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Association between vitamin D status and age-related macular degeneration by genetic risk

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Abstract

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

Objective—We examined 1) whether this association is modified by genetic risk for AMD and 2) if there is an association between AMD and single nucleotide polymorphisms (SNPs) of genes involved in vitamin D transport, metabolism and genomic function.

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Dr. Julie Mares had full access to all of the data in the study and takes responsibility for the integrity for the data and the accuracy of the data analysis.

Contribution of authors: AEM directed analyses, interpreted the data and wrote the manuscript. KJM and JAM aided in data analysis, interpretation of data and manuscript writing. JAM designed CAREDS and JAM, AEM and KJM oversaw data collection. ZL conducted all analyses, aided in interpretation of data and manuscript writing. KJM, CDE, and SKI informed the strategy for selecting SNPS within candidate genes and SKI conducted genotyping. CDE, RBW, ESL, LFT, SKI, JR and GES participated in interpretation of data analysis and manuscript writing.

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Design, Setting and Participants—Women were postmenopausal and participants of the Carotenoids in Age-Related Eye Disease Study (CAREDS) (54 to <75 years) with available serum 25(OH)D concentrations (assessed from 1994–1998), genetic data, and measures of AMD (n=142) assessed at CAREDS baseline from 2001–2004 (n=913).

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% confidence intervals (CIs) for AMD by the joint effects of 25(OH)D (<30, 30 to <50, 50 to <75, and 75 nmol/L) and risk genotype (noncarrier, one, or two risk alleles). The referent group was noncarriers with adequate vitamin D status (75 nmol/L). 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.

Results—We observed a 6.7-fold increased odds of AMD (95% CI=1.6, 28.2) among women with deficient vitamin D status (25(OH)D<30 nmol/L) and two risk alleles for complement factor H (*CFH*) Y402H (SI for additive interaction=1.4, 95% CI=1.1, 1.7; p for multiplicative interaction=0.25,. A significant additive (SI=1.4, 95% CI=1.1, 1.7) and multiplicative interaction (p=0.02) was observed for deficient women with two high risk complement factor I (*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 was highest in those with deficient vitamin D status and two risk alleles for *CFH* and *CFI g*enotype suggesting a synergistic effect between vitamin D status and complement cascade protein function. Limited sample size led to wide confidence intervals. Findings may be due to chance or explained by residual confounding.

INTRODUCTION

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

Vitamin D has anti-inflammatory and immune modulating properties[13] and is hypothesized to protect against development of AMD. [14] Animal models[15–18] and epidemiologic[19–21] studies of auto-immune disease support this hypothesis. *In vivo* research shows that both the proteins for 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 [1,25$(OH)_2$D]) are expressed in the retina. [22]

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Previous studies have found that decreased odds of AMD are associated with high compared to low concentrations of 25(OH)D,[14,23,24] vitamin D intake from foods,[23,25] and certain polymorphisms in genes involved in vitamin D metabolism,[26] although other studies do not support an association. [27–29] Research in the Carotenoids in Age-Related Eye Disease Study (CAREDS) showed that postmenopausal women <75 years of age had an increased odds of AMD if they had low versus high vitamin D status assessed with intake of vitamin D and 25(OH)D concentrations. [23] We proposed 1) to investigate whether this previously observed association between 25(OH)D and AMD was stronger in women with established AMD risk genotypes, including several which influence inflammatory pathways, and 2) to investigate the association between AMD and SNPs in genes involved in vitamin D transport, metabolism and genomic function.

METHODS

Study Sample

The Carotenoids in Age-Related Eye Disease Study (CAREDS) was conducted to study the association of lutein and zeaxanthin with age-related. [30–32] A total of 2,005 women enrolled in CAREDS between 2001 and 2004, an average of 6 years after WHI-OS baseline (1994–1998). This research study was approved at each institution by their institutional review board and the study procedures conformed to the Declaration of Helsinki.

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 1,853 of 1,894 women attending CAREDS' baseline study visits (2001–2004). Four additional women were included who had no retinal photographs, but provided a doctor's confirmation of AMD status. Of these, 1,230 had sufficient serum at WHI-OS baseline (1994–1998) for 25(OH)D assessment, gave approval for use of their genetic data, had sufficient DNA for genotyping, and passed quality assurance/control tests for genotyping.

Women enrolled in CAREDS but excluded (n=775) due to missing serum 25(OH)D and genetic data had a similar prevalence of AMD, and a similar mean intake of vitamin D compared to women with these data (n=1,230) (eTable 1). Women with compared to women without missing data were slightly older (p=0.01) and had healthier lifestyle scores (p=0.06).

The current analyses are limited to the sample of women 54 to <75 years of age (n=913), in whom we previously observed an association between vitamin D status and AMD[23]. We do not present data in women 75 years due to the small sample size (n=317) and potential selective mortality bias in this older sample[23]. A slightly larger sample (n=1,230) of women <75 years was available for the analysis of the association between AMD and SNPs of vitamin D related genes since women were not further excluded for missing 25(OH)D data.

