Joint Associations of Diet, Lifestyle, and Genes with Age-Related Macular Degeneration

Kristin J. Meyers, PhD, Zhe Liu, MS, Amy E. Millen, PhD, Sudha K. Iyengar, PhD, Barbara A. Blodi, MD, Elizabeth Johnson, PhD, D. Max Snodderly, PhD, Michael L. Klein, MD, Karen M. Gehrs, MD, Lesley Tinker, PhD, Gloria E. Sarto, MD, Jennifer Robinson, MD, Robert B. Wallace, MD, Julie A. Mares, PhD

Purpose: Unhealthy lifestyles have been associated with increased odds for age-related macular degeneration (AMD). Whether this association is modified by genetic risk for AMD is unknown and was investigated.

Design: Interactions between healthy lifestyles AMD risk genotypes were studied in relation to the prevalence of AMD, assessed 6 years later.

Participants: Women 50 to 79 years of age in the Carotenoids in Age-Related Eye Disease Study with exposure and AMD data (n = 1663).

Methods: Healthy lifestyle scores (0–6 points) were assigned based on Healthy Eating Index scores, physical activity (metabolic equivalent of task hours/week), and smoking pack years assessed in 1994 and 1998. Genetic risk was based on Y402H in complement factor H (CFH) and A69S in age-related maculopathy susceptibility locus 2 (ARMS2). Additive and multiplicative interactions in odds ratios were assessed using the synergy index and a multiplicative interaction term, respectively.

Main Outcome Measures: AMD presence and severity were assessed from grading of stereoscopic fundus photographs taken in 2001–2004. AMD was present in 337 women, 91% of whom had early AMD.

Results: The odds of AMD were 3.3 times greater (95% confidence interval [CI], 1.8–6.1) in women with both low healthy lifestyle score (0–2) and high-risk CFH genotype (CC), relative to those who had low genetic risk (TT) and high healthy lifestyle scores (4–6). There were no significant additive (synergy index [SI], 1.08; 95% CI, 0.70–1.67) or multiplicative (P_interaction = 0.94) interactions in the full sample. However, when limiting the sample to women with stable diets before AMD assessment (n = 728) the odds for AMD associated with low healthy lifestyle scores and high-risk CFH genotype were strengthened (odds ratio, 4.6; 95% CI, 1.8–11.6) and the synergy index was significant (SI, 1.34; 95% CI, 1.05–1.70). Adjusting for dietary lutein and zeaxanthin attenuated, and therefore partially explained, the joint association. There were no significant additive or multiplicative interactions for ARMS2 and lifestyle score.

Conclusions: Having unhealthy lifestyles and 2 CFH risk alleles increased AMD risk (primarily in the early stages), in an additive or greater (synergistic) manner. However, unhealthy lifestyles increased AMD risk regardless of AMD risk genotype. Ophthalmology 2015; 1–9 © 2015 by the American Academy of Ophthalmology.

Current treatment options available for age-related macular degeneration (AMD) are limited to antiangiogenic treatments to improve visual outcomes in persons with neovascular AMD and to the use of high-dose antioxidant supplements1−3 to slow the progression of intermediate to advanced disease. The results of the Age-Related Eye Disease Studies demonstrated that the disease process can be impacted by nutritional interventions.1−3 However, the benefits or safety of using high-dose antioxidants for long periods, as might be needed to prevent AMD or slow progression in the early stages, has not been established.4

A large body of scientific evidence indicates that healthy lifestyle modifications can lower processes thought to promote AMD, including oxidative stress, inflammation, blood lipoprotein disturbances, and hypertension.5−10 Consistent with this, a healthy diet,11−15 not smoking,7,16 and physical activity14,16 have been associated previously with lower occurrence of early or advanced AMD, or both, in epidemiologic studies. The magnitude of risk reduction associated with multiple healthy lifestyles, considered jointly, may be greater than the magnitude associated with individual healthy lifestyles, as suggested by results of a previous study.
in the Carotenoids in Age-Related Eye Disease Study (CAREDS) that indicated that women (50–74 years of age) who had a combination of healthy lifestyle factors (healthy diet, physical activity, and not smoking) had a 3-fold lower odds for early AMD relative to women who had unhealthy lifestyles.14

Genetic risk may modify the benefit of a healthy lifestyle. Strong genetic risk factors for AMD include advanced age and certain genetic variants. In particular, the Y402H allele of the complement factor I (CFI) gene and the A69S (rs10490924) variant within the age-related maculopathy susceptibility 2 (ARMS2) locus consistently confer the greatest risk for both early and late AMD in people of European ancestry,17,18 increasing risk 1.5- to 3-fold with each additional risk allele for early and late AMD, respectively.17,19 Additional complement pathway genes are well characterized for increasing risk for late AMD, including complement component 3 (C3), complement factor I (CFI), and a locus between complement component 2 and complement factor B (C2/CFB; previously reviewed20), but the effect sizes for variants within these genes are attenuated greatly for risk of early AMD.17

