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The *LOC387715* Polymorphism, Inflammatory Markers, Smoking, and Age-Related Macular Degeneration:

A Population-Based Case-Control Study

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Abstract

Objective—To assess combined effects on the risk of age-related macular degeneration (AMD) by the *LOC387715* polymorphism, smoking, and inflammatory or hemostatic factors.

Design—Population-based case-control study.

Participants—Two hundred seventy-eight AMD cases (224 early, 54 late) and 557 controls matched for age, gender, and smoking, drawn from the Blue Mountains Eye Study cohort.

Methods—Subjects were genotyped for the *LOC387715* Ala69Ser polymorphism (rs# 10490924). Smoking was self-reported. Serum high-sensitivity C-reactive protein (CRP), interleukin 6 (IL-6), soluble intercellular adhesion molecule 1 (sICAM-1), fibrinogen, homocysteine, plasminogen activator inhibitor 1 (PAI-1), von Willebrand factor, and white cell count (WCC) were measured. Combined effects of this genetic variant plus any of these study factors on AMD risk were assessed using logistic regression models, adjusted for age and smoking. We defined interaction if the influence of 2 factors departed from the multiplicative scale, confirmed by a statistically significant interaction term. Otherwise, the combined effect was used.

Main Outcome Measures—Age-related macular degeneration was graded using the Wisconsin grading system.

Results—Combined effects on the likelihood of early or late AMD were demonstrated for the *LOC387715* Ala69Ser G/T and T/T genotypes with the markers high-sensitivity CRP (odds ratios [ORs], 1.2 for the highest tertile alone, 1.6 for G/T and T/T genotypes alone, and 2.2 for both G/T and T/T genotypes plus the highest tertile, compared with the G/G genotype with the 2 lower tertiles), IL-6 (corresponding ORs, 1.1, 1.6, and 2.2), sICAM-1 (ORs, 1.0, 1.5, and 2.3, respectively), and PAI-1 (ORs, 1.3, 1.7, and 2.3, respectively), but not with WCC, fibrinogen, homocysteine, and von Willebrand factor. Findings were similar for early and late AMD separately. Current smokers with G/T and T/T genotypes had strong combined effects on late AMD risk compared with those who never smoked or past smokers with the G/G genotype (ORs, 1.2 for current smokers alone, 1.8 for G/T and T/T genotypes alone, and 6.1 for current smokers plus G/T and T/T genotypes).

Conclusions—We found no significant interaction but combined effects for the *LOC387715* genotypes with 3 inflammatory markers and PAI-1 on the risk of early or late AMD, and with current smoking on the risk of late AMD.

Age-related macular degeneration (AMD), also termed late maculopathy, is the leading cause of irreversible blindness and moderate visual impairment in older white persons^{1–10} and will remain an increasing threat to vision in coming decades.^{11,12} Research on the pathogenesis of AMD has progressed rapidly in recent decades.^{11,13–21} In particular, the identification of genetic and environmental factors that influence AMD risk has identified clearer future research directions.^{20,22,23} Combined genetic and environmental effects, gene–environment interactions, or both are key findings in understanding the mechanisms of this disease.^{24,25}

Recently, many studies using clinical cases and control samples have identified different AMD-related gene variants, including a complement factor H (*CFH*) polymorphism at chromosome 1q31^{26–32} and *LOC387715*^{24,33,34} and *HTRA1*^{35,36} at chromosome 10q26. The effects of interactions between *CFH* gene variants and some systemic and environmental factors on the risk of AMD were recently investigated in a clinic-based sample, the Age-Related Eye Disease Study,^{25,37} and a population-based sample in Netherlands.³⁸ Combined effects of smoking and the apolipoprotein E (*APOE*) gene³⁹ and interactions of smoking with *LOC387715* gene variants have also been investigated^{24,37} in studies of large case–control samples. Apart from smoking and body mass index,^{24,37} combined effects or potential interactions between *LOC387715* gene variants and other systemic or environmental factors on the risk of AMD have not previously been investigated in either clinic or population-based samples.

