CFH and *ARMS2* Genetic Polymorphisms Predict Response to Antioxidants and Zinc in Patients with Age-related Macular Degeneration

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Objective: The Age-Related Eye Disease Study (AREDS) demonstrated that antioxidant and zinc supplementation decreases progression to advanced age-related macular degeneration (AMD) in patients with moderate to severe disease. We evaluated the interaction of genetics and type of nutritional supplement on progression from moderate to advanced AMD.

Design: Genetic analysis of a randomized, prospective clinical trial.

Participants: White patients with AREDS category 3 AMD in 1 eye and AREDS categories 1 through 4 AMD in the fellow eye enrolled in the AREDS with available peripheral blood-derived DNA (995).

Methods: Subjects were evaluated for known AMD genetic risk markers and treatment category. The progression rate to advanced AMD was analyzed by genotypes and AREDS treatment group using Cox regression.

Main Outcome Measures: The effect of inherited gene polymorphisms on treatment group−specific rate of progression to advanced AMD.

Results: Over an average of 10.1 years, individuals with 1 or 2 complement factor H (*CFH*) risk alleles derived maximum benefit from antioxidants alone. In these patients, the addition of zinc negated the benefits of antioxidants. Treatment with zinc and antioxidants was associated with a risk ratio (RR) of 1.83 with 2 *CFH* risk alleles ($P = 1.03E-02$), compared with outcomes for patients without *CFH* risk alleles. Patients with age-related maculopathy sensitivity 2 (*ARMS2*) risk alleles derived maximum benefit from zinc-containing regimens, with a deleterious response to antioxidants in the presence of *ARMS2* risk alleles. Treatment with antioxidants was associated with an RR of 2.58 for those with 1 *ARMS2* risk allele and 3.96 for those with 2 *ARMS2* risk alleles ($P = 1.04E-6$), compared with patients with no *ARMS2* risk alleles. Individuals homozygous for *CFH* and *ARMS2* risk alleles derived no benefit from any category of AREDS treatment.

Conclusions: Individuals with moderate AMD could benefit from pharmacogenomic selection of nutritional supplements. In this analysis, patients with no *CFH* risk alleles and with 1 or 2 *ARMS2* risk alleles derived maximum benefit from zinc-only supplementation. Patients with one or two *CFH* risk alleles and no *ARMS2* risk alleles derived maximum benefit from antioxidant-only supplementation; treatment with zinc was associated with increased progression to advanced AMD. These recommendations could lead to improved outcomes through genotype-directed therapy.

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The Age-Related Eye Disease Study (AREDS) established the ability of supplementation with antioxidants and zinc to reduce progression rates of moderate to advanced age-related macular degeneration (AMD). Study subjects received placebo, antioxidants, zinc, or both antioxidants and zinc. The AREDS formulation of high-dose β-carotene, vitamin C, vitamin E, and zinc reduced the 5-year risk of progression from intermediate to advanced AMD by 25% and produced a 19% reduction in moderate vision loss in individuals at high risk of developing geographic atrophy or choroidal neovascularization.[1] The intake of these nutritional supplements is the only evidence-supported means of reducing the risk of progression to advanced AMD.

The biological features of known AMD genetic risk factors predicts interaction with components of the AREDS formulation. Complement factor H (*CFH*) binds zinc, which can neutralize its ability to inactivate complement component 3b.[2−4] Age-Related Maculopathy Sensitivity 2 (*ARMS2*) localizes to mitochondria, potentially affecting oxidative phosphorylation and the generation of oxygen free radicals

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that could interact with antioxidants such as vitamins C and E.[5,6] Hepatic lipase remodels low-density lipoprotein and high-density lipoprotein,[7] which affect uptake and transport into the retina of carotenoids by high-density lipoprotein.[8] The adenosine triphosphate (ATP) binding cassette subfamily A member 1 and hepatic lipase genetic variants correlate with macular pigment density, which is affected by dietary xanthophylls[9] and, when decreased, is associated with an increased risk of AMD.[10]

To test our hypothesis that the effect of nutritional supplementation for individuals with moderate AMD can be influenced by genetic risk factors for AMD we performed a pharmacogenetic analysis of AREDS patients. We studied genetic markers that account for almost all of the known population-attributable risk.[11] We sought to identify groups of patients in whom specific nutritional supplements were beneficial or deleterious.

