Treatment Response to Antioxidants and Zinc Based on CFH and ARMS2 Genetic Risk Allele Number in the Age-Related Eye Disease Study

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Objective: To evaluate the impact of complement factor H (CFH) and age-related maculopathy susceptibility 2 (ARMS2) risk alleles on the observed response to components of the Age-Related Eye Disease Study (AREDS) formulation.

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

Participants: White patients from the AREDS with category 3 or 4 age-related macular degeneration (AMD) with available DNA (n = 989).

Methods: Four genotype groups based on CFH and ARMS2 risk allele number were defined. Progression to advanced AMD was analyzed by genotype and treatment using Cox proportionate hazards estimates and 7-year events.

Main Outcome Measures: The effect of predefined genotype group on treatment-specific progression to advanced AMD.

Results: Patients with 2 CFH risk alleles and no ARMS2 risk alleles progressed more with zinc-containing treatment compared with placebo, with a hazard ratio (HR) of 3.07 (P = 0.0196) for zinc and 2.73 (P = 0.0418) for AREDS formulation (AF). Seven-year treatment-specific progression rates were: placebo, 17.0%; zinc, 43.2% (P = 0.023); and AF, 40.2% (P = 0.039). Patients with 0 or 1 CFH risk alleles and 1 or 2 ARMS2 risk alleles benefited from zinc-containing treatment compared with placebo, with an HR of 0.514 for zinc (P = 0.012) and 0.569 for AF (P = 0.0254). Seven-year treatment-specific AMD progression rates were as follows: placebo, 43.3%; zinc, 25.2% (P = 0.020); and AF, 27.3% (P = 0.011). Zinc and AF treatment each interacted statistically with these 2 genotype groups under a Cox model, with P values of 0.000999 and 0.00366, respectively. For patients with 0 or 1 CFH risk alleles and no ARMS2 risk alleles, neither zinc-containing treatment altered progression compared with placebo, but treatment with antioxidants decreased progression (HR, 0.380; P = 0.034). Seven-year progression with placebo was 22.6% and with antioxidants was 9.17% (P = 0.033). For patients with 2 CFH risk alleles and 1 or 2 ARMS2 risk alleles, no treatment was better than placebo (48.4%).

Conclusions: The benefit of the AREDS formulation seems the result of a favorable response by patients in only 1 genotype group, balanced by neutral or unfavorable responses in 3 genotype groups.

The Age-Related Eye Disease Study (AREDS) demonstrated that the AREDS formulation, a combination of high-dose antioxidants (β-carotene, vitamin C, and vitamin E) and high-dose zinc, reduced the 5-year risk of progression from intermediate to advanced age-related macular degeneration (AMD) by 25% and produced a 19% reduction in severe vision loss in individuals at high risk of geographic atrophy or choroidal neovascularization developing.

Similar results were obtained in the population-based Rotterdam Study, which found an above-median intake of dietary zinc and antioxidants to be associated with a 35% lower risk of incident AMD. Recently, the Age-Related Eye Disease Study 2 (AREDS2) found in its primary analysis that adding omega-3 fatty acids or the antioxidants lutein and zeaxanthin to the AREDS formulation had no additional overall effect on progression to advanced AMD. However, study participants treated with a formulation containing lutein plus zeaxanthin and no β-carotene had a slight reduction in risk of advanced AMD compared with those treated with a formulation containing β-carotene. The AREDS2 also evaluated the effects of a lower dose of zinc (25 vs 80 mg). There was no significant difference in AMD progression based on zinc dose, but a trend favoring the higher dose of zinc was observed. Based on these
results, the currently recommended AREDS2 formulation consists of the antioxidants lutein, zeaxanthin, vitamin C, and vitamin E, together with 80 mg zinc.

We reported recently the results of a genetic subgroup analysis of AREDS patients demonstrating that CFH and ARMS2 genetic polymorphisms have different effects on AMD progression risk in different AREDS-assigned treatment groups.3 We found that CFH and ARMS2 risk allele number significantly influenced the response to treatment with zinc, antioxidants, or both and reported the risk ratios associated with risk allele number in these settings. Using these risk ratios, we predicted the response to zinc, antioxidants, or both, the major components of the AREDS formulation, as influenced by individual genetic background, and we made recommendations for personalized nutritional treatment based on CFH and ARMS2 risk allele number.

