Clinical and Epidemiologic Research

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


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Purpose. We tested variants in genes related to lutein and zeaxanthin status for association 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 357 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). Increasing evidence suggests the dietary carotenoids lutein and zeaxanthin protect against pathogenic processes of AMD by absorbing an estimated 40% to 90% of incident blue light otherwise damaging the macula, and lowering oxidative stress and inflammation. Systemic antioxidant and
antiinflammatory effects of lutein also have been suggested, 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 and by secondary, but not primary, analyses in the Age-Related Eye Disease Study 2 (AREDS2), a multicenter, randomized controlled clinical trial of lutein and zeaxanthin supplements, and progression of AMD individuals with intermediate or advanced disease. A protective influence of lutein and zeaxanthin intake on early AMD sometimes, but not always, 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. 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 genetic factors. Results of a recent twin study suggest 27% of macular response to dietary carotenoids is heritable. Studies in animal models and humans support a role for genetic variation in determining carotenoid status in the retina or serum (see prior reviews). 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, and retinopathies associated with impaired macular pigment. These SNPs were studied previously for their relation to MP optical density. Relationships to serum concentration of lutein and zeaxanthin are reported 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. 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. 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 druse (≥125 µ) or extensive intermediate drusen (total area ≥ 565 µ when soft indistinct drusen were present or ≥ 650 µ when soft indistinct drusen were absent). 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.

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 as described previously.

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. 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 southeastern-Ashkenazi Jewish ancestry clines.

The SNPs were genotyped at Case Western Reserve University (Cleveland, OH) using an Illumina Custom Golden-Gate 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, resulting in exclusions of SNPs with Hardy-Weinberg equilibrium (HWE) > P < 1.0 × 10⁻⁷, MAF < 0.01, or genotype call rates < 95%. A total of 424 candidate
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 their individual genotyping call rate (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 357 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.47 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, 15′-monooxygenase 1 (BCMO1); ATP-binding cassette, subfamily A, member 1 (ABCA1) and subfamily G member 5 (ABCG5); scavenger receptor class B member 1 (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; GSTP1), 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 stAR-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 or serum lutein and zeaxanthin in the present sample, but previously associated with carotenoid status in another sample (apolipoprotein E; APOE65) 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), 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 357 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).

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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 ($P = 1.7 \times 10^{-5}$; 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>
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<td>Xanthophyll binding in retina</td>
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<td>STARD3</td>
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<td>838</td>
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<td>667</td>
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<td>AA</td>
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<td>–5%</td>
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<tr>
<td>SCARB1</td>
<td>rs10846744</td>
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<td>1192</td>
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<td>CC</td>
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<td>705</td>
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<td>GG</td>
<td>213</td>
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<td>6%</td>
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<tr>
<td>Genes previously related to maculopathies</td>
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<td>CC</td>
<td>22</td>
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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 β-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>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>rs2487714</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 (1.04, 1.76)</td>
<td>0.04</td>
</tr>
<tr>
<td>BCO2</td>
<td>rs2254047</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>1.33 (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.64,65 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.](image1)

![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).](image2)
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
Variants in Carotenoid Cleavage Enzyme Genes

Two variants each from \textit{BCMO1} and \textit{BCO2}, both carotenoid cleavage enzymes, were associated with AMD for the first time in the present study. The \(\beta\)-\textit{carotene}, 15,15'-
\textit{monooxygenase} 1 is a cytosolic enzyme that cleaves symmetrically and \(\beta\)-\textit{carotene}, 9',10'-
\textit{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 \textit{pro-vitamin A} carotenoids, like \(\beta\)-\textit{carotene}. \(P < 0.01\).

In the present sample, having one or two \textit{A} alleles in \textit{rs11645428} \textit{(BCMO1)} was associated with higher levels of lutein and zeaxanthin in serum (Table 1) and the macula, \(P < 0.01\), and a 20% lower odds for AMD. Women with another variant \textit{(rs6564851)} related to higher serum vitamin A carotenoids, like \(\beta\)-\textit{carotene}, and xanthophyll carotenoids for absorption in the intestine and into tissues. Several \textit{BCMO1} variants associated with high circulating levels of \(\beta\)-\textit{carotene} are associated with low circulating levels of lutein and zeaxanthin. \(P < 0.01\).

The opposing relationships between serum levels of \(\beta\)-\textit{carotene} and macular carotenoids are consistent with observations in some studies that \(\beta\)-\textit{carotene} supplementation leads to lower circulating levels of lutein and zeaxanthin. \(P < 0.01\). Further evidence of interactions between xanthophyll carotenoids and \(\beta\)-\textit{carotene} in relation to AMD risk were observed in secondary analyses conducted in AREDS2; lutein supplementation without \(\beta\)-\textit{carotene}, compared to with \(\beta\)-\textit{carotene}, was associated with lower risk for progression of advanced AMD. \(P < 0.01\).