Methods

Study procedures of the AREDS have been reported.[1] The AREDS dataset was provided by the database of genotypes and phenotypes under an investigator agreement. Patients had been characterized at AREDS enrollment, with retinal images classified by a central reading center.[1]

The AREDS participants varied at enrollment, ranging from those with normal eyes to those with advanced AMD. Disease was classified by the AREDS investigators based on the category of AMD in the patient's worse eye: AREDS category 1 (no AMD), fewer than 5 small (<63 μm) drusen; category 2 (mild AMD), multiple small drusen, nonextensive intermediate (63−124 μm) drusen, pigment abnormalities, or a combination; category 3 (intermediate AMD), at least 1 large (>125 μm) druse, extensive intermediate drusen, or geographic atrophy not involving the center of the macula; and category 4 (advanced AMD), central geographic atrophy or neovascular AMD in 1 eye or visual loss resulting from AMD, regardless of lesion type. All participants with mild AMD or worse had been randomized at AREDS entry to 1 of 4 treatment categories of dietary supplements: placebo; antioxidants (β-carotene 15 mg, vitamin C 500 mg, and vitamin E 400 IU); zinc (80 mg as zinc oxide and copper 2 mg); and antioxidants and zinc combined. Antioxidants plus zinc is the currently accepted AREDS formulation.[1]

Because the genetics of AMD have been studied most thoroughly in white persons, patients of other racial backgrounds were eliminated from our study. Because the AREDS found no benefit for nutritional supplementation for patients with AREDS categories 1 and 2 disease, we limited our analysis to patients with AREDS category 3 disease in 1 eye and AREDS category 1, 2, 3, or 4 disease in the fellow eye at enrollment. We defined disease progression as the development of AREDS category 4 disease in either eye of patients without AREDS category 4 disease at the time of enrollment or the development of bilateral category 4 disease in patients with unilateral category 4 disease at the time of enrollment.

Genotyping

DNA from all available white patients in AREDS with category 3 disease in at least 1 eye at enrollment were purchased from the Coriell Institute (Camden, NJ). Genotyping was performed using bidirectional sequencing by Beckman Coulter Genomics (Danvers, MA) according to good laboratory practices. We selected a comprehensive set of AMD genetic risk predictors from the literature, as outlined in Table 1. For markers with homozygous minor allele frequencies of less than 1%, we combined homozygous minor allele counts with heterozygotes.

Table 1. Age-Related Macular Degeneration−Associated Risk Genes, Reference Single Nucleotide Polymorphism Identification, and Chromosomal Location (Genome Reference Consortium Human Build 37 Patch Release 5)

Gene	Marker	Chromosome	Genome Reference Consortium Human Build 37 Patch Release 5
CFH	rs3766405	1	196695161
CFH	rs412852	1	196703707
C3	rs2230199	19	6718387
C2	rs4151669	6	31915144
CFB	rs522162	6	31919917
CFI	rs10033900	4	110659067
TIMP3	rs9621532	22	330845511
LPL	rs12678919	8	19844222
LIPC	rs493258	15	58687880
ABCA1	rs1883025	9	107664301
*ARMS2**	372_815del443ins54	10	124206868

ABCA1 = ATP-binding cassette transporter subgroup A member 1; *ARMS2* = age-related maculopathy sensitivity 2; *CFB* = complement factor B; *CFH* = complement factor H; *CFI* = complement factor I; *C3* = complement component 3; *C2* = complement component 2; *LPL* = lipoprotein lipase; *TIMP3* = tissue inhibitor of metalloproteinase 3.
*ARMS2 risk was assessed using the putative pathophysiologic 3′ insertion-deletion polymorphism that affects ribonucleic acid stability.

To analyze the genetic variability of the *CFH* locus, we prospectively selected a set of 5 common polymorphisms: rs1048663, rs3766405, rs412852, rs11582939, and rs1066420 (previously rs1280514) for genotyping that were reported by Li et al[13] to tag 4 common disease-associated *CFH* haplotypes. rs1066420 Was excluded from further analyses because of deviations from Hardy-Weinberg equilibrium in control individuals without AMD ($P < 0.001$). Linkage disequilibrium and tagging analysis of the remaining 4 single nucleotide polymorphisms revealed that any combination of 2 single nucleotide polymorphisms is sufficient to tag haplotypes (>1%) occurring within the *CFH* locus. For technical convenience, we selected rs3766405 and rs412852 for further haplotyping. We considered rs412852 homozygous cytosine with rs3766405 homozygous cytosine to be 2 risk copies of *CFH*. Patients heterozygous for risk at the *CFH* locus had either rs412852 (CC) and rs3766405 (CT) or rs412852 (CT) and rs3766495 (CT). All other genotype combinations designated low-risk alleles.[12] We abbreviated genotypes as C*X*A*X*, with C for *CFH*, A for *ARMS2*, and *X* indicating any number of risk alleles, or with *X* replaced by the actual number of risk alleles.

