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Endothelial Nitric Oxide Synthase Gene Variants and Primary Open-Angle Glaucoma: Interactions with Hypertension, Alcohol, and Cigarette Smoking

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Abstract

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

Methods—Two functional and two tagging single nucleotide polymorphisms (SNPs) (T-786C: rs2070744, Glu298Asp: rs1799983; rs7830; rs3918188) were evaluated in nested case-control studies from the Nurses' Health Study (followed 1980–2002) and the Health Professionals' Follow-up Study (followed 1986–2002). Participants were aged ≥ 40 years and Caucasian, who were followed biennially. We included 527 incident cases of POAG and 1539 controls, matched on cohort, age and eye exam at the matched cases' diagnosis dates. Cohort–specific relative risks (RR) were estimated using multivariable conditional logistic regression and pooled with meta-analyses.

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 (RR=1.45, 95% CI = 1.01, 2.08); however, among carriers of the variant (C) allele hypertension was not associated, or even showed protective associations (p-interaction = 0.007). Similarly, compared with CC homozygotes in the rs7830 tagging SNP who never smoked, CC homozygotes who were past or current smokers were at significantly higher risk (RR=1.63, 95% CI = 1.15, 2.31); however, among carriers of the variant allele (A), smoking was not associated (p-interaction=0.004). Interactions were not observed with alcohol intake.

Conclusions—The associations between hypertension and cigarette smoking in relation to POAG depended on NOS3 SNP polymorphisms.

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INTRODUCTION

Nitric oxide synthase 3 (NOS3) catalyzes the production of nitric oxide (NO), which influences the tone of luminal structures with smooth muscle.[1] NOS3 is of interest in glaucoma, because this isoform is in the human outflow pathway,[2] and the ocular endothelial cells in the vasculature for retinal ganglion cells.[3].

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 POAG patients, including both those with normal and elevated intraocular pressure (IOP), failed to exhibit flow-mediated vasodilation.[4] Among normal tension glaucoma cases, Henry et al.[5] demonstrated that the brachial artery failed to dilate in response to acetylcholine, which triggers endothelial cell-mediated relaxation. Feke and Pasquale[6] documented unstable retinal blood flow in response to physiologic alterations in ocular perfusion pressure, which implicates dysfunction in NO-mediated responses.[7–9] NO also influences trabecular meshwork cell volume and outflow facility[10–12] that in turn influences IOP, an established risk factor for POAG.

While single nucleotide polymorphisms (SNPs) in the NOS3 gene influence NO levels,[13, 14] exogenous factors such as systemic hypertension, cigarette smoking and alcohol consumption may also alter endothelial cell derived luminal tone, partially via NO-dependent mechanisms.[15–21] Therefore, we evaluated whether the associations with any of the factors of hypertension, alcohol intake and cigarette smoking and risk of primary open-angle glaucoma (POAG) depended on nitric oxide synthase gene (NOS3) variants. We used data from two large case control studies nested within the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS), where 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.[22] The HPFS started in 1986 with 51,529 US male health professionals (aged 40 to 75 years) who responded to a similar mailed health questionnaire. Participants have been followed with biennial questionnaires to update information on lifestyle factors and newly diagnosed illnesses, such as glaucoma.[23] Follow-up rates were high (> 95% of the total possible person-time through 2002). The Human Research Committees of Brigham & Women's Hospital, Massachusetts Eye and Ear Infirmary and the Harvard School of Public Health approved this study.

Blood and cheek sample collection

In 1989–1990, blood samples were collected from 32,826 (27%) women, and in 1993–1995, blood samples were collected from 18,225 (35%) men. In 2001–2004, among 29,684 women who did not provide a blood sample, buccal cell samples were collected. Follow-up has been >95% of the total possible person-time for both of these subcohorts.

Blood samples were collected with sodium heparin as the anticoagulant. They were returned by mail within 26 hours of blood draw, immediately centrifuged, aliquoted into plasma, red blood cells, and buffy coat components, and stored in liquid nitrogen freezers. All buccal cell samples were collected 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

We ascertained POAG cases first with the biennial questionnaires, where we asked whether participants received an eye exam and a diagnosis of "glaucoma". Second, for participants self-reporting glaucoma, we sought permission to retrieve their medical records. We contacted the diagnosing eye care provider 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, prior ophthalmic surgery, and any VF loss. Relevant medical records were also accepted in lieu of questionnaires. To determine case status, all the ophthalmic information from questionnaires/medical records and VFs were evaluated in a standardized manner by a glaucoma specialist (LRP).