Other investigators also have reported significant interaction between zinc treatment and CFH risk alleles in AREDS patients.1-6 However, we were the first to report an important adverse response to the AREDS formulation compared with placebo in patients with a particular genotype combination (those homozygous for CFH genetic risk and without ARMS2 genetic risk). Our findings and treatment recommendations were based on Cox regression analysis of outcomes in each of the 4 AREDS treatment groups, with treatment groups ranging in size from 232 to 272.

Given the far-reaching public health implications of our treatment recommendations, our prior publication has been met with both interest and skepticism. One frequent misconception is that our findings are based on an analysis of patients in each of the 9 combinations of CFH and ARMS2 risk allele number (there are 9 possible combinations of 0, 1, or 2 CFH and ARMS2 risk alleles). Because of the relative rarity of some genotypes, such an approach would be underpowered to evaluate certain subgroups for clinically important interactions of treatment and CFH or ARMS2 risk allele number. For example, there were fewer than 10 patients with the combination of 0 CFH risk alleles and 2 ARMS2 risk alleles. The technical inappropriateness of this method is strikingly illustrated by a recently published genetic evaluation of AREDS patients by Chew et al9 that used separate and isolated analyses of 27 subgroups (9 genetic combinations × 3 active AREDS treatments). Although the authors fail to detect an association between genetics and nutritional supplements for AMD prophylaxis, the lack of statistical power also results in data that fail to demonstrate the benefit of the AREDS formulation for patients in any of the 27 subgroups.

Having demonstrated in our prior publication that CFH and ARMS2 risk allele numbers are significant determinants of AMD progression within each AREDS treatment group, we now provide a direct observation of outcomes of AREDS patients based on logically derived and appropriately sized genetic subgroups. We defined 4 natural genotype groupings sufficiently sized to allow measurement of statistically meaningful outcomes. Using AREDS data, we compared actual progression rates within these genotype groups among patients who received placebo, antioxidants, zinc, or the AREDS formulation. We compared these actual outcomes with our previously published projections and we examined the potential impact of treatment recommendations based on these genotype groups.

**Methods**

Patients were derived from the AREDS population. Study procedures have been reported previously.1 Patient consent was given in the AREDS to permit genetic samples to be used for eye diseases only or for general research use. The AREDS data set was provided by the database of Genotypes and Phenotypes under an investigator agreement. Patients were characterized by AREDS investigators at enrollment, with time course retinal images classified by a central reading center, allowing determination of the interval from study enrollment to AMD progression.1

The progression risk of participants in the AREDS cohort varied based on initial AMD status. Disease was classified by AREDS investigators based on the category of AMD in each eye: AREDS category 1 (no AMD), fewer than 5 small drusen (<63 µm); category 2 (mild AMD), multiple small drusen, nonextensive intermediate drusen (63–124 µm), pigment abnormalities, or a combination thereof; category 3 (intermediate AMD), at least 1 large druse (<125 µm), extensive intermediate drusen, or geographic atrophy not involving the center of the macula; and, category 4 (advanced AMD in 1 eye only), central geographic atrophy or neovascular AMD or visual loss resulting from AMD regardless of lesion type. All participants with mild AMD or greater were randomized by AREDS investigators at study entry to 1 of 4 oral nutritional supplements consisting of (1) placebo; (2) antioxidants (β-carotene, 15 mg; vitamin C, 500 mg; and vitamin E, 400 IU); (3) zinc, 80 mg as zinc oxide, and copper, 2 mg; and (4) treatment 2 plus 3 consisting of both antioxidants and zinc. The AREDS investigators reported reduced progression to advanced AMD in a subgroup analysis of patients with category 3 or 4 AMD treated with the combination of antioxidants and zinc.6

We restricted our analysis to white patients because AMD genetics has been studied best in this group. Like the AREDS investigators, we selected those with category 3 or 4 AMD at the time of enrollment. Age-related macular degeneration progression was defined as the development of advanced AMD in either eye for those individuals with category 3 AMD at study entry or the development of bilateral advanced AMD for those who had category 4 AMD at study entry. Clinical record abstracts of patients (n = 989) meeting these criteria were used to determine the time to AMD progression and the total period of observation for nonprogressing patients.

