Genetic Evidence for Role of Carotenoids in Age-Related Macular Degeneration in the Carotenoids in Age-Related Eye Disease Study (CAREDS)


1Department of Ophthalmology and Visual Sciences, McPherson Eye Research Institute, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin
2Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio
3Department of Social and Preventive Medicine, School of Public Health and Health Professions, University at Buffalo, The State University of New York, Buffalo, New York
4Department of Ophthalmology, Oregon Health and Science University, Casey Eye Institute, Portland, Oregon
5Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts
6Department of Population Health Sciences, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin
7Department of Ophthalmology and Visual Sciences, University of Iowa Carver College of Medicine, Iowa City, Iowa
8Department of Cancer Prevention Research Program, Fred Hutchinson Cancer Research Center, Seattle, Washington
9Department of Epidemiology, University of Iowa College of Public Health, Iowa City, Iowa
10Departments of Epidemiology and Medicine, University of Iowa College of Public Health, Iowa City, Iowa
11Kaiser Center for Health Research, Portland, Oregon
12Department of Obstetrics and Gynecology, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin
13Moran Eye Center, University of Utah Health Care, Salt Lake City, Utah
14National Institutes of Health, National Eye Institute, Clinical Trials Branch, Bethesda, Maryland

Correspondence: Julie A. Mares, Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, 610 N. Walnut Street, 1063 WARE, Madison, WI 53726; jmarespe@wisc.edu.
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PURPOSE. We tested variants in genes related to lutein and zeaxanthin status with age-related macular degeneration (AMD) in the Carotenoids in Age-Related Eye Disease Study (CAREDS).

METHODS. Of 2005 CAREDS participants, 1663 were graded for AMD from fundus photography and genotyped for 424 single nucleotide polymorphisms (SNPs) from 24 candidate genes for carotenoid status. Of 537 AMD cases 91% had early or intermediate AMD. The SNPs were tested individually for association with AMD using logistic regression. A carotenoid-related genetic risk model was built using backward selection and compared to existing AMD risk factors using the area under the receiver operating characteristic curve (AUC).

RESULTS. A total of 24 variants from five genes (BCMO1, BCO2, NPC1L1, ABCG8, and FADS2) not previously related to AMD and four genes related to AMD in previous studies (SCARBI, ABCA1, APOE, and ALDH3A2) were associated independently with AMD, after adjusting for age and ancestry. Variants in all genes (not always the identical SNPs) were associated with lutein and zeaxanthin in serum and/or macula, in this or other samples, except for BCO2 and FADS2. A genetic risk score including nine variants significantly (P = 0.002) discriminated between AMD cases and controls beyond age, smoking, CFH Y402H, and ARMS2 A69S. The odds ratio (95% confidence interval) for AMD among women in the highest versus lowest quintile for the risk score was 3.1 (2.0–4.9).

CONCLUSIONS. Variants in genes related to lutein and zeaxanthin status were associated with AMD in CAREDS, adding to the body of evidence supporting a protective role of lutein and zeaxanthin in risk of AMD.

Keywords: macular degeneration, carotenoids, genes

Age-related macular degeneration (AMD) is a degenerative disease of the macula and the leading cause of blindness among the elderly in developed countries. Lutein and zeaxanthin, and the lutein metabolite meso-zeaxanthin, uniquely concentrate in the macula and comprise macular pigment (MP).1–4 Increasing evidence suggests the dietary carotenoids lutein and zeaxanthin protect against pathogenic processes of AMD5–7 by absorbing an estimated 40% to 90% of incident blue light8 otherwise damaging the macula,9 and lowering oxidative stress10–12 and inflammation.12–14 Systemic antioxidant and
antiinflammatory effects of lutein also have been suggested,\textsuperscript{15,16} which may influence the retina indirectly through general inflammatory processes, and the availability of antioxidants and antiinflammatory molecules.

Despite strong biological plausibility for a protective effect of macular carotenoids against AMD, the body of evidence from epidemiologic studies and clinical trials is inconsistent. A protective influence of lutein and zeaxanthin in the diet or blood on lower risk for advanced AMD is supported by the results of several epidemiologic studies\textsuperscript{17–22} and by secondary, but not primary, analyses in the Age-Related Eye Disease Study 2 (AREDS2), a multicenter, randomized controlled trial of lutein and zeaxanthin supplements, and progression of AMD individuals with intermediate or advanced disease.\textsuperscript{23} A protective influence of lutein and zeaxanthin intake on early AMD sometimes\textsuperscript{22,24–26} but not always\textsuperscript{22,24,27–29} is observed in epidemiologic studies. Thus, a role of dietary lutein and zeaxanthin in preventing and lowering progression of AMD is unclear.

One reason for inconsistency across previous studies may be that there is a variable macular pigment response to dietary intake of macular carotenoids.\textsuperscript{30–45} While lutein and zeaxanthin are acquired only through diet or supplements, the subsequent accumulation of these carotenoids in the retina is related to many factors, including several genetic factors.\textsuperscript{28,44–46,47} Results of a recent twin study suggest 27\% of macular response to dietary carotenoids is heritable.\textsuperscript{44} Studies in animal models and humans support a role for genetic variation in determining carotenoid status in the retina or serum (see prior reviews\textsuperscript{48,49}). Therefore, genetic variation associated with carotenoid status in the serum and retina can provide another line of evidence for the putative role of lutein and zeaxanthin in protecting against AMD, independent from dietary estimates of exposure to macular carotenoids. Relationships of diet or serum carotenoids and AMD also might reflect other unknown, and unadjusted for, aspects of diet and lifestyle related to AMD, while genetic measures of carotenoid status would not.

