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The effect of genetic variants in *SERPING1* on the risk of neovascular age-related macular degeneration

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Abstract

Purpose—Genetic factors influence an individual's risk for developing age-related macular degeneration (AMD), a leading cause of irreversible vision loss. Previous studies investigating the potential association between all AMD subtypes and the *SERPING1* gene, which encodes a key regulator of the classical complement pathway, have yielded conflicting results. The purpose of this study was to determine whether variations in *SERPING1* are associated with the neovascular form of AMD.

Methods—A total of 556 patients with neovascular AMD and 256 ethnically-matched controls were genotyped for polymorphisms in *SERPING1*. A tagging single nucleotide polymorphism (tSNP) approach was used to cover the *SERPING1* gene plus 2 kilobases (kb) on each side, spanning the promoter and the 3' untranslated regions. Ten SNPs with a minimum allele frequency of 0.10 were covered by three tSNPs (rs1005510, rs11603020, rs2511989).

Results—*SERPING1* SNPs rs1005510 and rs2511989 were significantly associated with neovascular AMD in our cohort, with rs1005510 conferring an adverse risk effect (OR 1.49, 95% CI 1.18–1.88) and rs2511989 conferring a protective effect (OR 0.73, 95% CI 0.59–0.90). For both tSNPs, logistic regression of individual genotypes demonstrated statistically-significant stepwise changes in the risk of developing AMD. Combined analysis of rs1005510 with variants in *CFH* and *HTRA1* confirmed an independent risk effect. The rs11603020 variant had no effect on AMD susceptibility in this study (OR 0.98, 95% CI 0.78–1.24).

Conclusions—This study comprehensively investigates the *SERPING1* gene (using three tSNPs) and evaluates these genetic variants in the largest neovascular AMD cohort to date. Our results support the hypothesis that *SERPING1* has a modest effect on the risk of neovascular AMD.

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COMPETING INTEREST

None declared.

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Keywords

SERPING1; Complement cascade; Age-Related Macular Degeneration; Choroidal Neovascularization

INTRODUCTION

Neovascular age-related macular degeneration (AMD), characterized by aberrant choroidal angiogenesis, accounts for the majority of irreversible vision loss among older individuals. [1] In addition to dietary and environmental risk factors, genetics influences AMD susceptibility. [2, 3] Single nucleotide polymorphisms (SNPs) in the complement factor H (*CFH*) gene and the *ARMS2/HTRA1* locus have been strongly linked to AMD, implicating the alternative complement system in AMD pathophysiology. [3, 4] Common variants in other complement genes, including factor B (*CFB*), component 2 (*C2*), and component 3 (*C3*), have also been associated with the development of AMD. [5, 6]

Recently, two studies examined the possible influence of SNPs in the *SERPING1* gene, which encodes the complement component 1 (C1) inhibitor, on AMD. In a screen of 32 candidate genes selected based on potential functional relevance to AMD, Ennis *et al.* found the *SERPING1* SNP rs2511989 to be significantly associated with AMD in two independent cohorts from the UK and the US. A secondary scan of the *SERPING1* locus revealed five additional SNPs to be associated with AMD. These findings seem plausible given that the C1 inhibitor, an important regulator of the classical complement pathway, was shown to be expressed in the neural retina, retina pigment epithelium, and choroidal tissues. [7–8] Associations between *SERPING1* SNPs and AMD were not seen, however, by Park *et al.* in their study of two cohorts from the Mayo Clinic and the Age-Related Eye Disease Study (AREDS). [9] Two subsequent studies investigating the rs2511989 variant found no significant association between this SNP and AMD. [10, 11]

The previous studies included patients with all subtypes of AMD or a small cohort of neovascular AMD patients. In this study, we investigated the possible link between *SERPING1* and neovascular AMD, as this late stage accounts for 90% of AMD-related vision loss. [12]

METHODS

Subjects

This retrospective cohort study was approved by the Washington University Human Research Protection Office and the Barnes Retina Institute Study Center. Research adhered to the tenets of the Declaration of Helsinki and was conducted in accordance with Health Insurance Portability and Accountability Act regulations. All participants signed written informed consent prior to participation. At the Barnes Retina Institute, a retina-specialty ophthalmology practice, eligible Caucasian participants were identified for case and control cohorts through chart review. All cases had been diagnosed with neovascular AMD in at least one eye, as determined by slit lamp biomicroscopy, indirect ophthalmoscopy, and fundus photography. Patients showing no clinical signs of AMD were recruited as controls.

