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CFH Gene Variant, Y402H, and Smoking, Body Mass Index, Environmental Associations with Advanced Age-Related Macular Degeneration

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Key Words

Case-control association analysis · Environmental risk factor · Epidemiologic approaches · Gene-environment interaction · Genotype · Macular degeneration

Abstract

Objectives: We tested the hypothesis that modifiable lifestyle factors alter the genetic susceptibility associated with a common coding variant in the complement factor H (CFH) gene, Y402H, for the leading cause of blindness among the elderly, age-related macular degeneration (AMD). ***Methods:*** In this case-control association analysis, Caucasian participants in the multicenter Age-Related Eye Disease Study with advanced AMD (n = 574 cases) or no AMD (n = 280 controls) were evaluated. AMD status was determined by grading of fundus photographs. Risk factors including cigarette smoking and body mass index (BMI) were assessed and DNA specimens were genotyped for the variant in the CFH gene. Unconditional logistic regression analyses were performed. Attributable risks and multivariable AMD risk scores were calculated. ***Results:*** The number of risk alleles for Y402H was associated with advanced AMD, with odds ratios (OR) of 2.7 (95% confidence interval (CI) 1.8–3.8) for the CT heterozygous genotype and OR 7.4 (4.7–11.8) for the homozygous CC risk genotype, after controlling for demographic and behavioral risk factors. Current cigarette smoking (OR 5.1) and high BMI ≥ 30 (OR 2.1) were independently related to AMD, controlling for genotype. The association between AMD and BMI varied dependent on genotype (P interaction = 0.006 for the CT vs. TT genotype). The CC genotype plus higher BMI (OR 5.9) or smoking (OR 10.2) conferred the greatest risks. Gene plus environment risk scores provided an area under the receiver operating characteristic (ROC) curve of 0.70–0.75. ***Conclusions:*** Genetic and environmental factors are independently related to advanced AMD, and modifiable factors alter genetic susceptibility. The AMD risk score identifies a highly susceptible population.

The US twin study of age-related macular degeneration (AMD) quantified the proportions of variance in this disease due to genetic and environmental factors as 46–71% and 19–37%, respectively [1]. Several environmental factors have been identified over the past decade including cigarette smoking [2, 3], higher body mass index (BMI) [4, 5], and nutritional factors [6–9]. A genetic effect has also been suggested for several years based on familial aggregation and linkage studies [10–14] and more recently confirmed by identification of the association between CFH polymorphisms with AMD [15–18]. Several studies

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have confirmed the relationship between the common CFH polymorphism, Y402H, and AMD [19–21]. It is important to determine the effect of known environmental risk factors on AMD controlling for genetic susceptibility, and to assess the overall impact of risk genotypes within categories of these modifiable factors. Knowledge of the extent to which a combination of genetic and environmental factors can predict AMD, as a risk score, can also help with management and prevention of AMD. We evaluated these associations in a large cohort of cases with advanced AMD, the most visually disabling form of the disease, and control subjects without AMD from a national, multicenter study of age-related eye disease.

Methods

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 progression of AMD which ended in December, 2005. Study procedures have been previously reported [5, 7]. Based on ocular examination and reading center photographic grading of fundus photographs, Caucasian participants in this study were divided into two main groups representing the most discordant phenotypes: no AMD with either no drusen or nonextensive small drusen (n = 280), or advanced AMD with visual loss (n = 574) [7]. The advanced form of AMD was then reclassified into the two subtypes of either non-central or central geographic atrophy (n = 145) or neovascular disease (n = 429), independent of visual acuity level, using the Clinical Age-Related Maculopathy Grading System [22, 23], to determine whether results differed between these two (advanced dry and wet) phenotypes. Risk factor data was obtained at the baseline visit from questionnaires and height and weight measurements. DNA samples were obtained from the AREDS Genetic Repository. The common single nucleotide polymorphism (SNP) in exon 9 of the CFH gene on chromosome 1q31 (rs1061170), a change 1277T→C, resulting in a substitution of histidine for tyrosine at codon 402 of the CFH protein, Y402H, was evaluated. Genotyping was performed using primer mass extension and MALDI-TOF MS analysis by the MassEXTEND methodology of Sequenom (San Diego, Calif., USA) at the Broad Institute Center for Genotyping and Analysis, Cambridge, Mass., USA.