To evaluate genetic evidence for relationships of lutein and zeaxanthin to AMD, we examined relationships of common single nucleotide polymorphisms (SNPs) from genes in pathways related to binding, metabolism, or transport of macular carotenoids for association with AMD. These include variants in genes related to cholesterol and carotenoid membrane transport proteins in the intestine and retina, high density lipoprotein levels in blood, carotenoid cleavage, omega-3 fatty acid status previously related to macular pigment,\textsuperscript{50} and retinopathies associated with impaired macular pigment. These SNPs were studied previously for their relation to MP optical density.\textsuperscript{47} Relationships to serum concentration of lutein and zeaxanthin are evaluated within.

**METHODS**

**Study Sample**

The sample included participants of the Carotenoids in Age-Related Eye Disease Study (CAREDS), an ancillary study within the Women’s Health Initiative Observational Study (WHI-OS), described previously.\textsuperscript{26,28} The CAREDS study visits were conducted between 2001 and 2004 in 2005 women from WHI-OS study centers in Madison, Wisconsin (n = 694), Iowa City, Iowa (n = 631), and Portland, Oregon (n = 680). Visits included ocular photography, measurement of the optical density of MP and questionnaires to assess risk factors for age-related eye diseases, including queries of diet, supplement use, sunlight exposure history, and eye health history. The WHI-OS study visits in 1995–1998 provided additional relevant information, including collection and storage of serum samples that were used later for genotyping and biomarker measurement, smoking history, food frequency questionnaires, physical activity, blood pressure, and anthropometrics. The 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.

**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.\textsuperscript{51} 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 μ) or extensive intermediate drusen (total area ≥ 360 μ when soft indistinct drusen were present or ≥650 μ when soft indistinct drusen were absent).\textsuperscript{51} Additional criteria for early AMD included having pigmented abnormalities; an increase or decrease in pigmentation, if accompanied by at least one druse ≥ 65 μ. Advanced AMD included geographic atrophy, neovascularization, or exudation in the center subfield. The reference group included women who had neither early nor advanced AMD; generally corresponding to AREDS categories 1 and 2.\textsuperscript{51}

**Serum Analyses of Lutein and Zeaxanthin**

Serum samples, obtained from participants in WHI baseline examinations (1994–1998) and stored at −80°C, were analyzed for levels of trans lutein and zeaxanthin at Tufts University by a reverse phase high performance liquid chromatography (HPLC) analysis\textsuperscript{52} as described previously.\textsuperscript{28}

**Genotyping**

Genotyping was attempted for 438 SNPs from 24 carotenoid pathway genes selected based on previous evidence that suggested their capacity to encode factors influencing carotenoid status.\textsuperscript{47,49,55–58} Specific SNPs within candidate genes were chosen based on previous literature or as tag SNPs for their respective gene. Tagging was conducted using the HapMap Genome Browser Release 27 (available in the public domain at http://hapmap.ncbi.nlm.nih.gov/) CEU reference population and filtering for a minor allele frequency (MAF) ≥ 0.05 and r2 ≥ 0.80. Tagging included a 20 kilobase (kb) pair window up- and downstream of each gene. Genotyping included an additional 190 ancestry informative markers (AIMs) for northwest-southeast European ancestry and south-eastern-Ashkenazi Jewish ancestry clines.\textsuperscript{59}

The SNPs were genotyped at Case Western Reserve University (Cleveland, OH) using an Illumina Custom 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 have been stored frozen at −80°C. Genotype calls were made using Illumina Genome Studio (Illumina, Inc.). The SNPs that could not be assayed successfully because of the unique chemistry on the custom Illumina assay (not designable) were genotyped using KASP Assay at LCG Genomics (Teddington, UK) and called via the KASP SNP Genotyping System. Standard quality control (QC) filters were applied,\textsuperscript{60} resulting in exclusions of SNPs with Hardy-Weinberg equilibrium (HWE) \(P < 1.0 \times 10^{-6}, \text{MAF} < 0.01\), or genotype call rates < 95\%. A total of 424 candidate SNPs were genotyped at the University of Wisconsin using the 2500 Genotyper System (Applied Biosystems, Foster City, CA). Standard QC filters were applied and genotyping was complete for 88\% of women.
SNPs and 176 AIMs passed these QC filters. For a list of 424 SNPs tested in association analyses, see the previously published Supplementary Table S1.47

Of the 2005 enrolled, DNA was requested for 1787 participants who also had data on AMD status. Of these women 1697 approved use of and had sufficient DNA for genotyping. Participants were removed from the analysis if their individual genotyping call rate was < 90% (n = 21), overall heterozygosity > 44.5% (n = 12), or genotype concordance between individuals > 95% (n = 6). These were not mutually exclusive filters and resulted in a total of 1665 CAREDS participants (98%) passing QC tests.

**Statistical Analysis**

Data management and statistical analyses were performed using a combination of SAS software version 9.2 (SAS Institute, Inc., Cary NC) and PLINK version 1.07.61 Of CAREDS participants, 98% are self-reported white. However, to minimize the risk of residual confounding due to population stratification within a sample of European ancestry, principal components analysis was conducted using 176 AIMs and the SmartPCA program in EIGENSOFT.62,63 The first two components accounted for 3.1% and 1.3% of the genotype variability, respectively, and were used to adjust for ancestry.

Single SNP associations with serum lutein and zeaxanthin were performed using linear regression, assuming an additive genetic model and adjusting for age and ancestry. In the case where <15 participants were homozygous for the minor allele, a dominant model was assumed. The SNPs associated with AMD (P ≤ 0.05) in the present model were retained for consideration in risk score.

