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'To test, or not to test? That is the question,' quoth Hamlet, never.

Personalized medicine, de rigueur in oncology, has made a grand entrance into retina as Luxturna (voretigene neparvovec-rzyl, Spark Therapeutics, Inc). However, when it comes to nonneovascular AMD, the adoption of genetic testing to drive treatment decisions has been widely contested—but not widely adopted.

The basic question remains as to whether our genotypes affect our responses to AREDS supplements, and whether we can discern which alleles lead down the path to amelioration and which to damnation.

Numerous investigations have attempted to answer this important question using AREDS data, but have given conflicting results. Some found a signal suggesting patients with combined high-risk *CFH* and low-risk *ARMS2* alleles (called *GTG2*) may be worse off on supplements. Others disagreed.

In a recent article published in the *Journal of VitreoRetinal Diseases*, Stephen Kaufman, MD, and colleagues took an interesting approach to this problem. They reasoned that should genetics *not* play a role, the ratio of patients with newly diagnosed neovascular AMD taking the supplements to those not taking the supplements should be evenly distributed among different genotypes.

In a clever observational trial, they found an association between supplements and progression to neovascular AMD in GTG2 patients. Here, Dr. Kaufman reviews this evidence and offers an opinion on the need for genetic testing to drive AREDS supplement recommendations. Further commentary is offered by Drs. Carl Awh and Emily Chew, who have published on this important topic.

— *Joel A. Pearlman, MD, PhD, FASRS*
Editor in Chief

New Analysis Suggests Role for Personalized Medicine in Dry-AMD Treatment

There is new evidence that the Age-Related Eye Disease Studies Formulation (AREDS-F) may be neither safe nor effective for some patients with a subset of age-related macular degeneration (AMD) genetic risk alleles. Those with combined high-risk *CFH* and low-risk *ARMS2* alleles have an increased probability of developing neovascular AMD (nvAMD) if they use AREDS-F. Perhaps the time has come for a personalized approach guided by genetics.

We have found an interaction between genotype and AREDS-F use and reported it in the *Journal of Vitreoretinal Diseases*.[1] Certain alleles of complement factor H (CFH) and age-related maculopathy susceptibility 2 (*ARMS2*) are strongly associated with advanced AMD.[2]

Zinc binds with CFH and affects its ability to neutralize complement component 3b, which is involved in inflammation.[3] ARMS2 localizes to mitochondria and affects oxidative phosphorylation and oxygen free-radical generation, where it may interact with vitamins C and E, as well as other antioxidants.[4]

While initial claims of a differential response to AREDS-F in genetically defined subgroups were met with skepticism, newer research has provided compelling evidence that AREDS-F may increase the risk of nvAMD in genetically predisposed individuals.

Using AREDS data,[5] in 2015 Awh et al[6] expanded and refined a previously reported data set.[7] They grouped subjects with combinations of *CFH* and *ARMS2* risk alleles into 4 genotype groups (GTGs). The researchers found that subjects in GTG 2, with high-risk *CFH* alleles and low-risk *ARMS2* alleles (about 15% of AMD patients) had increased risk of progression to advanced AMD if they took AREDS-F compared to subjects who took placebo. Meanwhile, those in GTG 3, with low-risk *CFH* alleles and high-risk *ARMS2* alleles (about 40% of AMD patients) had decreased risk.

These conclusions were controversial, and the original AREDS study was not powered to detect this relationship post hoc. A report in *Ophthalmology* disputed these findings and, using a smaller, independent database, suggested that all GTGs benefit from AREDS-F, regardless of genotype.[8]

'Zinc binds with CFH and affects its ability to neutralize complement component 3b, which is involved in inflammation.'

The signals in these post hoc, subgroup analyses were intriguing, if conflicting. They were based on efforts to extract a higher level of data than the original study was designed to provide. Still, there was a possibility that a genetically defined subgroup of patients who did poorly with the supplements was masked by the overall benefit of AREDS-F to the whole patient cohort.

