Age, mean, y
Women 64.2 64.2
Men 67.1 67.1
Family history of glaucoma, %
Women 35.4 12.5
Men 30.1 11.3
Diabetes, %
Women 7.7 5.2
Men 8.0 5.5
Obesity (BMI ≥30), %
Women 13.7 15.2
Men 9.5 10.9
Hypertension, %
Women 37.4 39.4
Men 37.7 34.8
≥30 pack-years of smoking, %
Women 17.4 18.6
Men 19.4 23.4
Caffeine intake, mean, mg/d
Women 310 307
Men 223 226
Alcohol intake, mean, g/d
Women 6.0 6.2
Men 11.3 12.9
Reported eye examinations, a mean, No.
Women 3.1 3.2
Men 3.2 3.2
Current postmenopausal hormone use, %
Women 39.6 42.4
Men NA NA

The characteristics of the case patients with POAG and the matched control participants (as of the index case patient’s date of diagnosis) were similar (Table 1). Compared with the control participants, the case patients had a higher frequency of family history of glaucoma and self-reported diagnosis of diabetes; however, the case patients were somewhat less likely to smoke, drink alcohol, or be obese. Compared with the female control participants, the female case patients were less likely to be current postmenopausal hormone users.

SUMMARY OF MAIN EFFECTS OF NOS3 SNPs

The main effects of the NOS3 SNPs in relation to POAG overall have been previously reported.11 Briefly, we observed that none were associated with overall POAG (Table 2). In secondary analyses, we found that the
Table 2. Effect Modification by Hypertension on the Associations of Selected NOS3 Single-Nucleotide Polymorphisms and Polymorphisms and Primary Open-Angle Glaucoma

<table>
<thead>
<tr>
<th>SNP and Genotype</th>
<th>Overall Association Between SNP Genotypes and POAG</th>
<th>Female Cases vs Female Controls, No.</th>
<th>Male Cases vs Male Controls, No.</th>
<th>Pooled RR (95% CI)</th>
<th>P Value for Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No Hypertension</td>
<td>Hypertension</td>
<td>No Hypertension</td>
<td>Hypertension</td>
</tr>
<tr>
<td>T-786C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>1 [Reference]</td>
<td>73 vs 258</td>
<td>64 vs 156</td>
<td>38 vs 110</td>
<td>27 vs 56</td>
</tr>
<tr>
<td>TC</td>
<td>1.05 (0.83-1.34)</td>
<td>112 vs 280</td>
<td>54 vs 178</td>
<td>32 vs 136</td>
<td>34 vs 79</td>
</tr>
<tr>
<td>CC</td>
<td>1.00 (0.64-1.55)</td>
<td>42 vs 100</td>
<td>20 vs 72</td>
<td>19 vs 51</td>
<td>3 vs 25</td>
</tr>
<tr>
<td>Glu298Asp(rs7830)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>1 [Reference]</td>
<td>97 vs 301</td>
<td>67 vs 178</td>
<td>37 vs 128</td>
<td>35 vs 75</td>
</tr>
<tr>
<td>GT</td>
<td>1.04 (0.82-1.32)</td>
<td>97 vs 252</td>
<td>46 vs 176</td>
<td>36 vs 115</td>
<td>24 vs 55</td>
</tr>
<tr>
<td>TT</td>
<td>1.33 (0.94-1.86)</td>
<td>35 vs 71</td>
<td>21 vs 41</td>
<td>11 vs 37</td>
<td>4 vs 15</td>
</tr>
<tr>
<td>rs3918188</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>1 [Reference]</td>
<td>114 vs 267</td>
<td>59 vs 164</td>
<td>42 vs 131</td>
<td>27 vs 60</td>
</tr>
<tr>
<td>CA</td>
<td>0.74 (0.59-0.94)</td>
<td>93 vs 301</td>
<td>64 vs 196</td>
<td>28 vs 134</td>
<td>25 vs 84</td>
</tr>
<tr>
<td>AA</td>
<td>0.99 (0.46-2.12)</td>
<td>24 vs 87</td>
<td>17 vs 59</td>
<td>20 vs 36</td>
<td>12 vs 6</td>
</tr>
<tr>
<td>rs7830</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>1 [Reference]</td>
<td>106 vs 273</td>
<td>66 vs 188</td>
<td>44 vs 127</td>
<td>21 vs 74</td>
</tr>
<tr>
<td>CA</td>
<td>0.89 (0.58-1.37)</td>
<td>94 vs 307</td>
<td>52 vs 192</td>
<td>39 vs 152</td>
<td>32 vs 56</td>
</tr>
<tr>
<td>AA</td>
<td>1.16 (0.82-1.64)</td>
<td>33 vs 75</td>
<td>22 vs 43</td>
<td>7 vs 20</td>
<td>11 vs 30</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; POAG, primary-open-angle glaucoma; RR, relative risk; SNP, single-nucleotide polymorphism.