Retinal photographs

Stereoscopic retinal fundus photographs were taken at CAREDS baseline (2001–2004) and graded by the University of Wisconsin Fundus Photography Reading Center using the Age-Related Eye Disease Study (AREDS) protocol. [33] The outcome for these analyses was

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presence of either early or late AMD (any AMD). Early AMD was classified similar to AREDS Category 3,[33] including the presence of one or more large drusen (125 microns) or extensive intermediate drusen (area 360 microns when soft indistinct drusen were present or 650 microns when soft indistinct drusen were absent). Different from the AREDS Category 3, presence of early AMD also included having pigmentary abnormalities; an increase or decrease in pigmentation if accompanied by at least one druse 63 microns. Late AMD included geographic atrophy (non-central or central), neovascularization, or exudation in the center subfield. eTable 2 describes the distribution of AMD cases by serverity. The majority of these cases (133 of 142 or 94%) were early AMD. The reference group included women who had neither early nor late AMD; generally corresponding to AREDS categories 1 and 2. [33]

Serum Vitamin D Assays

Fasting bloods collected at WHI-OS baseline (1994–1998) were assessed for serum concentrations of 25(OH)D using the Diasorin LIAISON® chemiluminescence method (Heartland Assays, Inc.). [23] We adjusted for season of blood draw by regressing 25(OH)D concentrations on month of blood draw using a nonparametric regression technique. [23,34] 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

Blood, obtained at WHI-OS baseline, was stored, frozen at −80°C until DNA was extracted from the buffy coats and genotyped. Within CAREDS, a 768 custom SNP panel was designed to genotype candidate genes for carotenoid status,[35] vitamin D status,[36] and risk for AMD. The panel included five established genetic variants for late AMD: Y402H (rs1061170) in complement factor H *(CFH)*,[7] rs10033900 in complement factor I (*CFI)*,[12] rs641153 in complement factor B/complement 2 (*CFB/C2)*,[8,9] rs2230199 in complement 3 (*C3)*,[10] and A69S (rs10490924) in the age-related maculopathy susceptibility 2 locus (*ARMS2)*. [8,37] SNPs from six vitamin D related genes, some of which have been previously described in CAREDS,[36] were also included: 7-dehydrocholesterol reductase *(DHCR7)*, cytochrome P450, family 2, subfamily R, polypeptide *1(CYP2R1)*, *CYP27B1, CYP24A1*, vitamin D receptor *(VDR)* and group-specific component/vitamin D binding protein *(GC)*.

Genotyping was conducted at Case Western Reserve University (Cleveland, OH). Genotyping of all noted SNPs above, but *CFH* Y402H, were conducted with an Illumina Custom GoldenGate Assay and genotype calls were made using Illumina Genome Studio. Genotyping of Y402H was conducted using the KASP Assay at LCG Genomics (Teddington, UK) and called via the KASP SNP Genotyping System. *CFH* Y402H genotypes were imputed using MACH (http://www.sph.umich.edu/csg/abecasis/MACH/index.html) when there was insufficient DNA for KASP genotyping after Illumina genotyping (~3%). Imputation was done using the available chromosome 1 SNPs from Illumina (n=14 SNPs) and 1000 Genomes Project European ancestry panel (CEU) as a reference. The resulting R^2 from imputing Y402H was 99.5%. All SNPs passed standard quality control filters[38] including Hardy-Weinberg Equilibrium χ^2 *P*-value > 1.0×10^{-6}, minor allele frequency > 0.01, and genotype call rates >95%.

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Statistical Analyses

We created four categories of vitamin D status based on 25(OH)D and the Institute of Medicine’s Dietary Reference Intakes[39] (deficient: <30; inadequate: 30 to <50; adequate 50 to <75; and adequate 75 nmol/L). We categorized women into three genotype groups for each SNP according to whether they had two high risk alleles, one, or were a noncarrier for each risk gene (additive genetic model). For *CFB/C2*, we combined women with one and two copies of the minor allele (A) due 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% confidence intervals (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 25(OH)D 75 nmol/L and a genotype indicative of low AMD risk. 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. [23] A p for ordinal trend across vitamin D status categories was conducted 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, 2 risk alleles) and vitamin D status categories as ordinal variables. A interaction term with a p-value <0.05 was considered a statistically significant. A deviation from an additive effect was examined using the Synergy Index (SI). [40] An SI >1.0 or <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 genotype of vitamin D related genes using an additive genetic model.

All analyses were conducted using SAS software, version 9.2® (SAS Institute Inc., Cary NC).

RESULTS

Women with deficient compared to adequate status were less likely to be Caucasian, were less likely to have high incomes, education beyond high school, were more likely to be nondrinkers, were less likely to have healthy diet patterns or engage in physically activity, had 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 presented by vitamin D status, genotypes of high risk AMD genes and vitamin D related genes. Women with deficient (<30 nmol/L) compared to adequate (75 nmol/L) status had an 2.6-fold increased odds of AMD (Table 2). There was an over 2-fold increased odds of AMD among women with two risk alleles for *CFH* (CC) or *ARMS2* (AA) compared to noncarriers (Table 3). The p-for trend for increasing number of

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risk alleles was 0.04 for *CFI*. SNPs in *CYP2R1* (rs11819875 and rs12418214) and three in *VDR* (rs11168275, rs2189480, and rs2239186) were associated with increased risk of AMD (eTable 2).