Genetic risk for AMD also has been observed to amplify the risk for AMD associated with several specific healthy lifestyles or phenotypes in some previous studies,21 but not others.22,23 No previous studies have evaluated associations of joint markers of multiple healthy lifestyles together with AMD risk genotypes. In this study, we investigated the interactions between genetic risk for AMD and a healthy lifestyle score, summing 3 lifestyle factors (diet, smoking, and physical activity histories) on the prevalence of AMD in a study sample (CAREDS) in which AMD cases mostly were early-stage disease and were assessed 6 years after assessment of lifestyle exposures. Two main strategies were used to evaluate interactions between lifestyle and genetic risk factors. One strategy was to compute a synergy index (SI) to determine whether the burden of AMD risk attributable to genetic and lifestyles together was more than the sum of the risk of each individually. This is also considered to be evidence of biological synergy,24,25 which may be expected if lifestyle and genetic factors both contribute to the same biological mechanism for AMD pathologic features, such as to promote inflammation. A second strategy was used to determine whether genetic risk factors may multiply the magnitude of AMD risks associated with unhealthy lifestyles, assessed by multiplicative interactions.25 Evidence of multiplicative interactions may supply stronger evidence to conclude that recommendations to patients for personalized preventive interventions customized to their specific genetic risk profiles may be warranted.

We also explored the extent to which these joint associations were explained by measures of lutein and zeaxanthin (LZ) status in the diet, blood, or retina uniquely available in this cohort. Lutein and zeaxanthin and isomers uniquely accumulate in the macula of the retina, where they comprise macular pigment and may protect the macula by absorbing potentially damaging blue light, in addition to the actions of these carotenoids on lowering oxidative stress and inflammation (recently reviewed19,20). Higher LZ levels in the diet, serum, retina, or a combination thereof seem to be influenced not only by levels of these carotenoids in the diet, but also by other aspects of healthy diets, lifestyle, and genetic factors,4,29,30 which may work jointly to lower AMD risk. A recent report provides evidence that lutein intake only lowers risk of AMD incidence among persons with 2 or more risk alleles from common CFH and ARMS2 variants.23

Methods

Study Sample

The CAREDS is a previously described29,31 ancillary study of the Women’s Health Initiative (WHI) Observational Study (OS). The primary goal of the CAREDS was to examine associations between LZ status in women 50 to 79 years of age and the prevalence of age-related eye diseases, including AMD, an average of 6 years later. Fifty percent of all women participating in the OS study centers in Madison, Wisconsin (n = 694), Iowa City, Iowa (n = 631), and Portland, Oregon (n = 680), were recruited, targeting women reporting the lowest (<28th percentile) and highest (>78th percentile) LZ intakes at WHI baseline. Women in the CAREDS did not differ significantly from WHI women with intakes of LZ between the 28th and 78th percentile in terms of numerous known or suspected AMD risk factors, including age, education, body mass index, smoking, use of supplements or hormone therapy, and history of diabetes or cardiovascular disease (data not shown).

The CAREDS visits were conducted from 2001 through 2004 in 2005 women and have been described previously.29,31 Briefly, visits included obtaining stereoscopic fundus photographs,31 which were graded for prevalent AMD classification. The CAREDS visits also measured the optical density of macular pigment via customized heterochromatic flicker photometry22 and included questionnaires to assess health history, supplement use, and sunlight exposure history. Food frequency questionnaires were used to estimate usual dietary intakes at the WHI OS baseline (6 years before the CAREDS visits, 1994–1998) and recalled for intakes 15 years before the CAREDS visits.31 The WHI OS visits also included collection and storage of blood samples, smoking history, physical activity, blood pressure, and anthropometrics. The stored blood samples have been accessioned for genotyping and measurement of serum carotenoids,26 among other biomarkers. Therefore, exposure assessment was antecedent to outcome assessment. Of the original 2005 CAREDS participants, 1857 had gradable fundus photographs available for AMD classification, and 1663 of these also had genetic data available for the present analysis. All CAREDS and WHI OS procedures conformed to the Declaration of Helsinki, informed consent was obtained from all participants, and approval was granted by the institutional review board at each university.

