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Response to AREDS Supplements According to Genetic Factors: Survival Analysis Approach Using the Eye as the Unit of Analysis

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Abstract

Background/aims—The Age-Related Eye Disease Study reported the impact of antioxidant and zinc supplements on risk of progression to advanced stages of age-related macular degeneration (AMD). We evaluated the role of genetic variants in modifying the relationship between supplementation and progression to advanced AMD.

Methods—Among 4124 eyes (2317 subjects), 882 progressed from no AMD, early, or intermediate AMD to overall advanced disease, including geographic atrophy (GA) and neovascular disease (NV). Survival analysis using individual eyes as the unit of analysis was used to assess the effect of supplementation on AMD outcomes, with adjustment for demographic, environmental, ocular, and genetic covariates. Interaction effects between supplement groups and individual *CFH* Y402H and *ARMS2* genotypes, and composite genetic risk groups combining the number of risk alleles for both loci, were evaluated for their association with progression.

Results—Among antioxidant and zinc supplement users compared to the placebo group, subjects with a nonrisk genotype for *CFH* (TT) had a lower risk of progression to advanced AMD (hazard ratio [HR]: 0.55, 95% confidence interval [CI]: 0.32–0.95, P=0.033). No significant treatment effect was apparent among subjects who were homozygous for the *CFH* risk allele (CC). A protective effect was observed among high risk *ARMS2* (TT) carriers (HR: 0.52, 95% CI: 0.33–0.82, P=0.005). Similar results were seen for the NV subtype but not GA.

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The authors' responsibilities were as follows - JMS and BR: designed and conducted the research; BR: analyzed the data or performed the statistical analysis; and all authors: wrote the manuscript and have primary responsibility for the final content.

Competing Interests
There are no competing interests to declare.

Conclusion—The effectiveness of antioxidant and zinc supplementation appears to differ by genotype. Further study is needed to determine the biological basis for this interaction.

Keywords

epidemiology; treatment other; genetics; macula

Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness, irreversible vision loss, and reduced quality of life among adults over age 65. The multifactorial etiology of AMD is encompassed by a complex web of risk factors, both heritable and modifiable, that influence progression to advanced stages of disease.[1] Combined demographic, behavioral, and genetic factors have been incorporated into validated, comprehensive risk models for progression.[2–5] Subsequent inclusion of newly identified genetic variants has enhanced the predictability of these models over time, [6, 7] and increasing evidence has emerged that supports plausible interactions between these genetic and modifiable factors.[8, 9] Understanding this interplay is of utmost importance when considering the preventive and therapeutic strategies involved in patient care.

The impact of nutritional supplements for patients within specific genotype groups has been a subject of debate. The controversy surrounding whether genetic testing should be required prior to selecting specific supplements has been particularly noteworthy, and complement factor H (*CFH*) and age-related maculopathy susceptibility 2 (*ARMS2*) have been of primary interest as genes associated with AMD and its progression.[10] The Age-Related Eye Disease Study (AREDS) originally evaluated the impact of supplements consisting of antioxidants (vitamin E, vitamin C, and beta-carotene) and zinc, and reported a 25% reduced risk of progression to advanced AMD over 5 years.[11] The first evidence of a differential treatment effect with combined antioxidant and zinc supplements compared to placebo according to genotype demonstrated that a lower proportion of nonrisk *CFH* subjects progressed to advanced disease compared to high risk subjects.[2, 12] More recent publications evaluated similar relationships between treatment and genotype; however, these studies revealed conflicting results.[13–15]

Given the emergence of personalized medicine and targeted therapies, it is important to consider the utility of evaluating individual genotypes in order to inform the selection of patient-specific strategies.[7] We therefore aimed to further evaluate the specific genotypes for *CFH* Y402H and *ARMS2* that modify the relationship between supplementation and progression. Our study differs from previous publications by the analytic method selected, namely the use of survival analysis that evaluates individual eyes, and includes a larger component of the AREDS population with a genetic specimen.