Table 4 examines the joint effects of genotypes by vitamin D status. The adjusted ORs presented in these tables differ minimally from a ORs adjusted only for age (data not shown). There was a 6.7-fold, 95% CI (1.6, 28.2), increased odds of AMD among vitamin D deficient women with two risk *CFH* alleles (CC) compared to noncarriers with adequate vitamin D status (75 nmol/L), with a SI (95% CI)=1.4 (1.1, 1.7). There was a 6.3-fold, 95% CI (1.6, 24.2), increased odds of AMD among vitamin D deficient women with two risk *CFI* alleles (AA) relative to noncarriers with adequate vitamin D status, with a SI (95% CI)=1.4 (1.1, 1.7) and a p for interaction=0.02. Further adjustment of models for education, principal components (PC) from PC analysis using 176 ancestry informative markers, BMI 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 4 also presents the p-for trend values for the odds of AMD across ordinal decreasing concentrations of 25(OH)D stratified by genotype. The p-for trend was <0.05 for women with two high risk *CFH* alleles (CC), two 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 versus 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 two high risk alleles (either *CFH* or *CFI*); that the burden is above that expected from the addition of these two exposures alone. [41] 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 two high risk *CFH* alleles (CC) (as compared to one or none) may to lead to a greater burden (excess fraction) of AMD cases in vitamin D deficient versus adequate women than in other *CFH genotypes*. [41,42] Additive interaction might also be considered evidence of biological synergy.

We hypothesize that vitamin D suppresses a pro-inflammatory state in the retina via its genomic functions. [43] Calcitriol is thought to modulate the adaptive immune response to suppress damaging inflammation[44] by decreasing immune cell pro-inflammatory cytokine production,[45–48] inhibiting dendritic cell maturation,[49] inhibiting T and B lymphocyte proliferation,[45,50,51] and inducing T-regulatory cell function. [52] Polymorphisms in proteins essential to the complement cascade, have been shown to increase risk for AMD. [7–12] The *CFH* Y402H polymorphism[53,54] results in a CFH protein with decreased C-reactive protein (CRP) binding at this site. [54,55] CRP and CFH form a protein complex involved in inhibition of the complement cascade which works less efficiently for those homozygous for the *CFH*

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Y402H risk allele.[54,56] CFI acts to inhibit the complement cascade by inactivating C3b and C4b[12,57] but this regulation requires the cofactor of CFH,[12,57] illustrating the interconnectedness of these two 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, that 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, showed that vitamin D administration led to reductions in complement component C3b retinal expression (Bruch's membrane) compared to controls.[58] Currently, a biologic mechanism for a synergistic interaction between vitamin D status and complement pathway protein function is unknown.

Some [14,25,26], but not all [28,29] 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 randomization, which is an increasingly used method in epidemiology to test unconfounded, causal associations between exposures and disease.[59] Morrison et al.[26] observed that variants (rs1570669, rs1570670, rs2274130, rs2296239, rs4809957) in the vitamin 1,25$(OH)_2$D catabolizing *CYP24A1* gene were associated with a decreased odds of AMD. We examined these SNPs, and also a SNP from a genome-wide association meta-analysis (rs6013897)[60] and another that is a non-synonymous (coding) SNP (rs35031736) in an effort to more broadly capture variation in this gene. We observed that two polymorphisms in the *CYP2R1* gene and three 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 two large genome wide association studies [60,61] or SNPs known to influence the function of the *VDR*.[62] Adjustment for multiple testing[63] would have resulted in no statistically significant findings. Discrepancies in study findings may be explained by differences in stages of AMD between study samples, as there was a large proportion of late AMD cases (36–38%) in the previous study[26] as compared to CAREDS (6%; n=4 neovascular, n=5 geographic atrophy).

Few studies have examined associations between dietary factors and genes on AMD risk ([64–69] [Meyers KJ, 2014 unpublished paper]), and none with 25(OH)D. Previous literature has observed that intake of fish (a rich food source of vitamin D) [69] and omega-3 docosahexaenoic fatty acids (a nutrient concentrated in fish)[68] 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.[23] A previous study showed the greatest odds for late AMD in persons with the high risk *CFH* Y402H genotype and high BMI[70]. Results in CAREDS showed that a healthy lifestyle score was associated with a reduced odds of AMD,[71] and that the greatest odds of disease were in women with the CC *CFH* Y402H genotype and low healthy lifestyle score (Meyers KJ, 2014 unpublished paper). These studies' findings parallel our findings with vitamin D status. We did adjust ORs for BMI and physical activity, but this did not influence our study conclusions. Difficulty in

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adjusting for highly correlated variables makes it impossible outside the context of a clinical trial to know if 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 confidence intervals. It is possible our findings are purely by chance as we did not adjust for multiple testing. The possibility of residual confounding cannot be excluded as this was an observational study. Although this study assessed prevalent AMD, ocular photographs were taken ~6 years after assessment of vitamin D status. Only 1.9% of CAREDS women with 25(OH)D, genetic data and fundus photographs self-reported having AMD at WHI-OS baseline (1994–1998). Early AMD is asymptomatic, so it is unlikely that behavior changes as a result of AMD occurred prior to the WHI-OS baseline blood assessment, resulting in reverse causality. Because our study included primarily highly educated, Caucasian women our findings may not be generalizable to other populations.