a The numbers of case patients and control participants may differ with each analysis because the numbers with missing genotype information for each SNP differed.

b The adjusted P value for the false discovery rate is .04.

T-786C polymorphism was associated with high-tension POAG only among the women (relative risk [RR], 1.80 [95% confidence interval {CI}, 1.14-2.85]; P = .02 for trend with increasing variant allele), and so were the polymorphisms in the tagging SNP rs3918188 (RR, 0.48 [95% CI, 0.28-0.82]; P = .001 for trend with increasing variant allele). In relation to normal-tension glaucoma, for the T-786C polymorphism, the pooled RR for the CC homozygote was 0.44 (95% CI, 0.22-0.87), and the P value for trend was .03. In all main analyses, associations were consistent in the NHS and the HPFS, and all results could be pooled using meta-analytic methods.

**EFFECT MODIFICATION WITH HYPERTENSION**

None of the 4 SNPs were associated with hypertension (P values ranged from .57 to .98). We observed that the association between hypertension and POAG differed by the promoter T-786C SNP (P interaction = .007). Compared with wild-type TT homozygotes without hypertension, wild-type TT homozygotes with hypertension were at significantly higher risk of POAG (pooled RR, 1.50 [95% CI, 1.04-2.18]) (Table 2). However, carriers of the variant (C) allele with hypertension were not at significantly elevated risk of POAG. In fact, among CC homozygotes, the RR of POAG for not having hypertension was 1.45 (95% CI, 0.97-2.16), whereas the RR for having hypertension was 0.60 (95% CI 0.13-2.82), suggesting that, in this group, having hypertension was inversely associated with POAG. None of the other SNPs showed interactions with hypertension.

We examined the RR for the genotype by hypertension interactions separately for high-tension POAG and normal-tension POAG (data not shown). Because approximately 70% of patients with POAG had high-tension POAG, the associations with high-tension POAG were similar to those of the main analyses. None of the interactions were significant for normal-tension glaucoma, likely owing to the smaller numbers of such cases.

We evaluated whether treatment for hypertension might affect the results by separately examining patients treated for hypertension (50% of patients in the NHS and 54% of patients in the HPFS) and patients untreated for hypertension (50% of patients in the NHS and 46% of patients in the HPFS). The separate results were very similar to the main analyses (data not shown).

**EFFECT MODIFICATION WITH CIGARETTE SMOKING**

With cigarette smoking, we observed that the associations with cigarette smoking status differed significantly by the rs7830 tagging SNP (P = .004) (Table 3). Compared with CC homozygotes who never smoked, CC homozygotes who were past or current smokers were at significantly higher risk of POAG (RR, 1.63 [95% CI, 1.15-2.31]); however, carriers of the variant allele (A) who were past or current smokers were not at significantly elevated risk of POAG.

**EFFECT MODIFICATION WITH ALCOHOL INTAKE**

We did not observe interactions with alcohol intake for the 4 NOS3 SNPs (Table 4). Red wine, which is high in resveratrol, has shown potential beneficial effects in an experimental study,32 so we also examined interactions...
with red wine consumption. In models controlling for total alcohol intake, greater red wine intake (at least 0.07 g of red wine per day, which was the daily median intake) did not show interactions with NOS3 SNPs ($P = .26-.79$).

