


## Original Investigation

# Association Between Vitamin D Status and Age-Related Macular Degeneration by Genetic Risk

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**IMPORTANCE** Deficient 25-hydroxyvitamin D (25[OH]D) concentrations have been associated with increased odds of age-related macular degeneration (AMD).

**OBJECTIVE** To examine whether this association is modified by genetic risk for AMD and whether there is an association between AMD and single-nucleotide polymorphisms of genes involved in vitamin D transport, metabolism, and genomic function.

**DESIGN, SETTING, AND PARTICIPANTS** Postmenopausal women (N = 913) who were participants of the Carotenoids in Age-Related Eye Disease Study (CAREDS) (aged 54 to <75 years) with available serum 25(OH)D concentrations (assessed October 1, 1993, to December 31, 1998), genetic data, and measures of AMD (n = 142) assessed at CAREDS baseline from May 14, 2001, through January 31, 2004, were studied.

**MAIN OUTCOMES AND MEASURES** Prevalent early or late AMD was determined from graded, stereoscopic fundus photographs. Logistic regression was used to estimate odds ratios (ORs) and 95% CIs for AMD by the joint effects of 25(OH)D (<12, ≥12 to <20, ≥20 to <30, and ≥30 ng/mL) and risk genotype (noncarrier, 1 risk allele, or 2 risk alleles). The referent group was noncarriers with adequate vitamin D status (≥30 ng/mL). Joint effect ORs were adjusted for age, smoking, iris pigmentation, self-reported cardiovascular disease, self-reported diabetes status, and hormone use. Additive and multiplicative interactions were assessed using the synergy index (SI) and an interaction term, respectively. To examine the association between AMD and variants in vitamin D-related genes, age-adjusted ORs and 95% CIs were estimated using logistic regression.

**RESULTS** Among the 913 women, 550 had adequate levels of vitamin D (≥20 ng/mL), 275 had inadequate levels (≥12 to <20 mg/mL), and 88 had deficient levels (<12 ng/mL). A 6.7-fold increased odds of AMD (95% CI, 1.6-28.2) was observed among women with deficient vitamin D status (25[OH]D <12 ng/mL) and 2 risk alleles for *CFH* Y402H (SI for additive interaction, 1.4; 95% CI, 1.1-1.7; *P* for multiplicative interaction = .25). Significant additive (SI, 1.4; 95% CI, 1.1-1.7) and multiplicative interactions (*P* = .02) were observed for deficient women with 2 high-risk *CFI* (rs10033900) alleles (OR, 6.3; 95% CI, 1.6-24.2). The odds of AMD did not differ by genotype of candidate vitamin D genes.

**CONCLUSIONS AND RELEVANCE** In this study, the odds of AMD were highest in those with deficient vitamin D status and 2 risk alleles for the *CFH* and *CFI* genotypes, suggesting a synergistic effect between vitamin D status and complement cascade protein function. Limited sample size led to wide CIs. Findings may be due to chance or explained by residual confounding.

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Age-related macular degeneration (AMD) is the leading cause of visual impairment and blindness in older Americans,<sup>1</sup> has no cure, and has only limited treatment options. Research indicates a role for inflammation in the pathogenesis of AMD.<sup>2</sup> Individuals with a history of inflammatory diseases have been found to have an increased risk of AMD,<sup>3,4</sup> and markers of systemic inflammation have been positively associated with late AMD.<sup>5</sup> Inflammatory molecules are found within drusen, suggesting that these accumulations elicit a local chronic inflammation.<sup>6</sup> In addition, variants in genes for inflammatory response proteins are associated with AMD risk.<sup>7-12</sup> The *CFH* Y402H single-nucleotide polymorphism (SNP) (rs1061170) increases risk 1.4- to 1.5-fold for early AMD<sup>11</sup> and 2.5- to 6.0-fold for late AMD.<sup>7</sup>

Vitamin D has anti-inflammatory and immune-modulating properties<sup>13</sup> and is hypothesized to protect against the development of AMD.<sup>14</sup> Animal models<sup>15-18</sup> and epidemiologic studies<sup>19-21</sup> of autoimmune disease support this hypothesis. In vivo research indicates that both the proteins for the vitamin D receptor (VDR) and the enzyme 1- $\alpha$ -hydroxylase, which converts the major circulating metabolite of vitamin D (25-hydroxyvitamin D [25(OH)D]) to its active hormone calcitriol (1,25-dihydroxyvitamin D), are expressed in the retina.<sup>22</sup>

