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

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Abstract

Purpose—Healthy diets and lifestyles are thought to protect against age-related macular degeneration (AMD), but whether the benefits vary across high risk AMD genotypes is unknown.

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The objective is to investigate the joint effects of healthy diet and lifestyle with genetic risk on the odds for AMD.

Design—Healthy lifestyles scores and their interactions with AMD risk genotypes were studied in relation to the prevalence of AMD, assessed six years later.

Participants—Women 50–79 years of age in the Carotenoids in Age-Related Eye Disease Study (CAREDS) with exposure and AMD data available (N=1,663).

Methods—Healthy lifestyle scores (0–6 points) were assigned based on Healthy Eating Index scores, physical activity (MetHrs/week), and pack years of smoking assessed between 1994–1998. Genetic risk was based on Y402H in complement factor H (*CFH*) and A69S in age-related maculopathy susceptibility locus 2 (*ARMS2*). Interactions between healthy lifestyle score and genotype in relation to the odds of AMD were assessed.

Main Outcome—Stereoscopic fundus photographs were taken and graded for AMD six years after exposure assessment (2001–2004). A total of 308 women had early AMD and 29 had late AMD).

Results—The odds of AMD were 3.3 times greater 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 healthy lifestyle scores of 4–6 (95% CI:1.8–6.1). There were no significant additive (SI=1.08, 95% CI: 0.70–1.67) or multiplicative ($P_{interaction}$=0.94) interactions in the full sample. Limiting the sample to those with stable diets prior to AMD assessment (n=728) strengthened the joint effects (OR=4.6, 95% CI: 1.85–11.6) and suggested high risk genotype and low lifestyle score combined had a stronger association than expected by simply adding the two effects (SI=1.34, 95% CI: 1.05–1.70). Adjusting for dietary lutein and zeaxanthin attenuated, and therefore partially explained the joint association. There was no significant evidence of additive or multiplicative interactions for *ARMS2* and lifestyle score.

Conclusions—These results, in a sample in which the majority of AMD cases were early, suggest the effects of high risk Y402H genotype and poor diets and lifestyles combine in at least an additive manner to influence odds for AMD, but may combine to be more than the sum of their individual effects.

INTRODUCTION

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 of time, 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, healthy diets [11–13,14,15]

not smoking [7,16] and physical activity [14,16] have been previously associated with lower occurrence of early and/or advanced AMD in epidemiological studies. The magnitude of risk reduction associated with several 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 lifestyles (healthy diets, physical activity and not smoking) had a 3-fold lower odds for early AMD, relative to women who had unhealthy lifestyles.[14]

Genetic risk might modify the benefit of healthy lifestyles. Strong genetic risk factors for AMD include advanced age and certain genetic variants. In particular, the Y402H (rs1061170) variant within the complement factor H gene (*CFH*) 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 greatly attenuated for risk of early AMD.[17]

Genetic risk for AMD has also 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 several different healthy lifestyles, together, with AMD risk genotypes. In the present report, we investigate the interactions between genetic risk for AMD and a healthy lifestyle score, summing three lifestyles (diet, smoking and physical activity histories) on the prevalence of AMD, in a study sample (CAREDS) in which AMD cases were mostly comprised of early stages and assessed six years after assessment of lifestyle exposures. Two main strategies were employed to evaluate interactions between lifestyle and genetic risk factors. One strategy was to compute a synergy index 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 might be expected if lifestyle and genetic factors both contribute to the same biological mechanism for AMD pathology, such as to promote inflammation. A second, more stringent, strategy was employed to determine whether genetic risk factors might ***multiply*** the magnitude of AMD risks associated with healthy lifestyles, assessing by the p-value for multiplicative interactions.[28] Evidence of multiplicative interactions might supply stronger evidence to conclude that recommendations to patients for personalized preventive interventions customized to their specific genetic risk profiles might be warranted.