As proof of concept for the ability of variation in lutein- and zeaxanthin-related genes to impact AMD risk beyond well-established predictors, we investigated the joint influence of the variants significantly related to AMD on the ability to classify persons correctly with AMD in this sample. The joint effect of individually significant SNPs in relation to AMD was assessed by constructing a lutein- and zeaxanthin-related genetic risk score, a linear combination of the number of risk alleles for each SNP, weighted by the respective logarithm of the odds ratio (OR).64,65 Model selection for the risk score was conducted beginning with all SNPs associated with AMD (P ≤ 0.05), and then implementing backward selection (exclusion P ≤ 0.10). Receiver operating characteristic (ROC) curves were plotted to assess whether the carotenoid-related genetic risk score significantly improved classification of AMD cases and noncases, beyond well-established AMD risk factors, including age, smoking (never, <7 pack-years, ≥7 pack-years), and complement factor H (CFH) Y402H (rs1061170) and age-related maculopathy susceptibility locus 2 (ARMS2) A69S (rs10490924) genotypes.

**RESULTS**

**Sample Characteristics**

The CAREDS sample,26 and determinants of lutein and zeaxanthin in the serum and macula28 have been described previously. In brief, the 1663 participants included in this analysis were on average 69 years of age at time of fundus photography (range, 53–86). The average body mass index was 28 kg/m² (range, 16–62), 6% reported having diabetes and only 2.4% were self-reported current smokers. There were 337 AMD cases, 91% (n = 308) of which were early stages of AMD. Distribution of AMD risk factors were consistent when comparing participants included in the present analysis (n = 1663), relative to the 342 excluded due to lack of genetic and/or phenotypic data. The exception was that women included consumed slightly less dietary lutein and zeaxanthin compared to those not included in the study (2.3 vs. 2.5 mg/d, P = 0.02).

**SNP Associations With Carotenoid Status**

Associations of SNPs from lutein and zeaxanthin pathway candidate genes related to MPOD in this sample were described previously.67 The SNPs associated with serum lutein and zeaxanthin are described in Table 1. Genes associated with MPOD and levels of these carotenoids in the serum included: β-carotene 15, 15α-monoxygenase 1 (BCMO1); ATP-binding cassette, subfamily A, member 1 (ABCA1) and subfamily G member 5 (ABCG5); scavenger receptor class B member1 (SCARB1); and retinal pigment epithelium-specific protein 65kDa (RPE65). The SNPs from candidate genes associated with MPOD, but not related to serum lutein and zeaxanthin, in this sample include genes related to xanthophyll binding in human macula (glutathione S-transferase pi 1; GSTPI), high density lipoprotein status (hepatic lipase; LIPC), long chain fatty acid status (fatty acid desaturase 1 [FADS1] and elongation of very long chain fatty acids protein 2 [ELOVL2], and maculopathies associated with MPOD aldehyde dehydrogenase 3 family, member A2 (ALDH3A2)]. Fatty acid desaturase 2 (FADS2) was related to MPOD, but not statistically significant after adjusting for other predictors. Genes associated uniquely with serum lutein and zeaxanthin (Table 1) and not MPOD were from sterol-related lipid transfer protein 3 (STARD3), ATP-binding cassette subfamily G member 8 (ABCG8), Niemann-Pick C1-like protein 1 (NPC1L1), and cholesteryl ester transfer protein (CETP). SNPs from some genes were not significantly and independently associated with MPOD on serum lutein and zeaxanthin in the present sample, but previously associated with carotenoid status in another sample (apolipoprotein E; APOE) or with genes previously associated with the accumulation of lutein and zeaxanthin in tissues of other mammals (β-carotene oxygenase 2; BCO2).55,56

**SNP Associations With AMD**

There were 24 SNPs from nine carotenoid-candidate genes associated with AMD (P ≤ 0.05) after adjusting for age and ancestry (Table 2). The SNPs with the greatest statistical significance for associations with AMD (reflecting effect size and higher minor allele frequencies) included rs2487714 downstream of ABCA1 (OR = 1.31; 95% confidence interval [CI], 1.10–1.56; P = 0.002), rs8069576 in ALDH3A2 (OR = 0.77; 95% CI, 0.64–0.92; P = 0.004), and rs2250417 in BCO2 (OR = 1.24; 95% CI, 1.04–1.48; P = 0.01). Other carotenoid-candidate genes with SNPs exhibiting significant associations with AMD included ABCG8, APOE, BCMO1, FADS2, NPC1L1, and SCARB1. Further adjusting for dietary lutein or for Y402H (CFH) and A69S (ARMS2), two well-known, strong genetic risk factors for AMD, did not significantly alter ORs and P values for each of these 24 SNPs or conclusions (data not shown).

**Genetic Risk Model**

To evaluate the potential impact of carotenoid-related genes on AMD risk, beyond known AMD genetic risk factors, we created a genetic risk score for AMD and tested the extent to which adding the risk score altered the percentage of AMD cases that
could be detected by a model containing age, smoking, Y402H, and A69S. Model selection resulted in the inclusion of the following 9 SNPs: rs2254884, rs2487714, and rs4149263 from ABCA1; rs8069576 from ALDH3A2; APOE e4; rs2250417 from BCO2; rs526126 from FADS2; rs10234070 from NPC1L1; and rs9919713 from SCARB1 (Table 3). The SNPs included in this model, the gene they are tagging, their respective weights (logarithm of the ORs) for each copy of the risk allele, and the individual statistical significance within the risk score are outlined in Table 3. The mean weighted genetic risk score was higher in AMD cases compared to noncases, 2.84 vs. 2.66, respectively \((P = 1.7 \times 10^{-5}; \text{Fig. 1})\). The odds of AMD for those in the highest quintile of genetic risk relative to the lowest quintile of risk was 3.15 (95% CI, 2.03–4.87; Fig. 2). Genes with the largest contribution to the score, based on likelihood ratio test statistic with degrees of freedom equal to number of SNPs respective to the gene were ABCA1 \((P = 6.7 \times 10^{-5})\) and SCARB1 \((P = 0.006)\).