Previous studies have found that decreased odds of AMD are associated with high compared with low concentrations of 25(OH)D,<sup>14,23,24</sup> vitamin D intake from foods,<sup>23,25</sup> and certain polymorphisms in genes involved in vitamin D metabolism,<sup>26</sup> although other studies<sup>27-29</sup> do not support an association. Research in the Carotenoids in Age-Related Eye Disease Study (CAREDS) revealed that postmenopausal women younger than 75 years had an increased odds of AMD if they had low vs high vitamin D status assessed with intake of vitamin D and 25(OH)D concentrations.<sup>23</sup> We proposed to investigate whether this previously observed association between 25(OH)D and AMD was stronger in women with established AMD risk genotypes, including several that influence inflammatory pathways, and to investigate the association between AMD and SNPs in genes involved in vitamin D transport, metabolism, and genomic function.

## Methods

### Study Sample

CAREDS was conducted to study the association of lutein and zeaxanthin with AMD.<sup>30-32</sup> A total of 2005 women enrolled in CAREDS from May 14, 2001, through January 31, 2004, a mean of 6 years after the Women's Health Initiative Observational Study (WHI-OS) baseline (October 1, 1993, to December 31, 1998). This research study was approved at all institutions by their institutional review boards, and the study procedures conformed to the Declaration of Helsinki. Written informed consent was obtained from all participants.

All CAREDS participants were administered questionnaires at WHI-OS and CAREDS baseline to assess demographic characteristics, family and medical history, and lifestyle habits inclusive of relevant AMD risk factors. Gradable retinal photographs were obtained from 1853 of 1894 women

### At a Glance

- Deficient vitamin D status has been associated with increased odds of age-related macular degeneration (AMD); modification by genetic risk for AMD is unknown.
- Women deficient in vitamin D, with 2 high-risk alleles (*CFH* Y202H or *CFI* [rs100333900]), were at increased odds of having AMD.
- Study limitations include small number of cases in joint effect cells and possible spurious findings from multiple testing.
- The possibility of residual confounding exists, and the use of prevalent AMD cases limits our ability to determine causality.
- Maintenance of adequate vitamin D status, especially in persons at high genetic risk, should be considered for AMD prevention.

attending CAREDS' baseline study visits (May 14, 2001, through January 31, 2004). Four additional women were included who had no retinal photographs but provided a physician's confirmation of AMD status. Of these, 1230 had sufficient serum amounts at WHI-OS baseline (October 1, 1993, to December 31, 1998) for 25(OH)D assessment, gave approval for use of their genetic data, had sufficient DNA for genotyping, and passed quality assurance and control tests for genotyping.

Women enrolled in CAREDS but excluded because of missing serum 25(OH)D and genetic data ( $n = 775$ ) had a similar prevalence of AMD and a similar mean intake of vitamin D compared with women with these data ( $n = 1230$ ) (eTable 1 in the Supplement). Women with compared with women without missing data were slightly older ( $P = .01$ ) and had healthier lifestyle scores ( $P = .06$ ).

The current analyses are limited to the sample of women aged 54 to younger than 75 years ( $N = 913$ ), in whom we previously observed an association between vitamin D status and AMD.<sup>23</sup> We do not present data in women 75 years or older because of the small sample size ( $n = 317$ ) and potential selective mortality bias in this older sample.<sup>23</sup> A slightly larger sample ( $n = 1230$ ) of women younger than 75 years was available for the analysis of the association between AMD and SNPs of vitamin D-related genes because women were not further excluded for missing 25(OH)D data.

### Retinal Photographs

Stereoscopic retinal fundus photographs were taken at CAREDS baseline (May 14, 2001, through January 31, 2004) and graded by the University of Wisconsin Fundus Photography Reading Center using the Age-Related Eye Disease Study (AREDS) protocol.<sup>33</sup> The outcome for these analyses was presence of early or late AMD (any AMD). Early AMD was classified similarly to AREDS category 3,<sup>33</sup> including the presence of one or more large drusen ( $\geq 125 \mu\text{m}$ ) or extensive intermediate drusen ( $\geq 360 \mu\text{m}$  when soft indistinct drusen were present or  $\geq 650 \mu\text{m}$  when soft indistinct drusen were absent). Different from the AREDS category 3, the presence of early AMD also included having pigmentary abnormalities or an increase or decrease in pigmentation if accompanied by at least one drusen  $63 \mu\text{m}$  or larger. Late AMD included geographic atrophy (noncentral or central), neovascularization, or exudation in the center subfield. eTable 2 in the Supplement lists the distribution of AMD cases by severity. Most

of these cases (133 of 142 [93.7%]) were early AMD. The reference group included women who had neither early nor late AMD, generally corresponding to AREDS categories 1 and 2.<sup>33</sup>

### Serum Vitamin D Assays

Fasting blood samples collected at WHI-OS baseline (October 1, 1993, to December 31, 1998) were assessed for serum concentrations of 25(OH)D using the Diasorin LIAISON chemiluminescence method (Heartland Assays Inc).<sup>23</sup> We adjusted for season of blood draw by regressing 25(OH)D concentrations on month of blood draw using a nonparametric regression technique.<sup>23,34</sup> Residuals were added to the sample 25(OH)D mean to obtain season-adjusted 25(OH)D concentrations, which are presented in the following analyses.

