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

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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 or 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.*

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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 supplements[1–3] 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 activity[14,16] 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

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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 (rs1061170) variant within the complement factor H (*CFH*) 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 reviewed[20]), 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,[21−24] but not others.[25,26] 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,[27,28] 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.[28] 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 reviewed[19,20]). 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,[14,29,30] 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 described[29,31] 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 photometer[32] 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 accessed for genotyping and measurement of serum carotenoids,[29] 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,[31] 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.[33] 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).[33] Additional criteria for early AMD included having pigmentary 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.[33]

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/years, >7 pack/years).[14] Details of the HLS development and distribution can be found elsewhere.[14] 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 LCG Genomics (Teddington, United Kingdom). Standard quality control filters were applied,[34] 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 R^2 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).[28,35] 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.[36] 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[31] 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)

	Overall Sample (n = 1663)				Stable Diet Group* (n = 727)			
Single Nucleotide Polymorphism	*No. (%)*	*No. of Age-Related Macular Degeneration Cases*	*Age-Adjusted Odds Ratio (95% Confidence Interval)*	*P Value*†	*No. (%)*	*No. of Age-Related Macular Degeneration Cases*	*Age-Adjusted Odds Ratio (95% Confidence Interval)*	*P Value*†
CFH (rs1061170)				<0.001				0.004
TT	606 (36)	92	Reference		275 (38)	34	Reference	
CT	791 (48)	163	1.38 (1.03–1.83)		338 (46)	58	1.46 (0.92–2.32)	
CC	266 (16)	82	2.41 (1.70–3.43)		114 (16)	28	2.30 (1.31–4.05)	
ARMS2 (rs10490924)				0.0001				0.19
CC	1017 (61)	179	Reference		456 (63)	70	Reference	
AC	575 (35)	136	1.51 (1.17–1.95)		247 (34)	45	1.29 (0.85–1.95)	
AA	68 (4)	22	2.18 (1.26–3.77)		22 (3)	5	1.50 (0.53–4.28)	
CFB/C2 (rs641153)				0.02				0.04
AA or AG	278 (17)	44	Reference		116 (16)	12	Reference	
GG	1385 (83)	293	1.52 (1.07–2.17)		611 (84)	108	1.94 (1.02–3.67)	
CFI (rs10033900)				0.28				0.19
GG	430 (26)	92	Reference		198 (27)	34	Reference	
GA	835 (50)	148	0.81 (0.60–1.09)		361 (50)	47	0.73 (0.45–1.18)	
AA	397 (24)	97	1.21 (0.87–1.69)		168 (23)	39	1.43 (0.85–2.40)	
C3 (rs2230199)				0.71				0.75
CC	69 (4)	17	Reference		29 (4)	9	Reference	
GC	540 (32)	106	0.78 (0.43–1.42)		237 (33)	32	0.38 (0.16–0.92)	
GG	1053 (64)	214	0.79 (0.45–1.42)		461 (63)	79	0.49 (0.21–1.12)	

*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.
†For 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

