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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. *Ophthalmology 2014;■:1–8 © 2014 by the American Academy of Ophthalmology.*

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.[1] 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.[2] 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

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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.[4–6] 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 patients.

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 al[7] 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.[8] 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.[8]

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.[3] 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 al[9] 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