

Genetic Determinants of Macular Pigments in Women of the Carotenoids in Age-Related Eye Disease Study

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PURPOSE. To investigate genetic determinants of macular pigment optical density in women from the Carotenoids in Age-Related Eye Disease Study (CAREDS), an ancillary study of the Women's Health Initiative Observational Study.

METHODS. 1585 of 2005 CAREDS participants had macular pigment optical density (MPOD) measured noninvasively using customized heterochromatic flicker photometry and blood samples genotyped for 440 single nucleotide polymorphisms (SNPs) in 26 candidate genes related to absorption, transport, binding, and cleavage of carotenoids directly, or via lipid

transport. SNPs were individually tested for associations with MPOD using least-squares linear regression.

RESULTS. Twenty-one SNPs from 11 genes were associated with MPOD ($P \leq 0.05$) after adjusting for dietary intake of lutein and zeaxanthin. This includes variants in or near genes related to zeaxanthin binding in the macula (*GSTP1*), carotenoid cleavage (*BCMO1*), cholesterol transport or uptake (*SCARB1*, *ABCA1*, *ABCG5*, and *LIPC*), long-chain omega-3 fatty acid status (*ELOVL2*, *FADS1*, and *FADS2*), and various maculopathies (*ALDH3A2* and *RPE65*). The strongest association was for rs11645428 near *BCMO1* ($\beta_A = 0.029$, $P = 2.2 \times 10^{-4}$). Conditional modeling within genes and further adjustment for other predictors of MPOD, including waist circumference, diabetes, and dietary intake of fiber, resulted in 13 SNPs from 10 genes maintaining independent association with MPOD. Variation in these single gene polymorphisms accounted for 5% of the variability in MPOD ($P = 3.5 \times 10^{-11}$).

CONCLUSIONS. Our results support that MPOD is a multi-factorial phenotype associated with variation in genes related to carotenoid transport, uptake, and metabolism, independent of known dietary and health influences on MPOD. (*Invest Ophthalmol Vis Sci.* 2013;54:2333-2345) DOI:10.1167/iov.12-10867

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The carotenoids lutein (L) and zeaxanthin (Z) from the diet, and meso-Z, synthesized in the retina from L,¹ concentrate in the inner retinal layer of the macula² where they comprise macular pigment. The density³ and concentration^{4,5} of these macular carotenoids vary more than 10-fold among individuals. There is interest in mechanisms to enhance macular pigment because of emerging evidence that they are important to ocular health. L and Z may lower the risk and/or delay onset of the common and costly condition of AMD.⁶⁻⁸ This may be due to the ability of macular pigment to absorb short wavelength (blue) light; depending on the density of the pigment, it is estimated that 40% to 90% of blue light is absorbed by these pigments.⁹ Blue light absorption may limit oxidative stress, photochemical damage, or damage due to the formation of photosensitizing pigments in the retina as byproducts of the visual cycle, which might otherwise act as inflammatory triggers. These carotenoid pigments are also thought to have other protective roles in the macula, including antioxidant and anti-inflammatory effects directly^{10,11} and indirectly.¹² Independent of protection against development or worsening of AMD, macular pigment might also contribute to better visual performance.¹³⁻¹⁷

Understanding the predictors of macular pigment optical density (MPOD) in large samples is one way to understand

conditions that favor the accretion of these carotenoids in the retina. Previous studies in the Carotenoids in Age-Related Eye Disease Study (CAREDS) indicated that women with higher levels of L and Z in diet or serum, have higher MPOD.³ These factors account for only 8% of the variability in MPOD, indicating the influence of other factors. Consistent with other factors playing an auxiliary role, the blood^{18–20} and retinal^{1,21–36} response to oral supplementation is known to be highly variable.

Results of a previous study in twins suggest that genetic factors may explain 67% of variability in MPOD³⁷ and 27% of variability in response to supplementation over 6 months.³⁴ Variation in candidate genes may influence the ability to accumulate macular pigments.³⁸ Candidates include genes encoding proteins that (1) bind carotenoids in the retina,^{39–41} (2) cleave carotenoids or influence metabolism of vitamin A,^{38,42–44} (3) have joint roles in transport of carotenoids and lipids through plasma membranes in the retina^{45,46} and intestine,^{46,47} (4) influence the circulating levels of high density lipoprotein (HDL) molecules^{48,49} that carry approximately half of circulating macular carotenoids,^{50,51} (5) influence omega-3 fatty acid levels in the blood or retina,^{52,53} and/or (6) genes that have been previously related to both macular pigment density and maculopathies.^{54–56}

The main objective of this paper is to examine and provide a broad overview of the hypothesized genetic determinants of MPOD in the CAREDS, an ancillary study of the Women's Health Initiative (WHI). CAREDS was designed to investigate the relationships of L and Z in the diet, serum, and retina to AMD^{57,58} and cataract presence and severity among postmenopausal women with low and high LZ intakes at three of the WHI clinical sites.⁵⁹

METHODS

All participants provided informed consent for participation in CAREDS, approval for research was granted by the institutional review boards at each participating university, and all procedures conformed to the Declaration of Helsinki. Detailed description of methods can be found in Supplementary Material S1 (see Supplementary Material and Supplementary S1, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10867/-DCSupplemental>). In brief, 1585 women of the 2005 women in CAREDS with macular pigment measures and genotype data were included in the present study. Previous manuscripts provide detailed descriptions of CAREDS design and sampling,³ macular pigment measurement protocols,⁶⁰ measures of serum carotenoids,³ and measures of dietary and lifestyle factors.³ A total of 473 SNPs tagging variation (minor allele frequency [MAF] \geq 5%, $r^2 \geq$ 0.80) from 26 candidate genes for carotenoid status, and 190 ancestry informative markers,⁶¹ were genotyped. After standard quality control procedures (see Supplementary Material and Supplementary S1, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10867/-DCSupplemental>), 440 SNPs were tested for association with MPOD (see Supplementary Material and Supplementary Table S2, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10867/-DCSupplemental> for complete list of SNPs). The focus of this report is on MPOD at 0.5° from foveal center, the location at which the within to between variability of MPOD was the lowest⁶⁰ and is most commonly measured across studies. Associations between SNPs and other eccentricities of MPOD (at 0.25°, 1°, and 1.75°) were examined, but were generally similar (data not shown) and will be the focus of future investigations. The ϵ 2, ϵ 3, and ϵ 4 alleles of apolipoprotein E (*APOE*) were measured as haplotypes of SNPs rs429358 and rs7412. Association analysis was performed using linear regression as implemented in PLINK version 1.07,⁶² assuming an additive genetic model and adjusting for global (genome-wide) ancestry, via the first two principal components, and LZ intake (from diet and supplements). Further adjustments for other