Demographic Covariates

Demographic and epidemiologic features were selected prospectively and were abstracted from AREDS enrollment tables and included age, educational attainment (high school or greater), body mass index, smoking history (current, past, or never), and gender.[11]

Statistical Analysis

The time from enrollment to progression to advanced AMD or last follow-up was determined. Patients receiving the same AREDS

Table 4. Cox Regression Analysis* within Individual Treatment Groups of Variables

	Covariate		
	β	*P Value*	*Risk Ratio*
Placebo (sample size = 235)			
CFH 1 allele	0.806	0.032†	2.239
CFH 2 alleles	0.655	0.095†	1.926
ARMS2 1 allele	0.562	0.010†	1.754
ARMS2 2 alleles	1.172	0.000†	3.230
Antioxidants (sample size = 256)			
ARMS2 1 allele	0.948	5.749E-05‡	2.581
ARMS2 2 alleles	1.377	2.219E-06‡	3.963
Zinc (sample size = 232)			
CFH 1 allele	0.781	4.161E-02‡	2.184
CFH 2 alleles	1.495	7.522E-05‡	4.461
Antioxidants + zinc (sample size = 272)			
CFH 2 alleles	0.606	1.026E-02‡	1.833
ARMS2 2 alleles	0.635	8.540E-04‡	1.887

ARMS2 = age-related maculopathy sensitivity 2; *CFH* = complement factor H.
*Cox regression analysis by risk allele number to study allele dosage effects.
†Four degrees of freedom.
‡Two degrees of freedom.

nutritional intervention were grouped for analysis. We parsed demographic continuous variables into categorical variables as follows: age (<75 years, 75−85 years, >85 years); body mass index (<25 kg/m^2, 25−30 kg/m^2, >30 kg/m^2); smoking (current smoker or nonsmoker); educational level (high school graduate or not); and sex. A forward stepwise Cox regression analysis was performed first using all genetic and demographic risk markers.[13] Markers found to be associated significantly with progression to advanced AMD within any treatment group were included in treatment group-specific multivariate Cox models.[13] In recognition of the division of our analysis by the 4 treatment groups, significance thresholds were adjusted for multiple testing using the Bonferroni correction with n = 4.[14] Association of disease progression with genetics was studied using an additive allele model for individual AMD risk genetic markers. Statistical analyses were performed using SAS version 9.2 (SAS Inc, Cary, NC), except for the calculation of absolute risk, which was performed using Microsoft Excel version 14.3.4 (Microsoft, Redmond, WA).

Results

Patients

Of patients enrolled in the AREDS (n = 4757), white persons with AREDS category 3 disease in at least 1 eye at the time of enrollment (n = 2258) were selected for study. DNA was available from a subset of these (n = 995) from the Coriell Institute repository, collected under general research use or eye diseases only consent conditions. These samples constituted the sample set for our analysis.

To ensure that this sample set (n = 995) was representative of all white patients in AREDS with category 3 disease in 1 eye and category 1 through 4 disease in the fellow eye (n = 2258), we compared sex, smoking history, body mass index, treatment category, educational level, and percentage progression to advanced AMD. The groups did not differ statistically with respect to these parameters. There was a clinically insignificant age difference of 0.6 years between our sample and the larger group. Treatment assignments for our study set did not differ from assignments within the AREDS study overall (Table 2, available at http://aaojournal.org). The distribution of the AREDS simple scale score[15] or *CFH* and *ARMS2* risk allele proportions did not vary by treatment group (Tables 2 and 3, both available at http://aaojournal.org).