Although accumulating evidence suggests a role for both local and systemic inflammation and aberrant complement activation in the pathogenesis of AMD,^{22,23} findings showing an association of AMD and inflammatory markers, including C-reactive protein (CRP) and its genetic variants, have been inconsistent.^{40–44}

In this study, we aimed to replicate the reported interaction between *LOC387715* gene variants and smoking on AMD risk and to explore combined effects and potential interactions between *LOC387715* gene variants and systemic inflammatory and hemostatic factors on the risk of AMD, in a nested case–control sample drawn from an older population, the Blue Mountains Eye Study (BMES).

Materials and Methods

Study Subjects

The BMES is a population-based cohort study of vision and common eye diseases in an urban population at least 49 years old residing in 2 postal code areas of the Blue Mountains region, west of Sydney, Australia. Details of the baseline survey methods have been described previously.⁴⁵ In brief, 3654 (82.4%) of 4433 eligible persons identified in a door-to-door census of the study area participated in the BMES baseline survey in 1992 through 1994 (BMES I). At the 5-year follow-up, 2335 of the 3654 participants (75.1% of survivors) were reexamined in 1997 through 1999 (BMES II-a), after excluding those who had died (14.9%), moved (10.5%), or declined (10.8%). A second door-to-door census was conducted in 1999 and identified 1378 additional eligible permanent residents who had moved into the study area or had reached 49 years of age during the intervening period. Of these newly eligible persons, 1174 (85.2%) participated (BMES II-b).

From the BMES II-a and II-b population samples (total n = 3509), we drew 630 subjects including 197 cases with any AMD (early or late) and 433 controls (matched for age within 5 years, and for gender and smoking status to AMD cases) for whom we had serum samples to

measure inflammatory markers and hemostatic factors in another study. Of these 630 subjects, 581 (188 cases, 393 controls) had single-nucleotide polymorphism (SNP) typing performed for *LOC387715* polymorphisms.⁴⁶ To increase our sample size for the current study, we selected a further 254 subjects (90 cases, 164 matched controls) with DNA available from the entire population for genotyping. This gave a total of 835 subjects (278 cases, 557 controls) in whom *LOC387715* genotyping was analyzed. As we had data on measurement of white cell count (WCC), plasma fibrinogen, and homocysteine in over 80% of the whole study population, these additional 254 subjects had these 3 tests but did not have the other inflammatory markers and hemostatic factors measured. Thus, the analyses of combined effects with serum high-sensitivity CRP, intercellular adhesion molecule 1 (ICAM-1), interleukin 6 (IL-6), plasma plasminogen activator inhibitor 1 (PAI-1), and von Willebrand factor were limited to the initial sample of 581 subjects, whereas the analyses for combined effects with WCC, fibrinogen, and homocysteine were performed for all 835 subjects.

The study was performed under the tenets of the Declaration of Helsinki and was approved by the University of Sydney and the Sydney West Area Health Service human research ethics committees. Signed informed consent was obtained from all participants.

Retinal photographs were taken of at least one eye in 98% of study participants at both surveys. Age-related macular degeneration was diagnosed from grading of the retinal photographs, with graders masked to participant identity. All late AMD cases were adjudicated and confirmed by a retinal specialist (PM), and all early AMD cases were adjudicated between the 2 senior graders, who graded AMD from both baseline and follow-up photographs. Late AMD was defined as the presence of neovascular or atrophic AMD. Neovascular AMD was defined as serous or hemorrhagic detachment of the sensory retina or retinal pigment epithelium (RPE), the presence of subretinal or sub-RPE hemorrhage, or subretinal fibrous scar tissue. Geographic atrophy was defined as a discrete area at least 175 μm in diameter, characterized by a sharp border and the presence of visible choroidal vessels.⁴⁵ Early AMD was defined in either eye by (1) presence of large ($\geq 125\text{-}\mu\text{m}$ diameter) soft indistinct or reticular drusen within the macular area or (2) presence of both large distinct soft drusen and retinal pigmentary abnormalities within the macula, in the absence of late AMD. This closely followed the definition of early AMD used in the Beaver Dam Eye Study.⁴⁷

Smoking history was obtained using an interviewer-administered questionnaire. Current smoking status was compared to noncurrent smoking (persons who never smoked or smoked in the past). Alternatively, “ever smoking” status was defined to include persons who were current or past smokers, by reference to persons who had never smoked.