### Genotyping

Serum, obtained at WHI-OS baseline, was stored and frozen at  $-80^{\circ}\text{C}$  until DNA was extracted from the buffy coats and genotyped. Within CAREDS, a custom 768-SNP panel was designed to genotype candidate genes for carotenoid status,<sup>35</sup> vitamin D status,<sup>36</sup> and risk of AMD. The panel included 5 established genetic variants for late AMD: Y402H (rs1061170) in *CFH*,<sup>7</sup> rs10033900 in *CFI*,<sup>12</sup> rs641153 in *CFB/C2*,<sup>8,9</sup> rs2230199 in *C3*,<sup>10</sup> and A69S (rs10490924) in *ARMS2*.<sup>8,37</sup> The SNPs from 6 vitamin D-related genes, some of which have been previously described in CAREDS,<sup>36</sup> were also included: *DHCR7*, *CYP2R1*, *CYP27B1*, *CYP24A1*, *VDR*, and *GC* (see footnotes in Table 2 and eTable 3 for database links to individual variants).

Genotyping was conducted at Case Western Reserve University. Genotyping of all noted SNPs above, but not *CFH* Y402H, were conducted with an Illumina Custom GoldenGate Assay, and genotype calls were made using Illumina Genome Studio (Illumina Inc). Genotyping of Y402H was conducted using the KASP Assay at LCG Genomics and called via the KASP SNP Genotyping System. *CFH* Y402H genotypes were imputed using MACH (<http://csg.sph.umich.edu/abecasis/MACH/index.html>) when there was insufficient DNA for KASP genotyping after Illumina genotyping (approximately 3%). Imputation was performed using the available chromosome 1 SNPs from Illumina ( $n = 14$  SNPs) and 1000 Genomes Project European ancestry panel as a reference. The resulting  $R^2$  from imputing Y402H was 99.5%. All SNPs passed standard quality control filters,<sup>38</sup> including Hardy-Weinberg equilibrium  $\chi^2 P > 1.0 \times 10^{-6}$ , minor allele frequency greater than 0.01, and genotype call rates greater than 95%.

### Statistical Analyses

We created 4 categories of vitamin D status based on 25(OH)D and the Institute of Medicine's Dietary Reference Intakes<sup>39</sup> (<12 [deficient],  $\geq 12$  to <20 [inadequate],  $\geq 20$  to <30 [adequate], and  $\geq 30$  [adequate] ng/mL [to convert to nanomoles per liter, multiply by 2.496]). We categorized women into 3 genotype groups for each SNP according to whether they had 1 or 2 high-risk alleles or were a noncarrier for each risk gene (additive genetic model). For *CFB/C2*, we combined women with 1 and 2 copies of the minor allele (A) owing to low number of homozygous women ( $n_{AA} = 10$ ) (dominant genetic model with respect to the minor allele).

Logistic regression was used to estimate age-adjusted odds ratios (ORs) and 95% CIs for AMD by (1) vitamin D status, (2) established AMD risk genotype, and (3) combined categories of vitamin D and risk genotype. For the joint analyses, the reference group was the hypothesized lowest-risk group, defined as women with a 25(OH)D concentration of 30 ng/mL or greater and a genotype indicative of low AMD risk. The ORs in the joint analysis were adjusted for the following covariates used in the previous vitamin D status and AMD analysis: smoking pack-years, iris pigmentation, self-reported cardiovascular disease, self-reported diabetes status, and hormone use status.<sup>23</sup> A  $P$  for ordinal trend across vitamin D status categories was determined within each genotype class.

To test for deviation from a multiplicative interaction, we examined the  $P$  value for the interaction term (25[OH]D\*genotype) using genotype (0, 1, or 2 risk alleles) and vitamin D status categories as ordinal variables. An interaction term with  $P < .05$  was considered statistically significant. A deviation from an additive effect was examined using the synergy index (SI).<sup>40</sup> An SI greater or less than 1.0 indicated that genotype and vitamin D status act jointly more than or less than additively, respectively.