Despite these limitations, minimal data exists 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. In conclusion, 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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REFERENCES

1. Friedman DS, O'Colmain BJ, Munoz B, et al. Prevalence of age-related macular degeneration in the United States. Arch Ophthalmol. 2004 Apr; 122(4):564–572. [PubMed: 15078675]
2. Barouch FC, Miller JW. The role of inflammation and infection in age-related macular degeneration. International ophthalmology clinics. 2007 Spring;47(2):185–197. [PubMed: 17450018]
3. Klein R, Klein BE, Tomany SC, Cruickshanks KJ. Association of emphysema, gout, and inflammatory markers with long-term incidence of age-related maculopathy. Arch Ophthalmol. 2003 May; 121(5):674–678. [PubMed: 12742845]
4. van Leeuwen R, Ikram MK, Vingerling JR, Witteman JC, Hofman A, de Jong PT. Blood pressure, atherosclerosis, and the incidence of age-related maculopathy: the Rotterdam Study. Invest Ophthalmol Vis Sci. 2003 Sep; 44(9):3771–3777. [PubMed: 12939290]
5. Hong T, Tan AG, Mitchell P, Wang JJ. A review and meta-analysis of the association between C-reactive protein and age-related macular degeneration. Surv Ophthalmol. 2011 May-Jun;56(3):184–194. [PubMed: 21420705]
6. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. Prog Retin Eye Res. 2001 Nov; 20(6):705–732. [PubMed: 11587915]
7. Thakkinstian A, Han P, McEvoy M, et al. Systematic review and meta-analysis of the association between complement factor H Y402H polymorphisms and age-related macular degeneration. Hum Mol Genet. 2006 Sep 15; 15(18):2784–2790. [PubMed: 16905558]
8. Ting AY, Lee TK, MacDonald IM. Genetics of age-related macular degeneration. Current opinion in ophthalmology. 2009 Sep; 20(5):369–376. [PubMed: 19587596]
9. Jakobsdottir J, Conley YP, Weeks DE, Ferrell RE, Gorin MB. C2 and CFB genes in age-related maculopathy and joint action with CFH and LOC387715 genes. PloS one. 2008; 3(5):e2199. [PubMed: 18493315]
10. Thakkinstian A, McKay GJ, McEvoy M, et al. Systematic review and meta-analysis of the association between complement component 3 and age-related macular degeneration: a HuGE review and meta-analysis. Am J Epidemiol. 2011 Jun 15; 173(12):1365–1379. [PubMed: 21576320]
11. Holliday EG, Smith AV, Cornes BK, et al. Insights into the genetic architecture of early stage age-related macular degeneration: a genome-wide association study meta-analysis. PloS one. 2013; 8(1):e53830. [PubMed: 23326517]

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12. Fagerness JA, Maller JB, Neale BM, Reynolds RC, Daly MJ, Seddon JM. Variation near complement factor I is associated with risk of advanced AMD. European journal of human genetics : EJHG. 2009 Jan; 17(1):100–104. [PubMed: 18685559]

13. Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. Nature reviews. Immunology. 2008 Sep; 8(9):685–698.

14. Parekh N, Chappell RJ, Millen AE, Albert DM, Mares JA. Association between vitamin D and age-related macular degeneration in the Third National Health and Nutrition Examination Survey, 1988 through 1994. Arch Ophthalmol. 2007 May; 125(5):661–669. [PubMed: 17502506]

15. Mathieu C, Waer M, Casteels K, Laureys J, Bouillon R. Prevention of type I diabetes in NOD mice by nonhypercalcemic doses of a new structural analog of 1,25-dihydroxyvitamin D3, KH1060. Endocrinology. 1995 Mar; 136(3):866–872. [PubMed: 7867594]

16. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. Proc Natl Acad Sci U S A. 1996 Jul 23; 93(15):7861–7864. [PubMed: 8755567]

17. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. J Nutr. 1998 Jan; 128(1):68–72. [PubMed: 9430604]

18. Tang J, Zhou R, Luger D, et al. Calcitriol suppresses antiretinal autoimmunity through inhibitory effects on the Th17 effector response. J Immunol. 2009 Apr 15; 182(8):4624–4632. [PubMed: 19342637]

19. Holick MF. Vitamin D: important for prevention of osteoporosis, cardiovascular heart disease, type 1 diabetes, autoimmune diseases, and some cancers. Southern medical journal. 2005 Oct; 98(10): 1024–1027. [PubMed: 16295817]

20. Mark BL, Carson JA. Vitamin D and autoimmune disease--implications for practice from the multiple sclerosis literature. J Am Diet Assoc. 2006 Mar; 106(3):418–424. [PubMed: 16503232]

21. Cutolo M, Otsa K, Uprus M, Paolino S, Seriolo B. Vitamin D in rheumatoid arthritis. Autoimmunity reviews. 2007 Nov; 7(1):59–64. [PubMed: 17967727]

22. Alsalem JA, Patel D, Susarla R, et al. Characterization of vitamin D production by human ocular barrier cells. Invest Ophthalmol Vis Sci. 2014 Apr; 55(4):2140–2147. [PubMed: 24576880]

23. Millen AE, Voland R, Sondel SA, et al. Vitamin D Status and Early Age-Related Macular Degeneration in Postmenopausal Women. Arch Ophthalmol. 2011 Apr; 129(4):481–489. [PubMed: 21482873]

24. Graffe A, Milea D, Annweiler C, et al. Association between hypovitaminosis D and late stages of age-related macular degeneration: a case-control study. Journal of the American Geriatrics Society. 2012 Jul; 60(7):1367–1369. [PubMed: 22788394]

25. Seddon JM, Reynolds R, Shah HR, Rosner B. Smoking, Dietary Betaine, Methionine, and Vitamin D in Monozygotic Twins with Discordant Macular Degeneration: Epigenetic Implications. Ophthalmology. 2011 May 25. May 25. [Epub ahead of print].

