

# Prediction Model for Prevalence and Incidence of Advanced Age-Related Macular Degeneration Based on Genetic, Demographic, and Environmental Variables

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**PURPOSE.** The joint effects of genetic, ocular, and environmental variables were evaluated and predictive models for prevalence and incidence of AMD were assessed.

**METHODS.** Participants in the multicenter Age-Related Eye Disease Study (AREDS) were included in a prospective evaluation of 1446 individuals, of which 279 progressed to advanced AMD (geographic atrophy or neovascular disease) and 1167 did not progress during 6.3 years of follow-up. For prevalent AMD, 509 advanced cases were compared with 222 controls. Covariates for the incidence analysis included age, sex, education, smoking, body mass index (BMI), baseline AMD grade, and the AREDS vitamin-mineral treatment assignment. DNA specimens were evaluated for six variants in five genes related to AMD. Unconditional logistic regression analyses were performed for prevalent and incident advanced AMD. An algorithm was developed and receiver operating characteristic curves and C statistics were calculated to assess the predictive ability of risk scores to discriminate progressors from nonprogressors.

**RESULTS.** All genetic polymorphisms were independently related to prevalence of advanced AMD, controlling for genetic factors, smoking, BMI, and AREDS treatment. Multivariate odds ratios (ORs) were 3.5 (95% confidence interval [CI], 1.7–7.1) for *CFH* Y402H; 3.7 (95% CI, 1.6–8.4) for *CFH* rs1410996; 25.4 (95% CI, 8.6–75.1) for *LOC387715* A69S (*ARMS2*); 0.3 (95% CI, 0.1–0.7) for *C2* E318D; 0.3 (95% CI, 0.1–0.5) for *CFB*; and 3.6 (95% CI, 1.4–9.4) for *C3* R102G, comparing the homozygous risk/protective genotypes to the referent genotypes. For incident AMD, all these variants except *CFB* were significantly

related to progression to advanced AMD, after controlling for baseline AMD grade and other factors, with ORs from 1.8 to 4.0 for presence of two risk alleles and 0.4 for the protective allele. An interaction was seen between *CFH* Y402H and treatment, after controlling for all genotypes. Smoking was independently related to AMD, with a multiplicative joint effect with genotype on AMD risk. The C statistic for the full model with all variables was 0.831 for progression to advanced AMD.

**CONCLUSIONS.** Factors reflective of nature and nurture are independently related to prevalence and incidence of advanced AMD, with excellent predictive power. (*Invest Ophthalmol Vis Sci.* 2009;50:2044–2053) DOI:10.1167/iovs.08-3064

Knowledge about genetic and epidemiologic associations with the leading cause of blindness among the elderly, age-related macular degeneration, has grown exponentially in recent years. Several genetic variants with strong and consistent associations with AMD have recently been identified.<sup>1–15</sup> We also know that in addition to age, ethnicity, and family history, there are modifiable factors: smoking,<sup>16–19</sup> nutritional antioxidants and omega-3 fatty acid intake,<sup>20–27</sup> and overall and abdominal adiposity.<sup>28–30</sup> However, it remains unknown whether all these genetic and environmental factors act independently or jointly and to what extent they as a group can predict the occurrence of AMD or progression to advanced AMD from early and intermediate stages. Such information may be useful for screening those at high risk due to a positive family history or having signs of early or intermediate disease, among whom some progress to advanced stages of AMD with visual loss. Early detection could reduce the growing societal burden due to AMD by targeting and emphasizing modifiable habits earlier in life and recommending more frequent surveillance for those highly susceptible to the disease. Treatment trials will also benefit from such information by enrolling participants with more homogeneous risks of developing the outcome in question. To develop and evaluate such a model, we assessed the relative contribution of genetic and environmental factors toward prevalence and incidence of advanced AMD in a well-characterized multicenter study population. We also developed an algorithm and receiver operating curve models to discriminate between individuals who developed advanced AMD and those who did not.

## METHODS

### Phenotypic Data

The Age-Related Eye Disease Study (AREDS) included a randomized clinical trial to assess the effect of antioxidant and mineral supplements on risk of AMD and cataract and a longitudinal study of AMD that ended in December 2005. The study procedures have been reported.<sup>20</sup> Based on ocular examination and AREDS reading center photographic grading of fundus photographs, Caucasian participants in the present study were defined as AREDS category 1 in both eyes (essentially free of age-related macular abnormalities), category 2 in the worst eye (mild

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changes including multiple small drusen, nonextensive intermediate drusen, and/or pigment abnormalities), category 3 in the worst eye (at least one large drusen of at least 125- $\mu$ m diameter, extensive intermediate drusen, and/or noncentral geographic atrophy), category 4 in one eye (advanced AMD, either neovascular or central geographic atrophy, or visual loss due to AMD regardless of phenotype), or category 4 in both eyes. Non-Caucasians were excluded because the distribution of advanced AMD in that population differs considerably from that among Caucasians.<sup>30</sup> Since group 4 in the original AREDS classification included both advanced forms of AMD and visual loss regardless of phenotype,<sup>20</sup> we reclassified this group into grades 4 and 5: grade 4 including both noncentral and central geographic atrophy and grade 5 including neovascular disease, independent of visual acuity level using the Clinical Age-Related Maculopathy Grading System (CARMS),<sup>31</sup> to determine whether results differed between these two advanced dry and wet phenotypes. Comparisons were also made between unilateral or bilateral advanced AMD according to the AREDS system.<sup>20</sup> Cases were defined as having advanced AMD and progression was defined as incident advanced AMD based on the AMD grade at the end of the clinical trial in 2001, with a mean follow-up time of 6.3 years. Progressors were those individuals with early or intermediate AMD at baseline who progressed to advanced AMD during follow-up, and individuals with advanced AMD in one eye at baseline who progressed to advanced AMD in both eyes.

Demographic and risk factor data, including education, smoking history, and body mass index, were obtained at the baseline visit from questionnaires and height and weight measurements. Antioxidant status was defined as taking antioxidants (antioxidants alone or antioxidants and zinc) or no antioxidants (placebo or zinc alone) in the clinical trial. AREDS treatment groups included placebo, antioxidants alone, zinc, and antioxidants plus zinc. The research protocol was approved by institutional review boards, and all participants signed informed consent statements. Research adhered to the tenets of the Declaration of Helsinki.