Assessment of Inflammatory Markers and Hemostatic Factors

On arrival at the laboratory, blood specimens were centrifuged into serum and plasma components and an aliquot of each sample was immediately taken for basic blood tests including WCC and testing for fibrinogen and homocysteine. Remaining serum and plasma were stored separately in cryogenic vials at -80°C . For the present study, aliquots of serum and plasma were taken for high-sensitivity CRP, ICAM-1 and IL-6, PAI-1, and von Willebrand factor and shipped on ice to the collaborating laboratories at a later date. All samples were handled in an identical masked fashion.

Inflammatory Markers—Serum high-sensitivity CRP was measured by rate nephelometry on an Immage automated nephelometer (Beckman Coulter, Fullerton, CA). Details of assay methods have been reported previously.⁴⁸ This high-sensitivity CRP assay, with a functional sensitivity of 0.15 mg/l and interassay coefficient of variation (CV) of 7.9% at 0.81 mg/l, has been shown to correlate well with other commonly used assays.⁴⁹ Assessment of serum ICAM-1 levels was performed by enzyme-linked immunosorbent assay using a commercial

kit assay (Chemicon International, Te-mecula, CA), with a sensitivity of 3.3 ng/ml, detection range of 25 to 100 ng/ml, and interassay CV of 7.7%. Serum IL-6 was measured by a Quantikine high sensitivity assay (R&D Systems, Minneapolis, MN) with a sensitivity of 0.156 pg/ml and interassay CV of 9% at 16 pg/ml. White cell counts were measured using an Advir 120 autoanalyzer (Bayer, Leverkusen, Germany).

Hemostatic Factors—Plasma fibrinogen was measured by the Von Clauss assay using an ACL 300 coagulometer (IL-Coulter, Sydney, Australia) with a sensitivity of 50 mg/dl and interassay CV of 10% at 200 mg/dl. Plasma homocysteine was assayed by fluorescence polarization immunoassay using an IMX analyser (Abbott Laboratories, Chicago, IL) with a sensitivity of <0.5 μ mol/l and interassay CV of 3.7% at 22 μ mol/l. Plasma PAI-1 assays were performed using a commercial kit method (TintElize) available from Biopool (Trinity Biotech, Wicklow, Ireland) with an interassay CV of 3.3% at 40 ng/ml and detection range of 0.5 to 120 ng/ml. Assays of von Willebrand factor antigen were performed by enzyme-linked immunosorbent assay using established commercial reagents (DakoCytomation, Sydney, Australia) as described previously,⁵⁰ with an interassay CV of 12% and detection range of 0% to 400%.

DNA Extraction

Construction of DNA Standard—Standard DNA SNP templates were made to serve as genotyping assay references. Genomic DNA of heterozygous *LOC387715* (rs# 10490924) was polymerase chain reaction (PCR) amplified using the primers 5'-GTGGAGAAG-GAGCCAGTGAC-3' and 5'-CAGTGTCTCAGGTGGTGCTGAG-3'. The 158-base pair PCR fragment was inserted into the pGEM-T Easy Vector (Promega, Madison, WI). The ligation product was transformed to JM109 high-efficiency competent cells (Promega). Ten colonies were collected to determine the specific SNP types using restriction fragment length polymorphism assay. The colonies corresponding to each allele were propagated in Luria-Bertani broth for extraction of plasmid DNA, which later served as the genotype-specific DNA standard. The mixture of plasmids containing 2 different alleles served as the heterozygous control.