**Genotyping**

Our approach for genotyping CFH and ARMS2 risk alleles was reported previously.1 All available DNA from white AREDS participants with AREDS category 3 or 4 AMD (n = 989) 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 (GLP). For this study, we examined genotypes at the CFH locus and the ARMS2 locus.

To analyze the common genetic variability of the CFH locus, we selected a set of 5 polymorphisms for genotyping that were reported by Li et al10 to tag 4 common, disease-associated CFH haplotypes: rs1048663, rs3766405, rs412852, rs11582939, and rs1066420 (previously rs1280514). rs1066420 was excluded from further analyses because of deviations from Hardy-Weinberg equilibrium in controls (P < 0.001). Linkage disequilibrium and tagging analysis of the remaining 4 SNPs revealed that any combination of 2 SNPs is sufficient to tag all common haplotypes (>1%) of this SNP haplotype.
block. We selected rs3766405 and rs412852 to tag the 2 major CFH haplotypes, as has been done previously using linked SNPs rs2274700 ($r^2 = 0.86$ with rs3766405) and rs1061170 ($r^2 = 0.82$ with rs412852). We defined the 2 SNP high-risk haplotypes to be rs3766405 CC and rs412852 CC, the heterozygous haplotype to be all combinations not designated as high or low risk, and the homozygous low-risk haplotype to be rs3766405 CT/rs412852 TT or rs3766405 TT/rs412852 TT.

Risk at the ARMS2 locus was determined by sequencing at the site of a disease-associated insertion or deletion polymorphism in the Site regulatory domain (372_815del443ins54) that affects RNA stability. This marker is in linkage disequilibrium with rs10490924, a commonly assayed ARMS2 risk marker. We abbreviated genotypes as CXX, with C for CFH, A for ARMS2, and X indicating the number(s) of CFH or ARMS2 risk alleles in the corresponding genotype group.

For each patient, we determined the number of AMD risk alleles at CFH and ARMS2 based on allele frequency. Given the relative rarity of homozygous CFH low-risk alleles and ARMS2 homozygous high-risk alleles in the study population, we grouped individuals homozygous for these rare alleles with individuals heterozygous for the corresponding risk alleles for the purpose of statistical analysis (Table 1, available at www.aaojournal.org). We defined genotype group (GTG) 1 as those patients with 0 or 1 CFH risk alleles and no ARMS2 risk alleles (C01A0), GTG 2 patients had 2 CFH risk alleles and no ARMS2 risk alleles (C01A2), GTG 3 patients had 0 or 1 CFH risk alleles and 1 or 2 ARMS2 risk alleles (C01A12), and GTG 4 patients had 2 CFH risk alleles and 1 or 2 ARMS2 risk alleles (C2A12).

Statistical Analysis
We determined the time from enrollment to progression to advanced AMD or censoring for each patient using data supplied to us by the database of Genotypes and Phenotypes. The effects of treatment on progression within GTG were determined by the Cox proportional hazards regression estimates. Analyses, including interaction studies, were performed using the stats package R for Mac OS X (available at: http://www.r-project.org) using the Rcmdr interface (accessed from the Comprehensive R Archive Network). Statistically significant differences were determined using the Z test.

Results

Patients
Of patients enrolled in the AREDS ($n = 4757$), white patients with category 3 or 4 AMD at the time of enrollment ($n = 2258$) were selected for study. Of these, DNA samples were available for 989 patients through the Coriell Institute repository (Camden, NJ; at the time of this study), and these patients constituted the sample set for our analysis.

To ensure that this sample set ($n = 989$) was representative of the entire AREDS white population with category 3 or 4 AMD at enrollment ($n = 2258$), we compared age, gender, smoking history, body mass index, and overall treatment category assignment within each treatment category and found no statistical differences.