## Genotyping

DNA samples, which were obtained beginning in 1998, were obtained from the AREDS Genetic Repository. The following six common single-nucleotide polymorphisms (SNPs) associated with AMD were evaluated: (1) complement factor H (*CFH*) Y402H (rs1061170) in exon 9 of the *CFH* gene on chromosome 1, region q31, with the change 1277T>C resulting in a substitution of a histidine for a tyrosine at codon 402 of the *CFH* protein; (2) *CFH* rs1410996, an independently associated SNP within intron 14 of *CFH*; (3) *LOC387715* A69S, ARMS2, (rs10490924 in the *LOC387715/HTRA1* region of chromosome 10), a nonsynonymous coding SNP variant in exon 1 of *LOC387715*, resulting in a substitution of the amino acid serine for alanine at codon 69; (4) complement factor 2 or *C2* E318D (rs9332739), the nonsynonymous coding SNP variant in exon 7 of *C2* resulting in the amino acid glutamic acid changing to aspartic acid at codon 318; (5) complement factor B or *CFB* R32Q (rs641153), the nonsynonymous coding SNP variant in exon 2 of *CFB* resulting in the amino acid glutamine changing to arginine at codon 32; and (6) complement factor 3 or *C3* R102G (rs2230199), the nonsynonymous coding SNP variant in exon 3 of *C3* resulting in the amino acid change of glycine to arginine at codon 102. For the genetic variant on chromosome 10, *LOC387715* A69S, whether the gene *HTRA1* adjacent to it is in fact the AMD-susceptibility gene on 10q26<sup>9-11</sup> remains debatable; however, the relevant SNPs in these two genes have been reported to be nearly perfectly correlated. Thus, although the other SNP is a promising candidate variant, rs10490924 used in this study can be considered a surrogate for the causal variant that resides in this region. For the *C2/CFB* genes, there are two independent associations to the *C2/CFB* locus, but because of linkage disequilibrium, we do not know which of the two genes or both are functionally affected. Genotyping was performed using primer mass extension and MALDI-TOF MS analysis (MassEXTEND methodology of Sequenom, San Diego, CA) at the Broad Institute Center for Genotyping and Analysis (Cambridge, MA).

## Statistical Analyses

Individuals with advanced AMD were compared to the control group of persons with no AMD, and progressors were compared to nonprogressors with regard to genotype and risk factor data. Multivariate unconditional logistic regression analysis was performed to evaluate the relationships between prevalence or progression of AMD and all the genotypes plus various risk factors, controlling for age (70 years or older versus younger than 70), sex, and education (high school or less versus more than high school), cigarette smoking (never, past, or current), and body mass index (BMI), which was calculated as the weight in kilograms divided by the square of the height in meters (<25, 25-29.9, and 30+). Baseline AMD grade was included in the progression models. The AREDS assignment in the randomized clinical trial was also added to the multivariate model (taking a supplement containing antioxidants or taking study supplements containing no antioxidants for the case-control comparison, or the four treatment groups for the progression analyses). Tests for multiplicative interactions between each of the genotypes versus smoking and BMI were calculated by using cross product terms according to genotype and the individual risk factors. Similar analyses were performed to assess gene-gene interactions for each combination of genes. ORs and 95% CIs were calculated for each risk factor and within the three genotype groups. Tests for trend for the number of risk alleles for each genetic variant (0, 1, or 2) were calculated.<sup>32</sup> Sensitivities and specificities for a variety of risk score cutoffs were evaluated to assess the optimal use of the model for individual risk prediction (i.e., sensitivities and specificities of at least 80%). The method for calculation of the AMD progression risk score based on all ocular, demographic, behavioral, genetic and treatment factors is explained in Table 1, and is illustrated for a randomly selected progressor and nonprogressor.

The area under the receiver operating characteristic (ROC) curve was obtained. In addition, an age-adjusted concordance (C) statistic based on the ROC curve was calculated for different combinations of these factors, to assess the probability that the risk score based on the group of risk factors in that model from a random progressor was higher than the corresponding risk score from a random nonprogressor within the same 10-year age group.<sup>33</sup> We obtained standard errors of estimated C statistics and compared C statistics from alternative risk prediction models, by using correlated ROC curve methods.<sup>34</sup>

## RESULTS

The mean ages ( $\pm$ SD) of cases, controls and progressors, non-progressors were 69.1 ( $\pm$ 5.2), 66.8 ( $\pm$ 4.2) years and 69.2 ( $\pm$ 5.0), 67.7 ( $\pm$ 5.0) years, respectively. There were no statistically significant associations between any of the genetic variants and the demographic, behavioral, or treatment variables. Relationships between pairs of genes among the controls were evaluated. *CFH* Y402H rs1061170 and *CFH* rs1410996 were significantly related ( $P < 0.001$ ) as a result of linkage disequilibrium between these sites, and *CFB* R32Q rs641153 was weakly related to *C3* R102G rs2230199 ( $P = 0.03$ ). No other associations between pairs of genetic variants were statistically significant.

Table 2 displays the unadjusted association between demographic, environmental, and genetic variables and incident advanced AMD as well as the sample sizes within the groups. All factors except gender were related to progression. Baseline macular status was strongly related to progression. Both modifiable factors (smoking and BMI) and genetic variants were also associated with worsening of macular disease over time. The antioxidant/mineral treatment group had a lower rate of progression.

Tables 3 and 4 show the multivariate adjusted models for prevalent and incident advanced AMD, respectively, after adjustment for demographic and behavioral factors and all six genetic variants.

TABLE 1. Calculation of AMD Progression Risk Score  $S = \alpha + \sum_{i=1}^{22} \beta_i X_i$ , where  $\beta_i$  and  $X_i$  are given as follows