Only participants with "definite" or "probable" POAG were included as cases. For definite POAG cases, we required documentation of gonioscopy showing that angles were not occludable in either eye, slit lamp biomicroscopy showing no indication in either eye of pigment dispersion syndrome, uveitis, exfoliation syndrome, trauma, or rubeosis, and ≥2 reliable VFs showing reproduced defects that were consistent with glaucoma. For probable POAG cases, the slit lamp exam and VF criteria were also required, but documentation of pupil dilation without subsequent adverse events was accepted instead of gonioscopy. Among cases, >70% met the criteria for "definite POAG". For VFs, there was no requirement for the type of perimetry performed; however, in 95% of cases, full static threshold testing was completed and in <1% of cases, kinetic VFs were used. For static threshold or suprathreshold testing, we considered the VF reliable if the fixation loss rate was ≤ 33%, the false positive rate was ≤ 20% and the false negative rate was ≤ 20%. For kinetic VFs, we considered the field reliable unless there is notation by the examiner to the contrary.

We included 527 glaucoma cases and 1539 controls (373 NHS cases and 1078 controls; 154 HPFS cases and 461 controls), who were at least 40 years in age and Caucasian (< 20 cases were of Latino ethnicity). The controls were matched on gender, type of DNA sample (blood or cheek cell), year of birth, ethnicity (Latino or not), and they were required to have had an eye exam at the same period as the diagnosis date of the matched case. Approximately three controls per case were matched to each case, using incidence density sampling.

Genotyping

Two functional SNPs (T-786C: rs2070744 and Glu298Asp: rs1799983) and two tagging SNPs (rs3918188, and rs7830) were genotyped. The tagging SNPs corresponded to the NOS3 linkage disequilibrium (LD) blocks, and were selected using Haploview (version 4.1) according to the HapMap data (release 22) from the CEU population,[25] with the minimum minor allele frequency set to 0.01. Along with the two functional SNPs, the two tagging SNPs (rs3918188 and rs7830) captured the majority (88%) of alleles at r^2 greater than 0.8 across the whole gene, including the 5′ and 3′ untranslated regions.

For DNA extraction, 50 μL of buffy coat or 20 μL of cheek cells were diluted with 150 μL of PBS and processed via the QIAmp (QIAGEN Inc., Chatsworth, CA), 96 spin blood kit protocol. Quantitative PCR approach (TaqMan Assay) was used for genotyping, according to the manufacturer's instructions. The RT-PCR amplification of genomic DNA was performed in 96 well plates with a sequence detection system (ABI Prism© 7000 Sequence Detection System; Applied Biosystems Inc., Foster City, CA). The thermal cycler (model 2720, Applied Biosystems Inc., Foster City, CA) was set at the following parameters: 50°C for 2 minutes, 95°C for 10 minutes, 92°C for 15 seconds, and 58°C for 1 minute for a total

of 60 cycles. Genotyping success rate was over 90% for all four SNPs included in this 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 >95% concordance on genotyping calls.

Assessment of hypertension, cigarette smoking, and alcohol intake

Hypertension—From the baseline questionnaires, participants have been asked whether they received a physician-diagnosis of hypertension during the preceding 2 years. Self-reported hypertension was previously validated in NHS[26] and HPFS[27].