We also explore the extent to which these joint associations were explained by measures of LZ status in the diet, blood or retina uniquely available in this cohort. LZ 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 levels of the carotenoids lutein and zeaxanthin (LZ) in the diet, serum and/or retina, appear to be influenced not only by levels of these carotenoids in the diet, but also by other aspects of healthy diets, lifestyles and genetic factors, [14,29,30] which might work jointly to lower AMD risk. A recent report provides evidence that lutein intake only lowers risk of AMD incidence among persons with two or more risk alleles from common CFH and ARMS2 variants.[23]

METHODS

Study Sample

CAREDS is a previously described[29,31] ancillary study of the Women's Health Initiative (WHI) Observational Study (OS). The primary goal of CAREDS was to examine associations between LZ status in women 50–79 years of age to the prevalence of age-related eye diseases, including AMD, an average of six years later. Fifty percent of all women participating in the OS study centers in Madison, WI (n=694), Iowa City, IA (n=631), and Portland, OR (n=680) were recruited, targeting women reporting the lowest (<28th percentile) and highest (>78th percentile) LZ intakes at WHI baseline. Women in 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).

CAREDS study visits were conducted from 2001–2004 in 2,005 women and have been previously described.[29,31] Briefly, visits included obtaining stereoscopic fundus photographs[31] which were graded for prevalent AMD classification. CAREDS study visits also measured the optical density of macular pigment via customized heterochromatic flicker photometer,[32] questionnaires to assess health history, supplement use, and sunlight exposure history. Food frequency questionnaires (FFQ) were used to estimate usual dietary intakes at WHI_OS baseline (six years prior to CAREDS study visits (1994–1998)) and recalled for intakes 15-years prior to CAREDS study visits. [31] WHI-OS study 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 2,005 CAREDS participants, 1,857 had gradable fundus photographs available for AMD classification and 1,663 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 CAREDS suggest fluctuations in the amount of LZ consumed at the time of WHI enrollment (six years prior to ocular photography) and in the time prior to enrollment in the WHI. Thus, to avoid bias resulting from including women with fluctuating diets just prior to exposure assessment, we conducted analysis in the full sample, and then after excluding women whose intakes of LZ changed more than 1 quintile categorization between the 1988–1992 (CAREDS 15-year recall FFQ) and 1994–1998

(WHI baseline FFQ) (n=356, 18%) and those who were candidates for recent diet change due to diagnoses of the following comorbid conditions for which diet changes are often recommended: cardiovascular disease, diabetes, macular degeneration, and/or a history of hypertension (n=579, 29%). The subsample for these analyses included 728 women with stable diets.

AMD 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 one or more large drusen (≥125 microns) or extensive intermediate drusen (total area ≥360 microns when soft indistinct drusen were present or ≥650 microns when soft indistinct drusen were absent).[33] Additional criteria for early AMD included having pigmentary abnormalities; an increase or decrease in pigmentation if accompanied by at least one druse ≥63 microns. 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 which gives equal weight to each of three, 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 MET 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 three level variable based on composite HLS scores of 0–2, 3, and 4–6, which divided the sample into approximate tertiles.

Genotyping

Genotyping for known and candidate AMD genes was done at Case Western Reserve University (Cleveland, OH) using a custom Illumina GoldenGate Assay. DNA was extracted from the buffy coats of blood obtained at WHI-OS baseline examinations (1994–1998) that have been stored frozen at −80°C. Genotype calls were made using Illumina Genome Studio. SNPs 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 (QC) filters were applied,[34] resulting in exclusions of SNPs with Hardy-Weinberg equilibrium (HWE) χ^2 *P*-value $<1.0\times10^{-6}$, MAF <0.01, or genotype call rates <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 (http://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 (CEU) 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; two SNPs established to increase risk for both early and late AMD. Data was also 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 as 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., two factors combine to be more or less than the sum of their individual effects) were tested using the Synergy Index (SI) and corresponding 95% CIs.[28,35] When estimating the SI, it has been recommended to recode protective factors so “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 score (4–6). An SI=1.0 indicates no interaction (i.e., the factors combine in a manner that is exactly additive), SI>1.0 indicates the two factors considered together combine to be more than the sum of their individual effects (i.e., biological synergism), and SI<1.0 indicates negative additive interaction, or that the effects of the two 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 additionally adjusted for 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 Institute 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 HLS scores (0–2) had AMD, compared to 19% of women with high scores (4–6) (P=0.13). Limiting the sample to those with stable diets resulted in 120 cases of AMD. 20% of women with low HLS had AMD compared to 16% of women with high HLS in this diet stable subsample (P=0.14). The distribution of AMD risk phenotypes by HLS score levels is given in Table 1. There were no differences in genotype distributions by HLS classification (Table 1). Limiting the sample to women with stable diets (n=728) kept risk factor differences stable across HLS strata, except that the

higher HLS scores among older women did not persist in the subgroup limited to stable diets.