To address our second study aim, logistic regression was used to estimate the age-adjusted ORs and 95% CIs for AMD by variants in vitamin D-related genes using an additive genetic model.

All analyses were conducted using SAS statistical software, version 9.2 (SAS Institute Inc).

## Results

Among the 913 women, 550 had adequate levels of vitamin D ( $\geq 20$  ng/mL), 275 had inadequate levels ( $\geq 12$  to <20 ng/mL), and 88 had deficient levels (<12 ng/mL). Women with deficient compared with adequate vitamin D status were less likely to be white, were less likely to have high incomes, were less likely to have an educational level beyond high school, were more likely to be nondrinkers, were less likely to have healthy diet patterns or engage in physical activity, were more likely to have lower self-reported ocular visible sun exposure, were more likely to be obese, and were less likely to use hormone replacement therapy (Table 1).

Age-adjusted odds of AMD are reported by vitamin D status and genotypes of high-risk AMD genes and vitamin D-related genes. Women with deficient (<12 ng/mL) compared with adequate ( $\geq 30$  ng/mL) status had a 2.6-fold increased odds of AMD (95% CI, 1.3-5.2), those with inadequate status ( $\geq 12$  to <20 ng/mL) had a 1.5-fold increase (95% CI, 0.8-2.6), and those with adequate status ( $\geq 20$  to <30 ng/mL) had a 1.6-fold increase (95% CI, 0.9-2.7) ( $P$  for trend = .01). There was a more than 2-fold increased odds of AMD among women with 2 risk alleles for *CFH* (CC) or *ARMS2* (AA) compared with noncarriers (Table 2). The  $P$  for trend = .04 for increasing number of risk alleles for *CFI*. The SNPs in *CYP2R1* (rs11819875 and rs12418214) and *VDR* (rs11168275, rs2189480, and rs2239186) were associated with increased odds of AMD (eTable 2 in the Supplement).

Table 3 details the joint effects of genotypes by vitamin D status. The adjusted ORs presented in these tables differ minimally from the ORs adjusted only for age. There was 6.7-fold

Table 1. Characteristics of Participants Younger Than 75 Years by Vitamin D Status Determined at WHI-OS Baseline (October 1, 1993, to December 31, 1998)<sup>a</sup>

Characteristic	No. (%) of Participants by Vitamin D Status Determined by 25(OH)D (N = 913)				P Value <sup>b</sup>
	Adequate (≥30 ng/mL) (n = 177)	Adequate (≥20 to <30 ng/mL) (n = 373)	Inadequate (≥12 to <20 ng/mL) (n = 275)	Deficient (<12 ng/mL) (n = 88)	
25(OH)D, median (range), ng/mL	34 (30-66)	24 (20-30)	16 (12-20)	10 (3-12)	NA
Demographic					
Age at eye photography, mean (SE), y	65.8 (0.4)	66.0 (0.3)	66.9 (0.3)	66.1 (0.5)	.08
White	174 (98.3)	367 (98.4)	267 (97.1)	82 (93.2)	.02
Annual Income ≥\$75 000	48 (27.6)	67 (18.8)	41 (15.8)	10 (12.1)	.001
Educational level					
High school or less	39 (22.0)	77 (20.6)	62 (22.6)	32 (36.4)	.005
College	78 (44.1)	184 (49.3)	136 (49.5)	40 (45.5)	
Postcollege	60 (33.9)	112 (30.0)	77 (28.0)	16 (18.2)	
Study site					
Iowa	65 (36.7)	127 (34.1)	99 (36.0)	33 (37.5)	.20
Oregon	46 (26.0)	121 (32.4)	88 (32.0)	31 (35.2)	
Wisconsin	66 (37.3)	125 (33.5)	88 (32.0)	24 (27.3)	
Smoking, pack-years <sup>c</sup>					
Never	103 (58.2)	213 (57.1)	151 (54.9)	46 (52.3)	.11
0-7	42 (23.7)	89 (23.9)	69 (25.1)	16 (18.2)	
>7	32 (18.1)	71 (19.0)	55 (20.0)	26 (29.6)	
Alcohol, g/wk					
Nondrinker	62 (35.0)	138 (37.0)	113 (41.1)	41 (46.6)	.009
0.4 to <4.0	55 (31.1)	114 (30.6)	87 (31.6)	29 (33.0)	
≥4 to <127	60 (33.9)	121 (32.4)	75 (27.3)	18 (20.5)	
Modified Healthy Eating Index 2005	63.5 (0.6)	63.9 (0.4)	62.0 (0.5)	59.2 (0.9)	<.001
Recreational physical activity, MET- h/wk					
0-3	31 (17.5)	85 (22.9)	77 (28.1)	37 (43.5)	<.001
3-10	37 (20.9)	69 (18.6)	68 (24.8)	17 (20.0)	
10-21	46 (26.0)	118 (31.8)	70 (25.6)	16 (18.8)	
≥21	63 (35.6)	99 (26.7)	59 (21.5)	15 (17.7)	
Ocular visible sun exposure in the last 20 y, Maryland sun-years, mean (SE) <sup>c</sup>	0.82 (0.03)	0.72 (0.02)	0.72 (0.02)	0.67 (0.04)	.006
Ocular and medical factors					
Iris color, blue <sup>c</sup>	74 (41.8)	150 (40.2)	124 (45.1)	33 (37.5)	.90
Family history of macular degeneration <sup>c</sup>	33 (18.6)	63 (16.9)	38 (13.8)	10 (11.4)	.06
BMI					
<22.5	48 (27.1)	69 (18.5)	25 (9.1)	8 (9.1)	<.001
≥22.5 to <25	42 (23.7)	73 (19.6)	44 (16.0)	14 (15.9)	
≥25 to <30	55 (31.1)	141 (37.8)	105 (38.2)	25 (28.4)	
≥30 to <35	29 (16.4)	65 (17.4)	53 (19.3)	21 (23.9)	
≥35	3 (1.7)	25 (6.7)	48 (17.5)	20 (22.7)	
Hypertension	42 (23.7)	85 (22.8)	66 (24.0)	31 (35.2)	.10
Cardiovascular disease	37 (20.9)	72 (19.3)	67 (24.4)	17 (19.3)	.58
Diabetes mellitus	2 (1.1)	10 (2.7)	6 (2.2)	3 (3.4)	.36
Hormone replacement therapy					
Never	36 (20.3)	117 (31.4)	81 (29.5)	34 (38.6)	.005
Past	22 (12.4)	41 (11.0)	35 (12.7)	11 (12.5)	
Current	119 (67.2)	215 (57.6)	159 (57.8)	43 (48.9)	
CRP concentration, mean (SE), mg/L	4.63 (0.43)	4.44 (0.29)	5.11 (0.34)	4.80 (0.61)	.36