Materials and Methods

Study Population and Definition of Progression

Data from AREDS, a randomized controlled clinical trial, were used in these analyses. Participants were randomly assigned to receive one of four treatment interventions. All treatment assignments were double-masked, and included oral daily supplementation as follows: (1) antioxidants (500 mg of vitamin C, 400 IU of vitamin E, and 15 mg of beta-carotene); (2) zinc (80 mg of zinc as zinc oxide and 2 mg of copper as cupric oxide); (3) the combination of antioxidants and zinc; (4) or placebo.[11] Phenotype information for all follow up visits was based on the AREDS AMD severity scale, and was used to classify individuals into grade 1 (no AMD), grade 2 (early AMD), grade 3 (intermediate AMD), and two advanced stages of disease: grade 4, including both central and non-central forms of geographic atrophy (GA), and grade 5, neovascular disease (NV).[16] Progression was defined as the transition from no, early, or intermediate AMD to three categories of advanced disease: GA, NV, and overall advanced AMD (either GA or NV). Eyes with advanced disease at baseline were excluded from all analyses. Subjects with no AMD (grade 1) in both eyes at baseline were also excluded as in the original AREDS treatment analyses.[11]

Demographic and behavioral covariates

Baseline demographic, behavioral, ocular, and genetic characteristics were determined for each subject. The following covariates were evaluated as risk factors for progression: age (55–64, 65–74, ≥75), sex, education (≤ high school, > high school), body mass index (BMI) (<25, 25–29, ≥ 30), and smoking status (never, past, current). Baseline AMD grade was determined for each eye, and drusen size (μm) was evaluated for each non-advanced eye (<63, 63 to 24, 125 to 249, and ≥ 250). The four AREDS treatment interventions (antioxidant, zinc, antioxidant and zinc, and placebo) were assessed.

Genotype data

DNA samples were purchased from the AREDS repository. Genotypes for *CFH* Y402H (rs1061170) and *ARMS2* A69S (rs10490924), two single nucleotide polymorphisms (SNPs) associated with AMD, were determined using array-based and gene sequencing platforms as previously described.[17–20] All SNPs had a high genotype call rate (>98%), none deviated from Hardy-Weinberg equilibrium in the control group ($P < 10^{3}$), and none failed a differential missing test between case and control groups. PLINK was used to perform all quality control steps.[21]

Statistical analysis

The distribution of each risk factor was evaluated for each of the four AREDS supplement groups. Incident AMD outcomes were analyzed over the duration of the AREDS clinical trial (mean follow up: 6.6 years). Progression to advanced AMD was evaluated using survival analysis methodology with the individual eye as the unit of analysis (using PROC PHREG with the covariance aggregate option in SAS 9.3, allowing for the use of correlated data in eye-specific analyses). Multivariate Cox proportional hazards models included age, sex, education, BMI, smoking, supplement group, AMD grade at baseline, drusen size, and the genotypes for *CFH* Y402H and *ARMS2*. Separate models were used to evaluate progression to GA, NV, and overall advanced AMD for subgroups with and without an available genetic specimen. Hazard ratios (HRs) were estimated and 95% confidence intervals (CIs) were calculated.

Interaction effects between AREDS supplement group and genotype were evaluated for association with progression using multivariate Cox proportional hazards models. *CFH* Y402H and *ARMS2* were assessed separately to evaluate the differential effect of AREDS treatment among specific genotypes, comparing the homozygous and heterozygous risk genotypes to the nonrisk genotype groups. Interaction effects between the AREDS supplements and composite genetic risk groups combining the number of risk alleles for *CFH* Y402H and *ARMS2* were also determined. Low risk was defined as having zero risk alleles for a given SNP, and high risk was defined as having one or two risk alleles. Composite genetic risk groups were classified as follows (for *CFH* Y402H, *ARMS2*, respectively): 1) low, low; 2) low, high; 3) high, low; and 4) high, high.

All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC). P values <0.05 were considered statistically significant.

Results

Table 1 displays the association between the four AREDS treatment interventions and AMD risk factors at baseline for 2317 subjects. None of these variables were significantly associated with any AREDS treatment.