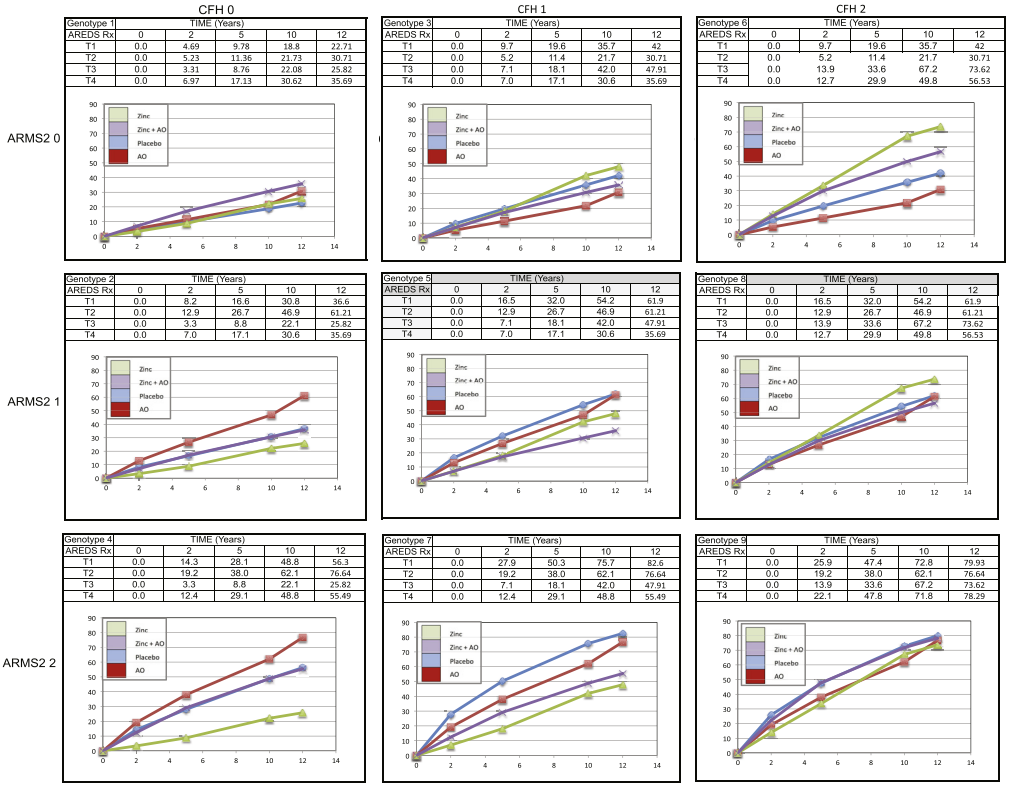
Age-Related Macular Degeneration Risk Parameters and Treatment

Forward Stepwise Cox Regression Analysis. To evaluate the determinants of AMD progression within each treatment group, a forward stepwise Cox regression analysis was performed within each treatment group. As expected, parameters with rare risk alleles (C2, CFB) or small effect sizes (ATP binding cassette subfamily A member 1, complement factor I, lipoprotein lipase) were not identified as significant predictors of progression risk in our population, whose size favored the identification of larger, clinically significant associations. Demographic and epidemiologic parameters did not improve the predictive value of the model.

Progression among placebo-treated subjects correlated with *CFH* and *ARMS2* risk alleles, as expected (P = 2.29E-4 and P = 2.30E-2, respectively). For individuals treated with antioxidants alone, only *ARMS2* risk allele status correlated with AMD progression (P = 3.31E-6). Among patients treated with zinc alone, only *CFH* risk allele status correlated with AMD progression (P = 2.41E-7). The combination of the *CFH* risk allele and the ARMS 2 risk allele covariates correlated maximally with progression in patients treated with both antioxidants and zinc (P = 2.35E-3 and P = 5.61E-3, respectively).

Cox Proportional Hazards Regression. We performed multivariate Cox proportional hazards regression analyses within treatment groups using as predictor variables risk alleles in *ARMS2* and *CFH* for patients receiving placebo, *ARMS2* risk alleles for those receiving antioxidants, *CFH* risk alleles for those receiving zinc alone, and both *CFH* and ARMS2 risk alleles for patients receiving antioxidants plus zinc (Table 4). In placebo-treated individuals, 1 copy of the *ARMS2* risk allele was associated with a risk ratio (RR) of 1.76 (P = 0.010), whereas 2 copies were associated with an RR of 3.23 (P = 2.55E-05). In placebo-treated individuals, 1 *CFH* risk allele was associated with an RR of 2.24 (P = 3.20E-2), and 2 *CFH* risk alleles were associated with an RR of 1.93 (P = 9.5E-2). Among individuals treated with antioxidants alone, 1 *ARMS2* risk allele was associated with an RR of 2.58 (P = 5.75E-05), and 2 copies were associated with an RR of 3.96 (P = 2.22E-06). *CFH* risk alleles did not influence the progression rate in antioxidant-treated patients. In contrast, *CFH* risk alleles were associated significantly with an increased progression rate in zinc-treated individuals (1 copy: RR, 2.18 and P = 4.16E-02; 2 copies: RR, 4.46 and P = 7.52E-05). *ARMS2* risk alleles did not influence the progression rate in zinc-treated patients. For those treated with antioxidants plus zinc (the AREDS formulation), the presence of homozygous *ARMS2* risk alleles was associated with an increased progression rate (RR, 1.83; P = 8.540E-04). Homozygous risk alleles at *CFH* also were significant predictors of an increased progression rate in patients treated with the AREDS formulation (RR, 1.83; P = 1.026E-02).