### Table 3. Effect Modification by Smoking Status on the Associations of Selected NOS3 Single-Nucleotide Polymorphisms and Primary Open-Angle Glaucoma

<table>
<thead>
<tr>
<th>SNP and Genotype</th>
<th>Never Smoked</th>
<th>Past or Current Smoker</th>
<th>Never Smoked</th>
<th>Past or Current Smoker</th>
<th>Never Smoked</th>
<th>Past or Current Smoker</th>
<th>$P$ Value for Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promoter T-786C</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TT</td>
<td>61 vs 198</td>
<td>76 vs 213</td>
<td>27 vs 76</td>
<td>37 vs 82</td>
<td>1 [Reference]</td>
<td>1.35 (0.92-1.97)</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>84 vs 206</td>
<td>80 vs 248</td>
<td>24 vs 103</td>
<td>39 vs 102</td>
<td>1.03 (0.60-1.79)</td>
<td>1.27 (0.88-1.84)</td>
<td>.60</td>
</tr>
<tr>
<td>CC</td>
<td>24 vs 79</td>
<td>38 vs 92</td>
<td>11 vs 27</td>
<td>11 vs 45</td>
<td>1.13 (0.88-1.87)</td>
<td>1.19 (0.51-2.75)</td>
<td></td>
</tr>
<tr>
<td>Glu298Asp (rs1799983)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>83 vs 210</td>
<td>79 vs 265</td>
<td>35 vs 89</td>
<td>25 vs 105</td>
<td>1 [Reference]</td>
<td>0.97 (0.68-1.38)</td>
<td></td>
</tr>
<tr>
<td>GT</td>
<td>60 vs 207</td>
<td>82 vs 218</td>
<td>17 vs 75</td>
<td>41 vs 87</td>
<td>0.79 (0.54-1.16)</td>
<td>1.27 (0.75-2.17)</td>
<td>.08</td>
</tr>
<tr>
<td>TT</td>
<td>26 vs 55</td>
<td>3 vs 53</td>
<td>7 vs 22</td>
<td>8 vs 26</td>
<td>1.01 (0.60-1.72)</td>
<td>1.56 (0.94-2.57)</td>
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<tr>
<td>rs3918188</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>78 vs 200</td>
<td>93 vs 225</td>
<td>27 vs 82</td>
<td>39 vs 99</td>
<td>1 [Reference]</td>
<td>1.23 (0.86-1.77)</td>
<td></td>
</tr>
<tr>
<td>CA</td>
<td>76 vs 227</td>
<td>80 vs 265</td>
<td>22 vs 97</td>
<td>30 vs 110</td>
<td>0.77 (0.54-1.09)</td>
<td>0.84 (0.59-1.21)</td>
<td>.91</td>
</tr>
<tr>
<td>AA</td>
<td>18 vs 69</td>
<td>3 vs 77</td>
<td>13 vs 29</td>
<td>19 vs 22</td>
<td>0.90 (0.39-2.07)</td>
<td>1.34 (0.48-3.69)</td>
<td></td>
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<tr>
<td>rs7830</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>68 vs 214</td>
<td>103 vs 241</td>
<td>24 vs 103</td>
<td>40 vs 87</td>
<td>1 [Reference]</td>
<td>1.63 (1.14-2.33)</td>
<td>.004b</td>
</tr>
<tr>
<td>CA</td>
<td>72 vs 227</td>
<td>72 vs 267</td>
<td>27 vs 80</td>
<td>42 vs 118</td>
<td>1.11 (0.67-1.84)</td>
<td>1.30 (0.55-3.03)</td>
<td></td>
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<tr>
<td>AA</td>
<td>32 vs 56</td>
<td>23 vs 62</td>
<td>11 vs 24</td>
<td>6 vs 25</td>
<td>1.77 (1.09-2.87)</td>
<td>1.17 (0.67-2.03)</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: CI, confidence interval; POAG, primary-open angle glaucoma; RR, relative risk; SNP, single-nucleotide polymorphism.

* The numbers of case patients and control participants may differ with each analysis because the numbers with missing genotype information for each SNP differed.

* The adjusted $P$ value for the false discovery rate is .04.

**Comment**

In a pooled analysis of 2 nested case-control studies from large prospective cohorts, we observed that the association between the factors of hypertension and cigarette smoking and the risk of POAG depended on NOS3 SNPs. We observed “crossover” interactions in which the relations between cigarette smoking and hypertension were in the opposite direction depending on the selected NOS3 SNP genotypes. Interactions were not observed with alcohol or red wine intake.