<i>i</i>	Variable Name ( $X_i$ )	Regression Coefficient $\beta_i$	Code	Nonprogression Subject A ( $X_{A_i}$ )	$\beta_i X_{A_i}$	Progression Subject B ( $X_{B_i}$ )	$\beta_i X_{B_i}$
	Intercept ( $\alpha$ )	-5.78		1	-5.78	1	-5.78
1	Age 70+ years	0.4116		1	0.41	0	0
2	Sex	0.0688	1, M/0, F	0	0	0	0
3	Education	-0.128	1, Some college/0, high school or less	1	-0.13	1	-0.13
	Baseline AMD Grade						
4	3	2.3944	1, Yes/0, No	0	0	1	2.39
5	4	2.9521	1, Yes/0, No	0	0	0	0
6	Current Smoking	1.1261	1, Yes/0, No	0	0	0	0
7	Past Smoking	0.1211	1, Yes/0, No	0	0	1	0.12
8	BMI 25-29	0.517	1, Yes/0, No	0	0	0	0
9	BMI 30+	0.4754	1, Yes/0, No	1	0.48	0	0
10	Antioxidant	-0.1299	1, Yes/0, No	0	0	0	0
11	Zinc	-0.3897	1, Yes/0, No	1	-0.39	0	0
12	Antioxidant/zinc	-0.4973	1, Yes/0, No	0	0	0	0
13	CFH:rs1061170 (Y402H) CT	0.2644	1, Yes/0, No	0	0	0	0
14	CFH:rs1061170 (Y402H) CC	0.6778	1, Yes/0, No	0	0	1	0.68
15	LOC387715:rs10490924 (A69S) GT	0.8396	1, Yes/0, No	0	0	0	0
16	LOC387715:rs10490924 (A69S) TT	1.3837	1, Yes/0, No	0	0	0	0
17	CFH:rs1410996 CT	0.5251	1, Yes/0, No	1	0.53	0	0
18	CFH:rd1410996 CC	0.8606	1, Yes/0, No	0	0	1	0.86
19	C2:rs9332739 (E318D) CG or CC	-1.051	1, Yes/0, No	0	0	0	0
20	CFB:rs641153 (R32Q) CT or TT	-0.2147	1, Yes/0, No	0	0	0	0
21	C3:rs2230199 (R102G) CG	0.3679	1, Yes/0, No	1	0.37	0	0
22	C3:rs2230199 (R102G) GG	0.597	1, Yes/0, No	0	0	0	0
	Risk score*				-4.52		-1.85

\* Includes the intercept of -5.7776. The probability of progression =  $\exp(\text{risk score})/[1 + \exp(\text{risk score})]$  For example, for the above two subjects of whom subject A did not progress and subject B progressed, the probability of progression =  $\exp(-4.52)/[1 + \exp(-4.52)] = .01$  for subject A and  $\exp(-1.85)/[1 + \exp(-1.85)] = .14$  for subject B.  $\alpha$  is the intercept and  $\beta_i$  is the regression coefficient for the *i*th risk factor

As seen in Table 3, there were positive associations between both *CFH* variants and the combined advanced AMD case group compared with the controls (Y402H: OR = 3.5, 95% CI = 1.7-7.1,  $P_{\text{trend}} = 0.0003$ ; rs1410996: OR = 3.7,  $P_{\text{trend}} = 0.0003$ ). In addition, there were positive associations between AMD and the *LOC387715* A69S variant (OR = 25.4,  $P_{\text{trend}} < 0.0001$ ) and *C3* (OR = 3.6,  $P_{\text{trend}} = 0.001$ ) and between both the *C2* variant (OR = 0.3,  $P = 0.003$ ) and the *CFB* variant (OR = 0.3,  $P < 0.0001$ ). There were positive independent associations with older age (OR = 2.8,  $P < 0.0001$ ), current smoking (OR = 3.9,  $P = 0.001$ ), and past smoking (OR = 1.9,  $P = 0.004$ ). A protective effect of higher education (OR = 0.6,  $P = 0.01$ ) was shown. A borderline positive association with BMI was present (OR = 1.5,  $P = 0.11$ ), and no significant association with sex or antioxidant treatment was seen. In general, similar associations between genes and AMD were seen for all subtypes of AMD, including unilateral and bilateral advanced AMD and dry and wet types of advanced AMD, although associations varied slightly for specific types of advanced AMD.

Table 4 displays the multivariate adjusted ORs for incident advanced AMD and shows that, after adjustment for genotypes, older age, smoking, and higher BMI were related to a higher rate of progression. Baseline grade was a strong predictor of incident advanced AMD, and antioxidant-mineral treatment was protective. The two *CFH* variants each independently increased risk of progression about two- to threefold, with similar increased risk for *C3*, comparing the homozygous risk and nonrisk genotypes. Variants in the two complement genes *C2* and *CFB* reduced risk, although the association with *CFB* was not significant for progression to incident advanced AMD. *LOC387715* (*ARMS2*) increased risk and was more strongly

associated with progression to neovascular disease (OR = 5.2, 95% CI = 2.9-9.4,  $P_{\text{trend}} < 0.001$ ) than to geographic atrophy (OR = 1.8, 95% CI = 0.8-4.2).

An assessment of interactions between genotypes and treatment groups on risk of progression to incident advanced AMD for all genetic variants is shown in Table 5. Only the *CFHY402H* variant showed a possible interaction with antioxidant-mineral treatment, with  $P = 0.057$  for the analysis including three genotype groups defined categorically and  $P = 0.016$  for analysis of number of alleles as a continuous variable. This result suggests that the antioxidant-mineral supplement is more effective for the homozygous low-risk or heterozygous *CFHY402H* genotype and less effective for the homozygous risk genotype, with adjustment for the other five genotypes and other nongenetic factors.

Interactions between each genotype versus smoking (ever/never) and BMI (25+/ $<25$ ) for prevalent AMD in the case-control comparison were evaluated, but no significant interactions were found between any of the genotypes and smoking or BMI (data not shown). However, there was a weak nonsignificant trend for a smaller effect of BMI among those with genotype *CFH* Y402H TT and an adverse effect of BMI for those with a risk allele (the CC and CT genotypes). Furthermore, within a given genotype, smoking and higher BMI increased risk of advanced AMD. For example, for the homozygous GG risk genotype for *C3*, the OR for advanced AMD was 3.3 (1.0-10.9) for never smokers and increased threefold to 9.8 (2.0-47.5) for individuals who had ever smoked, compared with the CC genotype among nonsmokers, indicating that there are main effects of both smoking and *C3* genotype, and the joint effects are consistent with a multiplicative model.

In Table 6, we present C statistics for models predicting progression to incident advanced AMD with different combi-

TABLE 2. Univariate Association between Baseline Demographic, Environmental, and Genetic Variables and Incident Advanced AMD