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

Alcohol—Semiquantitative food frequency questionnaires (FFQ) were administered every 4 years during the study period in NHS and HPFS. For alcohol assessment, the FFQ included separate items for beer (bottle or can, containing 13.2g of alcohol), wine (4 oz. glass with 10.8g of alcohol) and liquor (1 drink or shot with 15.1g of alcohol). From 1984 in the NHS and from 1986 in the HPFS, red and white wine was asked separately. The total alcohol intake for each subject 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 FFQ and the diet records were very similar (9.0 g/day), and the correlation between methods was 0.90 in NHS and 0.86 in the HPFS.[28]

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 (9.1.3) for analyses, and a value of $p < 0.05$ 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. For alcohol intake, we averaged the intake data from all the available FFQ's up to the date of the diagnosis of the index case. Potential confounders were family history of glaucoma, body mass index (<22, 22–23.9, 24–25.9, 26–27.9, 28–29.9, ≥30 kg/m^2), physical activity (quartiles of activity intensity/day), self reported history (yes/no) of diabetes, and cumulatively updated caffeine intake (0–149, 150–299, 300–449, 450–600, ≥600 g/day). In the women, we additionally controlled for age at menopause (20–44, 45–50, 50–54, 54+ years) and postmenopausal hormone status (never, past, current user).

In secondary analyses, we separately analyzed the risk of "high-tension" POAG, defined as those with maximum IOP ≥ 22 mm Hg before visual field loss (67.5% of all POAG cases) and "normal-tension" POAG, defined as those with maximum IOP < 22 mm Hg before visual field loss.

Effect modification with hypertension, cigarette smoking, and alcohol intake

To evaluate effect modification, we tested the significance of the pooled estimates of the interaction terms from the multivariable conditional logistic regression models, using Wald tests.

RESULTS

The characteristics of POAG cases and their matched controls as of the index case's date of diagnosis were similar (Table 1). Cases had a higher frequency of a family history of glaucoma and self-reported diagnosis of diabetes; however, the cases were somewhat less likely to smoke, drink alcohol or be obese. Among women, the cases were less likely to be current postmenopausal hormone users.

Summary of main effects of NOS3 SNPs

The main effects of the NOS3 polymorphisms in relation to POAG overall have been previously reported. [31] Briefly, we observed that none were associated with overall POAG (Table 2). In secondary analyses, we found that the T-786C polymorphism was associated with high-tension POAG only among the women (RR= 1.80 (95% 1.14, 2.85; p-trend with increasing variant allele= 0.02), as well as polymorphisms in the tagging SNP rs3918188 (RR= 0.48 (95% CI 0.28, 0.82); p-trend with increasing variant allele= 0.0008). 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 for trend was 0.03.

In all main analyses, associations were consistent in the NHS and HPFS, and all results could be pooled meta-analytically.

Effect modification with hypertension

None of the four SNPs were associated with hypertension (p values ranged from 0.57–0.98). In relation to POAG, we observed that the association between hypertension and POAG differed by the promoter T-786C SNP polymorphisms (p for interaction = 0.007). 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), while 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 (Supplementary Table 1). As ~70% of POAG cases were 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 due to the smaller numbers of such cases.

We evaluated whether treatment for hypertension might affect the results by separately examining treated (50% in NHS and 54% in HPFS) and untreated hypertension (50% in NHS and 46% in 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 by the rs7830 tagging SNP polymorphism (p=0.004) (Table 3; Supplementary Table 2). 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 the four NOS3 SNPs with alcohol intake (Table 4; Supplementary Table 3). 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-value= 0.26–0.79).

DISCUSSION

In a pooled analysis of two nested case-control studies from large prospective cohorts, we observed that the association with hypertension and cigarette smoking in relation to POAG depended on NOS3 SNPs polymorphisms. We observed “cross-over” interactions where 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.

NOS3 codes for nitric oxide (NO) derived from vascular endothelium. In POAG, high NO levels can induce beneficial vasodilation, which leads to increased optic nerve blood flow[33], but NO could also induce hyperperfusion damage and reactions that form peroxynitrite, a free radical that induces retinal ganglion cell death[34]. Thus, there may be biological plausibility to the observed significant “cross-over” interaction between hypertension and the promoter T-786C SNP, which influences nitric oxide levels[13, 35]. Among those 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 NO could also trigger peroxynitrate toxicity with hyperperfusion that leads to retinal ganglion cell apoptosis.[34] In contrast, among those with the variant allele (C), who have reduced NO production,[13] hypertension could increase blood flow to the optic nerve without peroxynitrate toxicity. Also, in those with normal blood pressure, slight elevations in IOP results in lower ocular perfusion,[38–42] which increases the risk of POAG, and this adverse effect may be worse in those with a NOS3 variant that might predispose to reduced retinal vessel diameters; thus hypertension may either have a net null effect or a mildly beneficial effect in this group. Accounting for NOS3 signaling may explain why both low blood pressure [41, 43–45] and high blood pressure has 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, due to 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 NO, increasing oxidative stress and reducing plasma antioxidant levels [54, 55]. In a previous study, we observed an overall weak non-significant inverse association between cigarette smoking on POAG risk.[56] 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.[57] Even though alcohol and resveratrol in red wine is known to upregulate NOS3 expression,[58, 59] we did not observe interactions between NOS3 SNP polymorphisms and alcohol or red wine. The levels of alcohol and red wine intake may have been too low to detect potential interactions in this study.