To study further the interaction between *CFH* risk alleles and zinc therapy, we determined the significance of interaction between treatment group and risk allele number using Cox regression analysis completed with both main effects and an interaction term. In a group consisting of patients treated with placebo and those receiving zinc alone, a P value for interaction between treatment group and *CFH* risk alleles of 0.0111 was observed using



Genotype 1	TIME (Years)				
AREDS Rx	0	2	5	10	12
T1	0.0	4.69	9.78	18.8	22.71
T2	0.0	5.23	11.36	21.73	30.71
T3	0.0	3.31	8.76	22.08	25.82
T4	0.0	6.97	17.13	30.62	35.69

Genotype 3	TIME (Years)				
AREDS Rx	0	2	5	10	12
T1	0.0	9.7	19.6	35.7	42
T2	0.0	5.2	11.4	21.7	30.71
T3	0.0	7.1	18.1	42.0	47.91
T4	0.0	7.0	17.1	30.6	35.69

Genotype 6	TIME (Years)				
AREDS Rx	0	2	5	10	12
T1	0.0	9.7	19.6	35.7	42
T2	0.0	5.2	11.4	21.7	30.71
T3	0.0	13.9	33.6	67.2	73.62
T4	0.0	12.7	29.9	49.8	56.53

Genotype 2	TIME (Years)				
AREDS Rx	0	2	5	10	12
T1	0.0	8.2	16.6	30.8	36.6
T2	0.0	12.9	26.7	46.9	61.21
T3	0.0	3.3	8.8	22.1	25.82
T4	0.0	7.0	17.1	30.6	35.69

Genotype 5	TIME (Years)				
AREDS Rx	0	2	5	10	12
T1	0.0	16.5	32.0	54.2	61.9
T2	0.0	12.9	26.7	46.9	61.21
T3	0.0	7.1	18.1	42.0	47.91
T4	0.0	7.0	17.1	30.6	35.69

Genotype 8	TIME (Years)				
AREDS Rx	0	2	5	10	12
T1	0.0	16.5	32.0	54.2	61.9
T2	0.0	12.9	26.7	46.9	61.21
T3	0.0	13.9	33.6	67.2	73.62
T4	0.0	12.7	29.9	49.8	56.53

Genotype 4	TIME (Years)				
AREDS Rx	0	2	5	10	12
T1	0.0	14.3	28.1	48.8	56.3
T2	0.0	19.2	38.0	62.1	76.64
T3	0.0	3.3	8.8	22.1	25.82
T4	0.0	12.4	29.1	48.8	55.49

Genotype 7	TIME (Years)				
AREDS Rx	0	2	5	10	12
T1	0.0	27.9	50.3	75.7	82.6
T2	0.0	19.2	38.0	62.1	76.64
T3	0.0	7.1	18.1	42.0	47.91
T4	0.0	12.4	29.1	48.8	55.49

Genotype 9	TIME (Years)				
AREDS Rx	0	2	5	10	12
T1	0.0	25.9	47.4	72.8	79.93
T2	0.0	19.2	38.0	62.1	76.64
T3	0.0	13.9	33.6	67.2	73.62
T4	0.0	22.1	47.8	71.8	78.29

Figure 1. Graphs showing estimated probabilities of progression as a function of genotype, treatment group, and time (years). Absolute progression rate over time for individuals is shown as determined through Cox regression analysis for individuals with 0 (left), 1 (middle), or 2 (right) complement factor H (*CFH*) risk alleles receiving either placebo (blue), antioxidants (red), zinc (green), or both antioxidants and zinc (purple). Similarly, treatment group-specific analysis of progression rates in individuals with 0, 1, or 2 age-related maculopathy sensitivity 2 (*ARMS2*) risk alleles are displayed in the top, middle, and bottom rows, respectively. Data are plotted in an array format to facilitate comparison. AO = antioxidants; AREDS = Age-Related Eye Disease Study; Rx = treatment; T1 = placebo; T2 = antioxidants; T3 = zinc/copper; T4 = antioxidants with zinc/copper.

continuous parameterization, which considers allele dose, and $P = 0.0118$ using categorical parameterization, which compares 1 or 2 *CFH* risk alleles to no risk alleles. Because *ARMS2* risk alleles affect progression rate to a similar degree in both placebo-treated and antioxidant-treated patients, there is no detectable significant interaction between antioxidant treatment and *ARMS2* risk status.