As previously described,12 data in the CAREDS suggest fluctuations in the amount of LZ consumed at the time of WHI enrollment (6 years before ocular photography) and in the time before enrollment in the WHI. Thus, to avoid bias resulting from including women with fluctuating diets just before exposure assessment, we conducted an analysis on the full sample, and then excluding women if their intake of LZ changed more than 1 quintile categorization between the 1988 and 1992 (CAREDS 15-year recall food frequency questionnaire) and 1994 and 1998 (WHI baseline food frequency questionnaire; n = 356; 18%) visits or if they were likely to have made a recent diet change because of diagnoses of the following comorbid conditions for which diet changes are often recommended: cardiovascular disease, diabetes,
macular degeneration, a history of hypertension, or a combination thereof (n = 579; 29%). The subsample for these analyses included 728 women with stable diets.

Age-Related Macular Degeneration Classification
Stereoscopic fundus photographs were graded by the University of Wisconsin Fundus Photograph Reading Center using the Age-Related Eye Disease Study (AREDS) protocol for grading maculopathy. For the present analysis, women were classified as having AMD if they had photographic evidence of either early or late stages of AMD. Early AMD was classified in part using criteria for AREDS category 3. This included the presence of 1 or more large drusen (>125 μm) or extensive intermediate drusen (total area, ≥360 μm when soft indistinct drusen were present or ≥650 μm when soft indistinct drusen were absent). Additional criteria for early AMD included having pigmented abnormalities and an increase or decrease in pigmentation if accompanied by at least 1 druse 63 μm or larger. Late AMD included geographic atrophy, neovascularization, or exudation in the center subfield. The reference group included women who had neither early nor late AMD, generally corresponding to AREDS categories 1 and 2.

Healthy Lifestyle Score
The healthy lifestyle score (HLS) is a 6-point variable that gives equal weight to each of 3, 3-level health habits queried at WHI baseline: diet assessed by a modified 2005 Healthy Eating Index (lowest 20%, 21%–80%, and highest 20%), physical activity measured in metabolic equivalent of task hours per week (lowest, second, and third tertile), and pack years of smoking (never, ≤7 pack/year, >7 pack/year). Details of the HLS development and distribution can be found elsewhere. For current analyses, HLS was classified into a 3-level variable based on composite HLSs of 0 to 2, 3, and 4 to 6, which divided the sample into approximate tertiles.

Genotyping
Genotyping for known and candidate AMD genes was carried out at Case Western Reserve University, Cleveland, Ohio, using a custom Illumina GoldenGate Assay (Illumina, Inc., San Diego, CA). DNA was extracted from the buffy coats of blood obtained at WHI OS baseline examinations (1994–1998) that were stored frozen at −80°C. Genotype calls were made using Illumina Genome Studio. Single nucleotide polymorphisms not designable to the custom Illumina assay, CFH Y402H being one, were genotyped using the KASP Assay at LGC Genomics (Teddington, United Kingdom). Standard quality control filters were applied, resulting in exclusions of single nucleotide polymorphisms (SNPs) with Hardy-Weinberg equilibrium chi-square P value of less than 1.0 × 10−6, minor allele frequency (MAF) less than 0.01, or genotype call rates less than 95%.

For individuals with an insufficient quantity of DNA for KASP genotyping after Illumina genotyping (n = 53 of the total CAREDS sample), CFH Y402H genotypes were imputed in MACH (available at: www.sph.umich.edu/csg/abecasis/MACH/index.html) using the available chromosome 1 SNPs from Illumina (14 SNPs) and the 1000 Genomes Project European ancestry panel as a reference. The resulting R² from Y402H imputation was 99.5%.

For the present analyses, genetic risk for AMD was defined by individual Y402H (CFH) or rs10490924 (A69S ARMS2) genotypes, 2 SNPs established to increase risk for both early and late AMD. Data also were available to explore joint effects for SNPs more strongly associated with late AMD: rs2230199 (C3), rs10033900 (CFI), and rs641153 (C2/CFB).