determinants of MPOD were tested to ascertain direct SNP effects on MPOD. Because of correlation between SNPs within a gene, conditional modeling, using forward selection, was performed to ascertain SNPs with independent effects within the gene. SNPs associated with MPOD were tested for interactions with LZ intake and waist circumference, two strong, modifiable predictors of MPOD. Statistical interactions were tested using an interaction term in the linear regression model where the SNP is coded additively and the environmental factor continuous. When the one degree of freedom test for interaction was suggestive ($P < 0.20$), SNP-MPOD associations were stratified by tertile of environmental variable to qualitatively understand dependency of SNP effects by waist circumference or dietary intake.

RESULTS

Sample Characteristics and Predictors of MPOD

In this sample of 1585 women, over 97% of which were self-reported Caucasian, the mean (\pm SD) MPOD at 0.5° from the foveal center in the right eye was 0.36 (\pm 0.21). Women were, on average 69 years of age, had a body mass index of 28 kg/m², and waist circumference of 88 cm. Six percent reported having diabetes, 45% were using hormone therapy at the time of MPOD measurement, 26% were using cholesterol lowering medication, and only 3% were self-reported current smokers. Correlates of MPOD in this subset of CAREDS participants, after adjustment for LZ intake, (Table 1) were consistent with previously reported predictors of MPOD in the CAREDS sample.³ Additional variables considered as potential physiological determinants of MPOD since our previous publication in 2006, informed either through new literature or the current analysis, included serum triglycerides ($r = -0.07$, $P = 0.003$), serum measures of β -carotene³ ($r = 0.13$, $P < 0.001$), C-reactive protein ($r = -0.07$, $P = 0.03$), and 25-hydroxyvitamin D⁶³ ($r = 0.05$, $P = 0.08$), the Healthy Eating Index-2005 score ($r = 0.08$, $P < 0.001$) and Healthy Lifestyle Score⁶⁴ ($r = 0.008$, $P = 0.001$).

Multivariable analyses of modifiable factors that might be physiologically related to lower MPOD resulted in the following model of independent predictors of MPOD (in order of model entry): LZ intake, serum LZ, waist circumference, dietary fiber, and diabetes status at CAREDS. This model explained 9.5% of the variability in MPOD and determined the full multivariable adjustment model used to test whether SNPs were associated with MPOD directly or indirectly via influence on these other determinants.

Overview of SNP Associations with MPOD

Adjusting for LZ intake and ancestry, 21 SNPs from 11 genes were associated with MPOD at P less than or equal to 0.05. Conditional modeling within each gene resulted in 14 SNPs from 11 genes maintaining independent associations with MPOD (Table 2). Further adjustment for other MPOD predictors had little or no influence. The few exceptions are noted below.

A multivariable SNP model containing 13 SNPs significantly associated with MPOD independent of other known predictors was able to explain 5.1% of the variability in MPOD (Table 3). SNPs contributing to the most variability included two in or near *BCMO1* and three in *SCARB1*, the gene for scavenger receptor class B, member 1. Jointly, these *BCMO1* and *SCARB1* SNPs explained approximately 2.3% of the variability in MPOD. Detailed results for SNPs from each class of candidate gene are described below. SNP associations were consistent across tertile of the largest two modifiable determinants of MPOD, LZ intake and waist circumference, with no evidence for statistical

TABLE 1. Mean Macular Pigment Optical Density (MPOD) by Level of Various Potential Predictors

Variable	N of Subjects	Adjusting for Dietary L and Z*		Additional Adjustment* for Waist Circumference, Diabetes Status, Fiber Intake, and Serum L and Z and 13 SNPs Associated with MPOD	
		Mean MPOD	% Diff. from Ref.	Mean MPOD	% Diff. from Ref.
Demographics, anthropometrics, and medical history					
Age group, y					
≤69	801	0.36	Ref.	0.36	Ref.
70 to 74	386	0.38	5	0.39	7
≥75	398	0.34	−5	0.34	−5
P value†		0.81		0.81	
Waist circumference, cm, quartile (median)					
1 (73.7)	440	0.40	Ref.	0.39	Ref.
2 (83.8)	378	0.39	−4	0.37	−3
3 (91.4)	361	0.34	−14	0.35	−10
4 (104.1)	406	0.31	−22	0.34	−12
P value†		<.0001		<.0001	
Presence of diabetes					
No	1482	0.37	Ref.	0.37	Ref.
Yes	103	0.28	−24	0.30	−17
P value†		<.0001		0.003	
Study visit site					
Iowa	535	0.38	Ref.	0.38	Ref.
Oregon	515	0.39	2	0.38	0
Wisconsin	535	0.32	−15	0.32	−16
P value†		<.0001		<.0001	
Education, WHI baseline					
High school	355	0.34	Ref.	0.35	Ref.
College	776	0.36	5	0.36	4
Postcollege	454	0.38	10	0.36	3
P value†		0.02		0.48	
Diet and lifestyle					
Dietary L+Z, μg, quintile (median)					
1 (797.9)	317	0.31	Ref.	0.33	Ref.
2 (1383.1)	317	0.33	8	0.34	2
3 (2120.5)	317	0.38	24	0.38	14
4 (3121)	317	0.39	27	0.37	12
5 (5330.9)	317	0.41	32	0.38	14
P value†		<.0001		0.18	
Physical activity, MetHrs/wk‡					
<3	369	0.34	Ref.	0.37	Ref.
3 to 10	348	0.36	5	0.37	0
10 to 21	440	0.36	4	0.35	−5
≥21	417	0.39	12	0.36	−3
P value†		0.14		0.09	
Dietary fiber, g/d, quintile (median)					
1 (7.9)	316	0.31	Ref.	0.33	Ref.
2 (12.1)	317	0.36	14	0.36	10
3 (16.0)	316	0.36	15	0.35	7
4 (20.1)	317	0.39	25	0.38	16
5 (27.0)	316	0.39	26	0.38	15
P value†		<.0001		0.004	
Dietary saturated fat, % of energy, quintile (median)					
1 (7.6)	316	0.38	Ref.	0.35	Ref.
2 (9.8)	317	0.38	0	0.37	5
3 (11.5)	316	0.36	−6	0.36	4
4 (13.6)	317	0.36	−6	0.36	3
5 (16.6)	316	0.35	−9	0.37	5
P value†		0.01		0.82	