Measurement of Absolute Progression Rate. To calculate the absolute risk of AMD progression as a function of treatment group and risk gene polymorphisms, we determined probabilities of progression using β coefficients from the fitted Cox models and baseline survival rates calculated from the average β coefficients in our cohort. For each treatment and genotype combination, excess risk (the product of Cox-specified mean progression proportions and the $\log_e$ of the difference between the risk allele−specific and group average β coefficients) was determined for specified intervals. The genotype-specific surviving proportion then was determined by the inverse $\log_e$ function of the excess risk over the entire period (sum of excess risk at each specified interval). Using this methodology, we calculated the absolute risk of progression for individuals with 0, 1, or 2 *ARMS2* risk alleles and 0, 1, or 2 *CFH* risk alleles who received placebo treatment. We also determined the progression risk for individuals with 0, 1, or 2 *ARMS2* risk alleles treated only with antioxidants. We estimated the progression risk for individuals with 0, 1, or 2 *CFH* risk alleles who were treated with zinc alone. Finally, we calculated the absolute risk for progression for individuals receiving the complete AREDS formulation with homozygous *CFH* and *ARMS2* risk alleles. We defined 9 combinations of *ARMS2* and *CFH* risk alleles (Table 5, available at http://aaojournal.org) and represented the 5-, 10-, and 12-year progression rate as a function of genotype and assigned treatment group (Fig 1).

Effect of CFH. The disease progression rate among zinc-treated patients increased as a function of the number of *CFH* risk alleles. Patients with genotype combinations containing 1 or 2 *CFH* risk alleles derived no benefit from zinc alone or from antioxidants plus zinc. Patients homozygous for *CFH* risk alleles and without *ARMS2* risk alleles (C2A0) treated with zinc had a 43% greater progression rate by 12 years compared with those treated with placebo (74% vs. 42%). This is consistent with the statistically significant interaction between zinc therapy and the presence of *CFH* risk alleles ($P = 0.0111$ using continuous parameterization). The interaction of *CFH* genetic risk and zinc treatment is illustrated further by the attenuated therapeutic effect of the AREDS formulation in patients with *CFH* risk alleles. Patients without *ARMS2* or *CFH* risk alleles (C0A0) who were treated with the AREDS formulation had a 36% progression rate at 12 years, but this increased to 57% for patients with 2 *CFH* risk alleles. The progression rate among those treated with antioxidants alone remained unchanged at 31%, regardless of *CFH* genotype status (genotypes 1, 3, and 6; Fig 1).

Effect of ARMS2. The disease progression rate among antioxidant-treated patients increased as a function of the number of *ARMS2* risk alleles. In patients with no *ARMS2* risk alleles (CXA0), antioxidant therapy was associated with 30.7% disease

Table 6. Optimal Treatment for the Study Population as a Function of Measured *CFH* and *ARMS2* Alleles

Risk Alleles		Best Age-Related Eye Disease Study Treatment*	Study Population Frequency (%)†
CFH	*ARMS2*		
0	0	—	5.86
0	1	Zinc	5.26
1	0	AO	22.5
0	2	Zinc	1.01
1	1	AO+zinc	22.6
2	0	AO	13.3
1	2	Zinc	6.57
2	1	—	16.4
2	2	—	6.67

AO = antioxidants; *ARMS2* = age-related maculopathy sensitivity 2; *CFH* = complement factor H.
*The treatment associated with the lowest progression rate for individuals with the indicated genetic risk profile. A dash indicates that there was no best treatment identified.
†The observed frequency of the genetic risk combinations in the study population.

progression by 12 years. The presence of 1 *ARMS2* risk allele (CXA1) was associated with a progression rate of 61.2%, and the presence of 2 *ARMS2* risk alleles (CXA2) was associated with a progression rate of 76.6%. Patients without *ARMS2* or *CFH* risk alleles who were treated with the AREDS formulation had a 36% progression rate at 12 years, but this increased to 55% in patients with 2 *ARMS2* risk alleles (C0A2). The progression rate for zinc-treated patients was not affected by the number of *ARMS2* risk alleles (Table 4).

Dual CFH and ARMS2 Risk Genotype. Consistent with the deleterious interactions of *CFH* and *ARMS2* risk alleles with zinc and antioxidant treatment, respectively, individuals with 2 copies of each risk allele fared comparatively poorly and derived minimal benefit from any therapy. Approximately three quarters of such individuals progressed to advanced AMD at 12 years and were not affected significantly by any therapy. This progression rate is twice that observed in patients without risk alleles.