The area under the curve (AUC) for a model containing age, smoking, CFH Y402H, and ARMS2 A69S was 0.69 (95% CI, 0.65–0.72). Addition of the genetic risk score, derived from variants in carotenoid candidate genes, significantly increased the AUC to 0.72 (95% CI, 0.69–0.75; \(P = 0.002\); Fig. 3).

### Table 3. SNPs Independently Associated With Serum Lutein and Zeaxanthin Within Each Gene, in CAREDS (N = 1643)

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotype</th>
<th>N</th>
<th>Mean Serum LZ</th>
<th>% Change From Reference</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Xanthophyll binding in retina</strong></td>
<td></td>
<td></td>
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<tr>
<td>STARD3</td>
<td>rs9892427</td>
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<td>1394</td>
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<td>Ref.</td>
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<td></td>
<td></td>
<td>AG or GG</td>
<td>249</td>
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<tr>
<td><strong>Carotenoid cleavage</strong></td>
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</tr>
<tr>
<td>BCO2</td>
<td>rs11645428</td>
<td>GG</td>
<td>726</td>
<td>0.25</td>
<td>Ref.</td>
<td>&lt;0.0001</td>
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<td></td>
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<td>732</td>
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<td>20%</td>
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<tr>
<td></td>
<td></td>
<td>AA</td>
<td>185</td>
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</tr>
<tr>
<td></td>
<td>rs6564851</td>
<td>CC</td>
<td>436</td>
<td>0.24</td>
<td>Ref.</td>
<td>&lt;0.0001</td>
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<td>391</td>
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<td>rs7500996</td>
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<tr>
<td></td>
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<td>GG</td>
<td>63</td>
<td>0.31</td>
<td>12%</td>
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<td><strong>HDL transport or status</strong></td>
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<tr>
<td>ABCA1</td>
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<td>1310</td>
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<td>1209</td>
<td>0.28</td>
<td>Ref.</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td>rs10205816</td>
<td>AA</td>
<td>838</td>
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<td>Ref.</td>
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<tr>
<td></td>
<td></td>
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<td>667</td>
<td>0.27</td>
<td>–5%</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
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<td>0.28</td>
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<tr>
<td></td>
<td>rs13405698</td>
<td>AA</td>
<td>835</td>
<td>0.28</td>
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<td>664</td>
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<td>rs4953028</td>
<td>GG</td>
<td>491</td>
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<td></td>
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<td>816</td>
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<tr>
<td></td>
<td></td>
<td>AA</td>
<td>336</td>
<td>0.27</td>
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<tr>
<td></td>
<td>rs217430</td>
<td>AA</td>
<td>954</td>
<td>0.28</td>
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<td>0.03</td>
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<td></td>
<td></td>
<td>AG</td>
<td>583</td>
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<td>3%</td>
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<tr>
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<td></td>
<td>GG</td>
<td>102</td>
<td>0.30</td>
<td>9%</td>
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</tr>
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<td>NPC1L1</td>
<td>rs217430</td>
<td>AA</td>
<td>954</td>
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<td>Ref.</td>
<td>0.03</td>
</tr>
<tr>
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<td>583</td>
<td>0.29</td>
<td>3%</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
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<td>0.30</td>
<td>9%</td>
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</tr>
<tr>
<td>CETP</td>
<td>rs708272</td>
<td>GG</td>
<td>525</td>
<td>0.29</td>
<td>Ref.</td>
<td>0.05</td>
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<tr>
<td></td>
<td></td>
<td>AA</td>
<td>313</td>
<td>0.28</td>
<td>–5%</td>
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</tr>
<tr>
<td><strong>Lipid and/or carotenoid absorption</strong></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>SCARB1</td>
<td>rs10846744</td>
<td>GG</td>
<td>1192</td>
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<td>Ref.</td>
<td>0.0001</td>
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<td></td>
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<td>0.30</td>
<td>9%</td>
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</tr>
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<td></td>
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<td>CC</td>
<td>35</td>
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<td>15%</td>
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<tr>
<td>CD36</td>
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<td>705</td>
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<td>Ref.</td>
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<td>AG</td>
<td>724</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>213</td>
<td>0.29</td>
<td>6%</td>
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</tr>
<tr>
<td><strong>Genes previously related to maculopathies</strong></td>
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<td></td>
<td></td>
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<td>RPE65</td>
<td>rs12744671</td>
<td>AA</td>
<td>1307</td>
<td>0.28</td>
<td>Ref.</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AC</td>
<td>310</td>
<td>0.27</td>
<td>–3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>22</td>
<td>0.23</td>
<td>–18%</td>
<td></td>
</tr>
</tbody>
</table>

Adjusted for WHI baseline dietary lutein and zeaxanthin and first two principal components from principal component analysis using 176 ancestry informative markers.
DISCUSSION

In this sample of over 1600 women over age 55, we observed that variation in multiple genes related to lutein and zeaxanthin physiology or status was associated with AMD. These results add to the increasing body of genetic and epidemiologic evidence suggesting a protective role of lutein and zeaxanthin against AMD, beyond evidence from previous observational studies of protective relationships between lutein and zeaxanthin in the diet or serum or macula.

Results of the present study primarily reflect relations to early AMD, which predominated among AMD cases in the present sample. Not accounting for single and multiple gene loci related to lutein and zeaxanthin in the serum and macula may have limited the ability to detect protective associations of lutein and zeaxanthin intake to early AMD in some previous studies. Protection of lutein and zeaxanthin against AMD progression is suggested by secondary, but not primary, analyses in AREDS2. In secondary analyses, participants with low dietary intake of these carotenoids at baseline and participants who consumed supplements that replaced \(\beta\)-carotene with lutein in the original AREDS formulation had lower progression from intermediate to advanced AMD.