Associations of \textit{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 \textit{A} alleles for \textit{rs11645428} had a higher mean MP optical density than those with one or no \textit{A} alleles \((0.34 \pm 0.03, 0.32 \pm 0.02, \text{ and } 0.31 \pm 0.02, \text{ respectively}; P < 0.01)\). Moreover, MP in women with two \textit{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). \(P < 0.01\).

Two minor alleles for \textit{rs2250417} in \textit{BCO2} was associated with almost a 50% increased risk for AMD. Having one or two minor alleles for \textit{rs1279611} was associated with an approximately 25% lower risk for AMD. Lutein and zeaxanthin are not thought to be substrates for \textit{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 \textit{pro-vitamin A} carotenoids, like \(\beta\)-\textit{carotene}, and xanthophyll carotenoids for absorption in the intestine and into tissues. Several \textit{BCMO1} variants associated with high circulating levels of \(\beta\)-\textit{carotene} are associated with low circulating levels of lutein and zeaxanthin. \(P < 0.01\).
zeaxanthin, and other carotenoids with a hydroxylated β-ionone ring (such as cryptoxanthin) are substrates for the BCO2 enzyme. Moreover, in mammals, BCO2 mutations result in accumulation of lutein and zeaxanthin in skin of livestock. 55,56 However, in the present study, BCO2 variants appear to be implicated to lutein and zeaxanthin accumulation in the blood and macular pigment, suggesting tissue specific influences. Studies in BCO2-deficient mice and human cells in culture indicate that excess carotenoids can impair respiration and induce oxidative stress in the mitochondria, and BCO2 may protect against this. 10 The fact that oxidative stress is known to promote AMD and the demonstrated role for BCO2 in limiting oxidative stress in mitochondria 100 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. 106

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 BCMO1 103 and BCO2 200 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 a proinflammatory cytokine 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 DHA 108 was added to lutein and zeaxanthin supplements. Results of previous studies suggest that DHA increases HDL and HDL subfractions, 109-111 so a mechanism for better accretion of macular pigments might be secondary to increased transport of lutein into the macula in HDLs. Alternatively, lower status for omega-3 fatty acids may have influenced the foveal architecture, which subsequently influenced the ability to accumulate macular pigments. In rhesus monkeys, dietary levels of omega-3 fatty acids affected the retinal pigment epithelium cell density and the response to xanthophyll supplementation. 112

An alternative explanation for relationships of FADS2 variants and AMD could be direct protection by DHA against the development or progression of AMD as has been observed in previous observational studies. 113,114 This may be secondary to known antiinflammatory effects of omega-3 fatty acids. 115 Although omega-3 supplements, with or without lutein did not prevent the progression to advanced AMD in the AREDS2 study in people supplementing with high dose antioxidants, 109 this study may have been too short (5 years), to observe a protective effect of long chain omega-3 fatty acids on disease progression. The FADS2 variants (and other unknown genetic influences on omega-3 fatty acids status) could influence DHA status over a lifetime. Whether this is related to or independent of macular carotenoids requires further study.

Variants Associated With Age-Related Maculopathies

Results of the present analysis of four common variants in ALDH3A2 related to AMD are consistent with a concurrent analysis in the AREDS cohort > 65 years of age in which different sequence variants in the same gene were observed to be related to advanced AMD over 12-years of follow-up. 116 Full search of results from the recent AMD Consortium analysis revealed two SNPs reported in Table 1 (rs1800869 and rs2072331) replicated with marginally significant associations with advanced AMD (P = 0.05), and consistent direction of effect. 117 Rare mutations in the ALDH3A2 gene result in Sjögren-Larsson Syndrome. 118 This condition results in neural and cutaneous defects as well as in macular dystrophy in the retinal ganglion cells and inner plexiform layer, and complete lack of macular pigment despite normal levels of carotenoids in serum. 119 We observed common variants within this gene related to MP, but not to levels of serum carotenoids in the present sample, suggesting influence is limited to the macula. 47 This gene encodes a lipid metabolic enzyme (aldehyde dehydrogenase 3 family member 2 protein), which catalyzes the oxidation of a variety of short and medium chain fatty aldehydes to fatty acids. In SLS patients, fatty aldehydes and alcohols accumulate in body tissues, and are thought to lead to Mueller cell degeneration with possible photooxidative stress as a result of a lack of macular pigment. 115 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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