Genotype	No. with Age-Related Macular Degeneration/ Total No. in Group	Most Healthy Lifestyles, Grades 4–6 (n = 769), Odds Ratio* (95% Confidence Interval)	Healthy Lifestyle Grade 3 (n = 454), Odds Ratio* (95% Confidence Interval)	Least Healthy Lifestyles, Grades 0–2 (n = 440), Odds Ratio* (95% Confidence Interval)	*P* Value†	*P* Value for Multiplicative Interaction	Synergy Index (95% Confidence Interval)
CFH (rs1061170)							
TT	92/606	1 [Reference]	1.24 (0.71–2.16)	1.35 (0.79–2.32)	0.26	0.94	1.08
TC	163/791	1.52 (0.99–2.34)	1.54 (0.95–2.48)	1.73 (1.07–2.80)	0.63		(0.70–1.67)
CC	82/266	2.43 (1.43–4.15)	2.84 (1.56–5.20)	3.30 (1.80–6.05)	0.23		
ARMS2 (rs10490924)							
CC	179/1017	1 [Reference]	1.22 (0.83–1.81)	1.33 (0.89–2.00)	0.15	0.63	0.9
AC/AA	158/643	1.70 (1.17–2.45)	1.83 (1.16–2.88)	1.97 (1.29–3.02)	0.52		(0.27–2.93)
ARMS2 (rs10490924)							
CC	179/1017	1 [Reference]	1.22 (0.83–1.81)	1.33 (0.89–2.00)	0.15	0.29	0.56
AC	136/575	1.54 (1.05–2.27)	1.66 (1.03–2.67)	2.13 (1.37–3.29)	0.19		(0.03–11.82)
AA	22/68	3.00 (1.44–6.25)	4.08 (1.34–12.47)	0.73 (0.16–3.38)	0.1		

*Adjusted for age (further adjustment for eye color and hormone therapy, or dietary lutein and zeaxanthin intake, did not alter odds ratios).
†For 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

Genotype	No. with Age-Related Macular Degeneration/ Total No. in Group	Most Healthy Lifestyles, Grades 4–6 (n = 353), Odds Ratio* (95% Confidence Interval)	Healthy Lifestyle, Grade 3 (n = 179), Odds Ratio* (95% Confidence Interval)	Least Healthy Lifestyle, Grades 0–2 (n = 196), Odds Ratio* (95% Confidence Interval)	*P* Value†	*P* Value for Multiplicative Interaction	Synergy Index (95% Confidence Interval)
CFH (rs1061170)							
TT	34/276	1 [Reference]	0.96 (0.39–2.39)	1.38 (0.58–3.26)	0.51	0.31	1.34
TC	58/339	1.69 (0.87–3.28)	1.20 (0.52–2.75)	1.70 (0.81–3.55)	0.93		(1.05–1.70)
CC	28/114	1.56 (0.65–3.71)	2.72 (0.97–7.65)	4.63 (1.85–11.60)	0.04		
ARMS2 (rs10490924)							
CC	70/457	1 [Reference]	0.67 (0.35–1.32)	1.09 (0.59–2.00)	0.94	0.16	1.65
AC/AA	50/269	0.88 (0.48–1.62)	1.40 (0.67–2.94)	1.75 (0.92–3.30)	0.07		(1.18–2.30)

*Adjusted for age (further adjustment for eye color and hormone therapy, or dietary lutein and zeaxanthin intake, did not alter odds ratios).
†For 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 ($P_{interaction} = 0.94$).

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; $P_{trend} = 0.04$, 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 could explain these associations between HLS and AMD. The joint effects of *CFH* genotype and dietary LZ intake similarly suggest at least additive effects of these 2 risk factors for AMD (Tables 5a and b, available at www.aaojournal.org).

We also explored whether higher intake of omega-3 fatty acids explained the association between HLS and AMD in women with the high-risk CC genotype. The OR and interaction terms were not influenced at all by this adjustment (data not shown).

Interactions between *ARMS2* Genotype and Healthy Lifestyle Score

The joint effect of poor lifestyle and *ARMS2* risk alleles was difficult to discern because of low sample sizes after cross-classification; only 18 individuals had both 2 A69S risk alleles and HLS between 0 and 2. Individuals with 1 or 2 A69S risk alleles were combined for subsequent analyses. In the full sample, the odds for AMD was twice as great in women who had at least 1 *ARMS2* risk allele and low HLS, relative to 0 risk alleles and high HLS (OR, 1.97; 95% CI, 1.29−3.02; Table 3). The SI suggests nonsignificant, subadditive joint effects of *ARMS2* genetic risk and poor lifestyle (SI, 0.9; 95% CI, 0.27−2.93). There was no evidence for a multiplicative interaction ($P_{interaction} = 0.63$). In the subsample of women with stable diets, the odds for AMD for women with both risk factors, relative to neither, was 1.75 (95% CI, 0.92−3.30; Table 4). The SI was statistically significant for more than additive effects in this reduced sample (SI, 1.65; 95% CI, 1.18−2.30). However, the SI is dependent on the reference group having the lowest odds for disease, which was not the case in this subsample (lowest odds was among women of low genetic risk and HLS, 3; OR, 0.67). Considering the totality of evidence presented here, there is no evidence to suggest deviations from either additive or multiplicative effects of *ARMS2* genotype and healthy lifestyles.