These data strengthen the body of evidence in support of a protective role of lutein and zeaxanthin against AMD development. This is because associations of AMD with carotenoid status-related genes would not be influenced by measurement error of dietary carotenoids, nor confounded by other unknown, and unadjusted for determinants of carotenoid status and dietary and lifestyle correlates that may influence associations in observational studies. Also, polymorphisms in genes related to carotenoid status in tissues could reflect conditions that influence macular carotenoid exposure over decades and protect against early AMD development on a subclinical level, whereas levels of carotenoids in the diet and blood change over time, which can mask causal relationships to AMD.

Several common variants in carotenoid candidate genes were related to the optical density of MP or serum carotenoids in the present sample, or independent samples. In the

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>OR*</th>
<th>95% CI</th>
<th>P Value</th>
<th>Minor Allele</th>
<th>Major Allele</th>
<th>Minor Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>rs2254884</td>
<td>1.24</td>
<td>(1.03, 1.50)</td>
<td>0.02</td>
<td>C</td>
<td>A</td>
<td>0.30</td>
</tr>
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<td></td>
<td>rs2297406</td>
<td>1.21</td>
<td>(1.01, 1.46)</td>
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<td>G</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>rs2472476</td>
<td>1.22</td>
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<td>G</td>
<td>A</td>
<td>0.37</td>
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<tr>
<td></td>
<td>rs2482432</td>
<td>0.79</td>
<td>(0.66, 0.94)</td>
<td>0.01</td>
<td>G</td>
<td>A</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>rs2487714</td>
<td>1.31</td>
<td>(1.10, 1.56)</td>
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<td>A</td>
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<td>rs2515614</td>
<td>0.81</td>
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<td>0.03</td>
<td>G</td>
<td>A</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>rs2740484</td>
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<td>(0.68, 0.98)</td>
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<td>ABCG8</td>
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<td>A</td>
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<td>ALDH3A2</td>
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<td>(1.02, 1.52)</td>
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<td>G</td>
<td>C</td>
<td>0.23</td>
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<tr>
<td></td>
<td>rs2072331</td>
<td>1.25</td>
<td>(1.03, 1.53)</td>
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<td>G</td>
<td>T</td>
<td>0.23</td>
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<tr>
<td></td>
<td>rs7215</td>
<td>0.79</td>
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<td>G</td>
<td>A</td>
<td>0.46</td>
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<tr>
<td></td>
<td>rs8069576</td>
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<tr>
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<td>(0.56, 0.95)</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCMO1</td>
<td>rs16164528</td>
<td>0.80</td>
<td>(0.66, 0.97)</td>
<td>0.02</td>
<td>A</td>
<td>G</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>rs16955008</td>
<td>1.31</td>
<td>(1.02, 1.68)</td>
<td>0.04</td>
<td>A</td>
<td>C</td>
<td>0.12</td>
</tr>
<tr>
<td>BCO2</td>
<td>rs12796114</td>
<td>0.80</td>
<td>(0.65, 0.99)</td>
<td>0.04</td>
<td>C</td>
<td>A</td>
<td>0.26</td>
</tr>
<tr>
<td>FADS2</td>
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<td>1.24</td>
<td>(1.04, 1.48)</td>
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<td>G</td>
<td>A</td>
<td>0.46</td>
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<tr>
<td>NPC1L1</td>
<td>rs10234070</td>
<td>0.75</td>
<td>(0.55, 0.99)</td>
<td>0.04</td>
<td>A</td>
<td>G</td>
<td>0.11</td>
</tr>
<tr>
<td>SCARB1</td>
<td>rs9919713†</td>
<td>0.60</td>
<td>(0.36, 0.98)</td>
<td>0.04</td>
<td>A</td>
<td>T</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* OR is the per-minor allele effect (additive genetic model).
† Dominant model is assumed.

Role of Carotenoids in AMD

Table 2. SNPs Associated With AMD (P ≤ 0.05) in CAREDS After Adjusting for Age and Ancestry

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>OR*</th>
<th>95% CI</th>
<th>P Value</th>
<th>Minor Allele</th>
<th>Major Allele</th>
<th>Minor Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCC8</td>
<td>rs4148222</td>
<td>1.27</td>
<td>(1.02, 1.60)</td>
<td>0.04</td>
<td>A</td>
<td>G</td>
<td>0.16</td>
</tr>
<tr>
<td>ALDH3A2</td>
<td>rs1800869</td>
<td>1.25</td>
<td>(1.02, 1.52)</td>
<td>0.03</td>
<td>G</td>
<td>C</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>rs2072331</td>
<td>1.25</td>
<td>(1.03, 1.53)</td>
<td>0.02</td>
<td>G</td>
<td>T</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>rs7215</td>
<td>0.79</td>
<td>(0.66, 0.94)</td>
<td>0.01</td>
<td>A</td>
<td>G</td>
<td>0.46</td>
</tr>
<tr>
<td>APOE</td>
<td>e-4</td>
<td>0.73</td>
<td>(0.56, 0.95)</td>
<td>0.02</td>
<td></td>
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<tr>
<td>BCMO1</td>
<td>rs16164528</td>
<td>0.80</td>
<td>(0.66, 0.97)</td>
<td>0.02</td>
<td>A</td>
<td>G</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>rs16955008</td>
<td>1.31</td>
<td>(1.02, 1.68)</td>
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<td>A</td>
<td>C</td>
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<tr>
<td>BCO2</td>
<td>rs12796114</td>
<td>0.80</td>
<td>(0.65, 0.99)</td>
<td>0.04</td>
<td>C</td>
<td>A</td>
<td>0.26</td>
</tr>
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<td>FADS2</td>
<td>rs2250417</td>
<td>1.24</td>
<td>(1.04, 1.48)</td>
<td>0.01</td>
<td>A</td>
<td>G</td>
<td>0.46</td>
</tr>
<tr>
<td>NPC1L1</td>
<td>rs10234070</td>
<td>0.75</td>
<td>(0.55, 0.99)</td>
<td>0.04</td>
<td>A</td>
<td>G</td>
<td>0.11</td>
</tr>
<tr>
<td>SCARB1</td>
<td>rs9919713†</td>
<td>0.60</td>
<td>(0.36, 0.98)</td>
<td>0.04</td>
<td>A</td>
<td>T</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Dominant genetic model assumed.