TABLE 1. Continued

Variable	N of Subjects	Adjusting for Dietary L and Z*		Additional Adjustment* for Waist Circumference, Diabetes Status, Fiber Intake, and Serum L and Z and 13 SNPs Associated with MPOD	
		Mean MPOD	% Diff. from Ref.	Mean MPOD	% Diff. from Ref.
Dietary polyunsaturated fat, % of energy, quintile (median)					
1 (4.34)	316	0.34	Ref.	0.33	Ref.
2 (5.66)	317	0.37	11	0.37	13
3 (6.67)	316	0.37	11	0.37	13
4 (7.82)	317	0.37	11	0.38	15
5 (9.78)	316	0.36	9	0.37	12
P value†		0.21		0.03	
Dietary total n-3 fatty acids, g/d, quintile (median)					
1 (0.61)	316	0.34	Ref.	0.35	Ref.
2 (0.91)	317	0.36	5	0.36	5
3 (1.16)	316	0.37	10	0.36	5
4 (1.49)	317	0.37	8	0.36	4
5 (2.10)	316	0.38	11	0.37	8
P value†		0.05		0.23	
Dietary retinol, µg/d, quintile (median)					
1 (196.2)	316	0.37	Ref.	0.37	Ref.
2 (338.8)	317	0.37	1	0.37	0
3 (479.0)	316	0.36	-1	0.36	-4
4 (641.4)	317	0.36	-2	0.36	-4
5 (919.1)	316	0.35	-4	0.35	-7
P value†		0.15		0.07	
Total HEI-2005 score, quintile (median)					
1 (61.4)	317	0.34	Ref.	0.37	Ref.
2 (67.0)	317	0.36	4	0.36	-2
3 (70.5)	317	0.36	4	0.36	-2
4 (73.5)	317	0.37	7	0.36	-2
5 (77.7)	317	0.39	15	0.37	1
P value†		0.001		0.70	
Healthy lifestyle score					
0 to 2	415	0.34	Ref.	0.37	Ref.
3	426	0.36	7	0.36	-2
4	368	0.37	8	0.36	-3
5	287	0.38	13	0.36	-3
6	89	0.40	18	0.35	-4
P value†		0.001		0.44	
Serum analytes					
L+Z, trans, µmol/L, quintile (median)					
1 (0.15)	313	0.25	Ref.	0.28	Ref.
2 (0.22)	314	0.35	36	0.35	27
3 (0.29)	313	0.37	46	0.37	32
4 (0.37)	313	0.41	59	0.39	40
5 (0.50)	314	0.44	71	0.42	51
P value†		<.0001		<.0001	
Retinol, µmol/L, quintile (median)					
1 (1.84)	313	0.36	Ref.	0.36	Ref.
2 (2.20)	314	0.35	-2	0.35	-4
3 (2.50)	313	0.38	8	0.39	7
4 (2.80)	314	0.37	4	0.36	-1
5 (3.31)	313	0.36	0	0.36	-1
P value†		0.96		0.70	
Retinyl palmitate, µmol/L, quintile (median)					
1 (0.0004)	195	0.37	Ref.	0.37	Ref.
2 (0.0010)	193	0.36	-3	0.36	-2
3 (0.0019)	191	0.38	3	0.38	2
4 (0.0032)	194	0.40	8	0.40	7

TABLE 1. Continued

Variable	N of Subjects	Adjusting for Dietary L and Z*		Additional Adjustment* for Waist Circumference, Diabetes Status, Fiber Intake, and Serum L and Z and 13 SNPs Associated with MPOD	
		Mean MPOD	% Diff. from Ref.	Mean MPOD	% Diff. from Ref.
5 (0.0070)	193	0.34	-7	0.33	-10
<i>P</i> value†		0.13		0.03	
Beta-carotene, all trans, μmol/L, quintile (median)					
1 (0.23)	313	0.29	Ref.	0.34	Ref.
2 (0.40)	314	0.36	21	0.37	9
3 (0.60)	313	0.38	30	0.37	11
4 (0.90)	314	0.39	31	0.36	8
5 (1.52)	313	0.40	37	0.37	12
<i>P</i> value†		<.0001		0.31	
Triglyceride, μmol/L, quintile (median)					
1 (0.90)	316	0.36	Ref.	0.34	Ref.
2 (1.25)	315	0.39	8	0.38	12
3 (1.57)	305	0.37	3	0.36	8
4 (2.07)	319	0.38	7	0.39	17
5 (2.96)	310	0.32	-12	0.34	0
<i>P</i> value†		0.003		0.48	
Vitamin D, adjusted for blood drawn month, nmol/L, quintile (median)					
1 (30.4)	235	0.35	Ref.	0.37	Ref.
2 (44.5)	236	0.36	2	0.37	0
3 (55.7)	235	0.36	3	0.36	-2
4 (67.2)	236	0.38	9	0.37	1
5 (84.9)	235	0.38	7	0.35	-6
<i>P</i> value†		0.07		0.52	
C-reactive protein, mg/L, quintile (median)					
1 (0.7)	221	0.38	Ref.	0.35	Ref.
2 (1.5)	219	0.38	-1	0.35	0
3 (2.7)	216	0.36	-6	0.36	3
4 (4.8)	225	0.36	-5	0.37	5
5 (9.9)	221	0.35	-8	0.38	8
<i>P</i> value†		0.03		0.35	

Diff., different.

* Exception to adjustment variables are when the respective variable is that being tested.

† *P* values are for trend of MPOD across continuous variable or categorical, where appropriate.

‡ MetHrs/Week is h/wk spent doing an activity by the intensity (the fold increase the activity is estimated to increase metabolic activity [oxygen consumption] relative to the resting state).

interactions between SNPs and either LZ intake or waist circumference ($P_{interaction} > 0.20$), except where noted below.

Xanthophyll-Specific Carotenoid Binding Proteins.