Statistical Analysis
Models were fit to estimate the joint effects of each genotype and HLS. Interactions were assessed on multiplicative and additive scales. Deviations from multiplicative interactions were tested based on the Wald test statistic for the interaction term in logistic regression models. This is a commonly used test for statistical interaction on a multiplicative scale because it tests whether the relative effect of an exposure of interest is constant across strata of another factor of interest. A P value for interaction less than 0.05 was considered suggestive. Deviations from additive interactions (i.e., 2 factors combine to be more or less than the sum of their individual effects) were tested using the SI and corresponding 95% confidence intervals (CIs). When estimating the SI, it has been recommended to recode protective factors so that exposure indicates risk and the joint effects stratum with the lowest risk is the reference group. Therefore, the joint reference group was women with low genetic risk and high HLS (range, 4–6). An SI of 1.0 indicates no interaction (i.e., the factors combine in a manner that is exactly additive), an SI of more than 1.0 indicates the 2 factors considered together combine to be more than the sum of their individual effects (i.e., biological synergism), and an SI of less than 1.0 indicates negative additive interaction, or that the effects of the 2 factors combine to be less than the sum of the individual effects. Because of small cell sizes when conducting joint analyses, a dominant genetic model was assumed for A69S (ARMS2) and rs641153 (CFB/C2). An additive genetic model was assumed for all other SNPs. Interaction analyses were adjusted for age. Additional adjustments for other risk factors previously identified to influence odds of AMD in CAREDS were tested, including blue iris color and current hormone therapy use. Smoking, diet, or physical activity were not adjusted for additionally because these variables are included within the healthy lifestyle score itself. Data management and statistical analyses were performed using SAS software version 9.2 (SAS Inc, Cary, NC).

Results
There were 337 cases of AMD in the full sample, of which 91% were early stages of AMD. After adjusting for age, 23% of women with low HLSs (range, 0–2) had AMD, compared with 19% of women with high scores (range, 4–6; P = 0.13). Limiting the sample to those with stable diets resulted in 120 cases of AMD. Twenty percent of women with a low HLS had AMD compared with 16% of women with a high HLS in this stable diet subsample (P = 0.14). The distribution of AMD risk phenotypes by HLS levels is given in Table 1 (available at www.aaojournal.org). There were no differences in genotype distributions by HLS classification (Table 1, available at www.aaojournal.org). Limiting the sample to women with stable diets (n = 728) kept risk factor differences stable across HLS strata, except that the higher HLSs among older women did not persist in the subgroup limited to stable diets.

Main Effects of Age-Related Macular Degeneration Genotypes in the Carotenoids in Age-Related Eye Disease Study
The odds for AMD increased with each additional copy of the Y402H CFH risk allele: women with 2 risk alleles had 2.4 times greater odds of AMD relative to women with 0 risk alleles (P < 0.0001; Table 2). Odds for AMD also increased with each
Table 2. Age-Adjusted Odds Ratios and 95% Confidence Intervals for Any Age-Related Macular Degeneration in the Carotenoids in Age-Related Eye Disease Study by Known Age-Related Macular Degeneration—Related Genes (Single Nucleotide Polymorphisms)

<table>
<thead>
<tr>
<th>Single Nucleotide Polymorphism</th>
<th>Overall Sample (n = 1663)</th>
<th>Stable Diet Group* (n = 727)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>Age-Adjusted Odds Ratio (95% Confidence Interval)</td>
</tr>
<tr>
<td>CFH (rs1061170)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>606 (36)</td>
<td>92 Reference</td>
</tr>
<tr>
<td>CT</td>
<td>791 (48)</td>
<td>165 1.38 (1.03–1.83)</td>
</tr>
<tr>
<td>CC</td>
<td>266 (16)</td>
<td>82 2.41 (1.70–3.43)</td>
</tr>
<tr>
<td>ARMS2 (rs10490924)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>1017 (61)</td>
<td>179 Reference</td>
</tr>
<tr>
<td>AC</td>
<td>575 (35)</td>
<td>136 1.51 (1.17–1.95)</td>
</tr>
<tr>
<td>AA</td>
<td>68 (4)</td>
<td>22 2.18 (1.26–3.77)</td>
</tr>
<tr>
<td>CFB/C2 (rs641153)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA or AG</td>
<td>278 (17)</td>
<td>44 Reference</td>
</tr>
<tr>
<td>GG</td>
<td>1385 (83)</td>
<td>293 1.52 (1.07–2.17)</td>
</tr>
<tr>
<td>CFH (rs10339900)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>430 (26)</td>
<td>92 Reference</td>
</tr>
<tr>
<td>GA</td>
<td>835 (50)</td>
<td>148 0.81 (0.60–1.09)</td>
</tr>
<tr>
<td>AA</td>
<td>397 (24)</td>
<td>97 1.21 (0.87–1.69)</td>
</tr>
<tr>
<td>C3 (rs2230199)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>69 (4)</td>
<td>17 Reference</td>
</tr>
<tr>
<td>GC</td>
<td>540 (32)</td>
<td>106 0.78 (0.43–1.42)</td>
</tr>
<tr>
<td>GG</td>
<td>1053 (64)</td>
<td>214 0.79 (0.45–1.42)</td>
</tr>
</tbody>
</table>

*Diet stable group was created after excluding women whose intakes of LZ changed more than 1 quintile categorization between the 1988-1992 and 1994-1998 visits, or those who were candidates for recent diet change because of diagnoses for which diet changes are indicated, cardiovascular disease, diabetes, macular degeneration, history of hypertension.