Statistical Analyses

Individuals with advanced AMD, as well as the separate types of advanced AMD, were compared to the control group of Caucasian persons with no AMD, with regard to genotype and risk factor data. Adjusted unconditional logistic regression analyses were performed to evaluate the relationships between AMD and these risk factors, controlling for age (70 or older, younger than 70), gender, and education (high school or less, more than high school). A multivariate logistic regression model was used to further adjust for and evaluate the effects of cigarette smoking (never, past, current), and BMI, which was calculated as the weight in kilograms divided by the square of the height in meters (<25, 25–29.9, and ≥30). 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). Tests for multiplicative interactions were calculated using cross product terms according to genotype and the individual risk factors. Odds ratios and 95% confidence intervals were calculated for each risk factor and within the three genotype groups. Tests for trend for the number of risk (C) alleles (0, 1, 2) were calculated [24]. An age-adjusted concordant or 'c' statistic (i.e. the area under the receiver operating characteristic (ROC) curve) was calculated for each model to assess the probability that the risk score based on the group of risk factors in that model from a random case was higher than the corresponding risk score from a random control within the same 10-year age group [25]. Three models were analyzed to assess advanced AMD as well as geographic atrophy and neovascular disease separately.

Results

The distribution of the variables according to CFH genotype, for controls and cases with geographic atrophy and neovascular disease are shown in table 1. Compared to controls, cases were older, had fewer years of education, were more likely to smoke, and had a higher body mass index.

Table 2 displays the odds ratios for the adjusted and multivariate models, comparing advanced AMD cases with controls, for the genetic, demographic, and behavioral risk factors. Controlling for age, gender, education, smoking, and body mass index, cases were more likely to have the homozygous CC genotype, with OR (multivariate 1 (MV1)) of 7.9 (95% confidence interval (CI) 4.2–14.8) for geographic atrophy, and OR (MV1) 7.5 (95% CI 4.6–12.3) for neovascular disease, compared with controls. For each additional 'C' allele, there was an increased risk of advanced AMD, and this 'dose effect' for the 'C' allele was highly significant (all p values <0.001). Controlling for genotype and other factors, age 70 years or older was associated with a significant 3-fold greater risk of advanced AMD. Compared to females, controlling for age, genotype, and other factors, males had a significantly lower risk of neovascular disease. Higher educational status was associated with a lower risk of AMD, with MV1 ORs ranging from 0.5 to 0.6 for education beyond high school compared with less education, and this association was marginally significant for neovascular disease in the multivariate models. Cigarette smoking was associated with a statistically significant increased risk of advanced AMD for both subtypes, controlling for genotype and other factors, with MV1 ORs for current smoking ranging from 4.1 to 5.1, and ORs 1.6 to 1.8 for past smoking. Body mass index of 30 kg/m^2 or higher increased risk for advanced

Table 1. Distribution of demographic and behavioral risk factors for advanced AMD according to CFH Y402H genotype

	Controls (n = 280)						Geographic atrophy (n = 145)						Neovascular AMD (n = 429)					
	TT		CT		CC		TT		CT		CC		TT		CT		CC	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
No. per genotype group	113		126		41		23		60		62		73		190		166	
Age, years																		
50–69	83	74	90	71	32	78	13	57	28	47	32	52	34	47	90	47	88	53
70–95	30	26	36	29	9	22	10	43	32	53	30	48	39	53	100	53	78	47
Mean (SD), years	66.9	3.9	66.9	4.1	65.7	4.5	66.9	6.7	69.0	5.2	68.8	5.2	68.9	5.6	69.7	4.8	69.2	5.1
Gender																		
Male	53	47	54	43	22	54	12	52	32	53	31	50	30	41	69	36	68	41
Female	60	53	72	57	19	46	11	48	28	47	31	50	43	59	121	64	98	59
Education																		
High school or less	5	4	5	4	2	5	2	9	3	5	7	11	5	7	19	10	24	14
College or more	108	96	121	96	39	95	21	91	57	95	55	89	68	93	171	90	142	86
Smoking																		
Never	51	45	69	55	18	44	9	39	21	35	21	34	20	27	80	42	68	41
Past	57	51	53	42	21	51	10	44	36	60	35	56	39	54	95	50	76	46
Current	5	4	4	3	2	5	4	17	3	5	6	10	14	19	15	8	22	13
Body Mass Index																		
<25	31	27	45	36	12	29	12	52	18	30	15	24	25	34	42	22	40	24
25–29.9	48	43	62	49	20	49	6	26	27	45	26	42	21	29	90	47	82	49
≥30	34	30	19	15	9	22	5	22	15	25	21	34	27	37	58	31	44	27
AREDS RX																		
No antioxidants	68	60	63	50	25	61	11	48	31	52	38	61	44	60	92	48	78	47
Antioxidants	45	40	63	50	16	39	12	52	29	48	24	39	29	40	98	52	88	53

Data are numbers (percentages rounded to nearest whole number) unless otherwise indicated.