26. Morrison MA, Silveira AC, Huynh N, et al. Systems biology-based analysis implicates a novel role for vitamin D metabolism in the pathogenesis of age-related macular degeneration. Human genomics. 2011 Oct; 5(6):538–568. [PubMed: 22155603]

27. Singh A, Falk MK, Subhi Y, Sorensen TL. The association between plasma 25-hydroxyvitamin D and subgroups in age-related macular degeneration: a cross-sectional study. PloS one. 2013; 8(7):e70948. [PubMed: 23923033]

28. Day S, Acquah K, Platt A, Lee PP, Mruthyunjaya P, Sloan FA. Association of vitamin D deficiency and age-related macular degeneration in medicare beneficiaries. Arch Ophthalmol. 2012 Aug; 130(8):1070–1071. [PubMed: 22893083]

29. Golan S, Shalev V, Treister G, Chodick G, Loewenstein A. Reconsidering the connection between vitamin D levels and age-related macular degeneration. Eye (Lond). 2011 Sep; 25(9):1122–1129. [PubMed: 21818133]

30. Mares JA, LaRowe TL, Snodderly DM, et al. Predictors of optical density of lutein and zeaxanthin in retinas of older women in the Carotenoids in Age-Related Eye Disease Study, an ancillary study of the Women's Health Initiative. Am J Clin Nutr. 2006 Nov; 84(5):1107–1122. [PubMed: 17093164]

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31. Parekh N, Voland RP, Moeller SM, et al. Association between dietary fat intake and age-related macular degeneration in the Carotenoids in Age-Related Eye Disease Study (CAREDS): an ancillary study of the Women's Health Initiative. Arch Ophthalmol. 2009 Nov; 127(11):1483–1493. [PubMed: 19901214]
32. Moeller SM, Parekh N, Tinker L, et al. Associations between intermediate age-related macular degeneration and lutein and zeaxanthin in the Carotenoids in Age-related Eye Disease Study (CAREDS): ancillary study of the Women's Health Initiative. Arch Ophthalmol. 2006 Aug; 124(8):1151–1162. [PubMed: 16908818]
33. The Age-Related Eye Disease Study system for classifying age-related macular degeneration from stereoscopic color fundus photographs: the Age-Related Eye Disease Study Report Number 6. Am J Ophthalmol. 2001 Nov; 132(5):668–681. [PubMed: 11704028]
34. SAS Institute Inc. SAS/STAT® 9.2 *User's Guide*. The LOESS Procedure (Book Excerpt). Cary, NC: SAS Institute Inc; 2008. http://support.sas.com/documentation/cdl/en/statugloess/61801/PDF/default/statugloess.pdf [Accessed January 7, 2015]
35. Meyers KJ, Johnson EJ, Bernstein PS, et al. Genetic determinants of macular pigments in women of the Carotenoids in Age-Related Eye Disease Study. Invest Ophthalmol Vis Sci. 2013 Mar; 54(3):2333–2345. [PubMed: 23404124]
36. Engelman CD, Meyers KJ, Iyengar SK, et al. Vitamin D intake and season modify the effects of the GC and CYP2R1 genes on 25-hydroxyvitamin D concentrations. J Nutr. 2013 Jan; 143(1):17–26. [PubMed: 23190755]
37. Conley YP, Jakobsdottir J, Mah T, et al. CFH, ELOVL4, PLEKHA1 and LOC387715 genes and susceptibility to age-related maculopathy: AREDS and CHS cohorts and meta-analyses. Hum Mol Genet. 2006 Nov 1; 15(21):3206–3218. [PubMed: 17000705]
38. Laurie CC, Doheny KF, Mirel DB, et al. Quality control and quality assurance in genotypic data for genome-wide association studies. Genetic epidemiology. 2010 Sep; 34(6):591–602. [PubMed: 20718045]
39. IOM (Institute of Medicine). Dietary Reference Intakes for Calcium and Vitamin D. Washington DC: The National Academy Press; 2011. Summary; p. 1-14.
40. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology. 1992 Sep; 3(5):452–456. [PubMed: 1391139]
41. Szklo, M.; Nieto, FJ. Epidemiology: Beyond the Basics. 3rd. Burlington, MA: Jones and Bartlett Publishers, LLC; 2014. Quality Assurance and Control; p. 185-223.
42. Zhang X, Smith-Warner SA, Chan AT, et al. Aspirin use, body mass index, physical activity, plasma C-peptide, and colon cancer risk in US health professionals. Am J Epidemiol. 2011 Aug 15; 174(4):459–467. [PubMed: 21673123]
43. Ramagopalan SV, Heger A, Berlanga AJ, et al. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome research. 2010 Oct; 20(10):1352–1360. [PubMed: 20736230]
44. Yin K, Agrawal DK. Vitamin D and inflammatory diseases. Journal of inflammation research. 2014; 7:69–87. [PubMed: 24971027]
45. Bhalla AK, Amento EP, Krane SM. Differential effects of 1,25-dihydroxyvitamin D3 on human lymphocytes and monocyte/macrophages: inhibition of interleukin-2 and augmentation of interleukin-1 production. Cellular immunology. 1986 Apr 1; 98(2):311–322. [PubMed: 3489547]
46. D'Ambrosio D, Cippitelli M, Cocciolo MG, et al. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3 Involvement of NF-kappaB downregulation in transcriptional repression of the p40 gene. J Clin Invest. 1998 Jan 1; 101(1):252–262. [PubMed: 9421488]
47. Lefebvre, d'Hellencourt C.; Montero-Menei, CN.; Bernard, R.; Couez, D. Vitamin D3 inhibits proinflammatory cytokines and nitric oxide production by the EOC13 microglial cell line. J Neurosci Res. 2003 Feb 15; 71(4):575–582. [PubMed: 12548714]
48. Rigby WF, Denome S, Fanger MW. Regulation of lymphokine production and human T lymphocyte activation by 1,25-dihydroxyvitamin D3 Specific inhibition at the level of messenger RNA. J Clin Invest. 1987 Jun; 79(6):1659–1664. [PubMed: 2884234]