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index (calculated as the weight in kilograms divided by height in meters squared); CAREDS, Carotenoids in Age-Related Eye Disease Study; CRP, C-reactive protein; MET, metabolic equivalent of task; NA, not applicable; WHI-OS, Women's Health Initiative Observational Study.

SI conversion factors: To convert CRP to nanomoles per liter, multiply by 9.524; 25(OH)D to nanomoles per liter, multiply by 2.496.

<sup>a</sup> Data are presented as number (percentage), and characteristics were assessed

at WHI baseline (1993-1998) unless otherwise noted.

<sup>b</sup> P values are for general associations. For categorical variables, the Cochran-Mantel-Haenszel statistic for a general association is used. For continuous variables, an analysis of variance to compare means by ordinal trend of serum vitamin D is used.

<sup>c</sup> Characteristics assessed at CAREDS baseline (2001-2004).

**Table 2. Prevalence of High-Risk AMD Genotypes and Age-Adjusted ORs of AMD Assessed at CAREDS Baseline (May 14, 2001, Through January 31, 2004) Among CAREDS Participants Younger Than 75 Years**

Genotype <sup>a</sup>	No. (%) of Participants (N = 913)	No. of Cases of AMD	Age-Adjusted OR (95% CI)	P Value for Trend <sup>b</sup>
<i>CFH</i> (rs1061170)				
TT	350 (38.3)	36	1 [Reference]	.001
CT	429 (47.0)	77	1.9 (1.2-2.9)	
CC	134 (14.7)	29	2.4 (1.4-4.1)	
<i>CFI</i> (rs10033900)				
GG	227 (24.9)	33	1 [Reference]	.04
GA	456 (50.0)	60	0.9 (0.6-1.5)	
AA	229 (25.1)	49	1.6 (1.0-2.7)	
<i>CFB/C2</i> (rs641153)				
AA/AG	148 (16.2)	22	1 [Reference]	.76
GG	765 (83.8)	120	1.1 (0.7-1.8)	
<i>C3</i> (rs2230199)				
CC	37 (4.0)	6	1 [Reference]	.87
GC	302 (33.1)	45	1.0 (0.4-2.5)	
GG	574 (62.9)	91	1.0 (0.4-2.5)	
<i>ARMS2</i> (rs10490924)				
CC	540 (59.3)	68	1 [Reference]	.002
AC	332 (36.4)	64	1.7 (1.1-2.4)	
AA	39 (4.3)	10	2.4 (1.1-5.1)	

Abbreviations: AMD, age-related macular degeneration; CAREDS, Carotenoids in Age-Related Eye Disease Study; OR, odds ratio.