AMD and this elevated risk was slightly higher among neovascular cases (OR 2.4, 95% CI 1.5–3.9), compared with geographic atrophy (OR 1.6, 95% CI 0.9–3.0), although this difference in ORs between the two advanced forms of AMD was not statistically significant. Additional adjustment for antioxidant status (multivariate 2 models) slightly strengthened these associations for neovascular AMD.

The probability that a random case had a higher risk score than a random control within each 10-year age group was 0.70–0.72 for the adjusted model (age, gender, education, and genotype). This score was somewhat higher with the addition of the environmental factors: 0.74–0.75. In contrast, the risk score with environmental factors only in the model without consideration of genotype was lower, with a 'c' statistic of 0.62–0.63.

Table 3 displays the effects of the modifiable risk factors, BMI and smoking, within each genotype, for each case group, as well as the p values for interaction between the risk factors and genotype. Higher BMI (25 or higher) did not increase risk of AMD with the TT genotype (OR 0.7, 95% CI 0.4–1.2), whereas higher BMI increased risk of advanced AMD for both the CT heterozygotes (OR 2.2, 95% CI 1.3–4.0) and CC homozygotes (OR 5.9, 95% CI 3.1–11.4). For individuals with BMI less than 25, only the homozygous CC genotype was associated with higher risk (OR 3.9, 95% CI 1.7–9.0). There was a significant interaction between BMI and genotype, with a difference in effect of BMI on AMD for the CT genotype compared with TT (P interaction = 0.006, 0.021, and 0.04 for advanced disease, geographic atrophy and neovascular disease, respectively). There was a non-significant trend in the same direction for the CC compared with the TT genotype. The interaction effect between number of C alleles and effect of BMI on risk of AMD was marginally statistically significant for advanced AMD and geographic atrophy (p interaction = 0.053, 0.047, respectively).

Increased risk of AMD was seen for smoking within each genotype, and for both types of advanced AMD. Increased risk associated with the presence of a C allele was

Table 2. Association between advanced AMD and demographic, genetic, and behavioral risk factors