Main Effects of AMD Genotypes in CAREDS

Odds for AMD increased with each additional copy of the risk Y402H *CFH* risk allele: women with two risk alleles had 2.4 times greater odds of AMD relative to women with zero risk alleles ($P<0.0001$) (Table 2). Odds for AMD also increased with each additional copy of the A69S risk allele; women with two copies had 2.2 times greater odds of AMD relative to women with zero 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*) nor 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 was 3.3 times greater in women who had both two high risk *CFH* alleles (CC) and low HLS, relative to low risk genotype (TT) and high HLS (OR=3.3, 95% CI: 1.80–6.05) (Table 3a). The joint effect of these two 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 to women with healthy lifestyle and low-risk genotype (OR=4.63, 95% CI: 1.85–11.60; Table 3b). The joint effect of these two 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 (0–2) relative to those with highest HLS (4–6) (OR=4.63 vs. 1.56, P_{trend} across HLS groups within genotype class=0.04; Table 3b). 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 MPOD (respectively), suggesting that better status for LZ could partially 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 two risk factors for AMD (Tables 4a and 4b; available at http://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. 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 due to low sample sizes after cross-classification, only 18 individuals had both two A69S risk alleles

and HLS between 0–2. Individuals with one or two A69S risk alleles were combined for subsequent analyses. In the full sample, the odds for AMD was 2 times greater in women who had at least one *ARMS2* risk allele and low HLS, relative to zero risk alleles and high HLS (OR=1.97, 95% CI: 1.29–3.02; Table 3a). The synergy index suggests non-significant, sub-additive 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 3b). The synergy index 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 is 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 AMD 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) were also explored (Tables 5a and 5b; available at http://aaojournal.org). Absent main effects for SNPs in *C3* and *CFI* (Table 2), along with a variable combination of genotype and HLS lending towards 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 Synergy Index) between *CFH* risk genotype and poor status for a broad lifestyle measure (including poor diet, low physical activity, and smoking). It thus appears that the attributable risk of AMD may be inordinately greater in women who have both high risk CFH genotype and these lifestyle characteristics. The majority (91%) of 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. While 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 of time [4], these data suggest that any success in physicians' attempts to persuade these patients to adopt healthy lifestyles at early ages could ultimately 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 increased risk for AMD (reviewed[37]). Individual factors comprising the HLS (broadly healthy diets, physical activity, and absence of smoking) are also known to be associated with reduced inflammation.[6,7,10,38]

The combined influence of poor lifestyles and genetic risk was, in part, explained by low LZ intake in the present study. This is despite the fact that we observed no significant interactions between lutein intake and CFH risk (Supplemental Tables 4A and 4B) 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[39] and a pooled analysis of the Rotterdam and Blue Mountain Eye Studies,[23] indicating interactions between LZ intake and high risk *CFH* and/or *ARMS2* genotypes for early AMD. The combined results from the present study and these two studies supports an augmentation of AMD genetic risk associated with lutein intake. Consistent with this, lutein has been demonstrated to have anti-inflammatory properties[40–42] and supplementation lowers circulating complement factor levels.[43] Within the sample used for this analysis, and in the overall CAREDS cohort,[14] higher HLS and dietary LZ intake were each associated with lower serum C-reactive protein, a systemic marker of inflammation, and higher vitamin D, also related to inflammatory conditions.[44–46] Protective associations between serum vitamin D, which also has anti-inflammatory properties, and AMD in women with a CC Y402H genotype[47] in this sample are described in a separate manuscript.[47]