One SNP, rs675679, near *GSTP1*, the gene for a human ocular Z-specific binding protein⁴⁰ was associated with MPOD independent of LZ intake ($P = 0.03$). The test for statistical interaction between rs675679 and LZ intake was suggestive ($P_{interaction} = 0.04$). Stratifying women by tertile of LZ intake demonstrated that while the direction of SNP effects were consistent, the SNP association was most significant at the lowest level of intake (Fig. 1). A combination of the two risk effects (low LZ intake and homozygosity for the common allele, AA) resulted in significant deviation from additivity; these women had 26% lower MPOD compared with women with at least one C allele and highest intake (Fig. 1). No significant relationships with MPOD were identified for SNPs in *STARD3*, the gene for StAR-related lipid transfer domain containing 3, a human ocular lutein specific binding protein,³⁹ even though the major allele (A) of rs9892427 was related to higher levels of LZ in serum ($P = 0.006$).

Carotenoid Cleavage and Retinoid Related Genes. Five SNPs in or near *BCMO1* were associated with MPOD (rs11645428, rs6564851, rs16955008, rs6564863, and rs9924371). The strongest associations were for two SNPs in weak linkage disequilibrium ($r^2 = 0.51$) upstream of *BCMO1*, rs11645428 ($P = 2.2 \times 10^{-4}$), and rs6564851 ($P = 0.003$). After adjustment for serum LZ concentration, the effects of these two SNPs on MPOD were modestly attenuated because both rs11645428 and rs6564851 were strongly associated with serum LZ in CAREDS ($P = 8.5 \times 10^{-25}$ and $P = 7.2 \times 10^{-23}$, respectively). Three other SNPs in *BCMO1* (rs9924371, rs6564863, and rs16955008) were associated with MPOD independent of serum LZ. However, conditional modeling of all five *BCMO1* SNPs suggests correlation of effects, as only rs11645428 and rs6564863 maintain independent associations (Table 2) and jointly explained 1% of the variability in MPOD (Table 3).

There was suggestive evidence for an interaction between rs1164528 and LZ intake ($P_{interaction} = 0.14$). The trend for increasing MPOD with increasing number of minor alleles was consistent across all levels of LZ intake; however, the effect of

TABLE 2. SNPs Independently Associated with MPOD within Each Gene, in CAREDS (N = 1585)

Gene	SNP	Genotype	N	Adjusted for Dietary L and Z and Ancestry*				Further Adjusted for Waist Circumference, Diabetes, and Fiber Intake		Final Adjustment for Serum L and Z	
				Mean MPOD	% Change from Reference	β †	P Value	β †	P Value	β †	P Value
Xanthophyll binding in retina											
<i>GSTP1</i>	rs675679	AA	1307	0.36	Ref.	0.028	0.033	0.027	0.035	0.027	0.031
		AC	263	0.39	8						
		CC	14	0.40	13						
Carotenoid cleavage											
<i>BCMO1</i>	rs11645428	GG	710	0.35	Ref.	0.029	2.2×10^{-4}	0.031	6.3×10^{-5}	0.017	0.027
		AG	694	0.36	5						
		AA	181	0.42	20						
	rs6564863	GG	686	0.35	Ref.	0.017	0.027	0.020	0.011	0.018	0.018
		AG	718	0.36	3						
		AA	176	0.40	12						
HDL transport or status											
<i>ABCA1</i>	rs1929841	AA	995	0.37	Ref.	-0.020	0.029	-0.024	0.007	-0.022	0.011
		AC	518	0.36	-2						
		CC	72	0.30	-19						
<i>ABCG5</i>	rs10179921	GG	1426	0.37	Ref.	-0.041	0.024	-0.038	0.028	-0.038	0.026
		AG or AA	153	0.33	-11						
<i>LIPC</i>	rs6078	GG	1480	0.37	Ref.	-0.044	0.041	-0.049	0.020	-0.043	0.038
		AG or AA	105	0.32	-12						
Lipid and/or carotenoid absorption											
<i>SCARB1</i>	rs10744182	AA	525	0.38	Ref.	-0.021	0.005	-0.021	0.004	-0.020	0.005
		AG	760	0.36	-5						
		GG	298	0.34	-11						
	rs838879	AA	768	0.38	Ref.	-0.018	0.020	-0.020	0.010	-0.018	0.016
		AG	647	0.35	-7						
		GG	169	0.35	-8						
	rs4379922	AA	651	0.37	Ref.	-0.016	0.036	-0.019	0.010	-0.016	0.025
		AG	711	0.36	-3						
		GG	220	0.34	-10						
Long-chain fatty acid status											
<i>ELOVL2</i>	rs1150561	GG	1407	0.36	Ref.	0.040	0.016	0.045	0.006	0.040	0.012
		AG or AA	178	0.40	11						
<i>FADS1</i>	rs174534	AA	711	0.35	Ref.	0.017	0.031	0.015	0.056	0.015	0.041
		AG	695	0.38	8						
		GG	177	0.37	7						
<i>FADS2</i>	rs2727271	AA	1244	0.36	Ref.	0.024	0.050	0.019	0.113	0.017	0.136
		AT	323	0.38	7						
		TT	15	0.40	12						
Genes previously related to maculopathies											
<i>RPE65</i>	rs4926339	GG	556	0.34	Ref.	0.018	0.020	0.017	0.021	0.015	0.038
		AG	784	0.37	10						
		AA	244	0.37	8						
<i>ALDH3A2</i>	rs8069576	GG	495	0.38	Ref.	-0.017	0.027	-0.016	0.026	-0.015	0.037
		AG	740	0.36	-6						
		AA	290	0.35	-9						

* First two principal components from principal component analysis using 176 ancestry informative markers.

† Beta is increase in MPOD per additional minor allele (additive genetic model).

the risk allele was greatest at the lowest level of intake (Fig. 2). Women both homozygous for the minor allele and in the highest tertile of LZ intake had a 32% higher MPOD than those with no minor alleles and intake in the lowest tertile.

Three SNPs (rs4926339, rs6688807, and rs11581095) in *RPE65*, the gene for retinal pigment epithelium (RPE)-specific protein 65 kDa, were associated with MPOD in CAREDS. The effects of these SNPs were correlated, only rs4926339

maintained independent associations with MPOD (Table 2) and explained 0.4% of the variability in MPOD (Table 3). No associations were found between MPOD and SNPs in either *RORA*, the RAR-related orphan receptor A gene, or *BCO2*, the beta carotene dioxygenase gene.