	Advanced AMD		Geographic atrophy		Neovascular AMD	
	OR (CI)	p value	OR (CI)	p value	OR (CI)	p value
No. of cases/controls	574/280		145/280		429/280	
Genotype						
Adjusted[a]						
TT	1.0		1.0		1.0	
CT	2.3 (1.6–3.3)	<0.001	2.3 (1.3–4.0)	0.003	2.3 (1.5–3.3)	<0.001
CC	6.6 (4.3–10.3)	<0.001	7.5 (4.0–13.8)	<0.001	6.5 (4.0–10.3)	<0.001
No. of C alleles		<0.001 (p-trend)		<0.001 (p-trend)		<0.001 (p-trend)
Multivariate 1[b]						
TT	1.0		1.0		1.0	
CT	2.7 (1.8–3.8)	<0.001	2.7 (1.5–4.8)	0.0009	2.7 (1.8–4.1)	<0.001
CC	7.4 (4.7–11.8)	<0.001	7.9 (4.2–14.8)	<0.001	7.5 (4.6–12.3)	<0.001
No. of C alleles		<0.001 (p-trend)		<0.001 (p-trend)		<0.001 (p-trend)
Multivariate 2[c]						
TT	1.0		1.0		1.0	
CT	2.3 (1.5–3.4)	0.001	2.0 (1.0–3.8)	0.03	2.4 (1.5–3.8)	<0.001
CC	6.6 (4.0–10.9)	<0.001	6.9 (3.4–14.1)	<0.001	6.6 (3.8–11.4)	<0.001
No. of C alleles		<0.001 (p-trend)		<0.001 (p-trend)		<0.001 (p-trend)
Age						
Adjusted[a]						
<70	1.0		1.0		1.0	
≥70	2.8 (2.0–4.0)	<0.001	2.8 (1.8–4.4)	<0.001	2.9 (2.1–4.1)	<0.001
Multivariate 1[b]						
<70	1.0		1.0		1.0	
≥70	3.1 (2.2–4.3)	<0.001	3.1 (1.9–4.9)	<0.001	3.2 (2.2–4.6)	<0.001
Multivariate 2[c]						
<70	1.0		1.0		1.0	
≥70	3.3 (2.3–4.8)	<0.001	2.9 (1.7–5.0)	<0.001	3.7 (2.5– 5.6)	<0.001
Gender						
Adjusted[a]						
Female	1.0		1.0		1.0	
Male	0.8 (0.6–1.1)	0.21	1.3 (0.8–2.0)	0.28	0.7 (0.5–0.96)	0.03
Multivariate 1[b]						
Female	1.0		1.0		1.0	
Male	0.7 (0.5–1.0)	0.07	1.2 (0.8–1.9)	0.46	0.6 (0.4–0.9)	0.01
Multivariate 2[c]						
Female	1.0		1.0		1.0	
Male	0.7 (0.5–1.0)	0.07	1.4 (0.8–2.3)	0.24	0.6 (0.4–0.9)	0.014
Education						
Adjusted[a]						
≤HS	1.0		1.0		1.0	
>HS	0.5 (0.2–0.9)	0.03	0.5 (0.2–1.3)	0.19	0.7 (0.5–0.96)	0.02
Multivariate 1[b]						
≤HS	1.0		1.0		1.0	
>HS	0.5 (0.3–1.0)	0.06	0.6 (0.2–1.4)	0.22	0.5 (0.2–0.96)	0.04
Multivariate 2[c]						
≤HS	1.0		1.0		1.0	
>HS	0.5 (0.2–1.2)	0.12	0.9 (0.3–2.9)	0.85	0.45 (0.2–1.0)	0.05

Table 2 (continued)

	Advanced AMD		Geographic atrophy		Neovascular AMD	
	OR (CI)	p value	OR (CI)	p value	OR (CI)	p value
Smoking						
Multivariate 1[b]						
Never	1.0		1.0		1.0	
Past	1.6 (1.2–2.3)	0.004	1.8 (1.1–2.9)	0.02	1.6 (1.1–2.13)	0.008
Current	5.1 (2.4–10.6)	<0.001	4.1 (1.6–11.0)	0.005	5.0 (2.3–10.6)	<0.0001
Multivariate 2[c]						
Never	1.0		1.0		1.0	
Past	1.8 (1.2–2.6)	0.003	1.4 (1.1–3.3)	0.02	1.7 (1.1–2.5)	0.013
Current	5.7 (2.6–12.4)	<0.001	3.8 (1.3–11.6)	0.01	5.9 (2.6–13.4)	<0.001
BMI						
Multivariate 1[b]						
<25	1.0		1.0		1.0	
25–29.9	1.1 (0.8–1.6)	0.52	0.9 (0.5–1.5)	0.72	1.3 (0.8–1.9)	0.26
≥30	2.1 (1.3–3.2)	0.001	1.6 (0.9–3.0)	0.13	2.4 (1.5–3.9)	0.0002
Multivariate 2[c]						
<25	1.0		1.0		1.0	
25–29.9	1.1 (0.7–1.7)	0.62	0.8 (0.5–1.6)	0.60	1.2 (0.8–2.0)	0.37
≥30	2.5 (1.5–4.0)	0.0003	1.8 (0.9–3.6)	0.10	2.8 (1.7–4.8)	0.0001
Area under ROC curve, adjusted in 10-year age groups						
Adjusted[a]	0.70 ± 0.02		0.72 ± 0.03		0.70 ± 0.02	
Multivariate 1[b]	0.74 ± 0.02		0.75 ± 0.03		0.74 ± 0.02	
Multivariate 2[c]	0.74 ± 0.02		0.75 ± 0.03		0.74 ± 0.02	
Environment only[d]	0.62 ± 0.02		0.62 ± 0.03		0.63 ± 0.02	

OR = Odds ratio; CI = 95% confidence interval; ROC = receiver operating characteristic (c statistic) [23].

[a] Adjusted for age (50–69, 70–95), gender, education (≤high school vs. >high school), genotype (TT, CT, CC).