If AMD protection by healthy lifestyles, was directly 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 due to 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 non-existent 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 the present 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,[48,49] these results have not been replicated.[50,51] While the current evidence is not strong enough to

justify recommendations for supplements or lifestyles to lower AMD risk which is tailored to genetic profiles, results of these five studies,[23,39,49–51] combined with the work presented here, highlight the fact that the individual-level benefit of diet, lifestyle, and/or supplements 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 six years later than exposure estimate, was a prevalence estimate. Some cases of AMD may have developed prior to the exposure assessment. Fluctuations in diet (and health behaviors) in the time prior to AMD assessment may not reflect long-term intake, leading to random or non-random 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 excluding women whose diets changed in a time period prior to WHI, or who were diagnosed with a chronic disease for which diet changes are often recommended (cardiovascular disease, diabetes, macular degeneration, and/or a history of hypertension). While minimizing bias, this approach 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; the majority of women with AMD in this study had early/intermediate stage (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 which 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 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 and/or have a family history for AMD. Confirmation of results in prospective studies and in a greater number of samples including men and other ethnicities are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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

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Table 1

Age adjusted distribution (mean±standard error or n(%)) of potential AMD risk factors among CAREDS participants by healthy lifestyle score.

Variables	Healthy Lifestyle Score, Full sample (n=1,663)			Healthy Lifestyle Score, Diet stable sample (n=728)		
	4 – 6	0 – 2	P-value	4 – 6	0 – 2	P-value
N	769	440		353	196	
Demographics						
Age group*, %			0.02			0.67
≤69	357 (46%)	230 (52%)		202 (57%)	115 (59%)	
70–74	199 (26%)	113 (26%)		76 (22%)	43 (22%)	
≥75	213 (28%)	97 (22%)		75 (21%)	38 (19%)	
Ethnicity, % white	746 (97%)	433 (99%)	0.07	341 (97%)	192 (96%)	0.20
Education, %			<.0001			<.0001
High school	125 (16%)	137 (30%)		56 (16%)	57 (27%)	
College	356 (47%)	224 (51%)		164 (46%)	104 (54%)	
Postcollege studies	288 (37%)	79 (18%)		133 (38%)	35 (17%)	
Lifestyle						
Physical activity, METs/week	23 ± 0.5	5 ± 0.6	<.0001	23 ± 1	6 ± 1	<.0001
Smoking, pack-years, %			<.0001			<.0001
0	592 (77%)	90 (21%)		266 (76%)	32 (15%)	
0–7	157 (20%)	117 (25%)		77 (22%)	55 (26%)	
>7	20 (3%)	233 (54%)		10 (3%)	109 (56%)	
Alcohol, g/day			0.07			5E-04
0	319 (41%)	169 (38%)		149 (42%)	62 (29%)	
>0 to <4	235 (31%)	118 (27%)		106 (31%)	49 (24%)	
≥ 4	215 (28%)	153 (35%)		98 (27%)	85 (44%)	
Total sunlight exposure (last 20 years), Maryland sun-years	0.83 ± 0.02	0.82 ± 0.02	0.75	0.8 ± 0.02	0.8 ± 0.03	0.94
Ocular factors						
Macular pigment density	0.38 ± 0.01	0.34 ± 0.01	0.002	0.40 ± 0.01	0.36 ± 0.02	0.05