Table 3. SNPs Included in Carotenoid Genetic Risk Score for AMD

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Beta</th>
<th>Risk Allele</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>rs2254884</td>
<td>0.27</td>
<td>C</td>
<td>1.31 (1.08, 1.59)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>rs2472476</td>
<td>0.27</td>
<td>G</td>
<td>1.31 (1.10, 1.56)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>rs4149263</td>
<td>0.27</td>
<td>G</td>
<td>1.30 (1.05, 1.62)</td>
<td>0.02</td>
</tr>
<tr>
<td>ALDH3A2</td>
<td>rs8069576</td>
<td>0.21</td>
<td>G</td>
<td>1.23 (1.03, 1.47)</td>
<td>0.02</td>
</tr>
<tr>
<td>APOE</td>
<td>e-4</td>
<td>0.29</td>
<td>G</td>
<td>1.34 (1.02, 1.76)</td>
<td>0.04</td>
</tr>
<tr>
<td>BCO2</td>
<td>rs2250417</td>
<td>0.21</td>
<td>A</td>
<td>1.24 (1.04, 1.47)</td>
<td>0.02</td>
</tr>
<tr>
<td>FADS2</td>
<td>rs526126</td>
<td>0.29</td>
<td>C</td>
<td>1.35 (1.07, 1.65)</td>
<td>0.01</td>
</tr>
<tr>
<td>NPC1L1</td>
<td>rs10234070</td>
<td>0.27</td>
<td>G</td>
<td>1.31 (0.97, 1.77)</td>
<td>0.08</td>
</tr>
<tr>
<td>SCARB1</td>
<td>rs9919713†</td>
<td>0.72</td>
<td>T</td>
<td>2.06 (1.24, 3.43)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Dominant genetic model assumed.
present sample, these SNPs only explained 5% of variation in MPOD. However, the percent variability explained could be underestimated due to our tagSNP approach, which does not include causal SNPs, exclusion of numerous unknown variants (rare and common) underlying this polygenic trait, and failure to account for interactions with other genes or environmental factors. Further exploration of joint and interacting genetic variables in relation to carotenoid status are the subject of continued investigation in this research group.

Relationships of individual tagSNPs to AMD were modest in the present study. However, when considered jointly in a risk score, there was a strong relationship with odds for AMD (OR = 3.15). Many studies have demonstrated that for complex traits, combining multiple loci, each with individually low to moderate effects, into a weighted genetic risk score improves case prediction. Our results in CAREDS are consistent with this polygenic model. Modest effects of some individual SNPs with AMD also may be explained by the indirect association they have with AMD via carotenoid status.

Improvement in Classification of AMD Risk

As further proof of concept that lutein and zeaxanthin status is related to AMD, we observed that a risk score combining nine SNPs from seven genes, which are related to levels of lutein and zeaxanthin in blood and macula, significantly increases the ability to classify AMD cases and noncases beyond four well-established AMD risk factors. While several carotenoid-related SNPs have been associated with AMD in previous studies (discussed below), variants from five genes (BCMO1, BCO2, NPC1L1, ABCG8, and FADS2) have not been related previously to AMD. We acknowledge that the criteria set for model

Figure 1. Distribution of the weighted genetic risk score in cases (red) and noncases (blue). P value for difference in mean weighted risk score between cases and noncases = 1.7 × 10⁻⁹.

Figure 2. OR (95% CI) for age-related macular degeneration in the CAREDS by quintile of the weighted genetic risk score (P value for trend across increasing quintile = 1.6 × 10⁻⁷).
selection may have influenced specific SNPs included in this model. For this reason, further evaluation of the SNPs in Table 2, in relation to early and advanced AMD risk in prospective studies, and in separate populations, is needed to continue evaluating specific SNPs, which might have predictive value in the general population.

Mechanisms Relating Genotypes to Lutein and Zeaxanthin Status and AMD

Genetic variants related to AMD in the present study were within genes related to: (1) cholesterol and carotenoid membrane transport proteins in the intestine and retina (SCARB1, NPC1L1, and ABCA1) and/or high density lipoprotein levels in blood (SCARB1, APOE, and ABCA1), (2) carotenoid cleavage enzymes (BCMO1 and BCO2), (3) omega-3 fatty acid status (FADS2), and (4) an inherited retinopathy associated with the complete absence of macular pigment (ALDH3A2). Different variants within many of these same genes were related previously to levels of lutein and zeaxanthin in serum (ABCA1, ABCG8, SCARB1, NPC1L1, and BCMO1, Table 1) or macula (ABCA1, SCARB1, BCMO1, and ALDH3A2). Because the tagSNP strategy used in CAREDS does not necessarily measure causal loci, the exact SNPs reported for associations with serum and macular levels of the carotenoids are not the same as those reported in association with AMD. The causal loci within these carotenoid-related genes, and mechanisms of their action in relation to carotenoid status and AMD risk, need to be determined.

Variants in Genes Related to Cholesterol and Carotenoid Membrane Transport

Carotenoids, like other fat soluble vitamins, are transported into the body and tissues in conjunction with membrane receptors, which influence the uptake of cholesterol and other lipids. Common variants in many genes encoding proteins related to cholesterol and carotenoid transport were related to AMD in the CAREDS sample (Table 2). Variants within SCARB1 and ABCA1 have been related to AMD in previous studies. To our knowledge, this is the first report of an association of variants in ABCG8 and NPC1L1 to AMD.