There were 280 patients (28.3%) in GTG 1 (C01A0), 131 patients (13.2%) in GTG 2 (C2A0), 351 patients (35.5%) in GTG 3 (C01A12), and 227 patients (23.0%) in GTG 4 (C2A12). As expected, the proportions of AMD genetic risk groups in a population with AMD deviated from the proportions observed in a reference European population not enriched for AMD (HapMap Utah residents with ancestry from northern and western Europe [CEU] sample set), with our study population containing proportionately more individuals with higher genetic risk (GTG 4) and fewer individuals with lower genetic risk (GTG 1; Table 2, available at www.aaojournal.org). These genotype groups did not vary with respect to age, body mass index, smoking history, or treatment assignment (Table 3).

### Age-Related Macular Degeneration Progression: Effect of Genotype Group

We determined univariate Cox proportional hazards regression estimations of AMD progression rates for individuals in each GTG (Table 4). Compared with patients in GTG 1, who had both low CFH and low ARMS2 genetic risk (C01A0), patients in GTGs 2, 3, and 4 had progressively increased AMD progression risks. Patients in GTG 2 had an observed hazard ratio (HR) for AMD progression of 1.87 ($P = 2.93E-03$), those in GTG 3 had an HR of 2.00 ($P = 2.94E-05$), and those in GTG 4 had an HR of 3.07 ($P = 4.53E-11$).

The Effect of Treatment Assignment within Genotype Groups

Table 5 lists the Cox regression hazard ratios and $P$ values within each GTG, as a function of AREDS-assigned nonplacebo treatment group, compared with outcomes of patients assigned to placebo. For patients in GTG 1 (C01A0), only treatment with antioxidants was associated with decreased AMD progression (HR, 0.380; 95% confidence interval [CI], 0.155–0.933; $P = 0.034$). Patients in GTG 2 (C2A0) showed significant increase in AMD progression if treated with zinc (HR, 3.07; 95% CI, 1.18–8.00; $P = 0.0200$) or with the zinc-containing AREDS formulation (HR, 2.73; 95% CI, 1.04–7.20; $P = 0.0418$). In contrast, patients in GTG 3 (C01A12) had decreased AMD progression if treated with zinc (HR, 0.514; 95% CI, 0.303–0.895; $P = 0.0177$).
Table 4. Risk for Age-Related Macular Degeneration Progression Associated with Genotype Groups 2, 3, and 4 Compared with Genotype Group 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTG 2</td>
<td>1.87</td>
<td>2.93E-03</td>
</tr>
<tr>
<td>GTG 3</td>
<td>2.00</td>
<td>4.93E-05</td>
</tr>
<tr>
<td>GTG 4</td>
<td>3.07</td>
<td>4.53E-11</td>
</tr>
</tbody>
</table>

GTG = genotype group.
Age-related macular degeneration progression risk increased with increasing risk allele number.

95% CI, 0.306–0.864; P = 0.012) or the AREDS formulation (HR, 0.569; 95% CI, 0.348–0.933; P = 0.0254). For patients in GTG 4 (C2A12), the GTG with the highest genetic risk, no treatment resulted in a significant decrease in AMD progression compared with placebo.

We performed an interaction analysis restricted to study patients in GTG 2 and GTG 3, the 2 groups with opposing levels of CFH and ARMS2 genetic risk: GTG 2 patients had highest CFH and lowest ARMS2 risk (C2A0), whereas those in GTG 3 had lowest CFH and highest ARMS2 risk (C0A12). A statistically significant interaction was observed between GTG and zinc-containing treatment with respect to their impact on AMD progression. A Cox regression interaction analysis within the GTG 2 and GTG 3 subpopulation yielded a statistically significant decrease in AMD progression if treated with zinc (25.2%; P = 0.011) or the AREDS formulation (27.2%; P = 0.011) compared with treatment with placebo (17%). Patients in GTG 3 had reduced AMD progression if treated with zinc (25.2%; P = 0.011) or the AREDS formulation (27.2%; P = 0.011) compared with treatment with placebo (43.3%). Patients in GTG 4 had an overall poor prognosis, with 44% of individuals overall progressing by 7 years and with no treatment group differing significantly in outcome compared with placebo.