[b] Adjusted for age (50–69, 70–95), gender, education (≤high school vs. >high school), smoking (never, past current), BMI (<25/25–29.9/30+), genotype (TT, CT, CC).

[c] Adjusted for variables in model 1 plus antioxidant treatment (taking study supplement containing antioxidants vs. taking study supplement containing no antioxidants).

[d] Adjusted for age (50–69, 70–95), gender, education (≤high school vs. >high school), smoking (never, past current), BMI (<25/25–29.9/30+).

seen for both smokers and non-smokers, although the risks for AMD were higher for smokers. The tests for interaction between genotype and cigarette smoking were not statistically significant. Compared with never smokers with the TT genotype, the ORs for advanced AMD associated with smoking were 1.7 (95% CI 0.9–3.1) for the TT genotype, 4.6 (95% CI 2.6–8.1) for CT heterozygotes, and 10.2 (95% CI 5.3–19.7) for CC homozygotes. The interaction effect between number of C alleles and effect of smoking was not statistically significant. Similar to BMI, results were not materially different for geographic atrophy and neovascular disease.

ORs associated with all three risk factors, including high BMI (≥25), smoking, and having the CC genotype was associated with a 17.4-fold increased risk of AMD, compared with being a non-smoker, with low BMI, and the TT genotype. In order to evaluate the potential impact of this polymorphism on risk of AMD after controlling for the effect of the other risk factors, we calculated the attributable risk (AR) for the presence of a C allele [(OR-1)/OR] × proportion exposed among cases = [(7.4 for CC genotype – 1)/7.4] × 0.397, plus [(2.7 for CT genotype –1)/2.7 × 0.436)] which was 62%, adjusting for smoking and BMI. The corresponding ARs for smoking and high BMI in this study population were 28% for smoking and 19% for BMI, respectively, adjusting for genotype. The combined AR's for the C (CC or CT) genotype plus smoking or high BMI (≥25) were 73% and 69.3%, respectively.

Table 3. Risk of age-related macular degeneration according to BMI, smoking, and genotype and assessment of interactions

	Genotype			
	TT	CT	CC	p (trend) for no. of C alleles
No. of cases				
Advanced AMD	96	250	228	
Geographic atrophy	23	60	62	
Neovascular AMD	73	190	166	
No. of controls	113	126	41	
BMI				
Advanced AMD				
<25	1.0	1.1 (0.6–2.1)	3.9 (1.7–9.0)	
25+	0.7 (0.4–1.2)	2.2 (1.3–4.0)	5.9 (3.1–11.4)	
P (interaction)		0.006 (CT vs. TT)	0.107 (CC vs. TT)	0.053
Geographic atrophy				
<25	1.0	1.0 (0.4–2.5)	3.2 (1.1–9.3)	
25+	0.4 (0.1–1.0)	1.6 (0.7–3.5)	4.7 (2.0–11.1)	
P (interaction)		0.021 (CT vs. TT)	0.050 (CC vs. TT)	0.047
Neovascular AMD				
<25	1.0	1.3 (0.6–2.6)	4.7 (2.0–11.4)	
25+	0.9 (0.4–1.7)	2.7 (1.5–5.2)	7.0 (3.5–14.3)	
P (interaction)		0.04 (CT vs. TT)	0.32 (CC vs. TT)	0.24
Smoking				
Advanced AMD				
Never	1.0	2.0 (1.1–3.5)	7.8 (3.9–15.8)	
Ever	1.7 (0.9–3.1)	4.6 (2.6–8.1)	10.2 (5.3–19.7)	
P (interaction)		0.40 (CT vs. TT)	0.58 (CC vs. TT)	0.63
Geographic atrophy				
Never	1.0	1.5 (0.6–3.7)	6.5 (2.4–17.4)	
Ever	1.2 (0.5–3.1)	4.0 (1.7–9.3)	9.9 (4.0–24.4)	
P (interaction)		0.19 (CT vs. TT)	0.74 (CC vs. TT)	0.86
Neovascular AMD				
Never	1.0	2.2 (1.2–4.3)	9.1 (4.2–19.5)	
Ever	1.9 (1.0–3.8)	5.1 (2.7–9.6)	10.6 (5.2–21.9)	
P (interaction)		0.71 (CT vs. TT)	0.31 (CC vs. TT)	0.31

OR = Odds ratio; CI = confidence interval.