	Progressors, n (%)	Nonprogressors, n (%)	Incidence Rates (%)	OR (95% CI)	P*
Total patients	279	1167			
Age (y)					
<70	137 (49)	743 (64)	16	1.0	
70+	142 (51)	424 (36)	25	1.8 (1.4-2.4)	<0.001†
Sex					
Female	163 (58)	694 (59)	19	1.0	0.74
Male	116 (42)	473 (41)	20	1.0 (0.8-1.4)	
Education					
≤High school	119 (43)	383 (33)	24	1.0	0.002
>High school	160 (57)	784 (67)	17	0.7 (0.5-0.9)	
Baseline AMD					
Grades					
2	8 (3)	446 (38)	2	1.0	
3	161 (58)	566 (48)	22	15.9 (7.7-32.6)	<0.001†
4	110 (39)	155 (13)	42	39.6 (18.9-83.0)	
Smoking					
Never	110 (39)	557 (48)	16	1.0	
Past	137 (49)	564 (48)	20	1.2 (0.9-1.6)	0.14
Current	32 (11)	46 (4)	41	3.5 (2.1-5.8)	<0.001
BMI					
<25	69 (25)	416 (36)	14	1.0	
25-29	130 (47)	484 (41)	21	1.6 (1.2-2.2)	0.003
30+	80 (29)	267 (23)	23	1.8 (1.3-2.6)	0.001
Treatment Group					
Placebo	74 (27)	264 (23)	22	1.0	
Antioxidants	77 (28)	295 (25)	21	0.9 (0.7-1.3)	0.70
Zinc	67 (24)	294 (25)	19	0.8 (0.6-1.2)	0.27
Antioxidants and zinc	61 (22)	314 (27)	16	0.7 (0.5-1.0)	0.056
CFH: rs1061170 (Y402H)					
TT	39 (14)	366 (31)	10	1.0	
CT	116 (42)	521 (45)	18	2.1 (1.4-3.1)	
CC	124 (44)	280 (24)	31	4.1 (2.8-6.1)	<0.001†
LOC387715:rs10490924 (A69S)(ARMS2)					
GG	67 (24)	612 (52)	10	1.0	
GT	138 (49)	446 (38)	24	2.8 (2.1-3.9)	<0.001†
TT	74 (27)	109 (9)	40	6.2 (4.2-9.1)	
CFH:rs1410996					
TT	8 (3)	158 (14)	5	1.0	
CT	74 (27)	472 (40)	14	3.1 (1.5-6.6)	<0.001†
CC	197 (71)	537 (46)	27	7.2 (3.5-15.0)	
C2:rs9332739 (E318D)					
GG	271 (97)	1075 (92)	20	1.0	
CG/CC	8 (3)	92 (8)	6	0.3 (0.2-0.7)	0.005
CFB:rs641153 (R32Q)					
CC	256 (92)	1023 (88)	20	1.0	
CT/TT	23 (8)	143 (12)	14	0.6 (0.4-1.0)	0.06
C3:rs2230199 (R102G)					
CC	124 (44)	652 (56)	16	1.0	
CG	130 (47)	456 (39)	22	1.5 (1.1-2.0)	
GG	25 (9)	59 (5)	30	2.2 (1.3-3.7)	<0.001†

OR, odds ratio; CI, confidence interval. AMD grade 2 was category 2 in one or both eyes (worst eye), grade 3 was AMD category 3 in one or both eyes (worst eye), grade 4 was AMD category 4 in one eye (the worst eye).

\* Referent category is that listed first under each variable.

† Test for linear trend.

nations of genetic, ocular, demographic, behavioral, and treatment variables.

There was a significant improvement in the C statistic when the behavioral factors smoking and BMI were added as additional risk variables in model 2 with a C statistic of  $0.757 \pm 0.016$  (model 1 vs. 2,  $P = 0.028$ ). In model 3, we added all six variants in this report, together with baseline AMD grade, age, sex, education, smoking, and BMI and found a C statistic of  $0.821 \pm 0.014$  which was a significant improvement over the model without genotypes (model 2 vs. 3,  $P < 0.001$ ). When treatment was added to model 3, the C statistic was unchanged with C statistic of  $0.822 \pm 0.014$ . When the treatment-CFH

Y402H interaction term was added to the full model, the C statistic significantly improved to  $0.831 (\pm 0.013)$ , but the change in the C statistic was small. Of note, these C statistics for prediction of AMD progression are similar to the Framingham risk score prediction model results for coronary heart disease (CHD).<sup>35</sup>

We then considered the possibility of using model 4 as shown in Table 6, for purposes of individual risk prediction. We calculated the sensitivity and specificity of model 4 in Table 6 using different cutoffs to denote potential screen-positive criteria. The ROC curve is presented in Figure 1.

Our goal was to identify a cutoff, if possible, where both the sensitivity and specificity would be at least 70%. This cutoff

TABLE 3. Association between Prevalent Advanced AMD and Demographic, Environmental, and Genetic Risk Factors