<sup>a</sup> Individual variants are searchable in the National Center for Biotechnology Information database dbSNP by the rs number identifier: <http://www.ncbi.nlm.nih.gov/SNP/>.

<sup>b</sup> P value for trend with increasing number of risk alleles.

(95% CI, 1.6-28.2) increased odds of AMD among vitamin D-deficient women with 2 risk *CFH* alleles (CC) compared with non-carriers with adequate vitamin D status ( $\geq 30$  ng/mL), with an SI of 1.4 (95% CI, 1.1-1.7). There was 6.3-fold (95% CI, 1.6-24.2) increased odds of AMD among vitamin D-deficient women with 2 risk *CFI* alleles (AA) relative to noncarriers with adequate vitamin D status, with an SI of 1.4 (95% CI, 1.1-1.7; *P* for interaction = .02). Further adjustment of models for educational level, principal components from principal component analysis using 176 ancestry informative markers, body mass index (BMI) (calculated as the weight in kilograms divided by height in meters squared), and recreational physical activity minimally influenced results. There was no evidence of an interaction between vitamin D status and the *ARMS2*, *CFB/C2*, and *C3* genotypes.

Table 3 also presents the *P* for trend values for the odds of AMD across ordinal decreasing concentrations of 25(OH)D stratified by genotype. There was a *P* for trend < .05 for women with 2 high-risk *CFH* alleles (CC), 2 high-risk *CFI* alleles (AA), and noncarriers of the *ARMS2* high-risk allele.

## Discussion

This study examined the joint effects of vitamin D status and high-risk genotypes on AMD. We observed a multiplicative interaction between vitamin D status and *CFI*, suggesting that the relative odds of AMD among women with deficient vs adequate vitamin D status differed by *CFI* genotype. We also observed additive interactions between vitamin D status and both *CFH* and *CFI* genotype. These additive interactions suggest that the greatest burden (attributable risk) of AMD may be in vitamin D-deficient women with 2 high-risk alleles (*CFH* or *CFI*),

indicating that the burden is above that expected from the addition of these 2 exposures alone.<sup>41</sup> Although the relative odds of AMD by vitamin D status may be similar in different *CFH* genotypes, the higher incidence of AMD among women with 2 high-risk *CFH* alleles (CC) (compared with one or none) may to lead to a greater burden (excess fraction) of AMD cases in vitamin D-deficient vs adequate women than in other *CFH* genotypes.<sup>41,42</sup> Additive interaction might also be considered evidence of biological synergy.

We hypothesize that vitamin D suppresses a proinflammatory state in the retina via its genomic functions.<sup>43</sup> Calcitriol is thought to modulate the adaptive immune response to suppress damaging inflammation<sup>44</sup> by decreasing immune cell proinflammatory cytokine production,<sup>45-48</sup> inhibiting dendritic cell maturation,<sup>49</sup> inhibiting T- and B-lymphocyte proliferation,<sup>45,50,51</sup> and inducing T-regulatory cell function.<sup>52</sup> Polymorphisms in proteins essential to the complement cascade increase the risk of AMD.<sup>7-12</sup> The *CFH* Y402H polymorphism<sup>53,54</sup> results in a CFH protein with decreased C-reactive protein binding at this site.<sup>54,55</sup> Both C-reactive protein and the CFH protein form a protein complex involved in inhibition of the complement cascade, which works less efficiently for those homozygous for the *CFH* Y402H risk allele.<sup>54,56</sup> The *CFI* acts to inhibit the complement cascade by inactivating C3b and C4b,<sup>12,57</sup> but this regulation requires the cofactor of CFH,<sup>12,57</sup> illustrating the interconnectedness of these 2 proteins in cascade inhibition. Our study's results suggest that being vitamin D deficient might impair one's ability to suppress a localized inflammatory response, which when coupled with a dysfunctional complement pathway response could lead to increased risk of AMD above that expected from either independent risk factor alone. A study in aged mice<sup>58</sup> found that vitamin D administration led to reductions in complement

**Table 3. ORs of Any AMD Assessed at CAREDS Baseline (May 14, 2001, Through January 31, 2004) by Vitamin D Status and High-Risk AMD Genotype Among CAREDS Participants Younger Than 75 Years<sup>a,b</sup>**