Limitations should be considered. First, our glaucoma definition was based on self-report with confirmation with medical records and visual fields. This definition has very high specificity, as we required documentation of reproducible defect on at least 2 reliable visual field tests. Given the insidiousness of glaucoma, some controls might have had undiagnosed glaucoma. However, it is unlikely to have had a major influence on our results, as the prevalence of glaucoma in adults over age 40 is 1.3% in Caucasians.[60] Furthermore, our controls were required to have had an eye exam as of the matched cases' diagnosis dates, and the average number of eye exams reported as of their selection as controls were three exams, implying that advanced glaucoma, if present, would likely have been detected. Any misclassifications of the disease would have biased the results towards the null. Third, our participants were generally healthy Caucasians and thus we are unable to make inferences to less healthy populations or minorities. Fourth, we lacked IOP data on the controls, 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 be due to chance, given the multiple comparisons. We did not explicitly correct for multiple comparisons, and therefore, these findings should be interpreted with caution and confirmed in future studies, particularly with different racial/ethnic groups.

The genetic determinants of the endothelial NO 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Characteristics of cases of primary open-angle glaucoma and their matched controls as of diagnosis date

		Cases	Controls
Number	Women	373	1078
	Men	154	461
Age (mean, yr)	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 (Body mass index ≥ 30 kg/m^2, %)	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 (mean, mg/day)	Women	310	307
	Men	223	226
Alcohol intake (mean, g/day)	Women	6.0	6.2
	Men	11.3	12.9
Number of reported eye exams[†]	Women	3.1	3.2
	Men	3.2	3.2
Current postmenopausal hormone use	Women	39.6	42.4
	Men	--	--

[†]Eye exams have been asked every two years, seven times from 1990 – 2002; the number represents the number of eye exams reported as of the period of the diagnosis date of the index case in matched case control sets.

Table 2

Effect modification by hypertension on the associations of selected NOS3 polymorphisms and primary open-angle glaucoma (POAG)*