Variable	All Advanced AMD			Unilateral Advanced AMD*			Bilateral Advanced AMD*			Geographic Atrophy†			Neovascular AMD†		
	OR (95% CI)	P	Cases/Controls (n)	OR (95% CI)	P	Cases/Controls (n)	OR (95% CI)	P	Cases/Controls (n)	OR (95% CI)	P	Cases/Controls (n)	OR (95% CI)	P	Cases/Controls (n)
Age (y)															
<70	1.0	<0.0001	202/222	1.0	0.001	307/222	1.0	<0.0001	156/222	1.0	0.001	373/222	1.0	<0.0001	
≥70	2.8 (1.8-4.2)			2.3 (1.4-3.8)			3.7 (2.2-6.2)		2.6 (1.5-4.6)			3.1 (1.9-4.9)			
Sex															
Female	1.0	0.62		1.0	0.85		1.0	0.55	1.0	0.89		1.0	0.5		
Male	0.9 (0.6-1.4)			1.0 (0.6-1.5)			0.9 (0.5-1.4)		1.0 (0.6-1.8)			0.9 (0.5-1.3)			
Education															
≤High school	1.0	0.01		1.0	0.01		1.0	0.07	1.0	0.18		1.0	0.01		
>High school	0.6 (0.4-0.9)			0.5 (0.3-0.9)			0.6 (0.4-1.0)		0.7 (0.4-1.2)			0.6 (0.3-0.9)			
Smoking															
Never	1.0	0.004		1.0	0.002		1.0	0.09	1.0	0.06		1.0	0.01		
Past	1.9 (1.2-2.9)			2.2 (1.3-3.6)			1.6 (0.9-2.6)		1.8 (1.0-3.1)			1.9 (1.2-3.1)			
Current	3.9 (1.7-8.9)			3.7 (1.5-9.6)			4.0 (1.5-10.7)		2.7 (0.8-8.9)			4.4 (1.9-10.4)			
BMI															
<25	1.0	0.72		1.0	0.53		1.0	0.99	1.0	0.97		1.0	0.65		
25-29	1.1 (0.7-1.8)			1.2 (0.7-2.1)			1.0 (0.6-1.8)		1.0 (0.5-1.9)			1.1 (0.7-1.9)			
30+	1.5 (0.9-2.6)			1.7 (0.9-3.2)			1.5 (0.8-2.9)		1.3 (0.6-2.8)			1.8 (1.0-3.2)			
Antioxidant															
No	1.0	0.25		1.0	0.29		1.0	0.42	1.0	0.77		1.0	0.14		
Yes	1.3 (0.8-1.9)			1.3 (0.8-2.1)			1.2 (0.7-2.0)		1.1 (0.6-1.9)			1.4 (0.9-2.2)			
CFH:rs1061170(Y402H)															
TT	1.0	0.02		1.0	0.01		1.0	0.08	1.0	0.16		1.0	0.01		
CT	1.8 (1.1-3.1)			2.2 (1.2-4.2)			1.8 (0.9-3.4)		1.7 (0.8-3.4)			2.3 (1.3-4.2)			
CC	3.5 (1.7-7.1)			4.2 (1.8-9.5)			3.4 (1.5-7.7)		3.3 (1.4-8.2)			4.1 (1.9-8.7)			
P trend	0.0003			0.0003			0.001		0.002			0.01			
LOC387715:rs10490924(A69S)(ARMS2)															
GG	1.0	<0.0001		1.0	0.002		1.0	<0.0001	1.0	0.001		1.0	<0.0001		
GT	3.1 (2.0-4.7)			2.1 (1.3-3.5)			5.1 (3.0-8.5)		2.7 (1.6-4.7)			3.7 (2.3-5.8)			
TT	25.4 (8.6-75.1)			13.4 (3.9-45.6)			64.9 (19.6-214.6)		21.7 (5.7-82.7)			31.2 (10.2-95.2)			
P trend	<0.0001			<0.0001			<0.0001		<0.0001			<0.0001			
CFH:rs1410996															
TT	1.0	0.2		1.0	0.46		1.0	0.34	1.0	0.28		1.0	0.53		
CT	1.6 (0.8-3.5)			1.4 (0.6-3.6)			1.6 (0.6-4.6)		2.0 (0.6-6.7)			1.3 (0.6-3.1)			
CC	3.7 (1.6-8.4)			2.4 (0.9-6.6)			5.5 (1.9-15.7)		4.2 (1.2-14.9)			3.3 (1.3-8.2)			
P trend	0.0003			0.0003			0.04		<0.0001			0.01			
C2:rs9332739(E318D)															
GG	1.0	0.003		1.0	0.11		1.0	0.002	1.0	0.02		1.0	0.02		
CG/CC	0.3 (0.1-0.7)			0.5 (0.2-1.2)			0.1 (0.04-0.5)		0.20 (0.05-0.8)			0.4 (0.2-0.8)			
CFB:rs641153(R32Q)															
CC	1.0	<0.0001		1.0	0.0001		1.0	0.0001	1.0	0.0004		1.0	<0.0001		
CT or TT	0.3 (0.1-0.5)			0.3 (0.1-0.5)			0.2 (0.1-0.5)		0.2 (0.1-0.5)			0.3 (0.1-0.5)			
C3:rs2230199(R102G)															
CC	1.0	0.02		1.0	0.08		1.0	0.01	1.0	0.03		1.0	0.03		
CG	1.6 (1.1-2.5)			1.5 (0.9-2.5)			1.9 (1.2-3.2)		1.9 (1.1-3.3)			1.7 (1.1-2.7)			
GG	3.6 (1.4-9.4)			5.0 (1.7-14.5)			3.7 (1.1-12.5)		4.9 (1.4-18.0)			4.3 (1.5-12.2)			
P trend	0.001			0.003			0.003		0.003			0.003			

OR, odds ratio; CI, confidence interval. OR's adjusted for age (<70, ≥70), sex, education (≤high school, >high school), smoking (never, past, current), BMI (25, 25-29, 30+), antioxidant treatment (yes, no), and all genetic variants and associated genotypes as listed in table.

\* Age-Related Eye Disease Study grades 4 (unilateral) and 5 (bilateral) advanced AMD.

† Clinical Age-Related Maculopathy Grading System grades 4 (noncentral and central geographic atrophy) and grade 5 (neovascular disease).

TABLE 4. Multivariate Association between Demographic, Environmental, and Genetic Risk Factors and Incident Advanced AMD