1For trend across genotypes.
Table 3. Joint Effects of Age-Related Macular Degeneration Genotype (Based on CFH or ARMS2) and Healthy Lifestyle Score in Association with Odds for Age-Related Macular Degeneration in Overall Sample

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. with Age-Related Macular Degeneration/Total No. in Group</th>
<th>Most Healthy Lifestyles, Grades 4–6 (n = 769), Odds Ratio* (95% Confidence Interval)</th>
<th>Healthy Lifestyle Grade 3 (n = 454), Odds Ratio* (95% Confidence Interval)</th>
<th>Least Healthy Lifestyles, Grades 0–2 (n = 440), Odds Ratio* (95% Confidence Interval)</th>
<th>P Value</th>
<th>P Value for Multiplicative Interaction</th>
<th>Synergy Index (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFH (rs1061170)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>92/606</td>
<td>1 [Reference]</td>
<td>1.24 (0.71–2.16)</td>
<td>1.35 (0.79–2.32)</td>
<td>0.26</td>
<td>0.94</td>
<td>1.08</td>
</tr>
<tr>
<td>TC</td>
<td>163/791</td>
<td>1.52 (0.99–2.34)</td>
<td>1.54 (0.95–2.48)</td>
<td>1.73 (1.07–2.80)</td>
<td>0.63</td>
<td>(0.70–1.67)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>82/266</td>
<td>2.43 (1.43–4.15)</td>
<td>2.84 (1.56–5.20)</td>
<td>3.30 (1.80–6.05)</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARMS2 (rs10490924)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>172/1107</td>
<td>1 [Reference]</td>
<td>1.22 (0.83–1.81)</td>
<td>1.33 (0.89–2.00)</td>
<td>0.15</td>
<td>0.63</td>
<td>0.29</td>
</tr>
<tr>
<td>AC/AA</td>
<td>158/643</td>
<td>1.70 (1.17–2.45)</td>
<td>1.83 (1.16–2.88)</td>
<td>1.97 (1.29–3.02)</td>
<td>0.52</td>
<td>(0.27–2.93)</td>
<td>0.56</td>
</tr>
<tr>
<td>CC</td>
<td>178/1017</td>
<td>1 [Reference]</td>
<td>1.22 (0.83–1.81)</td>
<td>1.33 (0.89–2.00)</td>
<td>0.15</td>
<td></td>
<td>(0.03–11.82)</td>
</tr>
<tr>
<td>AC</td>
<td>135/575</td>
<td>1.54 (1.03–2.27)</td>
<td>1.66 (1.03–2.67)</td>
<td>2.13 (1.37–3.29)</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>22/608</td>
<td>3.00 (1.44–6.25)</td>
<td>4.08 (1.34–12.47)</td>
<td>0.73 (0.16–3.38)</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| *Adjusted for age (further adjustment for eye color and hormone therapy, or dietary lutein and zeaxanthin intake, did not alter odds ratios).  
1For trend across lifestyle score group, within each genotype class.

Table 4. Joint Effects of Age-Related Macular Degeneration Genotype (Based on CFH or ARMS2) and Healthy Lifestyle Score in Association with Odds for Age-Related Macular Degeneration among Individuals with Stable Diets