Variables	Healthy Lifestyle Score, Full sample (n=1,663)			Healthy Lifestyle Score, Diet stable sample (n=728)		
	4 – 6	0 – 2	P-value	4 – 6	0 – 2	P-value
AMD						
Early, %	138 (17%)	83 (21%)	0.27	53 (15.0%)	37 (19.4%)	0.16
Large drusen, %	111 (15%)	63 (17%)	0.52	43 (12.5%)	27 (14.7%)	0.39
Pigmentary abnormalities, %	59 (8%)	47 (13%)	0.04	23 (7.37%)	20 (11.7%)	0.09
Advanced AMD, %	11 (1.3%)	12 (3.1%)	0.06	2 (0.51%)	2 (1.23%)	0.52
Early or Advanced AMD, %	149 (19%)	95 (23%)	0.13	55 (15.5%)	39 (20.3%)	0.14
Medical factors						
Body Mass Index (BMI), kg/m^2	26 ± 0.2	29 ± 0.3	<.0001	25.7 ± 0.3	28.5 ± 0.4	<.0001
Hormone therapy, %			0.16			1.00
Never	253 (32%)	147 (34%)		121 (34%)	63 (30%)	
Past	91 (12%)	66 (15%)		43 (12%)	29 (14%)	
Current	425 (56%)	227 (51%)		189 (54%)	104 (53%)	
Diet						
Modified Healthy Eating Index - 2005	68 ± 0.2	60 ± 0.3	<.0001	68.0 ± 0.3	59.7 ± 0.4	<.0001
Lutein and Zeaxanthin, mcg/day	2723 ± 60	1712 ± 80	<.0001	2759 ± 94	1614 ± 126	<.0001
Total omega-3 fatty acids, g/day	1.2 ± 0.02	1.4 ± 0.03	<.0001	1.19 ± 0.04	1.38 ± 0.05	0.002
Fruit intake, servings/day	2.6 ± 0.05	1.6 ± 0.1	<.0001	2.6 ± 0.1	1.6 ± 0.1	<.0001
Vegetable intake, servings/day	3.0 ± 0.1	2.0 ± 0.1	<.0001	3.0 ± 0.1	1.8 ± 0.1	<.0001
β-Carotene, µg/day	5282 ± 115	3346 ± 153	<.0001	5305 ± 166	3051 ± 223	<.0001
Vitamin C, mg/day	137 ± 2	93 ± 3	<.0001	135 ± 3	93 ± 5	<.0001
Vitamin D, mcg/day	6.4 ± 0.1	5.1 ± 0.2	<.0001	6.2 ± 0.2	4.9 ± 0.3	3E-04
Vitamin E, mg/day	8.8 ± 0.2	7.9 ± 0.2	0.001	8.7 ± 0.2	7.7 ± 0.3	0.01
Zinc, mg/day	12 ± 0.2	10 ± 0.2	<.0001	12.1 ± 0.3	9.8 ± 0.4	<.0001
Supplement use						
Multivitamin, %	351 (45%)	179 (41%)	0.13	168 (47%)	92 (45%)	0.94
Lutein or lutein + zeaxanthin†, %	17 (2.1%)	13 (3.4%)	0.31	5 (1%)	5 (2%)	0.30
Serum values						

Variables	Healthy Lifestyle Score, Full sample (n=1,663)			Healthy Lifestyle Score, Diet stable sample (n=728)		
	4 – 6	0 – 2	P-value	4 – 6	0 – 2	P-value
Lutein + zeaxanthin (trans), μmol/L	0.35 ± 0.01	0.27 ± 0.01	<.0001	0.36 ± 0.01	0.28 ± 0.01	<.0001
Genes						
CFH Y402H (rs1061170)			0.19			0.29
TT	294 (38%)	156 (36%)		138 (39%)	64 (33%)	
TC	360 (47%)	209 (47%)		156 (45%)	101 (49%)	
CC	115 (15%)	75 (17%)		58 (16%)	31 (15%)	
ARMS2 (rs10490924)			0.42			0.63
CC	465 (60%)	254 (58%)		219 (63%)	115 (58%)	
AC	266 (35%)	167 (38%)		117 (33%)	77 (38%)	
AA	36 (5%)	18 (4%)		15 (4%)	3 (1%)	
CFI (rs10033900)			0.53			0.39
GG	208 (27%)	114 (25%)		99 (29%)	51 (25%)	
GA	378 (49%)	215 (49%)		175 (49%)	95 (46%)	
AA	182 (24%)	111 (26%)		78 (22%)	50 (26%)	
CFB/C2 (rs641153)			0.53			0.24
AA/AG	123 (16%)	76 (18%)		51 (15%)	36 (20%)	
GG	646 (84%)	364 (82%)		301 (85%)	160 (78%)	
C3 (rs2230199)			0.87			0.23
CC	34 (4%)	16 (4%)		15 (5%)	9 (4%)	
GC	244 (32%)	143 (32%)		119 (34%)	55 (29%)	
GG	490 (64%)	281 (64%)		218 (62%)	132 (64%)	

*Not age adjusted.

†Use of single lutein, or lutein and zeaxanthin, supplements of dose ≥ 1 mg/day for ≥ 2 years.

Table 2

Age-adjusted odds ratios (OR) and 95% confidence intervals (CIs) for any AMD in CAREDS by known AMD-related genes (SNPs).