Genotype	Vitamin D Status Determined by 25(OH)D								P Value for Trend <sup>c</sup>	Synergy Index (95% CI)	P Value for Interaction
	Adequate (≥30 ng/mL)		Adequate (≥20 to <30 ng/mL)		Inadequate (≥12 to <20 ng/mL)		Deficient (<12 ng/mL)				
	OR (95% CI)	No. of AMD Cases/ Total No. of Participants	OR (95% CI)	No. of AMD Cases/ Total No. of Participants	OR (95% CI)	No. of AMD Cases/ Total No. of Participants	OR (95% CI)	No. of AMD Cases/ Total No. of Participants			
<b>CFH (rs1061170)</b>											
TT	1 [Reference]	5/62	1.2 (0.4-3.5)	16/159	1.0 (0.3-3.3)	10/97	1.8 (0.5-7.1)	5/32	.40		
CT	1.3 (0.4-4.3)	9/81	2.8 (1.0-7.5)	35/165	1.8 (0.7-5.1)	22/142	3.4 (1.1-10.9)	11/41	.35	1.4 (1.1-1.7)	.25
CC	1.8 (0.5-6.9)	5/34	2.1 (0.6-7.0)	8/49	4.4 (1.3-14.1)	11/36	6.7 (1.6-28.2)	5/15	.02		
<b>CFI (rs10033900)</b>											
GG	1 [Reference]	4/47	3.1 (0.9-9.9)	17/80	1.6 (0.5-5.5)	10/73	0.8 (0.1-4.9)	2/27	.87		
GA	1.9 (0.6-6.5)	11/85	1.7 (0.5-5.1)	24/200	1.3 (0.4-4.3)	15/135	4.6 (1.3-16.6)	10/36	.34	1.4 (1.1-1.7)	.02
AA	1.2 (0.3-5.1)	4/45	2.7 (0.8-8.5)	18/92	4.2 (1.3-13.6)	18/67	6.3 (1.6-24.2)	9/25	.01		
<b>ARMS2 (rs10490924)</b>											
CC	1 [Reference]	8/125	2.2 (0.9-5.0)	29/207	1.5 (0.6-3.7)	18/160	4.9 (1.8-13.1)	13/48	.02		
AC	2.6 (0.9-7.7)	7/43	3.4 (1.5-7.8)	28/148	3.3 (1.4-8.0)	22/107	3.9 (1.3-11.8)	7/34	.58	<sup>d</sup>	.09
AA	11.9 (2.5-56.9)	4/9	1.6 (0.3-8.3)	2/18	13.8 (2.4-81.1)	3/6	1.6 (0.1-16.5)	1/6	.68		
<b>ARMS2 (rs10490924)</b>											
CC	1 [Reference]	8/125	2.2 (1.0-5.0)	29/207	1.5 (0.6-3.7)	18/160	4.9 (1.9-13.2)	13/48	.02	<sup>d</sup>	.13
AC/AA	3.6 (1.3-9.6)	11/52	3.1 (1.4-7.2)	30/166	3.7 (1.6-8.7)	25/113	3.3 (1.1-9.8)	8/40	.90		
<b>CFB/C2 (rs641153)</b>											
AA/AG	1.0	4/30	0.9 (0.2-3.2)	8/68	0.6 (0.1-3.1)	3/30	3.3 (0.8-13.9)	7/20	.32	<sup>d</sup>	.48
GG	0.8 (0.2-2.5)	15/147	1.3 (0.4-3.9)	51/305	1.2 (0.4-3.6)	40/245	1.6 (0.5-5.5)	14/68	.16		
<b>C3 (rs2230199)</b>											
CC	1.0	2/9	<sup>d</sup>	0/12	1.9 (0.3-14.4)	4/12	<sup>d</sup>	0/4	.61		
GC	0.7 (0.1-3.8)	8/58	0.8 (0.1-4.1)	22/136	0.5 (0.1-2.6)	10/82	0.8 (0.1-5.4)	5/26	.89	1.3 (0.8-2.1)	.09
GG	0.3 (0.1-1.9)	9/110	0.7 (0.1-3.6)	37/225	0.7 (0.1-3.4)	29/181	1.4 (0.3-7.9)	16/58	.01		

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AMD, age-related macular degeneration; CAREDS, Carotenoids in Age-Related Eye Disease Study; OR, odds ratio.

<sup>a</sup> Adjusted for age, smoking pack-years, iris pigmentation, self-reported cardiovascular disease, self-reported diabetes status, and hormone use status.

<sup>b</sup> The single-nucleotide alleles are listed in order of increasing AMD risk.

<sup>c</sup> P value for ordinal trend across categorized concentrations of 25(OH)D within each genotype class.