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,[5−10] 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,[4] 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[37]). Individual factors comprising the HLS (broadly healthy diets, physical activity, and absence of smoking) also are known to be associated with reduced inflammation.[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[21] and a pooled analysis of the Rotterdam and Blue Mountain Eye Studies,[23] 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,[39–41] and supplementation lowers circulating complement factor levels.[42] Within the sample used for this analysis, and in the overall CAREDS cohort,[14] 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.[43–45] Protective associations between serum vitamin D, which also has anti-inflammatory properties, and AMD in women with a CC Y402H genotype[46] in this sample are described in a separate article.[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,[47,48] these results have not been replicated.[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,[23,38,48–50] 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.

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References

1. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E and beta carotene for age-related cataract and vision loss: AREDS report no. 9. Arch Ophthalmol 2001;119:1439–52.
2. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. JAMA 2013;309:2005–15.

3. Chew EY, Clemons TE, Agron E, et al. Long-term effects of vitamins C and E, β-carotene, and zinc on age-related macular degeneration: AREDS report no. 35. Ophthalmology 2013;120:1604–11.
4. Musch DC. Evidence for including lutein and zeaxanthin in oral supplements for age-related macular degeneration. JAMA Ophthalmol 2014;132:139–41.
5. Appel LJ, Moore TJ, Obarzanek E, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. N Engl J Med 1997;336:1117–24.
6. Moreto F, Kano HT, Torezan GA, et al. Changes in malondialdehyde and C-reactive protein concentrations after lifestyle modification are related to different metabolic syndrome-associated pathophysiological processes. Diabetes Metab Syndr 2015. pii: S1871−4021(15)00036-3, http://dx.doi.org/10.1016/j.dsx.2015.04.008 [Epub ahead of print].
7. Galor A, Lee DJ. Effects of smoking on ocular health. Curr Opin Ophthalmol 2011;22:477–82.
8. Esposito K, Marfella R, Ciotola M, et al. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA 2004;292:1440–6.
9. Bekkouche L, Bouchenak M, Malaisse WJ, Yahia DA. The Mediterranean diet adoption improves metabolic, oxidative, and inflammatory abnormalities in Algerian metabolic syndrome patients. Horm Metab Res 2014;46:274–82.
10. Tomaszewski M, Charchar FJ, Przybycin M, et al. Strikingly low circulating CRP concentrations in ultramarathon runners independent of markers of adiposity: how low can you go? Arterioscler Thromb Vasc Biol 2003;23:1640–4.
11. Van Leeuwen R, Boekhoorn S, Vingerling JR, et al. Dietary intake of antioxidants and risk of age-related macular degeneration. JAMA 2005;294:3101–7.
12. Chiu CJ, Mitchell P, Klein R, et al. A risk score for the prediction of advanced age-related macular degeneration: development and validation in 2 prospective cohorts. Ophthalmology 2014;121:1421–7.
13. Montgomery MP, Kamel F, Pericak-Vance MA, et al. Overall diet quality and age-related macular degeneration. Ophthalmic Epidemiol 2010;17:58–65.
14. Mares JA, Voland RP, Sondel SA, et al. Healthy lifestyles related to subsequent prevalence of age-related macular degeneration. Arch Ophthalmol 2011;129:470–80.
15. Amirul Islam FM, Chong EW, Hodge AM, et al. Dietary patterns and their associations with age-related macular degeneration: the Melbourne collaborative cohort study. Ophthalmology 2014;121:1428–34. e1422.
16. Klein R, Lee KE, Gangnon RE, Klein BE. Relation of smoking, drinking, and physical activity to changes in vision over a 20-year period: the Beaver Dam Eye Study. Ophthalmology 2014;121:1220–8.