Variations in NOS3 SNPs would affect levels of nitric oxide derived from the vascular endothelium. In POAG, high levels of nitric oxide can induce beneficial vasodilation, which leads to increased optic nerve blood flow.$^{33}$ But nitric oxide could also induce hyperperfusion damage and reactions that form peroxynitrites, free radicals that induce retinal ganglion cell death.$^{34}$ Thus, there may be biological plausibility to the observed significant crossover interaction between hypertension and the promoter T-786C SNP, which influences nitric oxide levels.$^{13,35}$ Among patients with the wild-type TT genotype, hypertension was significantly adversely associated with POAG. Hypertension may exacerbate nerve fiber layer damage caused by compensatory hyperperfusion, which has been observed in ocular hypertension$^{36}$ and early-stage POAG$^{37}$; moreover, the presence of abundant nitric oxide could also trigger peroxynitrite toxicity with hyperperfusion that leads to retinal ganglion cell apoptosis.$^{34}$ In contrast, among patients with the variant allele (C) who have reduced levels of nitric oxide,$^{33}$ hypertension could increase blood flow to the optic nerve without peroxynitrite toxicity. Also, in individuals with normal blood pressure, slight elevations in IOP result in lower ocular perfusion,$^{38-42}$ which increases the risk of POAG, and this adverse effect may be worse in individuals with a NOS3 variant that might predispose to reduced retinal vessel diameters; thus, hypertension may have either a net null effect or a mildly beneficial effect in this group. Accounting for NOS3 signaling may explain why both low blood pressure$^{11,15-45}$ and high blood pressure have been associated with POAG.$^{46,47}$ More confirmatory and mechanistic studies are warranted.

The interaction between the rs7830 tagging SNP and cigarette smoking was similar. The functional significance of the rs7830 NOS3 SNP is unknown, and the reason why other NOS3 SNPs did not interact with cigarette smoking in POAG overall is unclear. Cigarette smoking also may have both adverse and protective effects in POAG owing to the effects of nicotine on IOP, circulating endothelin-1 concentrations, vascular tone, and alterations in blood pressure.$^{48-53}$ Cigarette smoking also contributes to endothelial dysfunction through the uncoupling of the NOS3-mediated synthesis of nitric oxide, increasing oxidative stress and reducing plasma antioxidant levels.$^{54,55}$ In a previous study,$^{56}$ we observed an overall weak, nonsignificant inverse association between cigarette smoking and POAG. Given these findings, the relation between cigarette smoking and POAG may be complex.

Previously, we observed weak trends of protective associations with high consumption of alcohol.$^{37}$ Even
though alcohol and resveratrol in red wine is known to upregulate NOS3 expression, we did not observe interactions between NOS3 SNPs and alcohol or red wine intake. The levels of alcohol and red wine intake may have been too low to detect potential interactions in our study.

Limitations should be considered. First, our definition of glaucoma was based on self-reports, with confirmation from medical records and VF test results. This definition had a very high specificity because we required documentation of a reproducible defect on at least 2 reliable VF tests. This definition as of the matched case patients’ diagnosis dates, and the average number of eye examinations reported as of their selection as control participants was 3, implying that moderate or advanced glaucoma, if present, would likely have been detected. Any misclassifications of the disease would have biased the results toward the null. Third, our participants were generally healthy whites, and thus we are unable to make inferences to less healthy populations or minorities. Fourth, we lacked IOP data on the control participants, and thus we were not able to explore the interactions with perfusion pressure, which might be more etiologically relevant. Finally, it is possible that our results may not be due to chance, given the multiple comparisons. We corrected for multiple comparisons using an optimized false discovery approach; nonetheless, these findings should be interpreted with caution and confirmed in future studies, particularly with different racial/ethnic groups.

The genetic determinants of the endothelial nitric oxide signaling system may affect how other factors may contribute to POAG. Understanding the complex gene × environment interactions in POAG may serve to shed light on the etiology of this disease.

Submitted for Publication: May 11, 2010; final revision received October 18, 2010; accepted October 29, 2010. Correspondence: Louis R. Pasquale, MD, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston, MA 02114 (louis_pasquale@meei.harvard.edu).

Financial Disclosure: Dr Kang has received research funding (2008-2009) from Wyeth Pharmaceuticals.

Funding/Support: This work was supported by grants CA87969, CA55075, CA49449, EY09611, HL35464, and EY015473 from the National Institutes of Health. Dr Pasquale is also supported by a Research to Prevent Blindness Physician Scientist award.

Previous Presentation: Presented at the 2010 Association for Research in Vision and Ophthalmology Conference; May 2-6, 2010; Fort Lauderdale, Florida.

Additional Contributions: We thank the participants and staff of the Nurses’ Health Study and the Health Professionals Follow-up Study.

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Retinal Pigment Epithelial Tear in Vogt-Koyanagi-Harada Disease

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