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49. Penna G, Adorini L. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J Immunol. 2000 Mar 1; 164(5):2405–2411. [PubMed: 10679076]

50. Rigby WF, Stacy T, Fanger MW. Inhibition of T lymphocyte mitogenesis by 1,25-dihydroxyvitamin D3 (calcitriol). J Clin Invest. 1984 Oct; 74(4):1451–1455. [PubMed: 6332829]

51. Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. J Immunol. 2007 Aug 1; 179(3):1634–1647. [PubMed: 17641030]

52. Daniel C, Sartory NA, Zahn N, Radeke HH, Stein JM. Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile. The Journal of pharmacology and experimental therapeutics. 2008 Jan; 324(1):23–33. [PubMed: 17911375]

53. Giannakis E, Jokiranta TS, Male DA, et al. A common site within factor H SCR 7 responsible for binding heparin, C-reactive protein and streptococcal M protein. Eur J Immunol. 2003 Apr; 33(4): 962–969. [PubMed: 12672062]

54. Anderson DH, Radeke MJ, Gallo NB, et al. The pivotal role of the complement system in aging and age-related macular degeneration: hypothesis re-visited. Prog Retin Eye Res. 2010 Mar; 29(2): 95–112. [PubMed: 19961953]

55. Laine M, Jarva H, Seitsonen S, et al. Y402H polymorphism of complement factor H affects binding affinity to C-reactive protein. J Immunol. 2007 Mar 15; 178(6):3831–3836. [PubMed: 17339482]

56. Mihlan M, Stippa S, Jozsi M, Zipfel PF. Monomeric CRP contributes to complement control in fluid phase and on cellular surfaces and increases phagocytosis by recruiting factor H. Cell death and differentiation. 2009 Dec; 16(12):1630–1640. [PubMed: 19680263]

57. Catterall CF, Lyons A, Sim RB, Day AJ, Harris TJ. Characterization of primary amino acid sequence of human complement control protein factor I from an analysis of cDNA clones. The Biochemical journal. 1987 Mar 15; 242(3):849–856. [PubMed: 2954545]

58. Lee V, Rekhi E, Hoh Kam J, Jeffery G. Vitamin D rejuvenates aging eyes by reducing inflammation, clearing amyloid beta and improving visual function. Neurobiology of aging. 2012 Oct; 33(10):2382–2389. [PubMed: 22217419]

59. Willett, W. Nutritional Epidemiology. New York, NY: Oxford University Press; 2013. Chapter 14, Genetics in Dietary Analyses; p. 334-343.

60. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 2010 Jul 17; 376(9736):180–188. [PubMed: 20541252]

61. Ahn J, Yu K, Stolzenberg-Solomon R, et al. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet. 2010 Jul 1; 19(13):2739–2745. [PubMed: 20418485]

62. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene. 2004 Sep 1; 338(2):143–156. [PubMed: 15315818]

63. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ. 1995 Jan 21.310(6973):170. [PubMed: 7833759]

64. Klein ML, Francis PJ, Rosner B, et al. CFH and LOC387715/ARMS2 genotypes and treatment with antioxidants and zinc for age-related macular degeneration. Ophthalmology. 2008 Jun; 115(6):1019–1025. [PubMed: 18423869]

65. Wang JJ, Rochtchina E, Smith W, et al. Combined effects of complement factor H genotypes, fish consumption, and inflammatory markers on long-term risk for age-related macular degeneration in a cohort. Am J Epidemiol. 2009 Mar 1; 169(5):633–641. [PubMed: 19074778]

66. Ho L, van Leeuwen R, Witteman JC, et al. Reducing the genetic risk of age-related macular degeneration with dietary antioxidants, zinc, and omega-3 fatty acids: the Rotterdam study. Arch Ophthalmol. 2011 Jun; 129(6):758–766. [PubMed: 21670343]