17. Holliday EG, Smith AV, Cornes BK, et al. Insights into the genetic architecture of early stage age-related macular degeneration: a genome-wide association study meta-analysis. PLoS One 2013;8:e53830.
18. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. Science 2005;308:385–9.
19. Maller J, George S, Purcell S, et al. Common variation in three genes, including a noncoding variant in CFH, strongly influences risk of age-related macular degeneration. Nat Genet 2006;38:1055–9.
20. Priya RR, Chew EY, Swaroop A. Genetic studies of age-related macular degeneration: lessons, challenges, and opportunities for disease management. Ophthalmology 2012;119:2526–36.
21. Ho L, van Leeuwen R, Witteman JC, et al. Reducing the genetic risk of age-related macular degeneration with dietary antioxidants, zinc, and omega-3 fatty acids: the Rotterdam Study. Arch Ophthalmol 2011;129:758–66.
22. Schaumberg DA, Hankinson SE, Guo Q, et al. A prospective study of 2 major age-related macular degeneration susceptibility alleles and interactions with modifiable risk factors. Arch Ophthalmol 2007;125:55–62.
23. Wang JJ, Buitendijk GH, Rochtchina E, et al. Genetic susceptibility, dietary antioxidants, and long-term incidence of age-related macular degeneration in two populations. Ophthalmology 2014;121:667–75.
24. Reynolds R, Rosner B, Seddon JM. Dietary omega-3 fatty acids, other fat intake, genetic susceptibility, and progression to incident geographic atrophy. Ophthalmology 2013;120:1020–8.
25. Seddon JM, Reynolds R, Rosner B. Associations of smoking, body mass index, dietary lutein, and the LIPC gene variant rs10468017 with advanced age-related macular degeneration. Mol Vis 2010;16:2412–24.
26. Sofat R, Casas JP, Webster AR, et al. Complement factor H genetic variant and age-related macular degeneration: effect size, modifiers and relationship to disease subtype. Int J Epidemiol 2012;41:250–62.
27. Andersson T, Alfredsson L, Kallberg H, et al. Calculating measures of biological interaction. Eur J Epidemiol 2005;20:575–9.
28. Rothman K, Greenland S, eds. Modern Epidemiology. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1998.
29. Mares JA, LaRowe TL, Snodderly DM, et al. Predictors of optical density of lutein and zeaxanthin in retinas of older women in the Carotenoids in Age-Related Eye Disease Study, an ancillary study of the Women's Health Initiative. Am J Clin Nutr 2006;84:1107–22.
30. Meyers KJ, Johnson EJ, Bernstein PS, et al. Genetic determinants of macular pigments in women of the Carotenoids in Age-Related Eye Disease Study. Invest Ophthalmol Vis Sci 2013;54:2333–45.
31. Moeller SM, Parekh N, Tinker L, et al. Associations between intermediate age-related macular degeneration and lutein and zeaxanthin in the Carotenoids in Age-Related Eye Disease Study (CAREDS): ancillary study of the Women's Health Initiative. Arch Ophthalmol 2006;124:1151–62.
32. Snodderly DM, Mares JA, Wooten BR, et al. Macular pigment measurement by heterochromatic flicker photometry in older subjects: the Carotenoids and Age-Related Eye Disease Study. Invest Ophthalmol Vis Sci 2004;45:531–8.
33. Age-Related Eye Disease Study Research Group. The Age-Related Eye Disease Study system for classifying age-related macular degeneration from stereoscopic color fundus photographs: the Age-Related Eye Disease Study report number 6. Am J Ophthalmol 2001;132:668–81.
34. Laurie CC, Doheny KF, Mirel DB, et al. Quality control and quality assurance in genotypic data for genome-wide association studies. Genet Epidemiol 2010;34:591–602.
35. Hallqvist J, Ahlbom A, Diderichsen F, Reuterwall C. How to evaluate interaction between causes: a review of practices in cardiovascular epidemiology. J Intern Med 1996;239:377–82.
36. Knol MJ, VanderWeele TJ, Groenwold RH, et al. Estimating measures of interaction on an additive scale for preventive exposures. Eur J Epidemiol 2011;26:433–8.
37. Zipfel PF, Lauer N, Skerka C. The role of complement in AMD. In: Lambris JD, Adamis AP, eds. Inflammation and Retinal Disease: Complement Biology and Pathology,