Single-Nucleotide Polymorphism Typing—Typing of *LOC387715* (rs# 10490924) was performed by PCR-restriction fragment length polymorphism using the primers described above. The PCR annealing temperature was 61° C. Restriction fragment length polymorphism analysis was conducted using the enzyme *Fnu4HI*. DNA fragments were separated on 15% Tris-borate-ethylenediaminetetra-acetic acid polyacrylamide gels and visualized after ethidium bromide staining. Standard control DNA for both alleles and a heterozygous control were included on each genotyping plate. To confirm the accuracy of our genotyping method, 91% of all samples were also genotyped using the Taq-man SNP Genotyping Assay (no. C__29934973_20, Applied Bio-systems, Foster City, CA). The call rate was 98%. There was a 97% concurrence between results.⁴⁶

Statistical Analysis

The Hardy-Weinberg equilibrium for each SNP was tested using χ^2 procedures. Logistic regression was performed using SAS (release 9.1, SAS, Cary, NC) to compare adjusted odds ratios (ORs) of AMD for the *LOC387715* Ala69Ser G/T and T/T versus G/G genotypes, and also with and without other risk factors. Inflammatory markers and hemostatic factors were dichotomized as the highest tertile compared with the two lower tertiles. Estimated ORs are presented in the format recommended by Botto and Khoury,⁵¹ as 3 groups, 2 with either the genetic or the risk factor assessed alone and 1 with both the genetic and risk factors. Each of these three groups were compared to the group with neither factor. We tested for statistically significant interactions by adding a product term in the multivariable-adjusted logistic

regression models. We defined an interaction if the influence of 2 factors departed from the multiplicative scale of the influence of each factor alone, confirmed by a statistically significant interaction term. A *combined* or *add-on effect* existed if the influence of 2 factors was larger than the effect of each factor alone but the interaction product term was not significant.

Results

Major characteristics, genotypes, and mean levels and standard deviations of inflammatory markers and hemostatic factors were compared between cases and controls. Cases and controls were similar with respect to mean age and proportions who were current or ever smokers. Table 1 shows that cases, however, had higher mean values of high-sensitivity CRP, IL-6, WCC, homocysteine, and PAI-1 and a lower mean value of von Willebrand factor (all P s<0.01). The *LOC387715* G/T and T/T genotypes were more frequent among cases (48.2%) than controls (35.0%). The controls showed no significant deviation from the Hardy–Weinberg equilibrium with respect to the *LOC387715* polymorphism (P >0.05).

Presence of the *LOC387715* G/T and T/T genotypes alone conferred a 50% to 110% higher risk of AMD compared with the G/G genotype (Tables 2, 3). There was no significant interaction between the *LOC387715* G/T and T/T genotypes and any of the inflammatory markers or hemostatic factors assessed (all P s>0.05), after adjusting for age and current smoking status. Combined effects on increased likelihood of any (early or late) AMD were demonstrated for the *LOC387715* G/T and T/T polymorphisms with the following markers: high-sensitivity CRP, IL-6, ICAM-1, and PAI-1, but not with WCC, fibrinogen, homocysteine, and von Willebrand factor (Tables 2, 3). Findings were similar in analyses for early and late AMD separately, except that WCC and the *LOC387715* G/T and T/T genotypes appeared to have a modest joint effect on the risk of late AMD (Table 2). All joint effects with the 3 inflammatory markers (high-sensitivity CRP, IL-6, ICAM-1) or PAI-1 appeared stronger for late AMD than for early AMD (Tables 2, 3).