Lipid and Carotenoid Transport and Metabolism Genes. Four SNPs within *SCARB1* were associated with

TABLE 3. Final Multivariable SNP Model for SNPs Associated with MPOD in the CAREDS Population

Gene, SNP	β	P Value	Partial R ²
<i>BCMO1</i> , rs11645428	0.025	0.001	0.7%
<i>BCMO1</i> , rs6564863	0.018	0.021	0.3%
<i>SCARB1</i> , rs10744182	-0.024	0.002	0.6%
<i>SCARB1</i> , rs4379922	-0.014	0.061	0.3%
<i>SCARB1</i> , rs838879	-0.020	0.014	0.4%
<i>GSTP1</i> , rs675679	0.032	0.015	0.4%
<i>ABCA1</i> , rs1929841	-0.018	0.048	0.3%
<i>ABCG5</i> , rs10179921	-0.041	0.023	0.3%
<i>LIPC</i> , rs6078	-0.039	0.061	0.2%
<i>ELOVL2</i> , rs1150561	0.046	0.006	0.5%
<i>FADS1</i> , rs174534	0.020	0.011	0.4%
<i>RPE65</i> , rs4926339	0.019	0.014	0.4%
<i>ALDH3A2</i> , rs8069576	-0.018	0.018	0.4%
Full model R ²			5.1%
Full model P value			3.5×10^{-11}

MPOD: rs10744182, rs4379922, rs838879, and rs4238001. The most significant of these was rs10744182 ($P = 0.005$). When the conditional independence of these four SNPs was tested three (rs10744182, rs4379922, and rs838879) maintained independent effects within a multivariable SNP model (Table 2), and jointly explained 1.3% of the variability in MPOD in CAREDS (Table 3). There were no associations identified for SNPs in *SCARB2*, scavenger receptor class B, member 2, or *CD36*, cluster determinant 36.

Genes Related to HDL or Cholesterol Status. Variants from the following genes related to HDL or cholesterol status were genotyped: ATP-binding cassette, subfamily A, member 1 (*ABCA1*), ATP-binding cassette, subfamily G, member 5 (*ABCG5*), and ATP-binding cassette, subfamily G, member 8 (*ABCG8*), hepatic lipase (*LIPC*), cholesteryl ester transfer protein, plasma (*CETP*), Niemann-Pick C1-like protein 1 (*NPC1L1*), and *APOE*. From these genes, one SNP each from *ABCA1* (rs1929841), *ABCG5* (rs10179921), and *LIPC* (rs6078)

was associated with MPOD (Table 2). These three variants were able to explain 0.8% of the variability in MPOD (Table 3).

Synthesis or Metabolism of Long-Chain Fatty Acids in Blood or Retina. Tag SNPs from five genes related to status of long-chain fatty acids in the blood or retina including elongation of very long chain fatty acid proteins 2, 4, and 5 (*ELOVL2*, *ELOVL4*, and *ELOVL5*) and fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*), were genotyped. After adjustment for LZ intake, two SNPs within *ELOVL2* (rs1150561 and rs3846851), two SNPs in *FADS1* (rs174534 and rs2727270), and one SNP in *FADS2* (rs2727271) were associated with MPOD. Further adjustment for other predictors of MPOD slightly attenuated the *FADS2* relationship (Table 2). After testing independence of the SNPs in each gene, only rs1150561 (*ELOVL2*) and rs174534 (*FADS1*) maintained independent effects and explained 0.5% and 0.4% of the variability in MPOD, respectively (Table 3).

Genes with Known Variants Related to Macular Pigment and Maculopathies. We genotyped six tag SNPs within aldehyde dehydrogenase 3 family, member A2 (*ALDH3A2*), a gene with rare mutations known to cause the autosomal recessive condition Sjorgren-Larsson Syndrome, which among other clinical presentations, results in a complete lack of macular pigment.⁵⁴ Of these, rs8069576 was associated with MPOD in CAREDS, independent of other predictors such as waist circumference, diabetes, LZ intake, and fiber ($P = 0.047$; Table 2). We were not able to identify any single associations between complement factor H (*CFH*), age-related maculopathy susceptibility 2 (*ARMS2*), Htra serine peptidase 1 (*HTRA1*), or *APOE* and MPOD (data not shown).

DISCUSSION

This study, conducted in 1585 postmenopausal women, is the most extensive investigation of genetic predictors of MPOD, to date. Results from the present cross-sectional study support the previously proposed hypothesis,^{38,65} and results of twin studies,^{34,37,66} suggesting a role of genetic variation in macular pigment density.

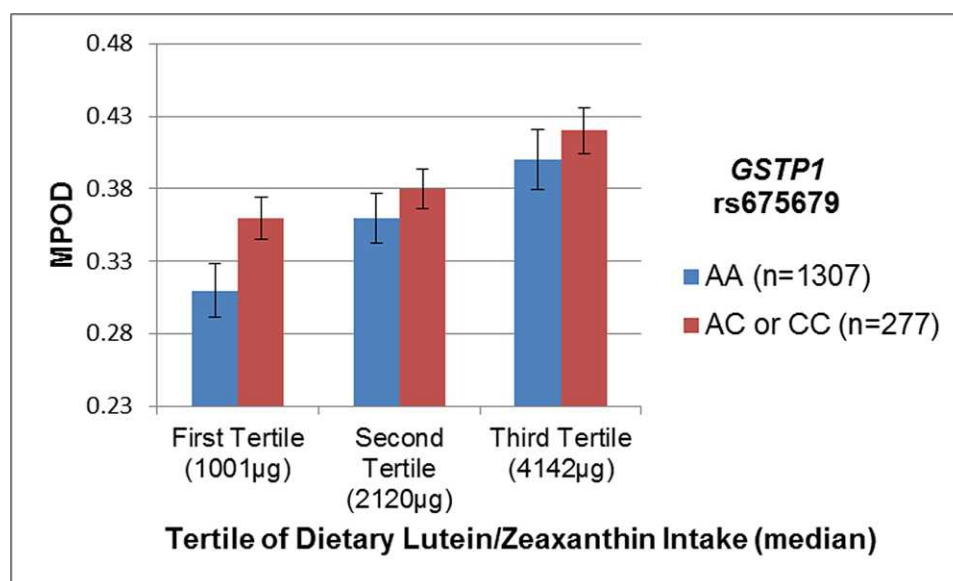


FIGURE 1. Joint effects of dietary lutein and zeaxanthin intake and *GSTP1* rs675679 on MPOD in CAREDS. The minor allele, C, of rs675679 was associated with higher mean MPOD in CAREDS. The direction of this relationship is consistent across all levels of dietary lutein and zeaxanthin intake, but significantly stronger at the lowest level of intake ($P_{interaction} = 0.04$).

