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Genetic Polymorphisms of *CFH* and *ARMS2* Do Not Predict Response to Antioxidants and Zinc in Patients with Age-Related Macular Degeneration

Independent Statistical Evaluations of Data from the Age-Related Eye Disease Study

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Purpose: Considerable controversy has erupted in recent years regarding whether genotyping should be part of standard care for patients with age-related macular degeneration (AMD) who are being considered for treatment with antioxidants and zinc. We aimed to determine whether genotype predicts response to supplements in AMD.

Design: Three separate statistical teams reanalyzed data derived from the Age-Related Eye Disease Study (AREDS), receiving data prepared by the AREDS investigators and, separately, data from investigators reporting findings that support the use of genotyping.

Participants: The population of interest was AREDS participants with AMD worse than category 1 and genotyping data available. Data from the 2 groups overlap imperfectly with respect to measurements made: the largest common set involved 879 participants for whom the same *CFH* and *ARMS2* single nucleotide polymorphisms were measured by both groups.

Methods: Each team took a separate but complementary approach. One team focused on data concordance between conflicting studies. A second team focused on replicating the key claim of an interaction between genotype and treatment. The third team took a blank slate approach in attempting to find baseline predictors of treatment response.

Main Outcome Measures: Progression to advanced AMD.

Results: We found errors in the data used to support the initial claim of genotype–treatment interaction. Although we found evidence that high-risk patients had more to gain from treatment, we were unable to replicate any genotype–treatment interactions after adjusting for multiple testing. We tested 1 genotype claim on an independent set of data, with negative results. Even if we assumed that interactions in fact did exist, we did not find evidence to support the claim that supplementation leads to a large increase in the risk of advanced AMD in some genotype subgroups.

Conclusions: Patients who meet criteria for supplements to prevent AMD progression should be offered zinc and antioxidants without consideration of genotype. *Ophthalmology 2018;125:391-397 © 2017 by the American Academy of Ophthalmology*

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The Age-Related Eye Disease Study (AREDS) was a large, multicenter, double-blind randomized trial to determine whether high-dose antioxidants, zinc, or their combination could reduce the risk of progression of age-related macular degeneration (AMD) in older patients. Excluding patients in AMD category 1, for whom the event rate was less than 1%, the combination of zinc and antioxidants was found to reduce the risk of progression to advanced AMD (odds ratio, 0.68; 95% confidence interval [CI], 0.49–0.93; $P = 0.002$).[1] The publication of the trial results led to rapid changes in practice, with at-risk patients routinely prescribed the zinc and antioxidant combination tested in the trial.

In 2008, Klein et al[2] published a pharmacogenomic study suggesting that the effects of antioxidants and zinc on AMD in AREDS may be influenced by genotype,

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specifically, the disease-related genes age-related maculopathy susceptibility 2 (*ARMS2*) and complement factor H (*CFH*), also known as *ARMS1*. For instance, there was a smaller difference between treatment and placebo in patients with the CC genotype for *CFH* Y402H (44% vs. 39%) compared with those with the TT genotype (34% vs. 11%; $P = 0.03$ for interaction). No interaction was found for *LOC387715/ARMS2*. The authors made only cautious conclusions, stating that "corroboration … is needed before considering modification of current management." Such corroboration seemed to come from Awh et al,[3] who examined the relative benefit of treatment across a wider set of genotypes from 11 disease-related markers before settling on 2 markers for *CFH* and 1 marker for *ARMS2*. Importantly, Awh et al claimed qualitative interactions between genotype and treatment outcome. The authors stated that the "data support a deleterious interaction between CFH risk alleles and high-dose zinc supplementation" such that patients with certain genotypes should be treated by antioxidants alone rather than by antioxidants plus zinc. The conclusions included "recommendations" that would lead to "improved outcomes through genotype-directed therapy."

These findings led the original study authors, Chew et al,[4] to attempt a replication. Measuring the genotype of a different subset of patients from AREDS, the authors did find the anticipated prognostic relationship between *CFH* and *ARMS2* genotype and risk of progression. However, they did not find any predictive relationship between genotype and treatment effect, with test results for interaction being nonsignificant. The authors concluded that "supplements reduced the rate of AMD progression across all genotype groups" and that genetic testing should not be used to determine treatment. These negative findings were challenged by Awh and Zanke,[5] who claimed that the study by Chew et al refutes any claim of overall benefit for supplementation and that a separate editorial, written by a well-known statistician and epidemiologist team (Wittes and Musch[6]) supported the genotyping. In response, Chew et al[7] claimed that Awh and Zanke had misinterpreted their study and that, in fact, the Wittes and Musch editorial favored their own position.