A total of 821 patients were genotyped for polymorphisms in *SERPING1* using DNA extracted from mouthwash samples. Each participant provided a buccal tissue sample by expectorating into a 50 ml conical tube (Falcon; BD Biosciences, San Jose, CA) after vigorously rinsing for 30 seconds with ~20 ml Scope mouthwash (Procter & Gamble, Cincinnati, OH). Genomic DNA was prepared from the collected buccal cells using the

Genra Puregene Buccal Cell Kit (qiagen.com, Valencia, CA) and quantified by absorbance at 260 nm (GeneQuant pro, GE Healthcare, Buckinghamshire, UK).

Tag single nucleotide polymorphism selection

A tagging SNP (tSNP) approach was used to cover the *SERPING1* gene plus 2 kilobases (kb) on each side, spanning the promoter and the 3' untranslated regions. We identified the SNPs in *SERPING1* with a minimum allele frequency of 0.10 using HapMap Project Build 36 (<http://hapmap.org>). The minimum r^2 value was set to 0.80, and genotypes for the CEU population (Utah residents with ancestry from northern and western European descent) were used. Three tSNPs (rs1005510, rs11603020, and rs2511989) covering these ten SNPs were identified using Tagger (<http://www.broad.mit.edu/mpg/tagger>). Study participants were genotyped for the three *SERPING1* tSNPs as well as for rs1061170 in *CFH* and rs10490924 in *HTRA1* using Sequenom MassARRAY technology (sequenom.com, San Diego CA). Only patients with genotypes graded A or B in quality by Sequenom for all five tSNPs were included in the analysis.

Data analysis

Descriptive statistics for all demographic and clinical variables were calculated, and comparisons were made using the t-test for continuous data (e.g., age) and the chi-square test for categorical data (e.g., gender). Genotyping data were loaded into Haploview in linkage format to generate allele frequencies, ratios, and p-values based on a chi-square test for association of alleles. [13] Evaluation of the effects of individual genotypes on the risk of AMD was performed by logistic regression with the normal haplotype dummy coded as reference. Statistics were performed with SAS (version 9.2). Adjustments were made for two potential confounders: age and gender. For all statistical analyses, $p < 0.05$ was considered to be significant, and multiple comparisons were adjusted for by the Bonferroni correction.

RESULTS

Genotypes from 556 neovascular AMD patients and 256 controls (98.9% of all recruited participants) yielded high-quality calls for all three tSNPs and were included in the analysis. The average ages of the cases and controls were 79.3 and 69.5, respectively ($p < 0.0001$), and 68.1% of cases and 54.7% of controls were female ($p = 0.0002$).

Allele and genotype distributions for the tSNPs are reported in Tables 1 and 2, respectively. All three tSNPs conformed to Hardy-Weinberg Equilibrium in both the case and control populations. After adjusting for multiple comparisons, the rs1005510 and rs2511989 variants were shown to be significantly associated with neovascular AMD (Table 1), with rs1005510 conferring an adverse risk effect (OR 1.49, 95% CI 1.18 – 1.88) and rs2511989 conferring a protective effect (OR 0.73, 95% CI 0.59 – 0.90). The rs11603020 allele had no effect on the risk of neovascular AMD in our cohort (OR 0.98, 95% CI 0.78 – 1.24).

Logistic regression of the individual genotypes demonstrated statistically-significant stepwise changes in the risk of developing AMD for both rs1005510 and rs2511989 (Table 2). The significance of our results was not affected by adjustments made for age and gender. Similarly, combined analysis of the risk tSNP rs1005510 with two polymorphisms known to be associated with AMD, rs1061170 in *CFH* and rs10490924 in *HTRA1*, showed that neither conferred any confounding effects on our results.

CONCLUSIONS

In the present study of 556 cases and 256 controls, two *SERPING1* tSNPs, rs2511989 and rs1005510, were found to be significantly associated with neovascular AMD, both showing appropriate stepwise changes in risk for each genotype. Previous studies investigating the relationship between *SERPING1* and AMD have yielded conflicting results. Ennis *et al.* found that the rs2511989 variant contributed significantly to risk of AMD in 479 cases and 479 controls recruited in the UK as well as in 248 cases and 252 controls from the University of Iowa ($p = 0.00372$). In a follow-up scan of the UK cohorts, the rs1005510 tSNP was also shown to be linked with AMD. [7] In contrast, no association was reported for rs2511989 by Park *et al.* in 476 cases and 310 controls recruited from the Mayo Clinic ($p = 0.46$) nor in 1241 cases and 300 controls from the AREDS ($p = 0.41$). [9] Allikmets *et al.* reported no significant association for rs2511989 in seven case-control studies involving patients with all AMD subtypes. [10] In a study of 94 neovascular AMD patients and 95 controls, Carter and Churchill also found no association for rs2511989. [11]