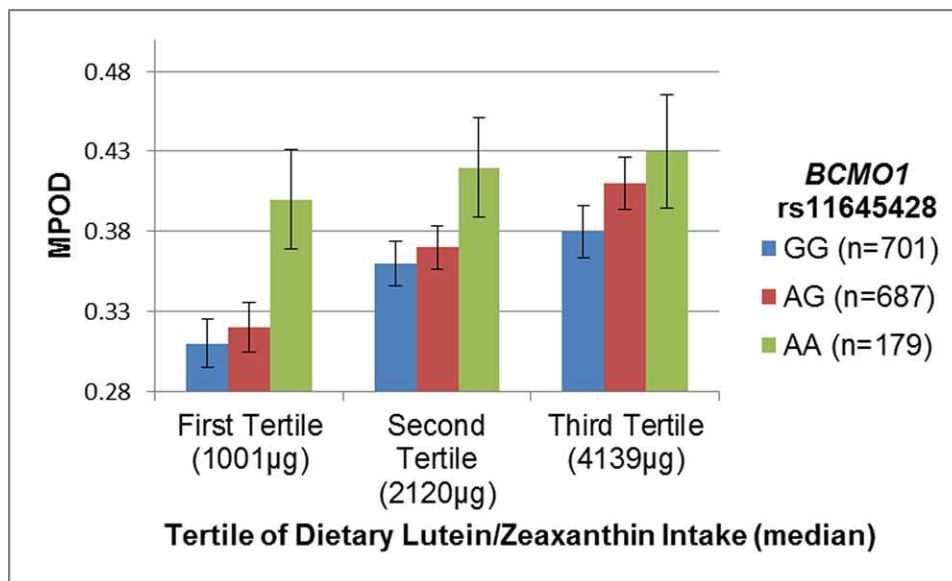


FIGURE 2. Joint effects of dietary lutein and zeaxanthin intake and *BCMO1* rs11645428. The minor allele, A, of rs11645428 was associated with higher mean MPOD in CAREDS. This relationship is consistent across all levels of dietary lutein and zeaxanthin intake, but less marked with increasing lutein intake ($P_{interaction} = 0.15$).

The genetic variation underlying MPOD appears to be polygenic involving common variation in numerous genes related to (1) carotenoid transport, uptake, and metabolism, (2) omega-3 fatty acid metabolism, and (3) some inherited maculopathies (discussed in detail below). Single genes that explain the greatest variability in MPOD were *SCARB1*, a scavenger receptor protein responsible for lipid and carotenoid uptake into gut and retina, and *BCMO1*, a carotenoid cleavage enzyme with preferred affinity for provitamin A carotenoids for synthesis of retinoids relative to xanthophyll carotenoids such as L and Z. *BCMO1* and *SCARB1* SNPs independently explained 2.3% of the variability in MPOD.

Evidence from this study suggests that the low percentage of variability in MPOD explained by dietary LZ in the present sample³ may be, in part, explained by genetic factors. For example, women in the lowest tertile of LZ intake who had two alleles of certain variants in *GSTP1* and *BCMO1*, had levels of macular pigment, which were higher than or nearly as high as women with intakes in the highest tertile (Figs. 1, 2). This suggests the possibility that having certain genotypes may compensate, to some degree, for low intake of macular carotenoids. It also indicates that LZ intake may explain a higher percentage of variability in MPOD in persons with certain genotypes. It is important to note that the low estimate of variability explained by LZ intake reflects *between person* variability of individual LZ intake; thus, does not constitute evidence that the level of LZ intake is unimportant on an individual level. The persistence of a strong relationship of serum LZ to MPOD, even after adjusting for dietary, genetic, and phenotype characteristics might reflect a residual influence of variability in levels of dietary intake of these carotenoids. LZ concentration in serum is not subject to variability in composition of LZ in the particular foods as dietary estimates are; however, serum LZ might also reflect additional unknown and unaccounted for genetic, phenotypic, or dietary factors that influence the uptake of LZ into the body and transport into the blood.

The variable response to supplementation observed across many studies might be, in part, due to genetic factors. Consistent with this, results of a recent study of 161 mono-

and dizygotic twin pairs estimated that 27% of the response to supplementation with 18 mg L and 2.4 mg Z for 6 months was due to heritable factors. Dietary supplementation with macular carotenoids has been observed to increase MPOD in 50%²⁸ to 95%⁶⁷ of subjects with many studies suggesting estimates between these two extremes.^{1,21-34} However, the magnitude of individual response in these studies is quite variable. We also note that the definition of macular “response” to intake of macular carotenoids is not standard; the magnitude of response in different regions of the macula needed to result in measurable differences in vision function or clinical outcomes remains to be further evaluated.

Variants in Genes Related to Carotenoid Uptake and Metabolism

We hypothesized variation in genes related to L and Z-specific binding proteins in the retina, StARD3,³⁹ and GSTP1,⁴⁰ respectively, might regulate retinal uptake or intracellular trafficking, thereby influencing MPOD. Variants in these genes have not yet been studied in relation to MPOD. We observed that one SNP, rs675679, near *GSTP1*, was associated with MPOD independent of known predictors of MPOD. No relationships with MPOD were identified for SNPs in *STARD3*, although variants in this gene were related to levels of LZ in serum.