67. Chiu CJ, Conley YP, Gorin MB, et al. Associations between genetic polymorphisms of insulin-like growth factor axis genes and risk for age-related macular degeneration. Invest Ophthalmol Vis Sci. 2011; 52(12):9099–9107. [PubMed: 22058336]

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68. Reynolds R, Rosner B, Seddon JM. Dietary omega-3 fatty acids, other fat intake, genetic susceptibility, and progression to incident geographic atrophy. Ophthalmology. 2013 May; 120(5): 1020–1028. [PubMed: 23481534]
69. Wang JJ, Buitendijk GH, Rochtchina E, et al. Genetic susceptibility, dietary antioxidants, and long-term incidence of age-related macular degeneration in two populations. Ophthalmology. 2014 Mar; 121(3):667–675. [PubMed: 24290803]
70. Seddon JM, George S, Rosner B. Cigarette smoking, fish consumption, omega-3 fatty acid intake, and associations with age-related macular degeneration: the US Twin Study of Age-Related Macular Degeneration. Arch Ophthalmol. 2006 Jul; 124(7):995–1001. [PubMed: 16832023]
71. Mares JA, Voland RP, Sondel SA, et al. Healthy lifestyles related to subsequent prevalence of age-related macular degeneration. Arch Ophthalmol. 2011 Apr; 129(4):470–480. [PubMed: 21149749]

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Table 1

Characteristics[*] (mean ± standard error [SE] or n [%]) of participants by vitamin D status determined at WHI-OS baseline (1994–1998): the CAREDS individual younger than age 75 (n=913).

Characteristic	Vitamin D status determined by 25-hydroxyvitamin D (25[OH]D) in nmol/L				p-value[†]
	Adequate (n=177)	Adequate (n=373)	Inadequate (n=275)	Deficient (n=88)	
	(75)	(50 to <75)	(30 to <50)	(<30)	
25(OH)D, nmol/L, median (range)	85.39 (75.19–165.33)	60.50 (50.02–74.96)	40.88 (30.13–49.96)	24.32 (8.20–29.92)	-
Demographic					
Age at eye photography, year	65.8 ± 0.4	66.0 ± 0.3	66.9 ± 0.3	66.1 ± 0.5	0.08
Ethnicity (white)	174 (98.3%)	367 (98.4%)	267 (97.1%)	82 (93.2%)	**0.02**
Income (75,000/year)	48 (27.6%)	67 (18.8%)	41 (15.8%)	10 (12.1%)	**0.001**
Education					**0.005**
High School or less	39 (22.0%)	77 (20.6%)	62 (22.6%)	32 (36.4%)	
College	78 (44.1%)	184 (49.3%)	136 (49.5%)	40 (45.5%)	
Post College	60 (33.9%)	112 (30.0%)	77 (28.0%)	16 (18.2%)	
Study site					0.20
Iowa	65 (36.7%)	127 (34.1%)	99 (36.0%)	33 (37.5%)	
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%)	
Lifestyle					
Smoking, pack-years[‡]					0.11
Never	103 (58.2%)	213 (57.1%)	151 (54.9%)	46 (52.3%)	
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/week					**0.009**
Nondrinker	62 (35.0%)	138 (37.0%)	113 (41.1%)	41 (46.6%)	
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%)	

Characteristic	Vitamin D status determined by 25-hydroxyvitamin D (25[OH]D) in nmol/L				p-value[†]
Modified Healthy Eating Index – 2005	63.5 ± 0.6	63.9 ± 0.4	62.0 ± 0.5	59.2 ± 0.9	**<0.0001**
Recreational physical activity, MET, hrs/week					**<0.0001**
0–3	31 (17.5%)	85 (22.9%)	77 (28.1%)	37 (43.5%)	
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%)	
Average ocular visible sun exposure in the last 20 years, Maryland sun-years[‡]	0.82 ± 0.03	0.72 ± 0.02	0.72 ± 0.02	0.67 ± 0.04	**0.006**
Ocular and medical factors					
Iris color (blue)[‡]	74 (41.8%)	150 (40.2%)	124 (45.1%)	33 (37.5%)	0.90
Family history of macular degeneration[‡]	33 (18.6%)	63 (16.9%)	38 (13.8%)	10 (11.4%)	0.06
Body mass index, kg/m^2					**<0.0001**
<22.5	48 (27.1%)	69 (18.5%)	25 (9.1%)	8 (9.1%)	
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 (yes)	42 (23.7%)	85 (22.8%)	66 (24.0%)	31 (35.2%)	0.10
Cardiovascular disease (yes)	37 (20.9%)	72 (19.3%)	67 (24.4%)	17 (19.3%)	0.58
Diabetes (yes)	2 (1.1%)	10 (2.7%)	6 (2.2%)	3 (3.4%)	0.36
Hormone replacement therapy					**0.005**
Never	36 (20.3%)	117 (31.4%)	81 (29.5%)	34 (38.6%)	
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, mg/L	4.63 ± 0.43	4.44 ± 0.29	5.11 ± 0.34	4.80 ± 0.61	0.36

[*] Means and standard errors (SEs) are presented for continuous variables within each vitamin D status category. The n and the percent (%) are presented for categories of categorical variables within each vitamin D status category. Characteristics were assessed at WHI baseline (1994–1998) unless otherwise noted.

[†] 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.

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‡Characteristics assessed at CAREDS baseline (2001–2004).