The association between AREDS treatment and genetic risk factors and progression to incident GA, NV, and overall advanced AMD for individual eyes is reported in Table 2. Analyses adjusted for age, sex, education, smoking status, BMI, baseline AMD grade, and baseline drusen size were conducted separately for the cohorts with and without a genetic specimen. Among 4543 eyes included in the total cohort, 995 progressed to advanced AMD. There was a significant beneficial effect of the combination antioxidant and zinc treatment on progression to NV (HR: 0.73, 95% CI: 0.56–0.97, P=0.028). A protective effect of the antioxidant alone treatment was noted for progression to overall advanced AMD (HR: 0.81, 95% CI: 0.67–0.99, P=0.039). No significant treatment effect was seen for the GA endpoint. These results were also present in the cohort with genetic data. There was a higher rate of progression among the homozygous risk genotype for both *CFH* Y402H (CC) (HR: 1.64, 95% CI: 1.30–2.07, P<0.0001) and *ARMS2* (TT) (HR: 2.44, 95% CI: 1.96–3.02, P<0.0001) compared to subjects who were homozygous for the nonrisk allele. This relationship was also observed for progression to the GA and NV endpoints. The association between other known AMD risk factors and progression to each advanced outcome is shown in Supplementary Table 1.

Associations between AREDS treatment groups and progression to advanced disease stratified by *CFH* Y402H and *ARMS2* genotypes are shown in Table 3. There was a significant protective effect of the combination antioxidant and zinc treatment in the *CFH* nonrisk (TT) group for progression to overall advanced AMD (HR: 0.55, 95% CI: 0.32–0.95, P=0.033) and progression to NV (HR: 0.34, 95% CI: 0.16–0.70, P=0.004). There was no apparent benefit of the combination supplement treatment for the *CFH* risk (CC) group. The interaction between this treatment and genotype was significant for comparisons of the high risk *CFH* genotype group to the nonrisk genotype group for progression to NV ($P_{interaction}$=0.019), with a suggestive, non-significant result in the same direction for overall advanced AMD ($P_{interaction}$=0.069). For the *ARMS2* genetic variant, there was a significant protective effect of antioxidant and zinc treatment in the high risk (TT) group for progression to overall AMD (HR: 0.52, 95% CI: 0.33–0.82, P=0.005) and NV (HR: 0.38, 95% CI: 0.20–0.72, P=0.003). No apparent benefit was observed in the nonrisk (GG) group. There was a significant interaction observed when comparing the high risk to the nonrisk *ARMS2* genotype group for both outcomes ($P_{interaction}$=0.024 and 0.009, respectively). If a Bonferroni adjustment is performed, the $P_{interaction}$ for *CFH* Y402H (CC vs. TT) is 0.038, and the $P_{interaction}$ for *ARMS2* (TT vs. GG) is 0.048 for overall advanced AMD and 0.018 for NV. Results related to the antioxidant alone and zinc alone treatments are reported in Supplementary Table 2.

Table 4 shows the association between the combination antioxidant and zinc treatment versus placebo and progression to advanced disease stratified by the composite genotypes for *CFH* Y402H and *ARMS2* A69S. Subjects with the nonrisk genotype for both SNPs (low, low group) had a lower risk of progression with combination treatment versus placebo (HR: 0.32, 95% CI: 0.09–1.12, P=0.075). Risk of progression to overall advanced AMD was also reduced for subjects with zero risk alleles for *CFH* and one or two risk alleles for *ARMS2* (low, high group) (HR: 0.52, 95% CI: 0.28–0.94, P=0.031). Similar results were observed for progression to NV. Subjects with high risk genotypes for both SNPs (high, high group) demonstrated a protective treatment effect for the NV endpoint (HR: 0.65, 95% CI: 0.44–0.95, P=0.026). In addition, for progression to overall advanced AMD, there was a difference between the treatment effect for the high risk *CFH* and low risk *ARMS2* subjects (high, low group), compared to the treatment effect for subjects with the nonrisk genotype for both SNPs (low, low group) (HRs: 1.23 and 0.32, $P_{interaction}$=0.039). Similar results were seen for the NV endpoint. Finally, a three-way interaction between treatment, *CFH*, and *ARMS2* genotype was evaluated, and results suggested that the differential *CFH* treatment effect was not modified significantly by *ARMS2* genotype (data not shown).

Discussion

The effectiveness of the antioxidant and zinc supplement treatment compared to placebo differed according to genotype, and subjects with a nonrisk genotype for *CFH* and subjects with the homozygous risk genotype for *ARMS2* had a lower risk of progression to overall advanced AMD. Individuals in both genotype groups using this combination supplement also had a lower risk of progression to NV. No significant treatment effect was observed for GA.