SNP	Overall Sample (n=1,663)				Diet Stable** (n=727)			
	No. (%)	No. of AMD Cases	Age-adjusted OR (95% CI)	p-for trend*	No. (%)	No. of AMD Cases	Age-adjusted OR (95% CI)	p-for trend*
CFH (rs1061170)				<0.001				0.004
TT	606 (36%)	92	Ref.		275 (38%)	34	Ref.	
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	Ref.		456 (63%)	70	Ref.	
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	Ref.		116 (16%)	12	Ref.	
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	Ref.		198 (27%)	34	Ref.	
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	Ref.		29 (4%)	9	Ref.	
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)	

*p for trend across genotypes.

Table 3a

Joint Effects of AMD Genotype (based on *CFH* or *ARMS2*) and Healthy Lifestyle Score in Association with Odds for AMD in overall sample

Genotype	No. with AMD/Total No. in Group	Most Healthy Lifestyles 4–6 (n=769)	3 (n=454)	Least Healthy Lifestyle 0–2 (n=440)	P-value†	P-value for Multiplicative Interaction	Synergy Index (95% CI)
		OR* (95% CI)	OR* (95% CI)	OR* (95% CI)			
CFH (rs1061170)							
TT	92/606	1 [Reference]	1.24 (0.71, 2.16)	1.35 (0.79, 2.32)	0.26		1.08 (0.70, 1.67)
TC	163/791	1.52 (0.99, 2.34)	1.54 (0.95, 2.48)	1.73 (1.07, 2.80)	0.63	0.94	
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		0.9 (0.27, 2.93)
AC/AA	158/643	1.70 (1.17, 2.45)	1.83 (1.16, 2.88)	1.97 (1.29, 3.02)	0.52	0.63	
ARMS2 (rs10490924)							
CC	179/1017	1 [Reference]	1.22 (0.83, 1.81)	1.33 (0.89, 2.00)	0.15		0.56 (0.03, 11.82)
AC	136/575	1.54 (1.05, 2.27)	1.66 (1.03, 2.67)	2.13 (1.37, 3.29)	0.19	0.29	
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 LZ intake, did not alter ORs)

†P-values are for trend across lifestyle score group, within each genotype class.

Table 3b

Joint Effects of AMD Genotype (based on CFH or ARMS2) and Healthy Lifestyle Score in Association with Odds for AMD among diet stable individuals

Genotype	No. with AMD/Total No. in Group	Most Healthy Lifestyles 4–6 (n=353)	3 (n=179)	Least Healthy Lifestyle 0–2 (n=196)	P-value†	P-value for Multiplicative Interaction	Synergy Index (95% CI)
		OR* (95% CI)	OR* (95% CI)	OR* (95% CI)			
CFH (rs1061170)							
TT	34/276	1 [Reference]	0.96 (0.39, 2.39)	1.38 (0.58, 3.26)	0.51		1.34 (1.05, 1.70)
TC	58/339	1.69 (0.87, 3.28)	1.20 (0.52, 2.75)	1.70 (0.81, 3.55)	0.93	0.31	
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 (1.18, 2.30)
AC/AA	50/269	0.88 (0.48, 1.62)	1.40 (0.67, 2.94)	1.75 (0.92, 3.30)	0.07		

*Adjusted for age (further adjustment for eye color and hormone therapy, or dietary LZ intake, did not alter ORs)

†P-values are for trend across lifestyle score group, within each genotype class.

Table 4a

Joint Effects of AMD Genotype (based on *CFH* or *ARMS2*) and Dietary Lutein and Zeaxanthin (LZ) Intake in Association with Odds for AMD in overall sample