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Table 2

Age-adjusted odds ratios (OR) and 95% confidence intervals (CIs) for any AMD, assessed at CAREDS baseline (2001–2004), by vitamin D status, assessed at WHI-OS baseline (1994–1998), among a subset of CAREDS participants <75 years of age with serum 25(OH)D concentrations and genetic data (n=913).

	Vitamin D status determined by 25(OH)D, nmol/L			
	Adequate (referent)	**Adequate**	**Inadequate**	**Deficient**
	(75 nmol/L)	**(50 to <75 nmol/L)**	**(30 to <50 nmol/L)**	**(<30 nmol/L)**
No. (%)	177 (19%)	373 (41%)	275 (30%)	88 (10%)
No. Cases AMD	19	59	43	21
Age-Adjusted OR (95% CI)	1.0	1.6 (0.9–2.7)	1.5 (0.8–2.6)	**2.6 (1.3–5.2)**
p-for trend *				**0.01**

* P-for trend calculated using continuous 25(OH)D concentrations.

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Table 3

Prevalence of high risk AMD genotypes and age-adjusted odds ratios (OR) and 95% confidence intervals (CIs) for any AMD assessed at CAREDS baseline (2001–2004) among a subset of CAREDS participants <75 years of age with serum 25(OH)D concentrations and genetic data (n=913)

	Sample < 75 years (n=913)			
	No. (%)	**No. Cases of AMD**	**Age-adjusted OR (95% CI)**	**p-for trend***
***CFH* (rs1061170)**				**0.001**
TT	350 (38%)	36	1.0 (reference)	
CT	429 (47%)	77	**1.9 (1.2–2.9)**	
CC	134 (15%)	29	**2.4 (1.4–4.1)**	
***CFI* (rs10033900)**				**0.04**
GG	227 (25%)	33	1.0 (reference)	
GA	456 (50%)	60	0.9 (0.6, 1.5)	
AA	229 (25%)	49	1.6 (1.0, 2.7)	
***CFB/C2* (rs641153)**				0.76
AA/AG	148 (16%)	22	1.0 (reference)	
GG	765 (84%)	120	1.1 (0.7, 1.8)	
***C3* (rs2230199)**				0.87
CC	37 (4%)	6	1.0 (reference)	
GC	302 (33%)	45	1.0 (0.4, 2.5)	
GG	574 (63%)	91	1.0 (0.4, 2.5)	
***ARMS2* (rs10490924)**				**0.002**
CC	540 (59%)	68	1.0 (reference)	
AC	332 (37%)	64	**1.7 (1.1, 2.4)**	
AA	39 (4%)	10	**2.4 (1.1, 5.1)**	

*p for trend with increasing number of risk alleles.

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Table 4

Adjusted* odds ratios (OR) and 95% confidence intervals (CIs) for any AMD assessed at CAREDS baseline (2001–2004) by vitamin D status and high risk AMD genotype† among CAREDS participants < 75 years of age with serum 25(OH)D concentrations and genetic data (n=913).

	Vitamin D status determined by 25(OH)D, nmol/L						
	Adequate (75)	**Adequate (50 to < 75)**	**Inadequate (30 to <50)**	**Deficient (<30)**			
Genotype	**OR (95% CI) N(AMD)/N(Total)**	**OR (95% CI) N(AMD)/N(Total)**	**OR (95% CI) N(AMD)/N(Total)**	**OR (95% CI) N(AMD)/N(Total)**	**P for trend‡**	**Synergy Index (95% CI)**	**P for interaction**
***CFH* (rs1061170)**							
TT	1.0 (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	0.40	**1.4 (1.1, 1.7)**	0.25
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**	0.35		
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**	**0.02**		
***CFI* (rs10033900)**							
GG	1.0 (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	0.87	**1.4 (1.1, 1.7)**	**0.02**
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**	0.34		
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**	**0.01**		
***ARMS2* (rs10490924)**							
CC	1.0 (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**	0.02	--§	0.09
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**	0.58		
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	0.68		
***ARMS2* (rs10490924)**							
CC	1.0 (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**	**0.02**	--§	0.13
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**	0.90		
***CFB/C2* (rs641153)**							

	Vitamin D status determined by 25(OH)D, nmol/L						
	Adequate (75)	**Adequate (50 to < 75)**	**Inadequate (30 to <50)**	**Deficient (<30)**			
Genotype	**OR (95% CI) N(AMD)/N(Total)**	**OR (95% CI) N(AMD)/N(Total)**	**OR (95% CI) N(AMD)/N(Total)**	**OR (95% CI) N(AMD)/N(Total)**	**P for trend‡**	**Synergy Index (95% CI)**	**P for interaction**
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	0.32	--§	0.48
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	0.16		
C3 (rs2230199)							
CC	1.0 2/9	--§ 0/12	1.9 (0.3, 14.4) 4/12	--§ 0/4	0.61	1.3 (0.8, 2.1)	0.09
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	0.89		
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	0.01		

*Adjusted for age, smoking pack years, iris pigmentation, self-reported cardiovascular disease, self-reported diabetes status, hormone use status

†SNP alleles are shown in order of increasing AMD risk.

‡p for ordinal trend across categorized concentrations of 25(OH)D within each genotype class.

§Synergy Index could not be estimated.

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