For example, AREDS Report No. 38 concluded that "AREDS supplements reduce the rate

of AMD progression across all genotype groups."[9] However, the data suggested a possible deleterious effect in those with 2 *CFH* and no *ARMS2* risk alleles, though this was not statistically significant. Conversely, the data did not show a benefit from AREDS-F use either, with nearly identical odds of progression to advanced AMD among those receiving AREDS-F and those receiving placebo in this subgroup.[10]

A study by Seddon et al helped to refine this issue.[11] They reported that the interaction between genotype and AREDS-F tracked with progression to nvAMD but not geographic atrophy (GA). This accorded with the AREDS results, which found benefit from AREDS-F for preventing progression to advanced AMD due to nvAMD but not GA.[5]

Several years later, further analysis of the original datasets endeavored to confirm or refute the contribution of genotype to driving the response to AREDS-F supplementation, but investigators continued to arrive at conflicting conclusions.[12,13]

Inspired by Seddon et al, Vavvas et al tested the hypothesis that there is a clinically relevant interaction between the GTGs and AREDS-F use in progression to nvAMD.[14] Their working hypothesis was that subjects in GTG 2 receiving the AREDS-F had an increased the risk of nvAMD compared to those taking placebo, and those in GTG 3 had a decreased risk of nvAMD. Of critical importance, they studied a "validation set" of genetic samples and clinical data from 299 AREDS subjects who were not available to the earlier researchers.

Their findings were impressive. The hazard ratio of progression to nvAMD for GTG 2 vs placebo was 4.9 ($P = .021$) and for GTG 3, it was 0.36 ($P = .003$). In other words, patients in GTG 2 who were exposed to AREDS-F had substantially greater risk of nvAMD, while those in GTG 3 had substantially reduced risk.

This effect was also seen using 0.632 bootstrap analysis,[15] a widely used method to confirm the validity of a statistical model. Bootstrapping involves running the model multiple times using a random resampling of the original dataset. It can identify whether a genotype-treatment interaction is spurious or is clinically relevant. Using all available AREDS genetic data, bootstrap analysis confirmed the increased risk to patients in GTG 2.

Still, the issue remained controversial. In an effort to clarify whether or not genotype groups interact with AREDS-F in progression to nvAMD, we performed a case-only study

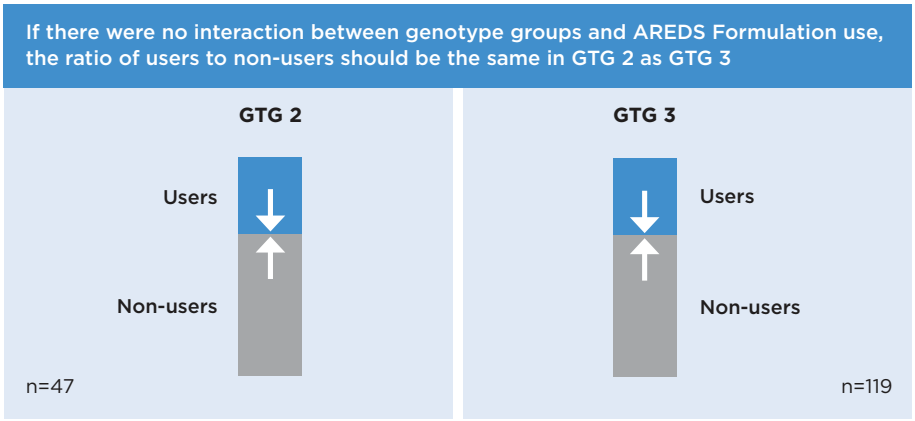

Figure 1. No-interaction scenario.

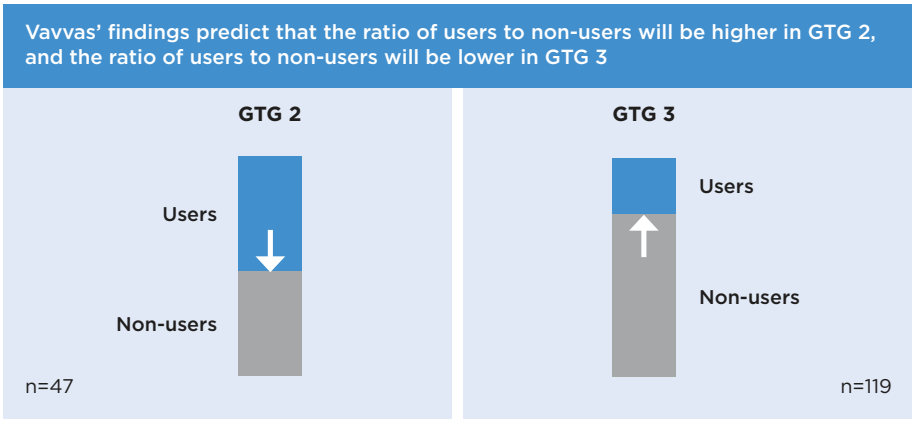

Figure 2. Scenario if Vavvas et al's findings were valid.