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. with Age-Related Macular Degeneration/Total No. in Group</th>
<th>Most Healthy Lifestyles, Grades 4–6 (n = 353), Odds Ratio* (95% Confidence Interval)</th>
<th>Healthy Lifestyle, Grade 3 (n = 179), Odds Ratio* (95% Confidence Interval)</th>
<th>Least Healthy Lifestyle, Grades 0–2 (n = 196), Odds Ratio* (95% Confidence Interval)</th>
<th>P Value</th>
<th>P Value for Multiplicative Interaction</th>
<th>Synergy Index (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFH (rs1061170)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>34/276</td>
<td>1 [Reference]</td>
<td>0.96 (0.39–2.39)</td>
<td>1.38 (0.58–3.26)</td>
<td>0.51</td>
<td>0.31</td>
<td>1.34</td>
</tr>
<tr>
<td>TC</td>
<td>58/339</td>
<td>1.69 (0.87–3.28)</td>
<td>1.20 (0.52–2.75)</td>
<td>1.70 (0.81–3.55)</td>
<td>0.93</td>
<td>(1.05–1.70)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>28/114</td>
<td>1.56 (0.65–3.71)</td>
<td>2.72 (0.97–7.65)</td>
<td>4.63 (1.85–11.60)</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARMS2 (rs10490924)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>70/457</td>
<td>1 [Reference]</td>
<td>0.67 (0.35–1.32)</td>
<td>1.09 (0.59–2.00)</td>
<td>0.94</td>
<td>0.16</td>
<td>1.65</td>
</tr>
<tr>
<td>AC/AA</td>
<td>50/269</td>
<td>0.88 (0.48–1.62)</td>
<td>1.40 (0.67–2.94)</td>
<td>1.75 (0.92–3.30)</td>
<td>0.07</td>
<td>(1.18–2.30)</td>
<td></td>
</tr>
</tbody>
</table>
| *Adjusted for age (further adjustment for eye color and hormone therapy, or dietary lutein and zeaxanthin intake, did not alter odds ratios).  
1For trend across lifestyle score group, within each genotype class.
additional copy of the A69S risk allele; women with 2 copies had 2.2 times greater odds of AMD relative to women with 0 copies of the risk allele (P = 0.0001). Homozygosity for the G allele of rs641153 (CFB/C2) was associated with increased odds of AMD (P = 0.02). No main effect of rs10033900 (CFI) or rs2230199 (C3) was observed within CAREDS. Similar trends were observed in the subsample of women with stable diets.

Interactions between CFH Genotype and Healthy Lifestyle Score

In the full sample, the odds for AMD were 3.3 times greater in women who had both 2 high-risk CFH alleles (CC) and low HLS, relative to low-risk genotype (TT) and high HLS (odds ratio [OR], 3.3; 95% CI, 1.80–6.05; Table 3). The joint effect of these 2 factors was the same as the sum of their individual effects (SI, 1.08; 95% CI, 0.70–1.67). There was no evidence for multiplicative interaction (Pinteraction = 0.94).

In the subsample of women with stable diets, the odds for AMD associated with having both poor lifestyle score and the high-risk CFH genotype was 4.6 times greater compared with women with healthy lifestyle and low-risk genotype (OR, 4.63; 95% CI, 1.85–11.60; Table 4). The joint effect of these 2 factors in this subsample was more than the sum of their individual effects, implying synergy (SI, 1.34; 95% CI, 1.05–1.70). The greatest increase in odds for AMD associated with poor lifestyle scores was among women with high genetic risk. In women with the high-risk CFH genotype (CC), the odds of AMD were 3 times higher for those with the lowest HLS (range, 0–2) relative to those with highest HLS (range, 4–6; OR, 4.63 vs. 1.56; Psynerg = 0.04, across HLS groups within genotype class; Table 4). We explored whether better status for LZ among women with high versus low HLS explained the association between HLS and AMD in women with the high-risk CC genotype. Indeed, the ORs for AMD comparing high versus low HLS in those with highest genetic risk were attenuated by 30%, 9%, and 15% when adjusting for LZ in the diet, serum, and macular pigment optical density (MPOD), respectively, suggesting that better status for LZ partially explained the association between HLS and AMD.

Interactions between Other Age-Related Macular Degeneration Risk Genotypes and Healthy Lifestyle Score

Joint effects of lifestyle score and variants in additional complement pathway genes (CFB/C2, C3 rs2230199, and CFI rs10033900) also were explored (Tables 6a and 6b, available at www.aaojournal.org). Absent main effects for SNPs in C3 and CFI (Table 2), along with a variable combination of genotype and HLS lending toward the lowest odds for AMD, resulted in unreliable estimates of synergy for these genotypes and HLS.

Discussion

In the present study, a low score for a combination of healthy lifestyles was observed to have added to odds for AMD associated with having high-risk CFH risk alleles. In women who had maintained stable diets, we also observed the first evidence of potential biological synergy (significant SI) between CFH risk genotype and poor status for a broad lifestyle measure (including poor diet, low physical activity, and smoking). Thus, it seems that the attributable risk of AMD may be inordinately greater in women who have both a high-risk CFH genotype and these lifestyle characteristics. Most (91%) AMD cases in the present report were in the early stages (large drusen or worse).

Public health interventions targeting such individuals might show great promise in lowering the number of people who have early AMD, potentially preventing or delaying the onset of advanced AMD. Given that there was no evidence of multiplicative interactions, the potential benefit of healthy lifestyles in lowering AMD risk may apply across women of different genotypes, so genotyping to identify persons at high risk may not be clinically necessary. Overall, these data should encourage physicians to recommend adoption of healthy lifestyles at early ages in people who have a family history of AMD and may motivate patients to follow such recommendations. Although benefit has yet to be proven in clinical trials, a large body of evidence, including data from clinical trials, suggests that these lifestyle changes lower blood pressure, oxidative stress, and inflammation, which are thought to promote AMD and are associated with lower risk for a large number of chronic diseases. Given a lack of evidence that high-dose antioxidant supplements prevent AMD and the unknown safety of consuming high-dose antioxidants for long periods, these data suggest that any success in physicians’ attempts to persuade these patients to adopt healthy lifestyles at early ages ultimately could benefit those patients significantly.