<sup>d</sup> Synergy index could not be estimated.

component C3b retinal expression (Bruch membrane) compared with controls. Currently, a biologic mechanism for a synergistic interaction between vitamin D status and complement pathway protein function is unknown.

Some<sup>14,25,26</sup> but not all<sup>28,29</sup> epidemiologic studies support a role of vitamin D in AMD. In addition to vitamin D intake or 25(OH)D, vitamin D status can also be inferred from genotypes previously associated with serum 25(OH)D. This is an underlying tenet of the method known as mendelian ran-

domization, which is an increasingly used method in epidemiology to test unconfounded, causal associations between exposures and disease.<sup>59</sup> Morrison et al<sup>26</sup> observed that variants (rs1570669, rs1570670, rs2274130, rs2296239, rs4809957) in the vitamin 1,25(OH)<sub>2</sub>D catabolizing *CYP24A1* gene were associated with a decreased odds of AMD. We examined these SNPs and also an SNP from a genome-wide association meta-analysis (rs6013897)<sup>60</sup> and another that is a nonsynonymous (coding) SNP (rs35031736) in an effort to more broadly cap-

ture variation in this gene. We observed that 2 polymorphisms in the *CYP2R1* gene and 3 in the *VDR* gene were associated with increased odds of AMD. However, these SNPs were not found to be predictors of 25(OH)D status in 2 large genome-wide association studies<sup>60,61</sup> or SNPs known to influence the function of the *VDR*.<sup>62</sup> Adjustment for multiple testing<sup>63</sup> would have resulted in no statistically significant findings. Discrepancies in study findings may be explained by differences in stages of AMD between study samples because there was a large proportion of late AMD cases (36%-38%) in the previous study<sup>26</sup> compared with CAREDS (6%; n = 4 neovascular, n = 5 geographic atrophy).

Few studies<sup>64-69</sup> have examined associations between dietary factors and genes on AMD risk (K.J.M., unpublished data, 2014) and none with 25(OH)D. Previous literature has observed that intake of fish (a rich food source of vitamin D)<sup>69</sup> and  $\omega$ 3 docosahexaenoic fatty acids (a nutrient concentrated in fish)<sup>68</sup> were protective against progression to late AMD in those with high genetic risk. Vitamin D status may partly explain these findings.

It is also possible that vitamin D status is a marker of overall healthy lifestyle, and findings with respect to 25(OH)D are confounded by such factors as diet, body fatness, and physical activity, factors correlated with vitamin D status.<sup>23</sup> A previous study<sup>70</sup> found the greatest odds for late AMD in persons with the high-risk *CFH* Y402H genotype and high BMI. Results in CAREDS revealed that a healthy lifestyle score was associated with a reduced odds of AMD<sup>71</sup> and that the greatest odds of disease was in women with the CC *CFH* Y402H genotype and low healthy lifestyle score (K.J.M., unpublished data, 2014). The findings of these studies parallel our findings with vitamin D status. We adjusted ORs for BMI and physical activity, but this did not influence our study conclusions. Difficulty in adjusting for highly correlated variables makes it impossible outside the

context of a clinical trial to know whether vitamin D status causally influences risk for AMD.

This study was limited by a small sample size for the investigation of interactions, with small numbers of cases within joint effect cells leading to wide CIs. It is possible our findings are purely by chance because we did not adjust for multiple testing. The possibility of residual confounding cannot be excluded because this was an observational study. Although this study assessed prevalent AMD, ocular photographs were taken approximately 6 years after assessment of vitamin D status. Only 1.9% (23 of 1230) of CAREDS women with 25(OH)D data, genetic data, and fundus photographs self-reported having AMD at WHI-OS baseline (October 1, 1993, to December 31, 1998). Early AMD is asymptomatic, so it is unlikely that behavior changes as a result of AMD occurred before the WHI-OS baseline blood assessment, resulting in reverse causality. Because our study included primarily highly educated white women, our findings may not be generalizable to other populations.

## Conclusions

Despite these limitations, minimal data exist on interactions between dietary and genetic factors in the context of age-related eye disease. To the best of our knowledge, effect modification of genetic risk by vitamin D status has not previously been explored. This cohort of postmenopausal women is very well defined, with detailed data on AMD risk factors and graded retinal photographs for age-related eye disease, and adds to the existing body of literature. Our study provides evidence of a suggestive joint effect between vitamin D status and genotypes of complement factor genes. Maintenance of an adequate vitamin D status and likely an overall healthy lifestyle may reduce the total burden of early AMD to the greatest extent in those with high genetic risk for genes in the complement cascade.

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**Study concept and design:** Millen, Meyers, Mares.  
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