		OVERALL ASSOCIATION BETWEEN SNP GENOTYPES AND POAG	ASSOCIATIONS OF SNP * HYPERTENSION INTERACTIONS AND POAG			
		Pooled	Women	Men	Pooled	
SNP	Genotype	All	All	All	No hypertension	Hypertension
		RR (95% CI)	Number of Cases/Controls by Hypertension status†	Number of Cases/Controls by Hypertension status†	RR (95% CI)	RR (95% CI)
Promoter −786C/T	TT	1.00 (ref)	HTN−: 73/258 HTN+: 64/156	HTN−: 38/110 HTN−: 27/56	1.00	1.50 (1.04, 2.16)
	TC	1.05 (0.83, 1.34)	HTN−: 112/280 HTN+: 54/178	HTN−: 32/136 HTN−: 34/79	1.20 (0.75, 1.91)	1.30 (0.81, 2.07)
	CC	1.00 (0.64, 1.55)	HTN−: 42/100 HTN+: 20/72	HTN−: 19/51 HTN−: 3/25	1.45 (0.97, 2.16)	0.60 (0.13, 2.82)
		P-trend=0.94				**P-interaction =0.007‡**
Glu298Asp (rs1799983)	GG	1.00 (ref)	HTN−: 97/301 HTN+: 67178	HTN−: 37/128 HTN+: 35/75	1.00	1.42 (0.84, 2.40)
	GT	1.04 (0.82, 1.32)	HTN−: 97/252 HTN+: 46/176	HTN−: 36/115 HTN+: 24/55	1.21 (0.89, 1.64)	1.29 (0.58, 2.88)
	TT	1.33 (0.94, 1.86)	HTN−: 35/71 HTN+: 21/41	HTN−: 11/37 HTN+: 4/15	1.50 (0.99, 2.29)	1.42 (0.79, 2.54)
		P-trend=0.16				**P-interaction =0.18**
rs3918188	CC	1.00 (ref)	HTN−:114/267 HTN+: 59/164	HTN−: 42/131 HTN+: 27/60	1.00	1.05 (0.74, 1.50)
	CA	0.74 (0.59, 0.94)	HTN−: 93/301 HTN+:64/196	HTN−: 28/134 HTN+: 25/84	0.72 (0.53, 0.96)	0.82 (0.58, 1.15)
	AA	0.99 (0.46, 2.12)	HTN−:24/87 HTN+:17/59	HTN−: 20/36 HTN+: 12/16	0.96 (0.49, 1.89)	1.12 (0.30, 4.18)
		P-trend=0.56				**P-interaction =0.95**
rs7830	CC	1.00 (ref)	HTN−:106/273 HTN+: 66/188	HTN−: 44/127 HTN+: 21/74	1.00	0.82 (0.58, 1.15)
	CA	0.89 (0.58, 1.37)	HTN−: 94/307 HTN+:52/192	HTN−: 39/152 HTN+: 32/56	0.74 (0.55, 0.99)	1.14 (0.42, 3.14)
	AA	1.16 (0.82, 1.64)	HTN−: 33/75 HTN+:22/43	HTN−: 7/20 HTN+: 11/30	0.97 (0.61, 1.54)	1.21 (0.72, 2.03)

		OVERALL ASSOCIATION BETWEEN SNP GENOTYPES AND POAG	ASSOCIATIONS OF SNP * HYPERTENSION INTERACTIONS AND POAG			
		Pooled	Women	Men	Pooled	
SNP	Genotype	All	All	All	No hypertension	Hypertension
		RR (95% CI)	Number of Cases/Controls by Hypertension status†	Number of Cases/Controls by Hypertension status†	RR (95% CI)	RR (95% CI)
		P-trend=0.99				P-interaction =0.13

*Abbreviation: POAG: primary-open angle glaucoma; HTN: hypertension

†The number of cases and controls may differ with each analysis, as the numbers with missing genotype information for each SNP differed

‡The adjusted p-value for false discovery rate = 0.04

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Table 3

Effect modification by smoking status on the associations of selected NOS3 polymorphisms and POAG

				Pooled	
SNP	**Genotype**	**Women**	**Men**	**No Smoking**	**Past or Current Smoking**
		Number of Cases/Controls by Smoking status	**Number of Cases/Controls by Smoking status**	**RR (95% CI)**	**RR (95% CI)**
Promoter −786C/T	TT	SMK−: 61/198 SMK+: 76/213	SMK−: 27/76 SMK−: 37/82	1.00	1.35 (0.92, 1.97)
	TC	SMK−: 84/206 SMK+: 80/248	SMK−: 24/103 SMK−: 39/102	1.03 (0.60, 1.79)	1.27 (0.88, 1.84)
	CC	SMK−: 24/79 SMK+: 38/92	SMK−: 11/27 SMK−: 11/45	1.13 (0.68, 1.87)	1.19 (0.51, 2.75)
					P-interaction =0.60
*Glu298Asp (*rs1799983)	GG	SMK−: 83/210 SMK+: 79/265	SMK−: 35/89 SMK+: 35/105	1.00	0.97 (0.68, 1.38)
	GT	SMK−: 60/207 SMK+: 82/218	SMK−: 17/75 SMK+: 41/87	0.79 (0.54, 1.16)	1.27 (0.75, 2.17)
	TT	SMK−: 26/55 SMK+: 30/53	SMK−: 7/22 SMK+: 8/26	1.01 (0.60, 1.72)	1.56 (0.94, 2.57)
					P-interaction =0.08
rs3918188	CC	SMK−:78/200 SMK+: 93/225	SMK−: 27/82 SMK+: 39/99	1.00	1.23 (0.86, 1.77)
	CA	SMK−: 76/227 SMK+:80/265	SMK−: 22/97 SMK+: 30/110	0.77 (0.54, 1.09)	0.84 (0.59, 1.21)
	AA	SMK−:18/69 SMK+:23/77	SMK−: 13/29 SMK+: 19/22	0.90 (0.39, 2.07)	1.34 (0.48, 3.69)
					P-interaction =0.91
rs7830	CC	SMK−: 68/214 SMK+: 103/241	SMK−: 24/103 SMK+: 40/87	1.00	1.63 (1.14, 2.33)
	CA	SMK−: 72/227 SMK+:72/267	SMK−: 27/80 SMK+: 42/118	1.11 (0.67, 1.84)	1.30 (0.55, 3.03)
	AA	SMK−: 32/56 SMK+:23/62	SMK−: 11/24 SMK+: 6/25	1.77 (1.09, 2.87)	1.17 (0.67, 2.03)
					P-interaction =0.004[‡]