Kaplan-Meier survival estimate curves further illustrate the divergent effect of zinc-containing treatment (zinc or the AREDS formulation), compared with placebo, on AMD progression for patients in GTG 2 (C2A0) and GTG 3 (C0A12; Fig 2). Zinc-containing treatment was detrimental for patients in GTG 2 (P = 0.0042) and was beneficial for patients in GTG 3 (P = 0.0106).

Table 5. Risk of Age-Related Macular Degeneration Progression within Each Genotype Group as a Function of Age-Related Eye Diseases Study–Assigned Treatment Group Compared with Progression If Treated with Placebo

<table>
<thead>
<tr>
<th>Genotype Group</th>
<th>Hazard Ratio (95% Confidence Interval)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidants</td>
<td>0.38 (0.16–0.93)</td>
<td>0.03</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.03 (0.50–2.14)</td>
<td>0.93</td>
</tr>
<tr>
<td>AF</td>
<td>0.80 (0.39–1.63)</td>
<td>0.54</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidants</td>
<td>1.33 (0.48–3.96)</td>
<td>0.61</td>
</tr>
<tr>
<td>Zinc</td>
<td>3.07 (1.18–8.20)</td>
<td>0.02</td>
</tr>
<tr>
<td>AF</td>
<td>2.73 (1.04–7.20)</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidants</td>
<td>0.72 (0.44–1.18)</td>
<td>0.19</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.51 (0.31–0.86)</td>
<td>0.01</td>
</tr>
<tr>
<td>AF</td>
<td>0.57 (0.35–0.93)</td>
<td>0.03</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidants</td>
<td>0.85 (0.49–1.48)</td>
<td>0.56</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.93 (0.53–1.65)</td>
<td>0.82</td>
</tr>
<tr>
<td>AF</td>
<td>0.88 (0.50–1.55)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

AREDS F = Age-Related Eye Diseases Study formulation (i.e., antioxidants + zinc).

Absolute Survival Determinants

To measure outcomes in each GTG directly, we performed a Cox proportional hazards estimate of observed 7-year AMD progression for each AREDS-assigned treatment group within each GTG.

We chose to analyze 7-year outcomes to parallel closely the design of AREDS report number 8, which reported the probability of progression to advanced AMD during years 2 through 7 of the AREDS. Calculated AMD progression for patients assigned to each nonplacebo treatment was compared with progression for patients assigned to placebo (Fig 1).

Patients in GTG 1, the group with the lowest average genetic risk, had a statistically significant reduction in 7-year AMD progression if treated with antioxidants (7% vs. 23% if treated with placebo; P = 0.033). Patients in GTG 2 (C2A0) had increased 7-year AMD progression if treated with zinc (43.2%; P = 0.023) or the AREDS formulation (40.2%; P = 0.039) compared with treatment with placebo (17%). Patients in GTG 3 had reduced AMD progression if treated with zinc (25.2%; P = 0.011) or the AREDS formulation (27.2%; P = 0.011) compared with treatment with placebo (43.3%). Patients in GTG 4 had an overall poor prognosis, with 44% of individuals overall progressing by 7 years and with no treatment group differing significantly in outcome compared with placebo.

The relationship between CFH genotype and effectiveness of AREDS supplements was suggested first by Klein et al., who found evidence of an interaction between CFH genotype and treatment with antioxidants plus zinc when compared with placebo. Klein et al stated that the observed interaction (P = 0.004) in the zinc-treated AREDS patients versus AREDS patients taking no zinc suggests that the genotype—treatment interaction in participants with the high-risk CFH genotype may be related primarily to the zinc component of the supplements. They observed that an argument may be made for genetic screening to identify those patients most and least likely to benefit from nutritional therapy, but they did not propose routine screening, largely because of their observation that “some benefit is derived by individuals in all CFH genotype groups.” However, a subsequent analysis of the data published by Klein et al revealed that there was no demonstrated treatment benefit for patients with the high-risk CFH genotype. More recently, Rotterdam Study investigators reported that high dietary intake of nutrients with antioxidant properties reduces the risk of early AMD in those at high CFH and ARMS2 genetic risk.