Genotype	No. with AMD/Total No. in Group	High LZ Intake ≥2000 mcg/day (n=827) OR* (95% CI)	Low LZ Intake <2000 mcg/day (n=836) OR* (95% CI)	P-value for trend	P-value for Multiplicative Interaction	Synergy Index (95% CI)
CFH (rs1061170)						
TT	92/606	1 [Reference]	1.10 (0.70, 1.72)	0.7		1.08 (0.55, 2.11)
TC	163/791	1.46 (0.98, 2.19)	1.42 (0.95, 2.13)	0.83	0.91	
CC	82/266	2.34 (1.41, 3.87)	2.71 (1.66, 4.41)	0.39		
ARMS2 (rs10490924)						
CC	179/1017	1 [Reference]	0.96 (0.69, 1.33)	0.81		1.44 (0.67, 3.12)
AC/AA	158/643	1.44 (1.01, 2.04)	1.65 (1.17, 2.32)	0.49	0.47	
ARMS2 (rs10490924)						
CC	179/1017	1 [Reference]	0.96 (0.69, 1.33)	0.81		1.22 (0.53, 2.82)
AC	136/575	1.32 (0.91, 1.91)	1.63 (1.15, 2.32)	0.29	0.72	
AA	22/68	2.48 (1.18, 5.24)	1.79 (0.79, 4.06)	0.46		
CFB/C2 (rs641153)						
AA/AG	44/278	1 [Reference]	0.69 (0.36, 1.35)	0.24		1.85 (1.03, 3.33)
GG	293/1385	1.21 (0.75, 1.95)	1.37 (0.85, 2.20)	0.35	0.18	
CFI (rs10033900)						
GG	92/430	1 [Reference]	1.58 (0.97, 2.55)	0.05		0.12 (2.7E-5, 535.7)
GA	148/835	1.13 (0.72, 1.75)	0.96 (0.61, 1.51)	0.37	0.16	
AA	97/397	1.55 (0.95, 2.55)	1.56 (0.95, 2.56)	0.95		
C3 (rs2230199)						
CC	17/69	1 [Reference]	2.39 (0.72, 7.91)	0.15		0.13 (1.9E-4, 89.0)
GC	105/540	1.30 (0.47, 3.60)	1.34 (0.48, 3.73)	0.79	0.34	
GG	214/1053	1.34 (0.49, 3.61)	1.35 (0.50, 3.66)	0.95		

*Adjusted for age (further adjustment for eye color, smoking, and hormone therapy did not alter ORs)

Table 4b

Joint Effects of AMD Genotype and Dietary Lutein and Zeaxanthin (LZ) Intake in Association with Odds for AMD among diet stable individuals

Genotype	No. with AMD/Total No. in Group	High LZ Intake ≥2000 mcg/day (n=358) OR* (95% CI)	Low LZ Intake <2000 mcg/day (n=370) OR* (95% CI)	P-value for trend	P-value for Multiplicative Interaction	Synergy Index (95% CI)
CFH (rs1061170)						
TT	34/275	1 [Reference]	1.59 (0.76, 3.31)	0.22		1.49 (0.65, 3.42)
TC	58/339	1.95 (0.98, 3.89)	1.78 (0.90, 3.54)	0.74	0.63	
CC	28/114	1.81 (0.74, 4.42)	4.37 (1.97, 9.68)	0.06		
ARMS2 (rs10490924)						
CC	70/457	1 [Reference]	1.51 (0.90, 2.54)	0.11	0.39	0.27 (3.3E-4, 222.8)
AC/AA	50/269	1.57 (0.86, 2.87)	1.67 (0.94, 2.96)	0.9		
CFB/C2 (rs641153)						
AA/AG	12/116	1 [Reference]	1.55 (0.46, 5.29)	0.57	0.78	0.98 (0.15, 6.32)
GG	108/612	2.14 (0.80, 5.71)	2.77 (1.05, 7.33)	0.23		
CFI (rs10033900)						
GG	34/199	1 [Reference]	1.54 (0.73, 3.27)	0.25		0.52 (0.08, 3.65)
GA	47/361	0.73 (0.35, 1.52)	1.09 (0.55, 2.17)	0.2	0.37	
AA	39/168	1.83 (0.85, 3.94)	1.74 (0.82, 3.68)	0.94		
C3 (rs2230199)						
CC	29-Sep	1 [Reference]	3.60 (0.59, 22.05)	0.17		1.01 (0.02, 57.10)
GC	32/237	0.97 (0.19, 4.85)	0.80 (0.16, 4.03)	0.68	0.97	
GG	79/462	0.91 (0.19, 4.37)	1.33 (0.28, 6.36)	0.14		