All models adjusted for age (50–69, 70–95), gender, education (≤high school vs. >high school), smoking (never, past current), BMI (<25/25–29.9/30+), and genotype (TT, CT, CC).

Discussion

To our knowledge, this is the largest series of advanced cases of AMD evaluated for both CFH Y402H genotypes and multiple environmental factors simultaneously, and the first to assess BMI together with smoking. Both genetic and environmental factors were independently associated with advanced AMD, the leading cause of visual impairment and vision-related reduced quality of life among elderly individuals. After adjustment for demographic and behavioral factors, the commonly reported variant of the CFH gene located on chromosome 1, Y402H, was strongly associated with advanced AMD, and similar effects were seen for both geographic atrophy and neovascular disease. After controlling for genotype, the modifiable lifestyle factors, higher BMI and smoking, were both statistically significantly related to increased risk of advanced AMD. Furthermore, susceptibility to advanced AMD conferred by this common genetic polymorphism was modified by BMI. Compared with lean individuals

with the TT genotype, increased risk of AMD among these lean individuals with BMI <25 was seen only for the CC homozygotes. For heavier persons with BMI ≥ 25, the risk varied from a non-significant null or slightly protective association for the TT genotype, to a moderately high 2.2-fold increased risk for the heterozygotes, and a very high 5.9-fold increased risk for the CC homozygote state. This interaction between BMI and genotype related to risk of advanced AMD was statistically significant for the CT vs. TT genotype.

Smoking was independently related to both forms of AMD, after controlling for genotype and other factors. The CFH variant was related to AMD for both smokers and nonsmokers with as high as a 10.6-fold increased risk for AMD among those who smoked and also had the CC genotype, versus never smokers with the TT genotype. A statistical interaction between smoking and this genotype was not observed. Only two reports have been published, to our knowledge, which evaluated the relationship between smoking and this CFH polymorphism [26, 27]. Both of those studies found that the CFH Y402H variant was associated with AMD among smokers and nonsmokers, similar to our findings. Our study differs in that it is based on a larger population of advanced AMD cases from multiple centers in the US, includes other important covariates in addition to smoking which are related to AMD, most notably education and BMI, and presents combined risk scores as estimates of AMD risk.

These findings relay an important message. Although we cannot change our genotype, we can alter or modify the risk of AMD associated with genetic susceptibility to some extent, by controlling our weight and not smoking. Risks associated with being overweight and smoking were all somewhat higher compared to being lean and non-smoking within the CT or CC genotypes. Both of these modifiable lifestyle factors are among the most common variables associated with overall morbidity due to chronic diseases, and AMD, a leading cause of blindness, should be considered an important part of that disease burden.

The discriminatory ability of the AMD risk score which was calculated based on the age, gender, education, genotype, smoking history and BMI of the participants, was relatively high (0.74–0.75). This is a multivariable-based estimate of AMD risk which could be used to identify high risk individuals for targeted prevention and treatment strategies. For example, the combination of being overweight and a smoker, and having the CC genotype conferred over a 17-fold higher risk of AMD compared with the lean, non-smokers, who had the TT genotype. This AMD score was only slightly lower than the Framingham risk score for coronary heart disease (0.79 for women and 0.83 for men) [28], although the latter values were not a comparison among same sex people with the same age, which would tend to inflate the 'c' statistic. It will be informative to determine whether this AMD prediction score can be applied to other populations.

The attributable risk for the presence of a C allele was 62% controlling for smoking and BMI, and the combined gene plus environment ARs were 69–73%. These findings need to be interpreted in the context that other factors, whether genetic or environmental, may be acting in concert with these factors to increase susceptibility to AMD. Environmental factors or modifying genes may be needed for gene expression, and genetic factors are likely involved in the effects of behaviors. The attributable risk for smoking after adjusting for genotype, 28%, is similar to previous estimates of 29% [2] and 32% [29], which considered only non-genetic factors.