Discussion

The AREDS demonstrated a beneficial effect of the AREDS formulation on progression to advanced AMD (adjusted odds ratio, 0.68; 99% confidence interval, 0.49−0.93).[1] Although the average duration of treatment was 6.3 years, the beneficial effect of nutritional supplementation was sustained for at least a decade, suggesting value in lifelong therapy.[16]

We present evidence, based on a large genetic dataset of patients with AREDS category 3 disease in 1 eye and AREDS category 1 through 4 disease in the fellow eye, that supports the pharmacogenomic selection of nutritional supplements. We found that the addition of zinc seems to negate the beneficial effect of antioxidants among *CFH* risk genotype groups (Fig 1). Our data support a deleterious interaction between *CFH* risk alleles and high-dose zinc supplementation, suggesting that individuals with 1 or 2 *CFH* risk alleles and with fewer than 2 *ARMS2* risk alleles would benefit maximally from supplementation with antioxidants only, because we also found that the beneficial effect of antioxidants completely disappears in the presence of 2 *ARMS2* risk alleles (genotype groups C*X*A2). This pharmacogenomic categorization allows us to identify subgroups of patients who benefited from optimized nutritional treatment significantly more than the average patient with AREDS category 3 disease in 1 eye benefited from the AREDS formulation.

We expanded on the relationship between *CFH* genotype and treatment with the AREDS formulation shown previously. Klein et al[17] showed that the benefit of supplementation with antioxidants plus zinc in reducing progression was greater in those with the low-risk rs1066170 *CFH* TT genotype (68% reduction) compared with those individuals with high-risk *CFH* alleles (11% for CC genotype). They noted an interaction between *CFH* genotype and zinc supplementation and hypothesized that the differential benefit of treatment with respect to *CFH* genotypes may be related to the zinc component. We also demonstrate a statistical interaction of *CFH* risk alleles and zinc therapy and present evidence that this interaction actually may promote the progression to advanced AMD ($P = 0.0111$ for interaction).

These findings are consistent with the current understanding of the interactions of these nutritional supplements and the physiologic functions of CFH and ARMS2. Zinc binds CFH, inducing large multimeric forms that lose complement component 3b inhibitory activity as a function of zinc concentration.[4,2,18] The functional consequence of *CFH* risk genotypes is decreased targeting of CFH protein to sites of active complement activation,[19] which may be exacerbated through zinc supplementation.[20] The ARMS2 protein localizes to the mitochondrial outer membrane.[6] Mitochondrial dysfunction, which occurs with aging, can result in impaired energy metabolism and homeostasis and generation of reactive oxygen species and cellular apoptosis.[21−24] Photoreceptors and retinal pigment epithelium contain high levels of polyunsaturated fatty acids and are exposed to intense light and high levels of oxygen, providing an ideal environment for oxidative damage.[25,26] Our data suggest that oral antioxidants may work to reduce oxygen free radical−induced retinal damage through a mechanism that is dependent on *ARMS2* function.

Treatment Implications

For patients with AREDS category 3 disease in at least 1 eye, we showed that 49% derived more benefit from a treatment regimen other than the AREDS formulation (genotype groups C0A1, C1A0, C0A2, C2A0, and C1A2; Tables 6 and 7). For individuals with a relatively common genotype, C2A0 (13% of our study population), a 56% reduction of 10-year progression to advanced disease could be expected from treatment with antioxidants alone, rather than with the AREDS formulation (21.7% vs. 49.8%). Similarly, individuals with the C0A1 genotype could have a 28% reduction in progression rate if treated with zinc alone, rather than with the AREDS formulation (22.1% vs. 30.6%). A 29% reduction in progression rate (21.7% vs. 30.6%) could be expected for those with the C1A0 genotype

Table 7. Anticipated Improvement in Progression Rate with Genotype-Optimized Prophylaxis among Individuals with *CFH* and *ARMS2* Risk Alleles*

Marker*		Antioxidants + Zinc		Antioxidants Alone		Zinc Alone		
CFH	*ARMS2*	*5 Years*	*10 Years*	*5 Years*	*10 Years*	*5 Years*	*10 Years*	**Progression Difference at 10 Years (%)**
2	0	29.9	**49.8**	11.7	**21.7**	33.6	67.1	28.1
0	2	29.1	**48.8**	38.0	62.1	8.76	**22.1**	26.7
1	0	17.1	**30.6**	11.4	**21.7**	18.2	42.0	8.90
0	1	17.1	**30.6**	26.7	46.9	8.76	**22.1**	8.52
1	2	29.1	**48.8**	38.0	62.1	18.2	**42.0**	6.80