Absorption of carotenoids into the body from the intestine occurs partly by a facilitated process involving plasma membrane receptor proteins scavenger receptor class B type 1 (SR-B1), Niemann-Pick type C1 Like 1 (NPC1L1), and ATP-binding cassette transporter, subfamily A (ABCA1), which also transport cholesterol and other lipids. The SR-B1, a plasma membrane receptor for HDL encoded for by SCARB1, mediates cholesterol efflux and carotenoid uptake in the retinal pigment epithelium facilitating transport of macular xanthophylls over β-carotene. Consistently, variants in SCARB1 have been
associated with macular density of lutein and zeaxanthin in the CAREDS and serum levels of lutein in CAREDS (Table 1) and two independent cohorts.70

The gene ABCA1 encodes the adenosine triphosphate-binding cassette transporter A1 with a known role in cholesterol efflux from cells. Mutations in ABCA1 in chickens and in humans with Tangier’s disease lead to low levels of HDL.81,82 Half of xanthophyll carotenoids are carried on HDLs different from carotenoids which do not accumulate abundantly in the macula, such as β-carotene, which is carried only on LDLs.83 This may explain why mutations in this gene impact lutein transport, and result in carotenoid deficiencies in chickens.82,84 A common variant in ABCA1 (rs1929841) was associated with macular density of lutein and zeaxanthin, and different variants to serum lutein and zeaxanthin (Table 1) in this sample.47

Other HDL-related loci associated with AMD in previous studies include LPL,77,85 LIPC,74,75,77,85 CETP73,74,77 In the present study, one SNP each in LPL (rs12678919) and CETP (rs708272) were weakly associated with AMD (P = 0.2). Furthermore, a SNP within CETP was associated with serum lutein and zeaxanthin (Table 1), and one in LIPC was associated with MP75 providing evidence for potential mediation of AMD risk through lutein and zeaxanthin status.

The ABCG8 protein is part of an ABCG5/8 heterodimer complex, which is critical to sterol homeostasis. Two variants in the ABCG5 gene were independently related to levels of lutein and zeaxanthin in serum (Table 1), but none was related to MP in CAREDS. However, different variants in ABCG5 were related independently to lutein and zeaxanthin in the serum (Table 1) and macula (rs10179921).47 Allelic variation within ABCG5 has been related to plasma response to cholesterol and lutein after eating eggs.86

Different variants in NPC1L1 and ABCG8, along with variants from two other carotenoid-related genes BCMO1 and CD36, explained 25% of the variation in plasma lutein and 38% of the variation of MP optical density in a separate sample of 29 males.80 The NPC1L1 protein was demonstrated recently to be related to lutein transport in Caco-2 cells.87

Like several past studies,73,80,89 we observed that women who had one or two APOE ε4 alleles had lower risk for AMD. This seems counterintuitive, as having ε4 alleles is associated with higher risk of mortality and risk for other chronic diseases of the eye. In one previous study, having ε4 alleles was associated with higher MPOD.57 We observed ε4 alleles to be associated with lower MPOD, although this was not statistically significant. An explanation for the protective effect of ε4 on AMD that involves opposing of cellular cholesterol export from the retinal pigment epithelium and into Bruch’s membrane has been suggested.90

The protective associations with allelic variants in SCARB1 and ABCA1 to AMD in this study also might reflect processes other than carotenoid accumulation and transport. Variants in these genes have been related to serum HDL levels and the process of reverse cholesterol export, in which cholesterol from peripheral tissues is transported to the liver for elimination in bile.91 Therefore, it may be that the variants related to AMD in this study reflect this general process and the presence of conditions that prevent the accumulation of lipids in Bruch's membrane, thought to encourage the pathogenic process of AMD.32 Knock-out mice for SR-B13 fed high fat diets or ApoE deficient mice24 display retinal phenotypes characterized by subretinal lipid accumulations and damage, similar to dry AMD. In addition, new roles for HDL in inflammation have been proposed, as HDL also contains proteins associated with the acute phase response and complement regulation.91

Variants in Carotenoid Cleavage Enzyme Genes

Two variants each from BCMO1 and BCO2, both carotenoid cleavage enzymes, were associated with AMD for the first time in the present study. The β,β-carotene 15,15'-monooxygenase 1 is a cytosolic enzyme that cleaves symmetrically and β,β-carotene 9',10'-dioxygenase 2 is a mitochondrial enzyme that cleaves asymmetrically, resulting in different cleavage products with numerous, incompletely understood biological effects; one well-known product is retinal from cleavage of pro-vitamin A carotenoids, like β-carotene.95,96 In the present sample, having one or two A alleles in rs11645428 (BCMO1) was associated with higher levels of lutein and zeaxanthin in serum (Table 1) and the macula,57 and a 20% lower odds for AMD. Women with another variant (rs6564851) related to higher serum (Table 1) and macular levels75 also were less likely to have AMD, but this was not statistically significant (P = 0.16). Having these two BCMO1 variants were associated previously with higher circulating levels of lutein and zeaxanthin in other samples,75 and higher catalytic activity of the BCMO1 enzyme in women;98 that is, higher conversion of β-carotene to retinal leading to lower circulating levels of β-carotene. These SNPs are located upstream from the coding region of BCMO1, a region that may alter transcriptional activity.