The suggestive synergistic relationship between CFH genotype and healthy lifestyles may reflect common influences on inflammation. The risk variant of Y402H is known to contribute to uncontrolled and defective regulation of the alternative complement pathway, leading to sustained
inflammatory reactions and ultimately to increased risk of AMD (reviewed\(^\text{17}\)). Individual factors comprising the HLS (broadly healthy diets, physical activity, and absence of smoking) also are known to be associated with reduced inflammation.\(^\text{6,7,10,38}\)

The combined influence of poor lifestyle and genetic risk was explained in part by low LZ intake in the present study, despite our observing no significant interactions between lutein intake and CFH risk (Tables 5 and 6, available at www.aaojournal.org) or between lutein intake and combined risk alleles for CFH and ARMS2 genes (data not shown). The power to detect significant interactions between lutein intake and genotype in AMD risk was lower relative to previous prospective studies in the Rotterdam Study\(^\text{21}\) and a pooled analysis of the Rotterdam and Blue Mountain Eye Studies,\(^\text{37}\) indicating interactions between LZ intake and high-risk CFH or ARMS2 genotypes, or both, for early AMD. The combined results from the present study and these 2 studies supports an augmentation of AMD genetic risk associated with lutein intake. Consistent with this, lutein has been demonstrated to have anti-inflammatory properties,\(^\text{39–41}\) and supplementation lowers circulating complement factor levels.\(^\text{42}\) Within the sample used for this analysis, and in the overall CAREDS cohort,\(^\text{43}\) higher HLS and dietary LZ intake each were associated with lower serum C-reactive protein, a systemic marker of inflammation, and higher vitamin D, also related to inflammatory conditions.\(^\text{43–45}\) Protective associations between serum vitamin D, which also has anti-inflammatory properties, and AMD in women with a CC Y402H genotype\(^\text{46}\) in this sample are described in a separate article.\(^\text{46}\)

If AMD protection by healthy lifestyles was direct through regulation of the complement pathway, one might hypothesize consistent synergy with other complement pathway genes known to influence AMD risk, such as C3, C2/CFB, and CFI. We did not observe joint effects for these SNPs consistent with that observed with Y402H. This lack of consistency may be the result of differential power to detect associations across SNPs with varying minor allele frequency, differential impact of genes on early versus late stages of AMD, or small or nonexistent main effects of these SNPs within the broader CAREDS cohort. Similar analyses in large study samples with more cases of late AMD would provide further insight.

The results of this study cannot be extended to supplemental intake of dietary antioxidants or other nutrients. In the present study, too few women (17\%) reported using high-dose supplements for more than 5 years before AMD was assessed to permit adequate statistical power to evaluate these associations by genotype. Although some post hoc analyses of AREDS data suggest multiplicative interactions between high-dose antioxidant supplements and AMD risk genotype in people with intermediate or worse AMD,\(^\text{47,48}\) these results have not been replicated.\(^\text{49,50}\) Although the current evidence is not strong enough to justify recommendations for supplements or lifestyles to lower AMD risk that is tailored to genetic profiles, results of these 5 studies,\(^\text{2,3,8,48–50}\) combined with the work presented here, highlight the fact that the individual-level benefit of diet, lifestyle, supplements, or a combination thereof cannot be extrapolated from average estimates of benefit in study groups, who also differ in many respects relative to the larger population of people at risk for AMD.

Limitations to the evidence provided by the present study are as follows. The AMD outcome, although assessed 6 years later than exposure estimate, was a prevalence estimate. Some cases of AMD may have developed before the exposure assessment. Fluctuations in diet (and health behaviors) in the time before AMD assessment may not reflect long-term intake, leading to random or nonrandom error in effect estimates on AMD risk. However, 72\% of women determined to have AMD (primarily large drusen) by photography had not previously been told they had AMD. To avoid this potential bias, we conducted analysis in the full dataset and then excluded women whose diets changed in a period before WHI or who were diagnosed with a chronic disease for which diet changes often are recommended (cardiovascular disease, diabetes, macular degeneration, a history of hypertension, or a combination thereof). Although minimizing bias, this approach also reduces statistical power. Therefore, results have been presented for the full sample (which may include bias) and the reduced sample (which minimized bias, but reduced power). Second, the estimated lower risk for AMD associated with healthy lifestyles in this study may apply primarily to lowering risk for early stages of AMD; most women with AMD in this study had early or intermediate stage disease (large drusen or worse). Confirmation of these results is needed in larger, long-term population-based studies of newly developed AMD and progression of AMD to more advanced stages.