The association between CFH polymorphisms and AMD is biologically plausible, as previously described, because this gene is involved with inflammatory and immune pathways and is located within binding sites for C-reactive protein (CRP) [15–21, 30], a systemic inflammatory marker associated with AMD [31, 32]. These potential mechanisms involved in the origins of AMD are complex since smoking and higher BMI, factors associated with AMD, increase levels of inflammation and CRP [33], and antioxidants, which are known to reduce progression to advanced AMD [6–9], reduce inflammation [33]. Another interesting observation is the similarity in results between the two subtypes of advanced AMD, geographic atrophy (dry) and neovascular (wet) disease, despite their very different phenotypic appearances. This finding, together with two previous reports based on populations in Iceland [21] and the UK [26], supports a common etiology for the visually disabling forms of AMD, and is consistent with the manifestation of these disparate forms of the disease in members of the same family [34]. Perhaps other modifying genes and/or environmental factors could influence the pathways that lead to these late forms of the disease.

Unique features of this study include the large, well characterized population of Caucasian patients with and without advanced AMD from various geographic regions around the US. Further strengths include standardized collection of risk factor information, direct measurements of height and weight, classification of maculopathy by standardized ophthalmologic examinations and grading of fundus photographs. Misclassification was unlikely

since grades were assigned without knowledge of risk factors or genotype. Confounding is a concern in case-control studies. We controlled for known AMD risk factors, including age and education, as well as antioxidant status, in the assessment of BMI, smoking, and genotype. Both the environmental and genetic risk factors were independently associated with AMD, when considered simultaneously, after adjustment for these factors. There may be some other unmeasured and therefore uncontrolled factors that might still be confounding these relationships, but they would have to be highly related to genotype, smoking and BMI, and a strong risk factor for AMD to explain these results. Although this is a selected population, cases likely represent the typical patient with AMD, and the overall population is similar to others in this age range in terms of smoking and prevalence of obesity, as well as the distribution of the CFH genotype. This large sample size and well-characterized population provided a unique opportunity to evaluate gene-environment associations and interactions. Furthermore, the biological effects of CFH and the modifiable factors are not likely to differ in major ways among various Caucasian populations with AMD.

These results are clinically relevant and impact the management of this disease. People with the high risk genotype have higher susceptibility to AMD. They are not necessarily destined to develop the disease, since some people with AMD have the non-risk genotype and some people without AMD carry the risk genotype. However, individuals with the risk genotype, if identified and appropriately advised, may be more motivated to adhere to healthy lifestyles which are known to be related to a reduced risk of AMD. These include not smoking, maintaining a normal or lean weight, getting exercise, eating an antioxidant rich diet with fruits and vegetables, as well as fish, and getting exercise. Eventually a risk profile which includes both genetic and environmental factors, such as the one calculated herein, may lead to targeted screening for this common cause of visual loss.

The Age-Related Eye Disease Study, a randomized clinical trial, showed that an antioxidant/mineral supplement reduced the rate of progression to advanced AMD by 25% over 5 years among people with intermediate stages of AMD or advanced AMD in one eye [7]. Should individuals who have the risk genotype take these supplements even if they do not yet have signs of the disease? There is no data at this time on which to base such a clinical recommendation. However, clinical trials in the future will be able to refine analyses and include genotype as another predictive risk factor related to progression of disease and response to treatment. Individuals with the risk genotype may also need to be examined more frequently to monitor progression of the disease.

In summary, both genetic and environmental factors are independently associated with AMD. The US twin study of AMD estimated that up to 71% of advanced AMD is heritable, and environmental factors also contribute to the etiology of this disease [1]. Results of this study confirm that genetic factors play a substantial role as shown by the large attributable risks related to this genetic polymorphism. Our report also underscores the need to adhere to healthy lifestyles. We may be entering an era of 'personalized medical care' in which prevention strategies and treatments for AMD will be guided by our genotype as well as demographic and behavioral risk factors [35].

Author Contributions

Dr. Seddon and Dr. Rosner had full access to the data and take responsibility for the integrity and accuracy of the data analyses.
Study concept and design: Seddon, Rosner, Klein
Acquisition of data: Seddon, Klein
Analysis and interpretation of data: Seddon, Rosner, George
Drafting of manuscript: Seddon, Rosner, George
Critical revision of the manuscript for important intellectual content: Seddon, Rosner, George, Klein
Statistical expertise: Rosner, Seddon
Obtained funding: Seddon, Klein
Administrative, technical, or material support: Seddon, George
Study supervision: Seddon, Rosner

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Role of Sponsor

The funding organizations did not influence the design and conduct of the study; the collection, analysis, and interpretation of the data; or the preparation or approval of the manuscript.

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