We first reported the independent association of these two genetic variants with progression to advanced stages of AMD in 2007, demonstrating a seven times increase in risk among the combined homozygous risk genotypes.[10] An interaction was suggested between *CFH*

Y402H and the combination AREDS treatment (TT genotype, proportion progressing = 11% combination treatment, 34% placebo; CC genotype, proportion progressing = 39% combination treatment, 44% placebo), $P_{interaction}$ = 0.03.[2, 12] The interaction effect between genotype and treatment groups was included in a predictive model including five additional AMD SNPs, with an area under the curve statistic of 83%.[2] These initial studies evaluated individual subjects and used logistic regression analyses. The methodological approach applied in this study, specifically the analysis of individual eyes, enhances the person-based analyses of the worst eye by accounting for eye-specific covariates, namely baseline grade and drusen size, and differentiating between subjects who progress in a single eye compared to those who progress in both eyes. This report incorporates these methods with a resulting increase in statistical power.

Our present study further evaluates this potential interaction and underscores the differential effect of the combination antioxidant and zinc supplement by *CFH* genotype. Subjects with the nonrisk genotype had a significantly lower risk of progression after treatment, while those with one or two risk alleles did not benefit. We recently reported that subjects with a nonrisk allele for *CFH* Y402H demonstrated significantly lower risk of progression to advanced stages of AMD in a study of nutrition,[8] in which Merle et al. identified a significant interaction between *CFH* risk alleles and high adherence to an alternate Mediterranean diet. Subjects with at least one nonrisk allele had a relatively lower risk of progression to advanced stages of AMD and subjects homozygous for the risk allele did not benefit. In addition to this prospective analysis of dietary patterns, the Nutritional AMD Treatment 2 study evaluated progression to neovascular disease and response to supplementation with docosahexaenoic acid (DHA). A similar interaction was reported: there was a protective effect of DHA supplementation among patients who were homozygous for the nonrisk *CFH* allele.[22] A study of anti-vascular endothelial growth factor (VEGF) treatment in a clinic population revealed that subjects with a low *CFH* risk score demonstrated more improvement over time with respect to central foveal thickness and visual acuity.[9] These studies suggest that modifiable supplement, dietary, and treatment factors might achieve maximum benefit among patients with low risk genotypes for *CFH*.

Our results implicate a possible interaction with *ARMS2*, where a protective effect of the combined supplementation was observed among high risk *ARMS2* carriers. Other studies also support a differential impact of this genotype in conjunction with nutritional intake. Dietary DHA has been associated with lower risk of incident GA among subjects homozygous for the *ARMS2* risk allele.[23] Another study of progression to early AMD revealed a similar interaction with the beneficial effect of combined eicosapentaenoic acid (EPA) + DHA intake among the *ARMS2* risk genotype group. [24]

Previous analyses related to the differential effect of the AREDS supplements among genotype groups have been inconclusive.[13–15] An initial publication by Awh et al. [13] reported the benefit of zinc in reducing progression to advanced AMD among 995 subjects with zero or one risk allele for *CFH* and one or two risk alleles for *ARMS2*. A more recent publication from the same group [15] suggested a differential impact on disease progression according to number of risk alleles for these SNPs: the detriment posed by a *CFH* risk allele was exacerbated and the harmful effect of the *ARMS2* risk allele was alleviated in subjects receiving supplementation with zinc, both alone or as a component of the AREDS combination supplement. Chew et al. examined a larger sample (n=1237) and said there was no significant interaction between treatment with supplements and genetics.[14] Those studies used the subject rather than the eye as the unit of analysis and assessed outcomes based on smaller subgroups of the AREDS population. Our report is based on a larger sample of the AREDS population (n=2317). Subjects with no evidence of AMD in both eyes (fewer than five small drusen, <63 μm) were excluded as they did not receive supplementation with zinc and most did not progress to advanced stages of disease. This selection was consistent with the criteria used in the original AREDS study.[11]

It is apparent that genetic susceptibility modifies risk of progression to advanced AMD, can possibly affect response to anti-VEGF treatment and dietary patterns, and the effectiveness of combination antioxidant and zinc supplementation may also differ by genotype. In this era of personalized medicine, genetic factors may become relevant when selecting specific treatments. Additional studies are needed to determine the biologic mechanism for this interaction and its implications for the comprehensive management of AMD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1:

Associations between AREDS supplements and demographic, environmental, genetic, and ocular risk factors for age-related macular degeneration[a]