There was no statistically significant interaction of *LOC387715* genotypes with ever smoking ($P = 0.56$) or current smoking ($P = 0.50$) on AMD risk, after adjusting for age. However, a combined effect of current smoking with the G/T and T/T genotypes on late AMD risk was indicated by a much higher risk magnitude for current smokers if they had G/T or T/T genotypes than for never or past smokers with the G/G genotype (ORs, 1.2 for current smokers with the G/G genotype, 1.8 for never or past smokers with the G/T or T/T genotypes, and 6.1 for current smokers with the G/T or T/T genotypes) (Table 4). Analyses of ever (past or current) smoking subjects compared with those who had never smoked showed a weaker but significant combined effect on late AMD risk, with corresponding ORs of 1.3 (95% confidence interval [CI], 0.6–2.9) for ever smoking alone, 2.0 (95% CI, 0.9–4.6) for the genotypes alone, and 2.6 (95% CI, 1.2–5.7) for the 2 factors combined.

Further adjustment for ICAM-1 and PAI-1 (the 2 markers that significantly contributed to the models of late or any AMD) did not attenuate the combined effects on late AMD risk from current smoking and presence of the *LOC387715* G/T and T/T genotypes (OR, 10.2; 95% CI, 2.8–37.6).

Discussion

In this older population-based sample of AMD cases and controls, we could not replicate the reported significant interaction between smoking and the *LOC387715* polymorphisms²⁴ but detected a combined effect of the 2 factors on the risk of late AMD. We also found no significant interactions but combined effects of the *LOC387715* polymorphisms with the inflammatory

and hemostatic variables, high-sensitivity CRP, IL-6, ICAM-1, and PAI-1 on the risk of any AMD, particularly late AMD.

The etiology of AMD, like many chronic conditions, cannot be explained by a single genetic or environmental risk factor. With the completion of the human genome project and the shift towards understanding the genetic basis of disease, one of the key challenges is how to resolve the complex etiologies of chronic diseases such as AMD. Multiple susceptibility gene variants (polymorphisms), multiple environmental factors, and multiple possibilities of interactions or combined effects between genes (gene–gene interactions) and between genes and environmental factors (gene–environmental interactions) appear to work together to shape an individual's susceptibility to certain diseases. Understanding the combined effects and interactions among specific genotypes and risk factors may help to resolve the complex etiology of these conditions.

Although Schmidt et al²⁴ showed that smoking modified the risk of late AMD in persons with the *LOC387715* risk allele in their clinic-based sample (OR, 2.24 and 95% CI, 0.85–5.88 for AMD risk in nonsmoking patients with the risk allele compared with OR, 7.56 and 95% CI, 3.15–18.14 in smoking patients with the same risk allele), we could not confirm a statistically significant interaction of these 2 factors in our population-based nested case–control sample. However, it should be noted that in our case–control sample we matched controls to cases by age, gender, and smoking status, so would not have expected to find and, indeed, did not find an association between smoking and AMD in this sample. We did find that the magnitude of late AMD risk in persons with the *LOC387715* risk alleles who also smoked was substantially higher than the expected multiplicative combined effect (corresponding to the scale of estimates obtained from our logistic regression models), with an OR of 6.1 for both factors compared with an OR of 1.8 for those with the risk allele who did not smoke and 1.2 for those who smoked but who did not have the risk allele. Even though the interaction term was not statistically significant, these findings do suggest a likely synergistic effect of smoking and this genetic variant on late AMD risk. Similarly, in the Age-Related Eye Disease Study sample³⁷ the interaction between ever smoking and the *LOC387715* genotype was not statistically significant ($P = 0.996$). A substantially higher risk of advanced AMD was found, however, in persons who both smoked and had the risk genotype, supporting a likely synergistic effect of smoking and this genetic variant on late AMD risk (OR, 27.5 for both factors compared with 10.6 for those with the risk allele who did not smoke and 2.0 for those who smoked but who did not have the risk allele).³⁷ Compared with clinic-based case–control samples, our population-based sample consists mostly of early AMD cases, with much fewer late AMD cases. This could have limited our ability to detect statistically significant risk interactions for late AMD.