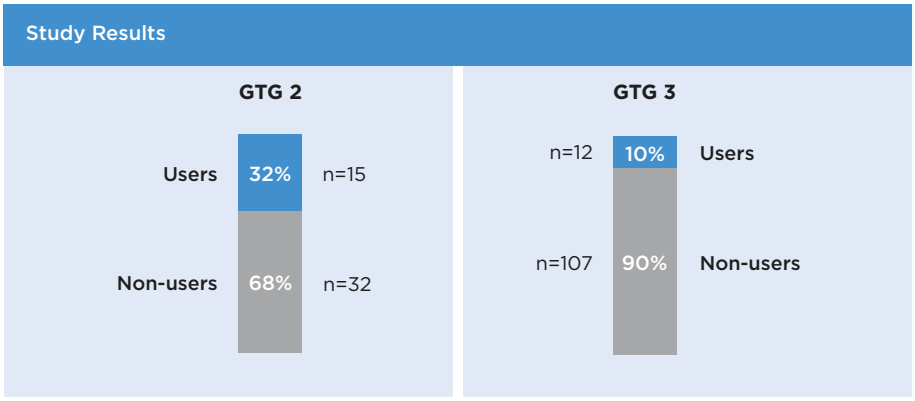

Figure 3. Interaction predicted by Vavvas et al confirmed.

of subjects with recently diagnosed nvAMD.[1] Patients with a reliable history of at least 5 years of AREDS-F use (AREDS-F users) or less than 1 month of AREDS-F use (AREDS-F nonusers) were genotyped. Those assessing the AREDS use history were masked to the patient's genotype, and those doing genetic testing were masked to AREDS-F use.

If AREDS-F use *increased* the risk of nvAMD among GTG 2 patients, we would expect a disproportionately increased number of AREDS-F users to be GTG 2. Similarly, if AREDS-F *decreased* the risk of nvAMD among GTG 3 patients, we would expect a disproportionately smaller number of AREDS-F users to be GTG 3, because AREDS-F use would have decreased their risk of nvAMD (Figures 1-2).

In our study of 265 patients, we compared the ratio of AREDS-F users to AREDS-F nonusers in GTG 2 and in GTG 3. If there were no interaction between GTGs and AREDS-F use, this ratio should be about 1.0. We found evidence for a strong interaction (Figure 3, Table 1, page 24) The simple ratio of

AREDS-F users divided by AREDS-F nonusers in GTG 2 compared to GTG 3 patients was 4.18 ($P < .004$). Regression analysis, correcting for known AMD risk factors, resulted in a log odds ratio of 4.81 ($P < .0006$)

Due to our study design, we could not prove that AREDS-F harms GTG 2 subjects or that it benefits GTG 3 subjects. We could show only that the response differed greatly between these 2 genetic groups. Because the direction of the interaction was the same as that of Vavvas et al, and because the effect size was comparable, our study adds credence to their findings that AREDS-F is remarkably protective against nvAMD for GTG 3 patients but increases the risk for GTG 2 patients.

While most of the genetic analyses were conducted using the original AREDS dataset, these findings can likely be extrapolated to the AREDS2 formulation. AREDS2 Report No. 18 studied the interaction between genetics and AREDS vs AREDS2 use in progression to advanced AMD.[16] It found that the AREDS and AREDS2 formulations are biologically equivalent when it comes to the interaction between genetics, AREDS-F use, and advanced AMD. This was not surprising, given that the AREDS formulation, with 80 mg zinc, and the AREDS2 formulation, with 25 mg zinc, have similar zinc bioavailability[17] and equivalent efficacy in terms of preventing advanced AMD.[18]

The American Academy of Ophthalmology (AAO) Age-Related Macular Degeneration Preferred Practice Pattern concludes that the "routine use of genetic testing [for AMD patients] is not supported by the existing literature and is not recommended at this time."[19] However, given the new, strong evidence for the utility of genetic testing to guide AREDS-F use and our low threshold to avoid potential harm, I think that the AAO should revisit this counsel.

The Preferred Practice Pattern also asserts, "one or more prospective clinical trials will need to demonstrate the value of genetic testing in AMD" before clinicians should recommend that it be performed.[19] While such a study would be scientifically desirable, it might not be possible.

Given the evidence for increased risk of nvAMD for patients in GTG 2, institutional review boards might not approve such a study, and both investigators and patients may be difficult to recruit. Because GTG 2 patients do not benefit from AREDS-F and may have increased risk, the Hippocratic principle of *primum non nocere*—ie, first, do no harm—favors genotype-guided treatment until there is compelling evidence that AREDS-F is safe and effective for these patients.