Although lutein and zeaxanthin are not thought to be substrates for BCMO1, even low levels of specificity for this substrate might lead to cleavage products that are responsible for a protective association with AMD. However, the body of evidence currently suggests an alternative explanation: a protective effect on AMD might be indirect and related to biological competition between pro-vitamin A carotenoids, like β-carotene, and xanthophyll carotenoids for absorption in the intestine and into tissues. Several BCMO1 variants associated with high circulating levels of β-carotene are associated with low circulating levels of lutein and zeaxanthin.97

The opposing relationships between serum levels of β-carotene and macular carotenoids are consistent with observations in some studies that β-carotene supplementation leads to lower circulating levels of lutein and zeaxanthin.23,99 Further evidence of interactions between xanthophyll carotenoids and β-carotene in relation to AMD risk were observed in secondary analyses conducted in AREDS2; lutein supplementation without β-carotene, compared to with β-carotene, was associated with lower risk for progression of advanced AMD.23 Associations of BCMO1 to AMD also might partly explain some inconsistency in observed relationships between diet lutein and AMD in past studies. Data in CAREDS suggests that variants in this gene may influence the macular response to dietary macular carotenoids: Women with lutein intakes in the lowest tertile (having a median lutein and zeaxanthin intake of 1.0 mg/d) and two A alleles for rs11645428 had a higher mean MP optical density than those with one or no A alleles (0.40 ± 0.03, 0.32 ± 0.02, and 0.31 ± 0.02, respectively; P < 0.01).47 Moreover, MP in women with two A alleles, despite low intake of lutein and zeaxanthin, was not significantly different than MP in women with much higher intakes (median 2.1 and 4.1 mg/d in the in the second and third tertiles for intake of lutein and zeaxanthin).47 This suggests the hypothesis that having either two A alleles for BCMO1 rs11645428 or intake of lutein and zeaxanthin > 2 mg/d is associated with higher carotenoids in the serum, macula, and with lower risk for AMD. Further, supplementation with macular carotenoids might lower AMD risk to a greater extent in persons without A alleles for rs11645428. This remains to be tested in trials.

Having two minor alleles for rs2250417 in BCO2 was associated with almost a 50% increased risk for AMD. Having one or two minor alleles for rs12796114 was associated with an approximately 25% lower risk for AMD. Lutein and
Role of Carotenoids in AMD

may protect against this.10 The fact that oxidative stress is known to induce oxidative stress in the mitochondria, and BCO2 variants in culture indicate that excess carotenoids can impair respiration to known antiinflammatory effects of omega-3 fatty acids. 115 Common BCO2 leptin, suggesting an obese phenotype.104 Obesity is known to be a state of chronic low-grade inflammation. 105 Common variants in BCO2 and IL8, which is near BCO2, also were related to serum concentrations of inflammatory cytokines in genome-wide association studies.106 One of these, rs2115763 in BCO2, is in weak linkage disequilibrium with rs2250417 (r2 = 0.51 in HapMap CEU population), which we report here to be associated with AMD.

Variants Related to the Synthesis of Long-Chain Omega-3 Fatty Acids

A variant in one gene that encodes an essential enzyme for the synthesis of docosahexaenoic acid (DHA)107 was associated with AMD in the present study. Associations between variants in this gene, FADS2, and AMD have not been reported previously, to our knowledge. Previous evidence suggests that this association might be related to better accumulation of macular pigments. Dietary omega-3 fatty acid intake and variants in other genes associated with long-chain fatty acid synthesis (FADS1 and ELOVL2) were associated with higher MP in this study sample 47 and higher plasma levels of long chain omega-3 fatty acids are related to higher MP in a separate sample.50 In one human trial, a trend for MP accretion in the foveal center was observed when DHA108 was added to lutein and zeaxanthin supplements. Results of previous studies suggest that DHA increases HDL and HDL subfractions,109–111 and zeaxanthin supplements. Results of previous studies suggests that these associations might reflect lower oxidative stress and damage to retinal pigment epithelium mitochondria. New evidence in mice suggests that BCO2 might be important for vitamin A synthesis from asymmetric carotenoids, like β-cryptoxanthin.101

Common BCMO1 and BCO2 variants also may be related to AMD via mechanisms involving an influence of activity of the enzymes they encode on lipid homeostasis and inflammation, although these lines of evidence are early in development. A BCMO1 variant previously identified as related to serum carotenoid levels, recently was associated with HDL in two populations.102 BCMO1103 and BCO2100 knockout mice have been observed to have hepatic lipid accumulation. The BCMO1-deficient mice have elevated levels of insulin and leptin, suggesting an obese phenotype.104 Obesity is known to be a state of chronic low-grade inflammation.105 Common variants in BCO2 and IL8, which is near BCO2, also were related to serum concentrations of inflammatory cytokines in genome-wide association studies.106 One of these, rs2115763 in BCO2, is in weak linkage disequilibrium with rs2250417 (r2 = 0.51 in HapMap CEU population), which we report here to be associated with AMD.

It is unclear whether a lack of MP could be a cause or consequence of mutation or genotypic variation in the ALDH3A2 gene.

Limitations

In addition to limitations addressed above, the following limit conclusions that can be drawn from evidence presented here. This study included only women and mainly persons of self-reported European ancestry. In addition, the availability of prevalent (existing) AMD as an outcome, rather than incident (newly developed AMD after exposure assessment) could result in associations that reflect confounding related to survivor bias or change in carotenoid exposure subsequent to AMD development. Further work is warranted in large, prospective studies of older men and women of varied ancestry to understand more fully the impact genes related to carotenoid status have on AMD status. In addition, there is a need to understand further the modifying influence, if any, of other suspected AMD risk factors.

Summary

The results demonstrated associations between genetic determinants of serum or macular carotenoids and AMD, which are independent of dietary lutein and zeaxanthin. Thus, they provided independent evidence to strengthen the existing body of evidence suggesting a role of carotenoids in the prevention of AMD, and provide direction for future work to better understand the direct and indirect effects these carotenoid-related genes have on AMD. The degree to which these observations have value in clinical prediction of AMD remains to be determined.
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