We reported previously that the impact of AREDS-assigned nutritional supplements on AMD progression is influenced by the number of CFH and ARMS2 risk alleles. In our earlier study, we showed that CFH risk allele number was a significant predictor of AMD progression for zinc-treated AREDS patients, whereas ARMS2 risk allele number had no predictive value for zinc-treated patients. Conversely, ARMS2...
The risk allele number was a significant predictor of AMD progression for patients who were treated with antioxidants, but not for those treated with zinc. Although we interpreted this as evidence of the impact of \( CFH \) risk in response to zinc-containing treatments and \( ARMS2 \) risk in response to antioxidant treatment, our current analysis suggests that these outcomes were the result of a differential impact of zinc on the effect of \( CFH \) or \( ARMS2 \) genetic risk alleles. Supplementation with high-dose zinc increases the deleterious impact of \( CFH \) risk alleles on AMD progression but diminishes the deleterious impact of \( ARMS2 \) risk alleles.

In our prior publication, we used the measured AMD progression risk associated with varying \( CFH \) risk allele number in patients treated with zinc or the AREDS formulation and the measured risk associated with varying \( ARMS2 \) risk allele number in patients treated with antioxidants to project 12-year AMD progression for each of the 4 AREDS-assigned treatment groups. By superimposing these treatment group projections over a matrix of the 9 possible combinations of 0, 1, or 2 \( CFH \) and \( ARMS2 \) risk alleles, we were able to compare the projected outcomes of each treatment for patients in each of the 9 genotype groups.

In this study, we combine the 9 distinct genotype combinations of 0, 1, or 2 \( CFH \) and 0, 1, or 2 \( ARMS2 \) risk alleles into 4 genotype groups with relatively balanced patient numbers. These groupings allow us to measure, with statistical validity, the actual outcomes of AREDS patients in these 4 genotype groups rather than projected outcomes. Like the AREDS itself, the absence of a similar independent data set impedes independent validation of our findings.

However, the presence of directly observed progression rate differences among AREDS treatment groups with different genetic backgrounds in this well-controlled data set provides reasonable input for clinical decision making.

The appropriateness of our genotype groupings is suggested by the diametric AMD progression rates of patients treated with a zinc-containing regimen who had opposite balances of \( CFH \) and \( ARMS2 \) genetic risk. For those with 2 \( CFH \) risk alleles and no \( ARMS2 \) risk alleles, treatment with a zinc-containing regimen was associated with an approximate 3-fold increase in AMD progression. An opposite therapeutic response to zinc occurred in patients with 0 or 1 \( CFH \) risk alleles and 1 or 2 \( ARMS2 \) risk alleles, for whom treatment with a zinc-containing regimen was beneficial, with an approximate halving of AMD progression rate.

The statistical interaction between genotype group and zinc treatment implies a gene–environment interaction that affects the underlying pathophysiology. Zinc seems to increase the harmful effect of \( CFH \) risk alleles, whereas it seems to ameliorate the risk associated with \( ARMS2 \) risk alleles.

Chew et al, in a reanalysis of AREDS data, failed to detect an association between genetics and nutritional supplements in AMD prophylaxis. The statistical model used in this interaction analysis divided 1237 AREDS patients into 27 subgroups for independent analysis, which severely limits the power to detect a treatment–gene interaction and obscures important relationships that could be detected through conventional model-building techniques that use the entire data set. Chew et al used a statistical model that including such nongenetic factors as age, gender, smoking.
Log-rank test. 4 had relatively high progression with all treatments, with minimally lower progression with antioxidants compared with placebo (P = 0.975). Our analysis suggests that the overall insignificant effect of the zinc-containing AREDS formulation they report is the result of a substantial benefit for patients in GTG 3 balanced by a deleterious treatment response for patients in GTG 2 (Fig 1).