Overall, our study results are consistent with previous research suggesting diets and lifestyles that limit oxidative stress and inflammation are protective against early AMD, and this may be most important for reducing AMD risk in individuals at high genetic risk. This suggests that interventions to consume plant-rich, high-lutein diets, reduce smoking, and encourage physical activity are reasonable strategies for AMD prevention, particularly in groups of people who are at high genetic risk, have a family history for AMD, or both. Confirmation of results in prospective studies and in a greater number of samples including men and other ethnicities are needed.

Acknowledgments. The Carotenoids in Age-Related Eye Disease Study is ancillary to the Women’s Health Initiative. A short list of investigators who have contributed to WHI science can be found at https://www.whi.org/researchers/Documents%20%20Write%20a %20Paper/WHI%20Investigator%20Long%20List.pdf. We thank the women who participated in the CAREDS study visits.

References


37. Zipfel PF, Lauer N, Skerka C. The role of complement in Retinal Disease: Complement Biology and Pathology,


Footnotes and Financial Disclosures

Originally received: August 25, 2014.
Final revision: July 23, 2015.
Accepted: July 27, 2015.

1 Department of Ophthalmology and Visual Sciences, McPherson Eye Research Institute, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin.

2 Department of Epidemiology and Environmental Health, School of Public Health and Health Professions, University at Buffalo, The State University of New York, Buffalo, New York.

3 Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio.

4 Jean Mayer USDA Human Nutrition, Research Center on Aging, Tufts University, Boston, Massachusetts.

5 Department of Neuroscience, University of Texas, Austin, Texas.

6 Casey Eye Institute, Department of Ophthalmology, Oregon Health & Science University, Portland, Oregon.

7 Department of Ophthalmology & Visual Sciences, University of Iowa Hospital & Clinics, Iowa City, Iowa.

8 Department of Cancer Prevention Research Program, Fred Hutchinson Cancer Research Center, Seattle, Washington.

9 Department of Obstetrics & Gynecology, School of Medicine & Public Health, University of Wisconsin, Madison, Wisconsin.

10 Department of Epidemiology, University of Iowa College of Public Health, Iowa City, Iowa.


Financial Disclosure(s): The author(s) have no proprietary or commercial interest in any materials discussed in this article.

Supported by the National Eye Institute, National Institutes of Health, Bethesda, Maryland (grant nos.: EY013018, EY016886); Research to Prevent Blindness, Inc, New York, New York; the Retina Research Foundation (Houston, TX); and the Carl and Mildred Reeves Foundation (Columbus, IN). The Women’s Health Initiative is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland (grant nos.: HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C).

Author Contributions:
Conception and design: Meyers, Liu, Millen, Iyengar, Blodi, Johnson, Snodderly, Klein, Gehrs, Tinker, Sarto, Robinson, Wallace, Mares
Analysis and interpretation: Meyers, Liu, Millen, Iyengar, Blodi, Johnson, Snodderly, Klein, Gehrs, Tinker, Sarto, Robinson, Wallace, Mares
Data collection: Meyers, Liu, Millen, Iyengar, Blodi, Johnson, Snodderly, Klein, Gehrs, Tinker, Sarto, Robinson, Wallace, Mares
Obtained funding: none
Overall responsibility: Meyers, Liu, Millen, Iyengar, Blodi, Johnson, Snodderly, Klein, Gehrs, Tinker, Sarto, Robinson, Wallace, Mares

Abbreviations and Acronyms:
AMERICAN = age-related macular degeneration; AREDS = Age-Related Eye Disease Study; ARMS2 = age-related maculopathy susceptibility locus 2; CAREDS = Carotenoids in Age-Related Eye Disease Study; CFH = complement factor H; CFI = complement factor I; CI = confidence interval; C2/CFB = complement component 2 and complement factor B; C3 = complement component 3; HLS = healthy lifestyle score; LZ = lutein and zeaxanthin; OR = odds ratio; OS = Observational Study; SI = synergy index; SNP = single nucleotide polymorphism; WHI = Women’s Health Initiative.

Correspondence:
Julie A. Mares, PhD, Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, 610 North Walnut Street, 1063 WARF, Madison, WI 53726. E-mail: jmarespc@wisc.edu.