Variable	Overall			Unilateral Advanced			Bilateral Advanced			Geographic Atrophy			Neovascular AMD		
	OR (95% CI)*	P		OR (95% CI)	P		OR (95% CI)	P		OR (95% CI)	P		OR (95% CI)	P	
Progressors/nonprogressors (n)	279/1167			123/1012			156/1167			64/584			119/1053		
Age (y)															
<70	1.0			1.0			1.0			1.0			1.0		
≥70	1.5 (1.1-2.0)	0.008		1.4 (0.9-2.2)	0.091		1.6 (1.1-2.5)	0.021		1.1 (0.6-1.9)	0.80		1.8 (1.2-2.7)	0.008	
Sex															
Female	1.0			1.0			1.0			1.0			1.0		
Male	1.1 (0.8-1.5)	0.68		0.9 (0.6-1.5)	0.81		1.3 (0.8-2.0)	0.29		1.3 (0.7-2.4)	0.35		1.1 (0.7-1.7)	0.63	
Education															
≤High school	1.0			1.0			1.0			1.0			1.0		
>High school	0.9 (0.6-1.2)	0.42		1.1 (0.7-1.7)	0.66		0.7 (0.5-1.0)	0.082		0.7 (0.4-1.2)	0.21		0.9 (0.6-1.5)	0.82	
Baseline Grade															
2	1.0			1.0			1.0			1.0			1.0		
3	11.0 (5.3-22.8)	<0.001		9.2 (4.2-20.2)	<0.001		24.4 (3.3-179.7)	0.002		†			8.4 (3.6-19.8)	<0.001	
4	19.1 (8.9-41.2)	<0.001					163.9 (22.3->999.9)	<0.001					9.1 (2.4-34.9)	0.001	
Smoking															
Never	1.0			1.0			1.0			1.0			1.0		
Past	1.1 (0.8-1.6)	0.47		1.2 (0.8-1.8)	0.48		1.1 (0.7-1.7)	0.75		1.0 (0.5-1.7)	0.90		1.3 (0.8-2.0)	0.30	
Current	3.1 (1.7-5.6)	<0.001		2.7 (1.1-6.7)	0.034		3.0 (1.4-6.3)	0.005		1.5 (0.4-6.1)	0.54		3.4 (1.4-8.7)	0.009	
BMI															
<25	1.0			1.0			1.0			1.0			1.0		
25-29	1.7 (1.2-2.4)	0.006		1.7 (1.0-2.7)	0.042		1.7 (1.0-2.9)	0.038		1.8 (0.9-3.6)	0.09		1.6 (1.0-2.7)	0.059	
30+	1.6 (1.1-2.4)	0.024		1.6 (0.9-2.8)	0.11		1.5 (0.9-2.7)	0.16		2.5 (1.2-5.1)	0.016		1.5 (0.8-2.6)	0.19	
Treatment Group															
Placebo	1.0			1.0			1.0			1.0			1.0		
Antioxidants	0.9 (0.6-1.3)	0.54		1.0 (0.6-1.9)	0.90		0.8 (0.4-1.4)	0.40		0.6 (0.3-1.4)	0.23		1.1 (0.6-2.0)	0.68	
Zinc	0.7 (0.4-1.0)	0.075		1.3 (0.7-2.3)	0.40		0.4 (0.2-0.7)	0.001		1.2 (0.6-2.5)	0.63		0.8 (0.4-1.5)	0.49	
Antioxidants and zinc	0.6 (0.4-0.9)	0.023		0.9 (0.5-1.6)	0.65		0.4 (0.2-0.8)	0.004		0.8 (0.4-1.7)	0.58		0.7 (0.4-1.3)	0.28	
CFH:rs1061170(Y402H)															
TT	1.0			1.0			1.0			1.0			1.0		
CT	1.3 (0.8-2.1)	0.29		1.6 (0.8-3.4)	0.22		1.1 (0.6-2.1)	0.77		1.0 (0.4-2.5)	0.98		1.5 (0.7-3.1)	0.28	
CC	2.0 (1.1-3.5)	0.019		2.7 (1.2-6.3)	0.019		1.5 (0.7-3.1)	0.33		1.9 (0.7-5.5)	0.21		2.1 (0.9-4.6)	0.08	
P trend		0.014			0.011			0.27			0.110			0.08	
LOC387715:rs10490924(A69S)(ARMS2)															
GG	1.00			1.0			1.0			1.0			1.0		
GT	2.3 (1.6-3.3)	<0.001		2.8 (1.7-4.4)	<0.001		2.0 (1.2-3.3)	0.007		1.9 (1.1-3.5)	0.03		2.7 (1.6-4.4)	<0.001	
TT	4.0 (2.6-6.1)	<0.001		3.1 (1.7-5.8)	<0.001		4.6 (2.6-8.2)	<0.001		1.8 (0.8-4.2)	0.16		5.2 (2.9-9.4)	<0.001	
P trend		<0.001			<0.001			<0.001			0.06			<0.001	
CFH:rs1410996															
TT	1.0			1.0			1.0			1.0			1.0		
CT	1.7 (0.7-4.0)	0.23		1.1 (0.4-3.5)	0.84		2.7 (0.7-10.8)	0.15		2.0 (0.4-10.4)	0.39		1.9 (0.5-7.2)	0.36	
CC	2.4 (1.0-5.8)	0.061		1.9 (0.6-6.2)	0.29		3.3 (0.8-13.6)	0.098		3.5 (0.6-19.4)	0.15		3.0 (0.8-12.0)	0.12	
P trend		0.029			0.083			0.16			0.09			0.037	
C2:rs9332739(E318D)															
GG	1.0			1.0			1.0			1.0			1.0		
CG/GC	0.4 (0.2-0.8)	0.010		0.5 (0.2-1.4)	0.19		0.2 (0.1-0.8)	0.021		0.2 (0.03-1.7)	0.15		0.4 (0.1-1.3)	0.12	
CFH:rs641153(R32Q)															
CT or TT	1.0			1.0			1.0			1.0			1.0		
CC	0.8 (0.5-1.4)	0.42		0.8 (0.4-1.6)	0.47		1.0 (0.5-1.9)	0.92		0.8 (0.3-2.1)	0.60		0.9 (0.5-1.9)	0.87	
CC	1.0			1.0			1.0			1.0			1.0		
CG	1.4 (1.1-2.0)	0.022		1.3 (0.8-2.0)	0.26		1.6 (1.0-2.4)	0.044		1.2 (0.7-2.1)	0.59		1.7 (1.1-2.7)	0.015	
GG	1.8 (1.0-3.2)	0.044		2.1 (1.0-4.5)	0.052		1.6 (0.7-3.6)	0.24		1.9 (0.7-5.3)	0.22		2.5 (1.2-5.4)	0.015	
P trend		0.006			0.050			0.049			0.31			0.002	

\* ORs adjusted for age (<70, ≥70), sex, education (≤high school, >high school), smoking (never, past, current), baseline AMD grade, BMI (<25, 25-29, 30+), and treatment groups (placebo, antioxidants, zinc, and antioxidants plus zinc), and all six genetic variants and associated genotypes as listed in the table.  
 † Analysis restricted to initial grade of 3.

TABLE 5. Assessment of Genotype-Treatment Interaction Effects on Risk of Incident Advanced AMD

	Treatment Groups				P-interaction (6 df)‡	P-interaction (3 df)§
	Placebo OR (95% CI)*	Antioxidant OR (95% CI)	Zinc OR (95% CI)	Antioxidant-Zinc OR (95% CI)		
CFH rs:1061170(Y402H)						
TT	1.0	0.8 (0.3-2.1)	0.2 (0.05-0.5)	0.2 (0.07-0.6)	0.057	0.016
CT	0.7 (0.3-1.5)	0.7 (0.3-1.5)	0.6 (0.3-1.3)	0.4 (0.2-1.0)		
CC	0.9 (0.4-2.2)	0.8 (0.3-1.9)	0.9 (0.4-2.0)	0.9 (0.4-2.1)		
LOC387715 rs:10490924(A69S) (ARMS2)						
GG	1.0	5.8 (2.5-13.5)	4.1 (1.7-9.8)	2.0 (0.8-4.8)	0.37	0.18
GT	3.5 (1.7-7.1)	2.5 (1.2-4.9)	1.9 (1.0-3.8)	1.7 (0.8-3.3)		
TT	5.0 (2.1-11.8)	1.0 (0.5-2.2)	0.8 (0.4-1.8)	1.1 (0.5-2.4)		
CFH:rs1410996						
TT	1.0	0.4 (0.06-2.6)	†	0.4 (0.06-2.3)	0.88	0.28
CT	0.9 (0.3-3.3)	0.9 (0.2-3.2)	0.5 (0.1-1.8)	0.5 (0.1-1.9)		
CC	1.1 (0.3-4.0)	1.0 (0.3-3.7)	1.0 (0.3-3.4)	0.7 (0.2-2.7)		
C2:rs9332739(E318D)						
GG	1.0	0.9 (0.6-1.3)	0.7 (0.4-1.0)	0.6 (0.4-0.9)		0.68
CG/CC	0.2 (0.02-1.3)	0.2 (0.05-1.2)	0.2 (0.05-1.2)	0.4 (0.1-1.5)		
CFB:rs641153(R32Q)						
CC	1.0	0.7 (0.2-1.9)	0.6 (0.2-1.9)	0.6 (0.3-1.5)		
CT/TT	0.3 (0.09-1.2)	0.8 (0.5-1.3)	0.6 (0.4-1.0)	0.5 (0.3-0.9)		0.45
C3:rs2230199(R102G)						
CC	1.0	1.0 (0.5-1.8)	0.7 (0.4-1.3)	0.7 (0.4-1.4)	0.56	0.54
CG	1.7 (0.9-3.3)	1.2 (0.6-2.2)	1.1 (0.6-2.0)	1.0 (0.5-1.8)		
GG	1.5 (0.5-4.8)	2.7 (1.0-7.9)	2.0 (0.6-6.5)	0.4 (0.07-1.9)		