[*] Abbreviation: POAG: primary-open angle glaucoma; SMK: cigarette smoking

[†] The number of cases and controls may differ with each analysis, as the numbers with missing genotype information for each SNP differed

‡The adjusted p-value for false discovery rate = 0.04

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Table 4

Effect modification by alcohol intake on the associations of selected NOS3 polymorphisms and POAG

				Pooled All Glaucoma	
SNP	**Genotype**	**Women**	**Men**	**No Drinking**	**Any Drinking**
		Number of Cases/Controls by Alcohol Drinking status	**Number of Cases/Controls by Alcohol Drinking status**	**RR (95% CI)**	**RR (95% CI)**
Promoter −786C/T	TT	ALC−: 32/85 ALC+: 105/329	ALC−: 12/21 ALC+: 53/145	1.00	0.82 (0.53, 1.26)
	TC	ALC−: 35/97 ALC+: 131/361	ALC−: 11/28 ALC+: 55/187	0.98 (0.57, 1.67)	0.90 (0.58, 1.38)
	CC	ALC−: 13/35 ALC+: 49/137	ALC−: 3/13 ALC+: 19/63	0.93 (0.32, 2.69)	0.86 (0.53, 1.41)
					P-interaction =0.86
Glu298Asp (rs1799983)	GG	ALC−: 36/92 ALC+: 128/387	ALC−: 27/26 ALC+: 37/177	1.00	0.89 (0.58, 1.36)
	GT	ALC−: 35/98 ALC+: 108/330	ALC−: 27/25 ALC+: 37/145	1.15 (0.68, 1.95)	0.92 (0.60, 1.41)
	TT	ALC−: 9/27 ALC+: 47/85	ALC−: 27/6 ALC+: 37/46	1.07 (0.47, 2.43)	1.28 (0.76, 2.13)
					P-interaction =0.08
rs3918188	CC	ALC−: 38/96 ALC+: 135/335	ALC−: 27/25 ALC+: 37/166	1.00	0.89 (0.58, 1.36)
	CA	ALC−: 36/102 ALC+: 121/395	ALC−: 27/29 ALC+: 37/189	0.79 (0.47, 1.35)	0.66 (0.43, 1.00)
	AA	ALC−: 8/28 ALC+: 33/118	ALC−: 27/8 ALC+: 37/44	0.62 (0.27, 1.45)	0.88 (0.38, 2.04)
					P-interaction =0.91
rs7830	CC	ALC−: 31/93 ALC+: 141/368	ALC−: 10/30 ALC+: 55/171	1.00	1.07 (0.70, 1.64)
	CA	ALC−: 38/102 ALC+: 108/397	ALC−: 13/20 ALC+: 58/188	1.31 (0.73, 2.35)	0.84 (0.54, 1.29)
	AA	ALC−: 14/32 ALC+: 41/86	ALC−: 3/12 ALC+: 15/38	0.99 (0.40, 2.48)	1.22 (0.72, 2.05)
					P-interaction =0.62

*Abbreviation: POAG: primary-open angle glaucoma; ALC: alcohol

†The number of cases and controls may differ with each analysis, as the numbers with missing genotype information for each SNP differed