The treatment recommendations we propose for each of the 4 genotype groups, based on 7-year outcomes in AREDS, are consistent with the treatment recommendations we proposed in our published statistical model.1 We recommended zinc or the zinc-containing AREDS formulation for patients with 0 or 1 CFH and 0 or 1 ARMS2 risk alleles. These patients are classified as members of GTG 3 in this analysis, a group whose 7-year outcomes were best if treated in this manner. We recommended antioxidants for patients with 1 CFH and 0 ARMS2 risk alleles, which is consistent with the outcomes of patients in GTG 1 (C01A0). We predicted that patients with 2 CFH risk alleles and 0 ARMS2 risk alleles would have higher AMD progression rates if treated with zinc or the AREDS formulation and should be treated with antioxidants, a prediction supported by the observed 3-fold increase in 7-year AMD progression associated with zinc or AREDS formulation treatment for patients in GTG 2 (C2A0). Finally, for patients with 2 CFH risk alleles and 1 or 2 ARMS2 risk alleles, with the highest genetic risk, we predicted that no treatment was of significant benefit. Patients in GTG 4 (C2A12) indeed had high rates of AMD progression, with no difference in outcome based on treatment.

Biochemical Interaction between Zinc, CFH, and ARMS2

Zinc has complex physiologic influences on oxidative stress, oxidative damage, and activation of the complement system. Although there has been general acceptance that zinc supplementation, as a component of the AREDS formulation, reduces AMD progression, zinc also has been implicated in the pathogenesis of AMD.15,16

Table 6. Genotype-Directed Therapy, the Treatment Associated with Lowest 7-Year Age-Related Macular Degeneration Progression for Patients in Each Genotype Group

<table>
<thead>
<tr>
<th>GTG</th>
<th>Percentage of Study Population (%)</th>
<th>Group with Lowest 7-Year Progression (%)</th>
<th>Progression (%) with Best Treatment vs. Placebo*</th>
<th>Progression (%) with Placebo*</th>
<th>P Value, Genotype-Directed Therapy vs. AREDS F*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (C01/A0)</td>
<td>28.3 Antioxidants</td>
<td>9.1</td>
<td>14.3</td>
<td>0.023</td>
<td>18.4</td>
</tr>
<tr>
<td>2 (C2A0)</td>
<td>13.2 Placebo</td>
<td>17.0</td>
<td>17.0</td>
<td>—</td>
<td>40.2</td>
</tr>
<tr>
<td>3 (C01A12)</td>
<td>35.4 Zinc 80 mg</td>
<td>25.2</td>
<td>43.3</td>
<td>0.005</td>
<td>27.3</td>
</tr>
<tr>
<td>4 (C2A12)</td>
<td>23.1 Antioxidants</td>
<td>42.6</td>
<td>48.4</td>
<td>0.768</td>
<td>44.4</td>
</tr>
</tbody>
</table>

Seven-year age-related macular degeneration progression percentages are shown for treatment with genotype-directed therapy, placebo, and Age-Related Eye Disease Study formulation (AREDS F). Patients in genotype group (GTG) 1 had decreased progression if treated with antioxidants compared with placebo (P = 0.0234, log-rank test) but not with AREDS F compared with placebo (P = 0.528). Patients in GTG 2 had increased progression if treated with AREDS F (P = 0.0305) or with zinc (P = 0.0227). Patients in GTG 3 had decreased progression if treated with zinc (P = 0.0052) or AREDS F (P = 0.0225). Patients in GTG 4 had relatively high progression with all treatments, with minimal lower progression with antioxidants compared with placebo (P = 0.540).

*Log-rank test.
In individuals with high-risk CFH alleles, zinc may promote inflammation. Zinc inhibits CFH-mediated regulation of C3b by promoting CFH to aggregate strongly, causing its inactivation.\textsuperscript{7,18} This results in increased complement activation. Given that patients homozygous for CFH risk have higher baseline complement activation, zinc supplementation and the resultant increase in complement-mediated inflammation may lead to increased AMD progression.