Table 1. Distribution of Genotype Among Age-Related Eye Disease Study Formulation Users and Nonusers.[a]

GTG	AREDS-F users	Non-AREDS-F users	Odds ratio users:nonusers
1 (n=37, 13.9%)	5	32	0.16
2 (n=47, 17.7%)	15	32	0.47
3 (n=119, 45.1%)	12	107	0.11
4 (n=62, 23.3%)	14	48	0.29

Abbreviations: AREDS-F, Age-Related Eye Disease Study formulation; GTG, genotype group.
[a]The odds ratio of GTG 2 vs GTG 3 for AREDS-F use to nonuse was 4.18 (P=.001).

From Kaufman SR, Yoganathan P, Small KW, et al. Genetics and Age-Related Eye Disease Study formulation interaction in neovascular age-related macular degeneration. *J VitreoRetinal Dis*. 2021;5(1):46-52. doi:10.1177/2474126420941713 *Reprinted with permission from the* Journal of VitreoRetinal Diseases *and SAGE Publishing*.

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Dr. Kaufman – None.

Consider Another Explanation of the Data

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In his Member Opinion piece, Stephen Kaufman, MD, explains his team's work on the interaction of genetics with response to Age-Related Eye Disease Study formulation (AREDS-F) supplementation, focusing exclusively on patients with neovascular AMD. While describing the controversy regarding the importance of genetic testing prior to AREDS supplement administration, one study was not cited.

The National Institutes of Health (NIH) tasked unpaid consultants from 3 independent academic institutions separately to repeat the analyses of the Artic Dx group and the AREDS research team. Each group concluded that they were unable to replicate any significant genotype-treatment interaction.[1]

The current report is an observational study—not a randomized controlled trial—of 265 patients who developed neovascular AMD with only 17% using AREDS-F. Why were less than 1 in 5 high-risk patients started on AREDS-F? It seems likely that those with the most-severe large drusen were felt to be at highest risk and started on treatment.

Patients with high-risk *CFH* alleles are more likely to have large drusen than those with high-risk *ARMS2*.[2] This would mean that the risk of being on AREDS-F was higher in the *CHF* high-risk groups, not that the risk of developing neovascular AMD was higher.

This is just one possible alternative explanation of the data. A randomized trial has the ability to control for both known and unknown confounders; an observational study does not. We do not think that this current study changes the conclusions that patients who are eligible for the AREDS supplement treatment should be treated without genetic testing. We would not change treatment recommendations.

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Dr. Chew – The National Eye Institute held a royalty-bearing license issued to Bausch+Lomb for the AREDS Supplement.

It's Time for a Clinical Trial

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Pharmacogenetics (PGx) is the study of how genes affect a person's response to drugs. PGx can dramatically impact drug safety and efficacy, and hundreds of Food and Drug Administration (FDA)-approved drugs have PGx labeling. Years ago, I wondered, is there a pharmacogenetic response to the Age-Related Eye Disease Studies Formulation (AREDS-F)?

In 2013, we published an analysis of AREDS data in *Ophthalmology* that demonstrated a PGx interaction.[1] Of subjects treated with AREDS-F, those with low *CFH* and high *ARMS2* genetic risk had reduced age-related macular degeneration (AMD) progression, but those with **high *CFH*** and **low *ARMS2*** genetic risk had *increased* progression to advanced AMD. Around 15% of AMD patients have this high-risk genotype.

We published more papers in *Ophthalmology* and the *Proceedings of the National Academy of Sciences*.[2,3] Our findings were consistent: Patients with AMD respond differently to AREDS-F due to differences in *CFH* and *ARMS2* genotype. The interaction is due to the zinc component of AREDS-F.

The AREDS Study Group insists that the PGx interaction doesn't exist and that the case is closed. They advise everyone with intermediate AMD to take AREDS2-F. The debate has been *energetic*.

I first met Steve Kaufman after one of my presentations. He was intrigued but skeptical. He asked tough questions. Unlike many, Steve Kaufman had read all the papers. Then he did something really atypical—he conducted his own trial!

Analyzing their own patients, Kaufman et al found evidence of a powerful PGx interaction with AREDS-F, with outcomes that support our findings. For some, taking AREDS-F may increase the risk of wet AMD.

AREDS-F has not been approved by the FDA, so it isn't regulated as a drug. It is a dietary supplement and regulated like food. We need a definitive clinical trial. The AREDS data set has been exhausted. AREDS2 had no placebo control, so AREDS2 data can neither confirm nor refute a PGx interaction.

A clinical trial may confirm an important pharmacogenetic interaction for the "eye vitamins" marketed to our patients. The case is far from closed.

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Financial Disclosures

Dr. Awh – Please see disclosures on page 2.