	Placebo	Antioxidant Alone	Zinc Alone	Combination Antioxidant and Zinc	P-value[b]
	N=545	N=576	N=599	N=597	
Risk Factors	N (%)	N (%)	N (%)	N (%)	
Age					0.556
75+	104 (19)	94 (16)	123 (21)	110 (18)	
65–74	346 (63)	375 (65)	384 (64)	387 (65)	
55–64	95 (17)	107 (19)	92 (15)	100 (17)	
Sex					0.797
Female	317 (58)	327 (57)	354 (59)	338 (57)	
Male	228 (42)	249 (43)	245 (41)	259 (43)	
Education					0.490
> High School	347 (64)	386 (67)	383 (64)	376 (63)	
≤ High School	198 (36)	190 (33)	216 (36)	221 (37)	
Smoking					0.356
Never	264 (48)	248 (43)	261 (44)	288 (48)	
Past	249 (46)	289 (50)	296 (49)	277 (46)	
Current	32 (6)	39 (7)	42 (7)	32 (5)	
BMI					0.636
<25	177 (33)	196 (34)	194 (32)	186 (31)	
25–29.9	215 (39)	237 (41)	254 (42)	265 (44)	
30+	153 (28)	143 (25)	151 (25)	146 (24)	
***CFH* Y402H rs1061170**					0.244
TT	145 (27)	164 (28)	168 (28)	169 (28)	
CT	260 (48)	250 (43)	248 (41)	279 (47)	
CC	140 (26)	162 (28)	183 (30)	149 (25)	
***ARMS2* A69S rs10490924**					0.853
GG	252 (46)	279 (48)	272 (45)	268 (45)	
TG	219 (40)	231 (40)	251 (42)	248 (42)	
TT	74 (14)	66 (11)	76 (13)	81 (14)	
Genetic risk group[c]					0.344
Low, low	69 (13)	95 (16)	71 (12)	79 (13)	
Low, high	76 (14)	69 (12)	97 (16)	90 (15)	
High, low	183 (34)	184 (32)	201 (34)	189 (32)	
High, high	217 (40)	228 (40)	230 (38)	239 (40)	
AMD Grade[d]					0.901

	Placebo	Antioxidant Alone	Zinc Alone	Combination Antioxidant and Zinc	P-value[b]
	N=545	**N=576**	**N=599**	**N=597**	
Risk Factors	**N (%)**	**N (%)**	**N (%)**	**N (%)**	
1,2	103 (19)	104 (18)	123 (21)	115 (19)	
1,3	30 (6)	39 (7)	25 (4)	38 (6)	
1,4	12 (2)	13 (2)	11 (2)	11 (2)	
2,2	68 (12)	77 (13)	65 (11)	63 (11)	
2,3	71 (13)	78 (14)	75 (13)	79 (13)	
2,4	24 (4)	24 (4)	25 (4)	22 (4)	
3,3	162 (30)	152 (26)	167 (28)	173 (29)	
3,4	69 (13)	75 (13)	94 (16)	85 (14)	
3,5	6 (1)	14 (2)	14 (2)	11 (2)	
Largest drusen size in non-advanced eye (microns)					0.793
<63	14 (13)	15 (12)	14 (10)	13 (10)	
63–124	29 (26)	29 (23)	32 (22)	30 (23)	
125–249	27 (24)	37 (29)	53 (37)	44 (34)	
≥250	41 (37)	45 (36)	45 (31)	42 (33)	
Drusen size - no advanced AMD in either eye					0.340
<63, <63	34 (8)	35 (8)	28 (6)	32 (7)	
63–124, 63–124	56 (13)	50 (11)	58 (13)	57 (12)	
63–124, <63	106 (24)	114 (25)	129 (28)	109 (23)	
125–249, 125–249	55 (13)	39 (9)	51 (11)	53 (11)	
125–249, 63–124	56 (13)	V 61 (14)	52 (11)	67 (14)	
125–249, <63	31 (7)	48 (11)	34 (7)	44 (9)	
≥250, ≥250	56 (13)	53 (12)	52 (11)	38 (8)	
≥250, 125–249	30 (7)	35 (8)	41 (9)	50 (11)	
≥250, ≤124	10 (2)	15 (3)	10 (2)	18 (4)	

[a] Analyses of individual subjects with an available genetic specimen

[b] P values were calculated using the chi-square test

[c] Genetic risk groups based on number of risk alleles for *CFH* Y402H rs1061170 and *ARMS2* A69S rs10490924: low, low = 0 risk alleles for *CFH* and 0 risk alleles for *ARMS2*; low, high = 0 risk alleles for *CFH* and 1 or 2 risk alleles for *ARMS2*; high, low = 1 or 2 risk alleles for *CFH* and 0 risk alleles for *ARMS2*; and high, high = 1 or 2 risk alleles for *CFH* and 1 or 2 risk alleles for *ARMS2*.