We particularly investigated potential combined effects or interactions between the *LOC387715* genotypes and smoking or inflammatory markers on the risk of early and late AMD. Our findings suggest that the combined or add-on effect of the *LOC387715* with smoking was only evident for late AMD, and that the add-on effects with high-sensitivity CRP, IL-6, ICAM-1, or PAI-1 also appeared stronger for late than early AMD (nearly double the risk magnitude of the point estimations for late vs. early AMD). The relatively small number of late AMD cases could have affected these risk estimates. These findings, therefore, need confirmation in other study populations. Alternatively, our findings could indicate that subjects with the susceptible gene variants were at a higher risk of late AMD if they also had any of these risk factors. Modifying these risk factors among subjects with the *LOC387715* G/T or T/T genotypes might reduce the risk of late AMD as well as the subsequent community and personal burden from this condition.

One previous study has shown that the frequency of the *LOC387715* risk allele is significantly higher in patients with late AMD than in those with early or intermediate AMD.³³ Our population-based sample appears to support this finding (the T/T genotype was present in 7.4% of late AMD cases and 4.5% of early AMD cases). The effect estimates from the *LOC387715* risk allele also appeared slightly weaker for early AMD (the OR ranged from 1.4 to 2.0) and late AMD (OR ranged from 1.8 to 3.4) in this sample.

A limitation of this study is that we did not have all of the relevant information available for the entire case–control sample. As all of our cases and controls were drawn from a general older Australian population, our sample covered the great majority of AMD cases found in this sample, and controls were selected from subjects matched to cases, we believe that our sample should be reasonably representative of white populations 49 years or older.

In summary, in this population-based case–control study we showed a stronger combined effect of the *LOC387715* T allele with smoking and some inflammatory markers on the risk of late AMD than that for early AMD. If confirmed by future studies, the implication of these findings may be that if preventive strategies (modifying health risk behaviors and risk markers) were applied to persons with AMD susceptible gene variants, the public health impact of this leading cause of blindness could be effectively reduced.

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Table 1
Comparison of Mean Age, Mean Levels of Inflammatory Markers and Hemostatic Factors, Proportions with a History of Smoking, and the *LOC387715* Genotypes between Age-Related Macular Degeneration (AMD) Cases and Controls

	Cases (n = 278)	Controls (n = 557)	P Values	Cases (n = 188)	Controls (n = 393)
Mean age (yrs) (SD)	75.6 (8.5)	74.9 (7.9)	0.1865		
White blood cell counts ($\times 10^9/l$) [mean (SD)]	7.0 (4.5)	6.6 (1.8)	0.1875		
Fibrinogen (mg/dl) [mean (SD)]	377.4 (99.3)	382.9 (93.1)	0.4330		
Homocysteine ($\mu\text{mol/l}$) [mean (SD)]	14.0 (5.2)	13.5 (6.0)	0.1823		
Current smokers (proportion)	8.5	9.0	0.64		
Ever smokers (proportion)	49.1	49.3	0.96		
<i>LOC387715</i> genotype [proportion (95% CI)] [any (early, late) AMD]					
G/G	51.8 (52.2, 50.0)	65.0	0.0002		
G/T	43.2 (43.3, 42.6)	32.1	0.0017		
T/T	5.0 (4.5, 7.4)	2.9	0.11		
High-sensitivity C-reactive protein (mg/l) [mean (SD)]			0.8687	4.1 (5.6)	4.0 (7.2)
Interleukin 6 (pg/ml) [mean (SD)]			0.2455	4.2 (10.6)	3.2 (5.1)
Intercellular adhesion molecule 1 (ng/ml) [mean (SD)]			0.0542	411.2 (141.5)	387.5 (139.4)
Plasminogen activator inhibitor 1 (ng/ml) [mean (SD)]			0.0985	86.6 (26.2)	83.1 (18.2)
von Willebrand factor (%) [mean (SD)]			0.4459	87.4 (43.5)	90.5 (51.5)

CI = confidence interval; SD = standard deviation.