To help resolve this debate, the Office of Intramural Research at the National Institutes of Health (NIH) asked our 3 biostatistical groups to re-examine independently the data used by Awh et al[3] and Chew et al[5] to determine whether genotyping should be part of the clinical decision of whether to use supplements for AMD prevention. Herein, we report our findings.

Methods

A research integrity officer at the NIH contacted both sets of investigators (Chew et al and Awh et al) and proposed that they provide data to be forwarded on to independent biostatisticians—whose names and affiliations were not revealed—for further analysis. The 2 groups agreed and sent their data to the research integrity officer, who forwarded it on to us. Neither the NIH nor any other outside group or investigator participated in the design of the statistical methods used, interpretation of the results, drafting of the manuscript, or manuscript review before submission. No direct funding or any other type of financial remuneration was provided by NIH to support the current work.

Clinical information on AREDS participants is available to qualified researchers through the Database of Genotypes and Phenotypes, and single nucleotide polymorphism (SNP) and sequencing data are available now for an ever-increasing subset, although significantly fewer data were available when the debate began. For their studies, Awh et al[3,8] focused on 979 patients for whom blood samples could be obtained from the Coriell biorepository. They used these samples to perform their own genotyping. They genotyped *CFH* at 2 SNPs, rs3766405 and rs412852, and assessed insertion/deletion (indel) status for *ARMS2* at 1 location. Chew et al[4,9] looked at data from 1237 patients for whom they had *CFH* and *ARMS2* genotype data at SNPs other than those used by Awh et al (rs1061170 and rs1410996 for *CFH* and rs10490924 for *ARMS2*; summarized in their Fig 1B) and from 1413 patients measured using exactly the same SNPs as those used by Awh et al (summarized in their Fig 1C). In all, genotype data from these 3 locations from Awh et al are available for 1523 participants: 879 were measured by both groups, 110 were measured only by Awh et al, and 534 were measured only by Chew et al. All data can be matched using anonymized AREDS patient identifiers.

The genotype data for patients measured at the above mentioned 3 SNPs underwent several levels of summarization. First, there were the raw genotype assessments (AA, AB, or BB) at each of the 3 SNPs. Second, results were expressed at the gene level in terms of the number of risk alleles for that gene (0, 1, or 2). This mapping is straightforward for *ARMS2* (measured at just 1 SNP), but requires more detailed specification for *CFH* to indicate how a pair of genotypes is reduced to a number. Third, the numbers of risk alleles for each of the 2 genes are used to assign patients to genotype groups (GTGs). Proposed treatment differentiation would occur at the GTG level.

The 3 statistical groups decided to work independently on 3 separate approaches to the replication problem. The MD Anderson Cancer Center group focused primarily on checking data and evaluating concordance between different data sets. The Duke University group's role was to replicate the key findings of Awh et al concerning interactions between genotype and outcome. The Memorial Sloan Kettering Cancer Center (MSKCC) group took a blank slate approach, using all baseline data, including both clinical variables and genotype data, to determine whether benefit from treatment could be predicted.

MD Anderson Cancer Center: Data Concordance

We received raw data on patients from AREDS[1] linking times to AMD disease progression to *CFH* and *ARMS2* genotypes and treatment group, from both Awh et al (Arctic)[3,8] and the AREDS investigators.[4,9] The data also contained various clinical covariates such as age, gender, race, body mass index (BMI), and smoking history.

Because unappreciated differences between data sets could explain some of the published inconsistencies, first we extensively checked the raw data supplied by both groups. We cross-tabulated genotype calls for rs3766405, genotype calls for rs412852, and the reported numbers of *CFH* risk alleles. We also checked progression data in each of the 2 data sets by examining the longitudinal data on AMD eye categories to identify the time point at which either progression to category 4 in either eye first occurs, if the patient's category values were less than 4 for both eyes at the outset, or progression to category 4 occurs in the non–category 4 eye if the patient has 1 eye rated as category 4 at the outset.