[d] Grade in each eye at baseline[16]: 1,2 (no AMD, early AMD); 1,3 (no AMD, intermediate AMD); 1,4 (no AMD, geographic atrophy); 2,2 (early AMD, early AMD); 2,3 (early AMD, intermediate AMD); 2,4 (early AMD, geographic atrophy); 3,3 (intermediate AMD, intermediate AMD); 3,4 (intermediate AMD, geographic atrophy); 3,5 (intermediate AMD, neovascular disease).

Table 2.

Multivariate associations between demographic, environmental, genetic, and ocular risk factors and progression to overall advanced age-related macular degeneration, geographic atrophy, and neovascular disease

	Overall Advanced AMD				Geographic Atrophy				Neovascular Disease			
	No genes[a]		With genes[b]		No genes		With genes		No genes		With genes	
	N=995/4543		N=882/4124		N=545/4543		N=485/4124		N=450/4543		N=397/4124	
Risk Factors	**HR (95% CI)[c]**	**P-value**	**HR (95%CI)**	**P-value**	**HR (95%CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
Treatment												
Placebo	1.00		1.00		1.00		1.00		1.00		1.00	
Antioxidant alone	0.81 (0.67 – 0.99)	0.039	0.79 (0.64 – 0.97)	0.025	0.82 (0.62 – 1.08)	0.157	0.80 (0.59 – 1.08)	0.140	0.81 (0.62 – 1.06)	0.126	0.78 (0.58 – 1.04)	0.090
Zinc alone	0.95 (0.78 – 1.16)	0.594	0.92 (0.74 – 1.13)	0.425	1.25 (0.95 – 1.64)	0.113	1.20 (0.90 – 1.61)	0.215	0.66 (0.50 – 0.88)	0.004	0.65 (0.48 – 0.87)	0.004
Antioxidant and Zinc	0.87 (0.71 – 1.05)	0.149	0.83 (0.67 – 1.03)	0.084	1.00 (0.76 – 1.32)	0.978	0.96 (0.72 – 1.29)	0.790	0.73 (0.56 – 0.97)	0.028	0.71 (0.53 – 0.96)	0.024
***CFH* Y402H rs1061170**												
TT			1.00				1.00				1.00	
CT			1.43 (1.14 – 1.79)	0.002			1.38 (1.00 – 1.92)	0.051			1.48 (1.09 – 2.00)	0.012
CC			1.64 (1.30 – 2.07)	<0.0001			1.62 (1.16 – 2.26)	0.005			1.66 (1.21 – 2.29)	0.002
***ARMS2* A69S rs10490924**												
GG			1.00				1.00				1.00	
GT			1.55 (1.30 – 1.85)	<0.0001			1.45 (1.14 – 1.85)	0.003			1.67 (1.29 – 2.17)	<0.0001
TT			2.44 (1.96 – 3.02)	<0.0001			2.17 (1.62 – 2.91)	<0.0001			2.83 (2.08 – 3.83)	<0.0001

[a] Sample includes individual eyes from all subjects.

[b] Sample includes individual eyes from the subgroup with an available genetic specimen.

[c] Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated by multivariate Cox proportional hazards models using the individual eye as the unit of analysis. In the analyses including all subjects, models are adjusted for age, sex, education, smoking status, body mass index, baseline grade, and baseline drusen size (μm). In the analyses including all subjects with an available genetic specimen, models are adjusted for all demographic, environmental, and ocular variables listed above as well as the genetic variables: *CFH* Y402H rs1061170 and *ARMS2* A69S rs10490924.

[d] Grade at baseline[16]: 1 (no AMD); 2 (early AMD); 3 (intermediate AMD).

Table 3.