\* ORs adjusted for age (<70, ≥70), sex, education (≤high school, >high school), smoking (never, past, current), baseline AMD grade, BMI (<25, 25-29, 30+), and treatment groups (placebo, antioxidants, zinc, and antioxidants plus zinc), and all genotypes.

† No cases among the zinc-TT genotype group.

‡ Interaction of treatment group by genotype group.

§ Interaction of treatment group by number of risk/protective alleles.

was approximately identified for risk score  $\geq -1.5$  as screen positive,  $< -1.5$  as screen negative, which yielded a sensitivity of 83% (232/279) and specificity of 68% (794/1167). Some examples of using this risk prediction algorithm on a random progressor and nonprogressor are given in Table 1.

Plotted histograms of scores for progressors and nonprogressors are shown in Figure 2, indicating a good separation between these two groups. Risk score distributions were substantially different with progressor scores tending to be higher than nonprogressors, although there was some overlap.

## DISCUSSION

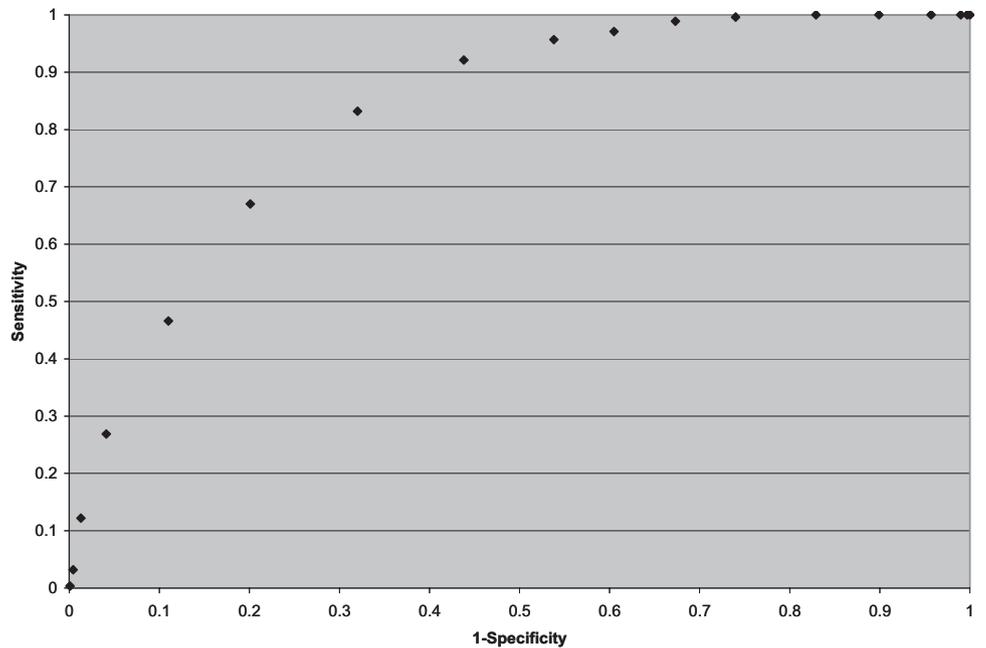
This article, for the first time to our knowledge, shows the independent association of six genetic variants with both prev-

alence and incidence of advanced AMD, with adjustment for all the genetic variants in addition to ocular, demographic, behavioral, and treatment factors. Discrimination between progressors and nonprogressors was excellent for the overall risk score. The predictive power of this composite of risk factors for progression to advanced AMD, with a C statistic score of 0.83, is comparable to the Framingham risk functions for CHD in which the C statistics were 0.79 for white men and 0.83 for white women in the Framingham study cohort and somewhat lower in several replication samples.<sup>35</sup> Clearly, genetic factors play a major role in this disease, as demonstrated by the large and consistent estimates of the effects of the genetic variants on various groups of advanced AMD, with adjustment for other known risk factors. However, modifiable factors also have an impact. Cigarette smoking increased risk for all genotypes. For

TABLE 6. C Statistics for Incident Advanced AMD Based on Models with Different Combinations of Genetic, Environmental, and Treatment Variables

Model	Genetic Variables	Demographic, Environmental, Treatment Variables	C Statistic ( $\pm$ SE)*
1		Age, gender, education, baseline grade	0.732 $\pm$ 0.017
2		Age, gender, education, baseline grade, smoking, BMI	0.757 $\pm$ 0.016
3	CFH Y402H, LOC387715 A69S(ARMS2), CFH 1410996, C2E318D, CFB R32Q, C3 R102G	Age, gender, education, baseline grade, smoking, BMI	0.821 $\pm$ 0.014
4	CFH Y402H, LOC387715 A69S(ARMS2), CFH 1410996, C2E318D, CFB R32Q, C3 R102G	Age, gender, education, baseline grade, smoking, BMI, treatment assignment	0.822 $\pm$ 0.014
5	LOC387715 A69S(ARMS2), CFH 1410996, C2E318D, CFB R32Q, C3 R102G, interaction between CFHY402H and treatment assignment	Age, gender, education, baseline grade, smoking, BMI	0.831 $\pm$ 0.013

\* Model 2 vs. 1,  $P = 0.028$ ; 3 vs. 2,  $P < 0.001$ ; 4 vs. 3,  $P = 0.52$ ; 5 vs. 4,  $P = 0.018$ ; 5 vs. 3,  $P = 0.019$ .



**FIGURE 1.** Sensitivities and specificities for a variety of risk score cutoffs and ROC curve for prediction of incident advanced age-related macular degeneration.