Table 2

Combined Effects* of *LOC387715* Genotypes and Inflammatory Markers on the Risk of Age-Related Macular Degeneration (AMD)

LOC387715 Genotype	Any AMD		Early AMD		Late AMD	
	Lower 2 Tertiles	Highest Tertile	Lower 2 Tertiles	Highest Tertile	Lower 2 Tertiles	Highest Tertile
G/G	1.0	1.19 (0.73–1.93)	High-sensitivity C-reactive protein 1.0 1.52 (0.95–2.45)			
G/T + T/T	1.63 (1.05–2.55)	2.22 (1.26–3.90)				
G/G	1.0	1.13 (0.69–1.85)	Interleukin 6 1.0 1.44 (0.89–2.32)			
G/T + T/T	1.58 (1.01–2.48)	2.16 (1.26–3.71)				
G/G	1.0	0.97 (0.59–1.57)	Intercellular adhesion molecule 1 1.0 1.39 (0.87–2.23)			
G/T + T/T	1.46 (0.95–2.27)	2.31 (1.30–4.11)				
G/G	1.0	1.03 (0.68–1.55)	White cell counts 1.0 1.83 (1.24–2.68)			
G/T + T/T	1.87 (1.30–2.70)	1.58 (1.02–2.46)				

Odds ratio (95% confidence interval).

* All models adjusted for age and current smoking status.

Table 3
Combined Effect* of LOC387715 Genotypes and Hemostatic Factors on the Risk of Age-Related Macular Degeneration (AMD)

LOC387715 Genotype	Any AMD		Early AMD		Late AMD	
	Lower 2 Tertiles	Highest Tertile	Lower 2 Tertiles	Highest Tertile	Lower 2 Tertiles	Highest Tertile
G/G	1.0	0.89 (0.59–1.35)	1.0 1.75 (1.19–2.57)	0.94 (0.60–1.46) 1.50 (0.92–2.45)	1.0 1.90 (0.95–3.78)	0.68 (0.28–1.67) 1.70 (0.73–3.97)
G/T + T/T	1.79 (1.25–2.56)	1.54 (0.98–2.43)				
G/G	1.0	1.50 (0.99–2.29)	1.0 1.95 (1.33–2.87)	1.30 (0.82–2.06) 1.48 (0.89–2.46)	1.0 3.42 (1.54–7.60)	2.65 (1.15–6.12) 2.79 (1.12–6.93)
G/T + T/T	2.13 (1.48–3.07)	1.67 (1.05–2.67)				
G/G	1.0	1.26 (0.78–2.04)	1.0 1.47 (0.91–2.37)	1.18 (0.70–1.99) 2.11 (1.17–3.83)	1.0 2.65 (1.11–6.34)	1.71 (0.63–4.60) 3.49 (1.15–10.59)
G/T + T/T	1.67 (1.07–2.60)	2.30 (1.31–4.02)				
G/G	1.0	1.05 (0.64–1.70)	1.0 1.63 (1.01–2.63)	1.10 (0.65–1.86) 1.60 (0.89–2.90)	1.0 2.67 (1.14–6.22)	0.84 (0.30–2.38) 1.63 (0.57–4.70)
G/T + T/T	1.81 (1.16–2.81)	1.61 (0.93–2.80)				

Odds ratio (95% confidence interval).

* All models adjusted for age and current smoking status.

Table 4

Combined Effect* of *LOC387715* Genotypes and Current Smoking on the Long-Term Risk of Age-Related Macular Degeneration

<i>LOC387715</i> Genotype	Never or Past Smoker	Current Smoker
Early AMD		
G/G	1.0 (reference)	1.01 (0.49–2.09)
G/T+T/T	1.75 (1.26–2.44)	1.17 (0.45–3.09)
Late AMD		
G/G	1.0 (reference)	1.21 (0.27–5.54)
G/T+T/T	1.77 (0.96–3.27)	6.06 (1.96–18.76)

* Models adjusted for age.