In addition to the uptake in the retina, we hypothesized that variants in genes coding for enzymes involved in carotenoid cleavage and vitamin A metabolism might also influence the levels of macular carotenoids. *BCMO1* encodes an enzyme active in the intestine and other tissues of the body that oxidatively cleave provitamin carotenoids to make vitamin A (retinal and retinol) and many biologically active retinoids, which are active in the visual cycle.⁶⁸ Variants in *BCMO1*, including the SNPs reported here, rs11645428 and rs6564851, have been associated with serum carotenoid levels in a large meta-analysis, genome-wide association study,⁶⁹ a result we were able to replicate in CAREDS. One previous report in a small sample of middle-aged men suggested rs7501331 in *BCMO1* was associated with MPOD.⁷⁰ In the CAREDS, we were

unable to replicate this association ($P = 0.56$). However, we did identify five other SNPs in *BCMO1* to be associated with MPOD. Although there is no evidence that L and Z are substrates for *BCMO1*,⁷¹ it has been previously suggested that low functioning of this enzyme might lead to higher circulating levels of levels of beta-carotene, which then might compete with L and Z for entry into tissues.⁷² Variation in *BCMO1* function may also have broader physiological effects, including influence on the accumulation of liver lipids and subsequent metabolic effects.^{42,73,74}

RPE65 belongs to the family of carotenoid cleavage enzymes⁷⁵; it isomerizes all-*trans* retinyl ester to 11-*cis*-retinal in the visual cycle.^{76,77} Mutations in *RPE65* are associated with Leber's congenital amaurosis and a recessive form of retinitis pigmentosa.⁷⁸ Variants in *RPE65* have not been previously studied in relation to MPOD; here, we identified rs4926339 as associated with MPOD. Increased RPE65 activity results in the accumulation of the fluorescent *bis*-retinoid A2E, which is a byproduct of this cycle. A2E, a major component of retinal lipofuscin, is nondegradable, accumulates with age and in Stargardt's disease^{76,79} and has a variety of toxic effects on RPE cells, including an increase in blue light photo damage and oxidative damage, which is thought to trigger the complement system and chronic inflammation in the macula.⁸⁰ Thus, common variation in RPE genes that increase its activity may lower MPOD by increasing A2E accumulation and oxidative stress.

Variants in Genes Related to Carotenoid and Cholesterol Transport

A larger proportion of L, Z, and other xanthophylls, are carried on HDLs (>50%), unlike other less polar carotenoids, such as lycopene and alpha- and beta-carotene, which are mainly carried on LDLs,^{50,51} suggesting that blood lipoprotein distribution may be an important indicator of xanthophyll transport and, ultimately, availability to tissue.

A large number of proteins with roles in HDL formation or metabolism, or that are related to HDL levels in the blood, are suspected of being able to influence the accumulation of L and Z in tissues. This includes *ABCA1*, sterol transporters, *ABCG5* and *ABCG8*, *LIPC*, *CETP*, *APOE*, and *SR-B1*. From the genes for these proteins, one SNP each from *ABCA1*, *ABCG5*, and *LIPC* was related to MPOD (Table 2). While variants in some of these genes have been previously characterized for importance in cholesterol status and/or AMD status,^{49,81-84} this is the first report describing the relationship between these genes and MPOD. Previous reports have supported the role of these genes in carotenoid status, as carriers of a dysfunctional allele in *ABCA1* have low levels and unstable HDL particles in humans with Tangiers disease,⁸⁵ as well as deficiencies in tissue carotenoids in chickens.^{65,86} Polymorphisms in *ABCG5*, which encodes a protein that directs cholesterol back into the intestinal lumen rather than into chylomicrons, which would carry cholesterol to the liver, have also been suggested to influence plasma response to both dietary cholesterol and carotenoids.⁸⁷

We observed three SNPs in *SCARB1*, the gene encoding for scavenger receptor class B type I (SR-B1) lipid transporter, independently related to MPOD. SR-B1 knock-out mice consuming an atherogenic diet display retinal abnormalities similar to dry AMD⁸⁸ and in humans, heterozygosity for a specific variant within *SCARB1* was related to AMD in one sample of people without high risk variants in the well-known AMD susceptibility genes *CFH* and *ARMS2*.⁸⁹ SR-B1, a plasma membrane receptor for HDL, mediates cholesterol efflux and carotenoid uptake in the RPE^{45,46} and intestine.^{46,47} The SR-B1 receptor, together with the Z-specific receptor *GSTP1*, may

explain the selective accumulation of xanthophylls in the retina, and saturation of these receptors might explain the plateau in MPOD as LZ intake increases in this sample.³

The gene for SR-B1 (*SCARB1*) has high homology to both the *ninaD* gene that, when mutated in *Drosophila*, results in blindness, and to cluster determinant 36 (CD36),⁹⁰ expressed in both human gut and retina. Each of these is related to the joint transport of lipids and carotenoids in these tissues.^{45,91} In the retina, the CD36 protein is involved in phagocytosis of rod outer segments and lipid transport.⁴⁶ Also, the silkworm analog of human CD36, *Cameo2*, is essential for LZ uptake into the insect's silk gland.⁹² In the present study, variants in the *CD36* gene were related to levels of LZ in the serum (data not shown), but not to MPOD, as had been previously reported in one small study.⁷⁰ However, expression of CD36 is also influenced by diet,⁹³ which might further modify the association between LZ status and MPOD.

Contrary to one previous report in 302 persons reporting higher MPOD with increasing $\epsilon 4$ count,⁵⁶ we observed no statistically significant differences in MPOD relative to *APOE* genotype. In fact, MPOD was lower, rather than higher, among women with $\epsilon 4/\epsilon 4$ genotype in our sample. However, the lower MPOD with higher $\epsilon 4$ count relative to $\epsilon 3/\epsilon 3$ was not significant ($P = 0.25$). Overall, there were no statistically significant differences of MPOD in any *APOE* genotype relative to $\epsilon 3/\epsilon 3$ (see Supplementary Material and Supplementary Table S3, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10867/-/DCSupplemental>). As a positive control for *APOE*-phenotype correlations in CAREDS, we observed the expected association between at least one copy of the $\epsilon 4$ allele and higher total cholesterol ($P = 0.01$).

Variants in Genes Related to Synthesis or Metabolism of Long-Chain Omega-3 Fatty Acids in Blood or Retina

Previous evidence suggested that long chain (LC) omega-3 (n-3) fatty acids in the diet,³ serum,⁵² and in supplements⁵³ were related to MPOD. Docosahexaenoic acid (DHA) is the most abundant LC n-3 fatty acid in rod outer segment membranes^{94,95} at a concentration that exceeds levels found elsewhere in the body⁹⁶ and is critical to retinal health. Furthermore, monkeys fed low n-3 fatty acid diets since birth have differences in foveal architecture relative to those fed adequate levels.⁹⁷ For this reason, and because low intake of LC n-3 fatty acids may be related to maculopathies, which subsequently result in lowering of MPOD, we hypothesized that common variants in genes related to better LC n-3 status were related to MPOD. Relationships between genetic determinants of LC n-3 status and MPOD had not been previously studied.