Advances in Experimental Medicine and Biology. Vol 703. New York: Springer; 2010:9–24.
38. Fung TT, McCullough ML, Newby PK, et al. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. Am J Clin Nutr 2005;82:163–73.
39. Wang MX, Jiao JH, Li ZY, et al. Lutein supplementation reduces plasma lipid peroxidation and C-reactive protein in healthy nonsmokers. Atherosclerosis 2013;227:380–5.
40. Bian Q, Gao S, Zhou J, et al. Lutein and zeaxanthin supplementation reduces photooxidative damage and modulates the expression of inflammation-related genes in retinal pigment epithelial cells. Free Radic Biol Med 2012;53:1298–307.
41. Izumi-Nagai K, Nagai N, Ohgami K, et al. Macular pigment lutein is antiinflammatory in preventing choroidal neovascularization. Arterioscler Thromb Vasc Biol 2007;27:2555–62.
42. Berendschot T, Tian Y, Murray I, et al. Lutein supplementation leads to a decreased level of circulating complement factors. IOVS 2013;54:4214.
43. Reid D, Toole BJ, Knox S, et al. The relation between acute changes in the systemic inflammatory response and plasma 25-hydroxyvitamin D concentrations after elective knee arthroplasty. Am J Clin Nutr 2011;93:1006–11.
44. Duncan A, Talwar D, McMillan DC, et al. Quantitative data on the magnitude of the systemic inflammatory response and its effect on micronutrient status based on plasma measurements. Am J Clin Nutr 2012;95:64–71.
45. Poole KE, Loveridge N, Barker PJ, et al. Reduced vitamin D in acute stroke. Stroke 2006;37:243–5.
46. Millen A, Meyers K, Liu Z, et al. Association between vitamin D status and age-related macular degeneration by genetic risk. JAMA Ophthalmology 2015 Aug 27. doi: 10.1001/jamaophthalmol.2015.2715. [Epub ahead of print]
47. Awh CC, Hawken S, Zanke BW. Treatment response to antioxidants and zinc based on *CFH* and *ARMS2* genetic risk allele number in the Age-Related Eye Disease Study. Ophthalmology 2015;122:162–9.
48. Awh CC, Lane AM, Hawken S, et al. *CFH* and *ARMS2* genetic polymorphisms predict response to antioxidants and zinc in patients with age-related macular degeneration. Ophthalmology 2013;120:2317–23.
49. Klein ML, Francis PJ, Rosner B, et al. *CFH* and *LOC387715/ARMS2* genotypes and treatment with antioxidants and zinc for age-related macular degeneration. Ophthalmology 2008;115:1019–25.
50. Chew EY, Klein ML, Clemons TE, et al. No clinically significant association between *CFH* and *ARMS2* genotypes and response to nutritional supplements: AREDS report number 38. Ophthalmology 2014;121:2173–80.

Footnotes and Financial Disclosures

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Abbreviations and Acronyms:
AMD = 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.

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