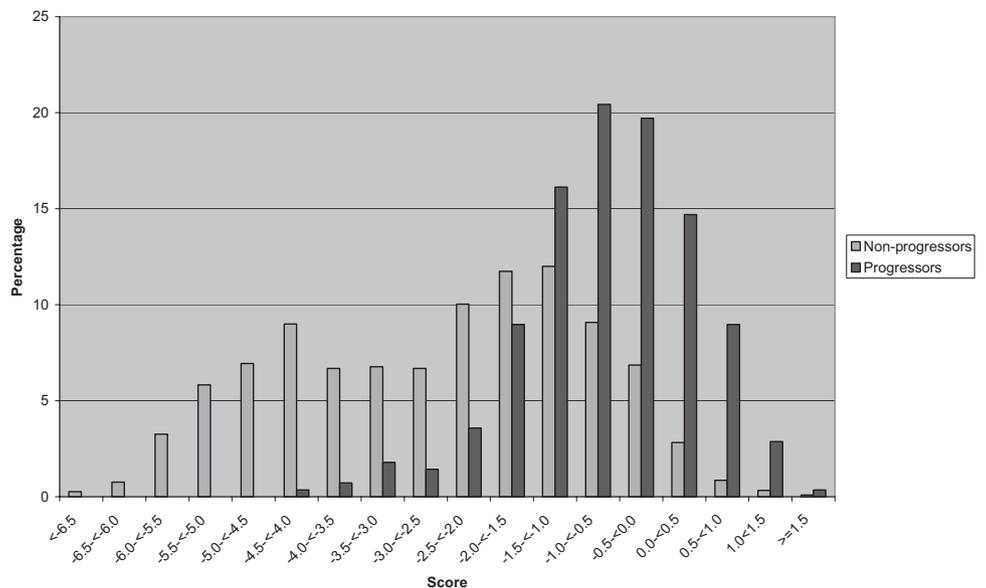
example, risk of advanced AMD increased from over 3-fold for nonsmokers to almost 10-fold for smokers among individuals with the same homozygous *C3* risk genotype compared with nonsmokers with the nonrisk genotype. Higher BMI also contributed to the risk profile for all genotypes.

We have previously presented the combined risk of AMD as a function of five of these genetic variants alone ranging from a less than 1% to a more than 50% lifetime risk.<sup>7</sup> This report expands and refines those observations in important and meaningful ways by adding a new genetic variant; incorporating ocular, demographic, behavioral, and treatment factors; calculating C statistics for advanced AMD based on models with different combinations of genetic and other variables; and evaluating the ability of the resultant risk scores to discriminate between individuals who progress and those who do not.

It is well known that the presence of intermediate AMD is related to progression to advanced AMD,<sup>20</sup> and results in our report underscore the importance of baseline patho-

logic features in the macula, including drusen and pigment irregularities. However, we have shown that two genetic variants also predict progression to advanced disease independent of the fundus appearance.<sup>12</sup> This current report expands on those results by adding four additional known genetic variants and adds new analyses. We show that a combined model with six genetic factors significantly contributes to the prediction of AMD progression, above and beyond knowledge of the ocular phenotype, as shown by the higher C statistic score when the genetic variables are added ( $P < 0.001$ ). In other words, the prediction of advanced AMD progressively improves beyond knowledge of only the demographic and ocular factors when the behavioral factors smoking and BMI are added to the model, and improves even more when genetic factors are known.

The relationship between treatment effect and two of these genotypes was evaluated previously.<sup>36</sup> In the current manuscript, we have expanded our set of genetic risk factors to



**FIGURE 2.** Incident advanced age-related macular degeneration. Risk scores for progressors and nonprogressors are based on all genetic variants as well as demographic, ocular, environmental, and treatment variables.

include four additional SNPs that are associated with AMD. We also considered interaction effects between genetic and environmental factors. We did find a significant interaction between the number of risk alleles for the *CFHY402H* variant and treatment, whereby patients with the CC (i.e., high risk) genotype are less likely to benefit from the antioxidant-mineral supplementation than are subjects with the TT and CT genotypes. However, inclusion of this interaction term increased the C statistic by less than 1% and had a small effect (approximately 1%) on the sensitivity and specificity, when a cutoff for test positive of  $\geq -1.5$  was used. Therefore, for the sake of simplicity, we elected not to include this interaction term in our risk prediction algorithm. It remains to be seen whether this interaction effect is reproducible in other study populations evaluated for progression of AMD.

Unique features of this study include the evaluation of the predictive power of several genetic and other variables based on a large, well-characterized population of Caucasian patients from various geographic regions around the United States. Further strengths include the standardized collection of risk factor information, direct measurements of height and weight, and classification of maculopathy by standardized ophthalmic examinations and grading of fundus photographs. Misclassification was unlikely, because grades were assigned without knowledge of risk factors or genotype. We controlled for known AMD risk factors, including age, education, BMI, smoking, baseline AMD grade, and treatment assignment in assessing the relationship between genetic variants and advanced AMD. Both the environmental and genetic risk factors were independently associated with AMD, when considered simultaneously. There may be some other unmeasured and therefore uncontrolled factors that confound these relationships, but, to explain these results, they would have to be highly related to genotype, smoking, BMI, and treatment assignment and a strong risk factor for AMD. Although this is a selected population, the subjects probably represent the typical patient with advanced AMD, and the overall population is similar to other clinic populations in this age range in terms of smoking and prevalence of obesity as well as the distribution of the genotypes. This large and well-characterized population provided a unique opportunity to evaluate gene-environment associations and interactions. Furthermore, the biologic effects of the genetic variants do not appear to differ in major ways among various Caucasian populations with AMD.

These analyses and results indicate the potential for individual prediction of risk for AMD. For example, in calculating the risk score, one could estimate "points" from the regression coefficients (Table 1) for current smoking (1.1), BMI 30+ (0.48), antioxidants and zinc (-0.50) and the various genetic variants (ranging from -1.0 to +1.4), to obtain an overall risk for an individual to progress to advanced AMD. This process could be refined as new genetic and environmental predictors are established. Targeting high-risk individuals could lead to heightened awareness and more frequent surveillance and clinical examinations, as well as identification of high-risk individuals for inclusion in clinical trials of new therapies. Other advantages of knowing such a risk score include the possibility of more targeted education and counseling about known modifiable factors. Screening would identify high-risk individuals who would be encouraged to follow a healthy lifestyle by not smoking, eating vegetables and fish, maintaining a normal weight and getting exercise, and taking antioxidant-mineral supplements. All of these factors are known to influence the inflammatory and immune pathways that are involved in the pathogenesis of AMD.<sup>37,38</sup>

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