Possible explanations for the discrepant findings with *SERPING1* include genotyping error and confounding effects. First, genotyping error seems unlikely because Ennis *et al.* and Park *et al.* both genotyped two independent replication cohorts with multiple sequencing platforms. In order to mitigate the risk of this source of error, we only included genotypes graded A or B by Sequenom standards at all five SNPs in data analysis. Second, statistically-significant variables (e.g., age, gender, and other SNPs known to be associated with AMD) could play confounding effects. We found our results were unaffected after adjusting for these three factors.

The previous studies included patients with all AMD subtypes or involved a small cohort of neovascular AMD patients. Although it remains unclear whether *SERPING1* is associated with all forms of AMD, our results suggest that this complement gene is linked to neovascular AMD. It is possible that the lower incidence of neovascular AMD in the Mayo and AREDS cohorts used by Park *et al.* (33.8% and 26.1%, respectively), as compared to the higher incidence in the UK cohort used by Ennis *et al.* (48.6%), contributed to the difference in their results. While the subtype analyses performed by Park *et al.* for the rs2511989 tSNP showed no evidence for association with any AMD subtype, results for the 161 neovascular AMD subjects from the Mayo Clinic did approach levels of significance ($p=0.05-0.66$) for the tested tSNPs. Because pathophysiology may differ between the subtypes of late-stage AMD, it is possible that *SERPING1* is only associated with the neovascular form.

Like Ennis *et al.*, [7] we found one protective SNP (rs2511989) and one risk SNP (rs1005510) in *SERPING1* to be associated with AMD. This result seems plausible, as polymorphisms may either positively or negatively affect C1 inhibitor function (and hence complement activity). Alternatively, these SNPs may simply be associated with functional variants in *SERPING1*.

In summary, the results of this study support the hypothesis that an association between the *SERPING1* gene and neovascular AMD exists. While other complement genes, namely *CFH*, *C3*, *C2*, and *CFB*, have previously been associated with AMD, *SERPING1* would be the first to implicate the role of the classical complement pathway in AMD development. The involvement of both the classical and alternative arms of complement activation in neovascular AMD further stresses the importance of the innate immune system and inflammatory response pathways in the pathogenesis of this disease. In light of recent findings, it appears that *SERPING1* has a modest effect on the risk of neovascular AMD and should be included in the growing list of genes that may be associated with AMD. Further

studies to replicate and confirm these results will be required before any conclusive statements of the association between *SERPING1* and neovascular AMD can be made.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE 1

Tagged single nucleotide polymorphisms (tSNPs) for *SERPING1*

	Function	Location	Frequency in Cases (n = 556)	Frequency in Controls (n = 256)	OR (95% CI)	p =
rs1005510	Intron 2	57123798			1.49 (1.18 to 1.88)	0.0026
G			738 (66.4%)	382 (74.6%)		
A			374 (33.6%)	130 (25.4%)		
rs11603020	Intron 6	57130908			0.98 (0.78 to 1.24)	1.000
C			790 (71.0%)	362 (70.7%)		
T			322 (29.0%)	150 (29.3%)		
rs2511989	Intron 6	57134901			0.73 (0.59 to 0.90)	0.011
G			699 (62.9%)	283 (55.3%)		
A			413 (37.1%)	229 (44.7%)		

Tests of significances were adjusted for multiple comparisons by Bonferroni.

TABLE 2Genotype distributions for rs1005510 and rs2511989 in *SERPING1*

	Cases (n = 556)	Controls (n = 256)	OD (95% CI)	p =
rs1005510				0.0010
GG	245 (44.1%)	145 (56.6%)	–	
GA	248 (44.6%)	92 (35.9%)	1.48 (1.17–1.87)	
AA	63 (11.3%)	19 (7.4%)	2.19 (1.37–3.50)	
rs2511989				0.0030
GG	213 (38.3%)	74 (28.9%)	–	
GA	273 (49.1%)	135 (52.7%)	0.72 (0.57–0.89)	
AA	70 (12.6%)	47 (18.4%)	0.51 (0.33–0.80)	