ARMS2 = age-related maculopathy sensitivity 2; *CFH* = complement factor H.
*The genetic risk allele numbers are indicated in the far left columns. The observed progression rate from Age-Related Eye Disease Study (AREDS) class 3/X or AREDS class 4/3 to AREDS class 3/4 or 4/4 disease, respectively, at 5 and 10 years for each genotype group is shown for individuals treated with the standard AREDS formula nutritional supplements consisting of antioxidants and zinc. For comparison, progression rates for individuals treated with antioxidants alone or zinc alone is provided. As an illustration of benefit associated with genotype-directed treatment, the difference between progression rate of standard therapy (antioxidants and zinc) and antioxidants alone or zinc alone is indicated in the far right column.

treated with antioxidants rather than with the AREDS formulation.

The AREDS 2 trial investigated the effect of supplementation with lutein and zeaxanthin and omega-3 fatty acids on the rate of progression to advanced AMD.[27] A secondary randomization studied variations of the original AREDS formulation: β-carotene versus no β-carotene and high-dose (80 mg) versus low-dose (25 mg) zinc. Because all patients in AREDS 2 received zinc, our analysis cannot be repeated with the AREDS 2 data.

The AREDS 2 found no significant difference in outcome with the lower or higher dose of zinc on the progression of AMD. This may indicate that in patients with *CFH* risk alleles, the adverse response to zinc is not dose related or that any dose-related response occurs outside the dose range evaluated by the AREDS 2. An alternate hypothesis for this AREDS 2 result is the existence of an opposing dose-related response to zinc for both groups of patients, that is, those who are harmed by zinc and those who benefit from zinc. In such a scenario, the similar outcomes in the low-dose versus high-dose zinc groups in AREDS 2 may result from reduced disease progression in patients with *CFH* risk alleles treated with low-dose zinc (improving outcome by reducing zinc intake) balanced by worsened outcomes in patients without *CFH* risk alleles treated with low-dose zinc (worsening their outcome by reducing zinc intake). Future subgroup analyses of the AREDS 2 results based on genotypes may address this hypothesis.

A secondary analysis suggested a small benefit in replacing β-carotene with lutein and zeaxanthin in the antioxidant formulation. *ARMS2* genotypes may have the same treatment interaction with an antioxidant regimen containing lutein and zeaxanthin because a specific interaction with β-carotene is unlikely. We believe that our conclusions are likely to apply equally to formulations containing either antioxidant regimen.

Our analysis revealed the following conclusions for AREDS patients with AREDS category 3 disease in 1 eye and AREDS category 1 through 4 disease in the fellow eye: (1) the AREDS formulation of antioxidants and zinc was maximally beneficial for patients with 1 *CFH* risk allele and 1 *ARMS2* risk allele (genotype C1A1); (2) with the exception of patients with genotype C1A1, zinc supplementation was maximally beneficial for patients with no more than 1 *CFH* risk allele and at least 1 *ARMS2* risk allele (genotypes C0A1, C0A2, and C1A2); (3) antioxidant supplementation was maximally beneficial for patients with at least 1 *CFH* risk allele and no *ARMS2* risk alleles (genotypes C1A0 and C2A0); and (4) there was no beneficial effect of any combination of the nutritional supplements studied in AREDS for patients with genotypes C0A0, C2A1, or C2A2.

We have estimated the potential benefit of using the maximally beneficial treatment, that is, genotype-directed nutritional therapy, for this same group of patients. Based on the distribution of genetic risk alleles (Table 6) and the observed outcomes for the different treatments (Fig 1), the average 10-year progression rate for this patient population if all were treated with placebo, AREDS formulation, or genotype-directed therapy would have been 47.0%, 40.5%, and 31.7%, respectively. We estimate that genotype-directed therapy of the study population would have more than doubled the reduction in AMD progression rate compared with treatment with the AREDS formulation.

Given the absence (to our knowledge) of an existing dataset appropriate for validation studies, confirmation of our findings in a different cohort is unlikely in the near future. Additional subgroup analyses of AREDS 2 data may add further insights to this observed pharmacogenomic association.

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Footnotes and Financial Disclosures

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CFH and *ARMS2* Genetic Polymorphisms Predict Response to Antioxidants and Zinc in Patients with Age-related Macular Degeneration

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Analysis of Age-Related Eye Disease Study data showed that complement factor H and age-related maculopathy susceptibility 2 risk polymorphisms eliminate the value of zinc supplementation and antioxidants, respectively, for age-related macular degeneration progression prophylaxis. This permits a personalized approach to nutritional supplementation.