*Adjusted for age (further adjustment for eye color, smoking, and hormone therapy did not alter ORs)

Table 5a

Joint Effects of Complement Pathway Genotype (*CFB/C2*, *CFI*, and *C3*) and Healthy Lifestyle Score in Association with Odds for AMD in overall sample

Genotype	No. with AMD/Total No. in Group	Most Healthy Lifestyles 4–6 (n=769)	3 (n=454)	Least Healthy Lifestyle 0–2 (n=440)	P-value†	P-value for Multiplicative Interaction	Synergy Index (95% CI)
		OR* (95% CI)	OR* (95% CI)	OR* (95% CI)			
CFB/C2 (rs641153)							
AA/AG	44/278	1 [Reference]	0.87 (0.40, 1.89)	0.84 (0.37, 1.93)	0.57	0.28	1.33 (1.11, 1.60)
GG	293/1385	1.24 (0.74, 2.06)	1.47 (0.86, 2.51)	1.66 (0.97, 2.83)	0.06		
CFI (rs10033900)							
GG	92/430	1 [Reference]	1.64 (0.93, 2.90)	1.41 (0.79, 2.52)	0.11		1.29 (0.78, 2.13)
GA	148/835	1.01 (0.65, 1.58)	1.11 (0.69, 1.81)	0.91 (0.54, 1.52)	0.7	0.61	
AA	97/397	1.33 (0.80, 2.19)	1.06 (0.57, 1.98)	2.43 (1.41, 4.19)	0.05		
C3 (rs2230199)							
CC	17/69	1 [Reference]	1.37 (0.31, 6.05)	6.02 (1.51, 24.05)	0.01		0.22 (8.7E-13, 5.7E10)
GC	105/540	1.58 (0.58, 4.35)	1.27 (0.44, 3.63)	1.42 (0.50, 4.08)	0.66	0.78	
GG	214/1053	1.26 (0.47, 3.39)	1.69 (0.62, 4.60)	1.68 (0.62, 4.59)	0.11		

*Adjusted for age (further adjustment for eye color and hormone therapy, or dietary LZ intake, did not alter ORs)

†P-values are for trend across lifestyle score group, within each genotype class.

Table 5b

Joint Effects of Complement Pathway Genotype (*CFB/C2*, *CFI*, and *C3*) and Healthy Lifestyle Score in Association with Odds for AMD among diet stable individuals

Genotype	No. with AMD/Total No. in Group	Most Healthy Lifestyles 4–6 (n=353)	3 (n=179)	Least Healthy Lifestyle 0–2 (n=196)	P-value†	P-value for Multiplicative Interaction	Synergy Index (95% CI)
		OR* (95% CI)	OR* (95% CI)	OR* (95% CI)			
CFB/C2 (rs641153)							
AA/AG	12/116	1 [Reference]	1.08 (0.24, 4.94)	1.38 (0.34, 5.63)	0.76	0.99	1.16 (0.48, 2.81)
GG	108/612	2.00 (0.75, 5.37)	1.86 (0.66, 5.25)	2.86 (1.04, 7.84)	0.19		
CFI (rs10033900)							
GG	34/199	1 [Reference]	1.03 (0.40, 2.61)	1.13 (0.46, 2.77)	0.78	0.18	1.43 (1.14, 1.80)
GA	47/361	0.73 (0.37, 1.47)	0.83 (0.37, 1.83)	0.73 (0.33, 1.65)	0.96		
AA	39/168	1.22 (0.57, 2.63)	0.71 (0.24, 2.10)	2.90 (1.31, 6.41)	0.05		
C3 (rs2230199)							
CC	29-Sep	1 [Reference]	0.86 (0.07, 11.11)	4.72 (0.75, 29.65)	0.10	0.95	1.08 (0.01, 169.2)
GC	32/237	0.85 (0.22, 3.31)	0.36 (0.08, 1.74)	0.65 (0.14, 2.92)	0.40		
GG	79/462	0.69 (0.18, 2.59)	0.90 (0.23, 3.54)	1.07 (0.28, 4.09)	0.13		

*Adjusted for age (further adjustment for eye color and hormone therapy, or dietary LZ intake, did not alter ORs)

†P-values are for trend across lifestyle score group, within each genotype class.