Multivariate associations between the combination antioxidant and zinc AREDS supplement and progression to advanced AMD, geographic atrophy, and neovascular disease stratified by genotype for *CFH* Y402H rs1061170 and *ARMS2* A69S rs10490924

		Overall Advanced AMD			Geographic Atrophy			Neovascular Disease		
		N=882/4124			N=485/4124			N=397/4124		
	Genotype	**HR (95% CI)[a]**	**P-value[b]**	**P-interaction[c]**	**HR (95% CI)**	**P-value**	**P-interaction**	**HR (95% CI)**	**P-value**	**P-interaction**
***CFH* Y402H**	TT	0.55 (0.32 – 0.95)	0.033		0.90 (0.39 – 2.06)	0.795		0.34 (0.16 – 0.70)	0.004	
	CT	0.92 (0.68 – 1.24)	0.571	0.112	1.03 (0.69 – 1.55)	0.875	0.763	0.80 (0.53 – 1.23)	0.315	0.045
	CC	1.01 (0.71 – 1.44)	0.954	0.069	1.05 (0.65 – 1.69)	0.859	0.753	0.98 (0.59 – 1.62)	0.930	0.019
ARMS2	GG	1.04 (0.70 – 1.55)	0.830		0.95 (0.56 – 1.60)	0.841		1.18 (0.67 – 2.09)	0.568	
	GT	0.88 (0.66 – 1.19)	0.415	0.508	1.10 (0.71 – 1.69)	0.672	0.673	0.70 (0.46 – 1.06)	0.093	0.149
	TT	0.52 (0.33 – 0.82)	0.005	0.024	0.71 (0.38 – 1.31)	0.271	0.481	0.38 (0.20 – 0.72)	0.003	0.009

[a]Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated by multivariate Cox proportional hazards models using the individual eye as the unit of analysis. All models are adjusted for age, sex, education, smoking status, body mass index, baseline AMD grade, and baseline drusen size.

[b]P value reports the difference in the effectiveness of the antioxidant and zinc treatment compared to placebo for each genotype.

[c]P interaction reports the difference in the effectiveness of the antioxidant and zinc treatment compared to placebo for the CC versus TT genotype and the CT versus TT genotype (for *CFH* Y402H) and for the TT versus GG genotype and the GT versus GG genotype (for *ARMS2* A69S).

Table 4.

Multivariate associations between composite genetic risk for *CFH* Y402H rs1061170 and *ARMS2* A69S rs10490924 and progression to overall advanced AMD, geographic atrophy, and neovascular disease among the AREDS combination antioxidant and zinc treatment compared to placebo

	Overall Advanced AMD			Geographic Atrophy			Neovascular Disease		
	N=882/4124			N=485/4124			N=397/4124		
Genetic risk group[a]	**HR (95% CI)**[b]	**P-value**[c]	**P-interaction**[d]	**HR (95% CI)**	**P-value**	**P-interaction**	**HR (95% CI)**	**P-value**	**P-interaction**
Low, low	0.32 (0.09–1.12)	0.075		0.55 (0.11–2.73)	0.466		0.15 (0.02–1.11)	0.063	
Low, high	0.52 (0.28–0.94)	0.031	0.473	0.86 (0.34–2.17)	0.748	0.605	0.32 (0.15–0.70)	0.004	0.467
High, low	1.23 (0.82–1.85)	0.326	0.039	1.01 (0.59–1.75)	0.961	0.467	1.54 (0.85–2.78)	0.154	0.024
High, high	0.81 (0.62–1.05)	0.12	0.161	0.99 (0.69–1.43)	0.960	0.490	0.65 (0.44–0.95)	0.026	0.161

[a] Genetic risk groups based on number of risk alleles for *CFH* Y402H rs1061170 and *ARMS2* A69S rs10490924: low, low = 0 risk alleles for *CFH* and 0 risk alleles for *ARMS2*; low, high = 0 risk alleles for *CFH* and 1 or 2 risk alleles for *ARMS2*; high, low = 1 or 2 risk alleles for *CFH* and 0 risk alleles for *ARMS2*; and high, high = 1 or 2 risk alleles for *CFH* and 1 or 2 risk alleles for *ARMS2*.

[b] Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated by multivariate Cox proportional hazards models using the individual eye as the unit of analysis. All models are adjusted for age, sex, education, smoking status, body mass index, baseline AMD grade, and baseline drusen size.

[c] P value reports the difference in the effectiveness of antioxidant and zinc treatment compared to placebo for each genetic risk group.

[d] P interaction reports the difference in the effectiveness of the antioxidant and zinc treatment compared to placebo for each genetic risk group compared to the low, low genetic risk group