Variants in *FADS1* and *ELOVL2*, two genes that encode proteins related to the metabolism of LC n-3 fatty acids, have been related to levels of n-3 fatty acids in red blood cell membrane phospholipids in previous large genome-wide association studies.^{98,99} SNPs tagging variation for *FADS1* and *ELOVL2* were independently related to MPOD in the present study. *FADS1* encodes a desaturase involved in converting the plant-derived essential fatty acid α -linolenic acid (ALA, 18:3n3) to the LC n-3 fatty acids eicosapentaenoic acid (EPA, 20:5n3) and the LC n-3 metabolite of EPA (docosapentaenoic acid-DPA) to docosahexaenoic acid (DHA, 22:6n3), the primary LC n-3 in the retina.^{98,99} The SNP in *FADS1* related to lower MPOD in the present study (rs174534), is related to lower levels of ALA and higher levels of EPA in circulating phospholipids in a large genome-wide association study in European populations.⁹⁹

FADS2 is an essential enzyme for DHA synthesis, as *FADS2* knock-out mice do not have DHA.¹⁰⁰ In the present study, *FADS2* was related to MPOD, but not significantly after further adjusting for other determinants of MPOD, suggesting the association with MPOD may, in part, reflect an influence of other determinants.

ELOVL2 encodes an enzyme responsible for the elongation of the very LCn-3 fatty acid EPA to DPA and, subsequently, DHA. In CAREDS, rs1150561, which tags variation downstream of *ELOVL2*, was associated with MPOD.

Variants in Known Genes for Maculopathies

Finally, we considered variants in genes related to either AMD or other maculopathies, which were also related to the status of carotenoids or retinoids in previous studies. Associations with *RPE* were discussed above. We also hypothesized that common variants within *ALDH3A2* may impact MPOD in the general population. Rare mutations within *ALDH3A2*, an aldehyde dehydrogenase, are known to cause Sjorgren-Larsson Syndrome, which results in numerous phenotypic effects including macular dystrophy, photophobia, and a complete lack of macular pigment.⁵⁴ In the first report of common variants and MPOD, we found the minor allele of rs8069576 to be associated with lower mean MPOD.

Similar to a recent report,⁵⁵ we were not able to identify any single associations between *CFH* or *ARMS2* variants and MPOD. However, this same report by Loane and colleagues suggested individuals homozygous for the risk allele at both Y402H and A69S may be at higher risk for lower MPOD.⁵⁵ Six women in CAREDS were homozygous at both these SNPs and their mean MPOD was 0.25. While lower mean MPOD relative to the rest of our sample (0.36) and consistent with the report by Loane et al,⁵⁵ it was not statistically significant ($P = 0.14$), likely due to low number of women homozygous at both genes.

Limitations

Overall, SNPs identified through our current candidate gene association study explained 5.1% of the variability in MPOD (Table 3), which falls short of the 67% heritability predicted in a previous twin study.³⁷ “Missing heritability” is a common phenomenon in human genetics research,^{101,102} and in CAREDS is likely reflective of (1) the small number of genetic variants tested (relative to genome-wide association studies), (2) the focus on variants with a minor allele frequency greater than 5%, when the majority of variation within the genome occurs at frequencies less than 5%,¹⁰³ (3) the exclusion of other types of genetic variants such as copy number variants, and/or (4) the exclusion of the complex interplay of physiological factors likely to influence MPOD such as gene-gene and gene-environment interactions. Evidence of an interaction between LZ intake and genetic factors (Figs. 1, 2) was detected, suggesting that while the genetic factors measured here only explained 5% of the variability in MPOD in the CAREDS population, the relevance of certain genes may shift under various environmental conditions, importantly in this case dietary intake of carotenoids. More complex statistical modeling of interactions and joint effects may reveal additional insights.

Some of the SNPs related to MPOD in the present study may be chance findings, given the large number of associations studied. Under the Bonferroni multiple testing correction, a P value less than 1×10^{-4} would be required to claim statistical significance. Only rs11645428 in *BCMO1* could be considered in this scenario. However, this correction is overly conservative as it assumes independence of tests, which is violated given the

correlation of SNPs within genes, and results of conditional SNP modeling within genes. Therefore, the consistency of associations across levels of other major determinants of MPOD (dietary intake and waist circumference), and support of biological plausibility of relationships with previous evidence, lessens the possibility of false positives. Replication in additional, independent cohorts, which are as, or better, powered to detect genetic effects will aid in understanding the nature of these results. The sample in CAREDS is comprised of largely white women from the United States (US), older than 55 years of age, who have high socioeconomic status, relative to the US population. It is unknown the extent to which these genotypes would be related to MPOD among men or among persons with ancestry that evolved under conditions of higher light exposure. Studies of genetic determinants of MPOD in additional study samples of diverse composition will aid in better understanding the genetic architecture underlying MPOD.

Another possible limitation is the focus on MPOD at 0.5° from the foveal center. The SNPs reported here may not reflect differences in the spatial distribution of macular pigment between individuals. An enhanced macular pigment at approximately 0.7°, which gives the appearance of a ring structure, has been related to lower occurrence of macular degeneration in some past studies.^{104,105} Persons with this ring have lower MPOD at 0.25° and 0.5° and higher at 1° and 2°.¹⁰⁵ Results of a recent twin study indicated macular pigment *peak density* to be more strongly related to genetic factors than macular pigment *spatial distribution*, which was more strongly related to environmental factors.⁶⁶ Although results in CAREDS were generally similar at all four eccentricities measured (not shown), further exploration of genetic determinants of the spatial distribution of MPOD in this sample are warranted.

CONCLUSIONS

In conclusion, this study in 1585 postmenopausal women is the most extensive investigation of genetic predictors of MPOD to date and suggests variation in genes related to the metabolism of carotenoids, LC n-3 fatty acids and a maculopathy related to absence of macular pigment is related to variation in the density of macular pigments. These findings, together with nondietary determinants of MPOD identified in this and other studies, likely contribute to the high variability in MPOD levels across different people and variable response to the intake of macular carotenoids. Further investigation of complex gene-gene and gene-environment joint effects and interactions might explain additional variability in MPOD. If macular pigment is found to protect against age-related maculopathies and optimize vision, this information about determinants of MPOD could be used to design intervention trials aimed at directly studying strategies to enhance macular pigment.

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