Although the role of the ARMS2 gene in the pathogenesis of AMD is unknown at present, we hypothesize that our observation that zinc ameliorates ARMS2-associated AMD risk is related to altered oxidative stress. Yang et al\textsuperscript{19} reported that the AMD-associated ARMS2 insertion or deletion polymorphism impairs the ability of the retinal pigment epithelium to defend against aging-related oxidative stress. They demonstrated in stem cell–derived retinal pigment epithelium that mitochondrial superoxide dismutase (SOD) 2–mediated antioxidative defense is impaired in the presence of ARMS2 risk alleles. Whereas SOD2 function is manganese dependent, SOD1, located in cytoplasm, is zinc and copper dependent, and SOD1-deficient mice show macular degeneration.\textsuperscript{20} If SOD1 and SOD2 affect a common pathophysiologic pathway, perhaps the deleterious effects of SOD2 deficiency can be minimized by increased activation of SOD1 or a similar antioxidant mechanism enhanced by zinc supplementation.

Treatment Recommendations

We propose GTG-directed nutritional treatment for white patients with AREDS category 3 (intermediate in at least 1 eye) or category 4 (advanced AMD in 1 eye only) AMD (Table 6). For patients in GTG 1 (low CFH and low ARMS2 risk; 28% of the study population), AMD progression may be minimized with antioxidant-only treatment. For patients in GTG 2 (high CFH risk and no ARMS2 risk; 13% of the study population), zinc-containing regimens seem to be harmful, associated with a doubling of AMD progression risk at 7 years compared with treatment with antioxidants or placebo. For patients in GTG 3 (low CFH and high ARMS2 risk; 35% of the study population), treatment with the AREDS formulation or zinc results in significant reduction in AMD progression. For the patients in GTG 4 (high CFH and high ARMS2 risk; 23% of the study population), no AREDS-assigned treatment is beneficial, and these patients can avoid the cost and infrequent adverse effects associated with nutritional supplements. These results suggest that most patients with AREDS category 3 or 4 AMD would be managed better with a treatment other than the AREDS formulation. These conclusions, based on actual outcomes of 989 patients followed up for 7 years in the AREDS, are consistent with our earlier projection analysis—based treatment recommendations. Our recommendations, based on an understanding of AMD genetics unknown at the time of the initial AREDS analysis and recommendations, may lead to improved outcomes for patients with AMD similar to those in our study group.

The AREDS recommendation for zinc and antioxidant treatment of patients with moderate AMD was based on a retrospective subgroup analysis of 2516 AREDS patients.\textsuperscript{4} Our study is a retrospective subgroup analysis of 989 patients with category 3 or 4 AMD followed up in the AREDS. DNA was not collected from all AREDS patients. Our analysis is based on all DNA from white AREDS patients with category 3 or 4 AMD available through the National Eye Institute—AREDS genetic repository. The GTG-based analysis of AREDS2 patients treated with the original AREDS formulation may demonstrate a similar difference in AMD progression between patients in GTG 2 and GTG 3, although the absence of a placebo group in AREDS2 prevents an exact comparison. Outcomes in AREDS2 patients assigned to other treatment regimens containing high-dose zinc also may reveal differences in AMD progression related to genotype. Because AREDS2 patients were randomized to either 25 or 80 mg of zinc, AREDS2 data also may reveal a dose-dependent interaction of zinc and genetic risk.

Validation by an independent data set would be helpful, but no such data set exists, and a replication trial would take many years. The recommendations of the AREDS were widely adopted without a validating study. Our recommendations, based on dramatic differences in outcomes of AREDS patients in easily identified GTGs, further illuminate the important findings of AREDS investigators and may serve as a basis for genotype-directed nutritional therapy for patients with moderate AMD.

References


Footnotes and Financial Disclosures

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Abbreviations and Acronyms:

AMD = age-related macular degeneration; AREDS = Age-Related Eye Disease Study; AREDS2 = Age-Related Eye Disease Study 2; ARMS2 = age-related maculopathy susceptibility 2; CFH = complement factor H; CI = confidence interval; GTG = genotype group; HR = hazard ratio; SOD = superoxide dismutase.

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